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**Variation in fine root traits reveals nutrient-specific acquisition strategies in agroforestry systems**

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24 **Abstract**

25 Aims: Root functional traits are increasingly used to assess nutrient acquisition strategies of  
26 plants across environmental conditions, focusing heavily on root trait expression within complex  
27 communities or along gradients of singular nutrients. In this field study, we investigate intra-root  
28 functional trait expression with six soil macro- and micro-nutrients in *Theobroma cacao*  
29 agroforestry systems.

30 Methods: Using image, chemical, and spatial analysis, fine root distribution, architecture, and  
31 morphology of *T. cacao* were compared to localized soil nutrients on two-dimensional soil  
32 profiles with conspecific and heterospecific neighbours.

33 Results: Fine-scale variation in soil nutrients was observed within the range of *T. cacao* root  
34 systems. Higher  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$  was associated with greater root length and biomass densities,  
35 coupled with greater investment to individual roots, expressed as increased fine root tissue  
36 density and diameter and lower specific root length. Conversely,  $\text{NO}_3^-$  had the opposite effect.  
37 Overall, roots tended towards higher acquisitive trait values when next to a shade tree.

38 Conclusions: Plants generally employ several concomitant and at times opposing strategies for  
39 nutrient acquisition in heterogeneous soils. We show that fine-scale root plasticity is highly  
40 linked to localized nutrient-specific and neighbour-specific effects, driving patterns of nutrient  
41 acquisition in agroforestry systems.

42

43 **Keywords**

44 functional traits; intercropping; nutrient distribution; root foraging; root system; *Theobroma*  
45 *cacao*

46

47 **Abbreviations**

48 A:T = ratio of absorptive to transport fine root length

49 D = average root diameter

50 FRLD = fine root length density

51 FRBD = fine root biomass density

52 RTD = fine root tissue density

53 SRL = specific root length

54

## 55 **Introduction**

56 Agroforestry is a prime example of applied ecology: species combinations are, in  
57 principle, chosen to enhance niche complementarity and/or facilitation, and thus improve  
58 nutrient cycles. The success of these interactions largely depends on differences in plant root  
59 functions and/or spatial distributions that, when compared to monocultures, permit more  
60 complete acquisition of soil nutrients (Brooker et al. 2015; Cardinael et al. 2015a). However,  
61 plant nutrient acquisition patterns are not static, and phenotypic plasticity in root systems, from  
62 whole plant to lateral root scales, can transform belowground interactions with neighbouring  
63 plants (Li et al. 2006; Cahill et al. 2010). The extent of root scale phenotypic plasticity and  
64 subsequent root foraging success is highly species-specific (Blair and Perfecto 2004; Malamy  
65 2005; Chen et al. 2018). Yet, in humid tropical agroforestry systems, where plant nutrient  
66 demand is high and supply is constrained, little is known on root system foraging patterns under  
67 extremely complex conditions.

68 Plants can benefit from localized areas of high nutrient availability in soil, i.e. soil  
69 nutrient hotspots (Chen et al 2018), by modifying their root systems via signalling mechanisms  
70 in roots that encounter elevated concentrations of nutrients (Forde and Lorenzo 2001; Malamy  
71 2005). Although typically at the plant scale there is relatively higher allocation of biomass to  
72 roots in nutrient-poor environments (Wright et al. 2011), in heterogeneous but nutrient-limited  
73 soil environments there is generally greater allocation of root biomass to locations in soil where  
74 nutrients are more abundant (Drew 1975; Hutchings and de Kroon 1994; Hodge 2004).  
75 Additionally, studies on root morphological traits across soil nutrient gradients indicate higher  
76 investment to fine root organs given increased soil nutrients, with construction of longer-lived  
77 roots characterized by thicker diameter (D), higher root tissue density (RTD), and lower specific  
78 root length (SRL) (Ostonen et al. 2007). Alternatively, the reverse has been observed where roots  
79 grow more rapidly with higher turnover to exploit nutrient-rich soil and, thus, show increased  
80 absorptive area per unit of biomass (e.g., higher SRL), while in nutrient-poor soil roots develop  
81 morphologies that limit nutrient losses (e.g., thicker D) (Fort et al. 2016). In sum, there is  
82 evidence that plants generally employ several concomitant and at times opposing strategies to  
83 increase the nutrient acquisition in heterogeneous soils by altering root initiation and growth and  
84 patterns of root morphology. These plastic responses can be nutrient specific, presumably  
85 influenced by the mobility of the nutrient in the soil matrix, the signalling and uptake pathways

86 employed by roots, and the capacity to translocate the nutrient within the plant (Drew 1975; Mou  
87 et al. 1995; López-Bucio et al. 2003; Hodge 2004), and are further contingent on the overall  
88 nutrient status of the plant and localized distribution of nutrients within the range of the root  
89 system (López-Bucio et al. 2003; de Kroon et al. 2009).

90 In agroforestry, trees that are retained from previous forest or are later planted in  
91 agroecosystems can strongly influence the overall nutrient status of soil and crops. Organic  
92 deposits from aboveground sources (e.g., leaf litter) (Xia et al. 2015) and belowground sources  
93 (e.g., root turnover and exudation, and microbial activity) (Mommer et al. 2016) can modify soil  
94 nutrient availability at a range of scales (Jackson and Caldwell 1993; Xia et al. 2015). At the  
95 same time, roots from neighbouring plants generally deplete nutrients in localized areas, and root  
96 development patterns are expected to reflect integrated responses to soil nutrient levels and  
97 competition with neighbours (Cahill et al. 2010; Mommer et al. 2012). Numerous studies that  
98 manipulate soil conditions and neighbour interactions under controlled conditions show dramatic  
99 plasticity of root growth and placement in response to soil nutrients and competitors within  
100 localized patches (Mahall and Callaway 1992; Cahill et al. 2010; Semchenko et al. 2014).  
101 However, little is known on how root traits vary in relation to multiple co-limiting nutrients, nor  
102 on how this variation is expressed within a plant's root system in naturally heterogeneous soil.  
103 Indeed, there is a general lack of empirical evidence for modular plasticity within root systems of  
104 individual plants in field conditions.

105 Plasticity of root systems in agroforestry systems can increase crop access to  
106 heterogeneous nutrient availability in soil but can also mitigate competitive effects from  
107 neighbouring trees (McGrath et al. 2001; Li et al. 2006; Isaac et al. 2014; Cardinael et al. 2015b;  
108 Isaac et al. 2017). This is particularly important when there are few external nutrient inputs,  
109 which is generally the case for the tropical tree crop *Theobroma cacao* L. – the focal species in  
110 our study – that is commonly grown under the canopy of larger heterospecific neighbour trees  
111 (i.e., shade trees) on smallholder farms. While trees with more complementary root distributions  
112 can be preferentially planted with crops (i.e., tree species with deeper rooting profiles), typically  
113 there will be overlap of root systems in upper soil layers where nutrients are most abundant  
114 (Isaac et al. 2014; Borden et al. 2017b). To this end, we sought to capture two-dimensional  
115 distributions of cocoa root systems (rather than vertical zonation only) to account for more

116 nuanced root allocation patterns (e.g., Sudmeyer et al. (2004), Li et al. (2006), and Laclau et al.  
117 (2013)).

118 In this study, we examined fine root functional trait expression and distribution of *T.*  
119 *cacao* in relation to soil nutrients and neighbour roots. We used two-dimensional vertical soil  
120 interfaces situated in three species combinations: at the interface with conspecific neighbours in  
121 monoculture and with two heterospecific neighbouring shade trees of distinctive growth  
122 strategies (early vs. late successional). We hypothesized that within these soil interfaces (i)  
123 higher nutrient availability (characterized by six soil macro- and micro-nutrients) will have more  
124 *T. cacao* roots, in fine root length and biomass density, (ii) and these roots will express root traits  
125 associated with root longevity. Additionally, (iii) systematic patterns of root trait variation with  
126 soil nutrients will be moderated by heterospecific neighbour roots based on differences in  
127 nutrient dynamics among species combinations.

128

## 129 **Materials and methods**

### 130 *Study site and species combinations*

131 The study was carried out in South Formangso, Ashanti Region, Ghana (6°36' N, 0°58'  
132 W) at a cocoa research station managed by the Forestry Research Institute of Ghana. The 2-ha  
133 site is situated on previously secondary forest that was cleared for cultivation and was left to  
134 fallow until the cocoa agroforestry system was established in 2001. *T. cacao* hybrid planting  
135 stock from the Cocoa Research Institute of Ghana was planted at a spacing of 3 × 3 m and, in  
136 agroforestry treatments, shade trees were planted in replacement of *T. cacao* at 12 × 12 m  
137 spacing. No fertilizer had been applied to the research site prior to the study. Soils are Acrisols  
138 with bulk density of  $1.22 \pm 0.02 \text{ g cm}^{-3}$  and soil pH ranging from  $6.2 \pm 0.1$  near the soil surface  
139 to  $4.9 \pm 0.0$  near 60 cm depth. The site is in a moist semi-deciduous forest zone with mean  
140 annual rainfall of 1528 mm and mean annual temperature of 26.2 °C. Sampling was completed in  
141 the on-set of the rainy season, during *T. cacao* flowering and cocoa pod production and, thus,  
142 when nutrient demands were high (van Vliet and Giller 2017).

143 Study *T. cacao* trees (DBH =  $14.6 \pm 1.1$  cm; mean  $\pm$  SE) were selected from pre-  
144 established blocks of species combinations at the site, providing three replications of each  
145 species combination. The two shade tree species used in this study, *Terminalia ivorensis* Chev.  
146 (DBH =  $58.8 \pm 3.8$  cm) and *Entandrophragma angolense* (Welw.) C. DC. (DBH =  $19.9 \pm 1.4$

147 cm), are commonly used in this region to provide upper canopy shade (< 25% shade) for *T.*  
148 *cacao* cultivation. *T. ivorensis* is a fast-growing, early successional tree species and was the  
149 larger of the two heterospecific neighbour species. This species is characterized by many shallow  
150 lateral roots and has been shown to affect fine root length density (FRLD) of *Coffea arabica* L.  
151 (van Kanten et al. 2005) and was assumed to have strong belowground competitive effects due to  
152 high SRL ( $34.7 \pm 9.3 \text{ m g}^{-1}$ ;  $n = 30$ ). Slower-growing, late-successional *E. angolense* is perceived  
153 by farmers to be deeper rooted and had lower SRL ( $29.7 \pm 6.2 \text{ m g}^{-1}$ ;  $n = 30$ ; measured from the  
154 study site; data not shown).

155

#### 156 *T. cacao* and neighbour soil interfaces: sampling on soil trenches

157         Nine soil trenches 1 m wide and at least 60 cm deep were manually excavated (three  
158 trenches per species combination). The exposed soil ‘interfaces’ in the trenches were  
159 perpendicular to transects connecting *T. cacao* with another *T. cacao*, or *T. cacao* with a shade  
160 tree, and located halfway between the trees’ stems (i.e., 1.5 m from each stem) (Fig. 1). The  
161 location and size of the soil interfaces were selected to represent an area occupied by an  
162 individual *T. cacao* root system and with limited root system interactions from non-study *T.*  
163 *cacao* trees (Isaac et al. 2014; Borden et al. 2017a), while sampling scale and intensity was first  
164 assessed from preliminary soil profiles that were tested for soil nutrients (data not shown; Soils  
165 Institute of Ghana, Kumasi, Ghana). In each of the present study’s soil interfaces, 40 soil cores (5  
166 cm diameter; 100 cm<sup>3</sup> volume) were taken horizontally and in a stratified random sampling  
167 scheme. Samples were taken such that the soil core was centred at 2.5, 7.5, 15, 27.5 cm depths  
168 (i.e., y direction) to capture the dominant rooting zone of *T. cacao* (i.e., to 30 cm) and centred at  
169 57.5 cm depth to capture root strategies in deeper soils. This vertical sampling scheme was  
170 repeated every 20 cm intervals (at 0, 20, 40, 60, 80, 100 cm) across the length of the trench (i.e.,  
171 x direction) followed by 10 additional samples taken at random, non-sampled locations in the  
172 soil interface, recorded using an x, y coordinate systems. Thus, in sum, samples were taken from  
173 five depths at six horizontal locations and an additional 10 randomly located on each of the nine  
174 interfaces for a total of 360 samples. In the lab, samples were gently homogenized by hand and  
175 then divided into two approximately equal volumes of soil, with half of each sample (~50 cm<sup>3</sup>)  
176 used for fine root analysis and the other half used for soil chemical analysis. Samples were stored  
177 in polyethylene bags and frozen until further processing.

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*Fine root analysis*

Roots were removed using forceps from soil samples passed through sequential sieving with water. Collected roots were then placed in water to further loosen and remove soil from roots. Fine roots were separated by species through visual inspection using a stereoscopic microscope. *T. cacao* fine roots are distinctly reddish-brown, whereas the shade tree roots were lighter in colour. We removed dead roots, characterized by their lack of turgor, black colouring, and easy separation of stele from cortex. Fine roots ( $\leq 2\text{mm}$ ) were then scanned using a flatbed scanner (STD4800; Regent Instruments Inc., Canada) at 600 dpi. From these images, average fine root diameter, number of root tips, fine root length, and fine root volume (approximated as cylindrical roots) from each core sample were measured using WinRhizo (Reg. 2016a; Regent Instruments, Canada). Fine root dry weights were measured after 48 hours of drying at  $65\text{ }^{\circ}\text{C}$ . These data were used to calculate five root traits that characterized either the root density in each soil sample: fine root length density (FRLD;  $\text{cm cm}^{-3}$ ) and fine root biomass density (FRBD;  $\text{mg cm}^{-3}$ ) of an individual *T. cacao*, or the morphology of the roots in each  $100\text{ cm}^3$  sample: specific root length (SRL;  $\text{m g}^{-1}$ ), root tissue density (RTD = [dry root mass/ fresh root volume];  $\text{mg cm}^{-3}$ ), and average root diameter (D; mm). We also estimated the ratio of the length of absorptive fine roots in relation to the length of fine transport roots (A:T) that were in each  $100\text{ cm}^3$  sampling unit. A:T captures the relative amount of fine root length that is predominantly responsible for nutrient uptake and was calculated using a diameter cut off that captured the majority of the first three orders based on *T. cacao* root data from this site: fine roots of *T. cacao* below a cut-off of 0.50 mm did not exhibit secondary growth and represented  $85.2 \pm 0.07\%$  ( $\pm$  SD;  $n = 30$ ; data not shown) of absorptive (root orders 1 to 3) length (Freschet and Roumet 2017). This diameter cut off for very fine roots (Roumet et al. 2016) was used as root samples are challenging to identify by root order when root topology is lost from sampling a small soil volume. A correction factor of 0.5 for FRLD and FRBD was used for *T. cacao* in monoculture to adjust for assumed presence of two *T. cacao* root systems. Dry weight biomass of shade tree fine roots was used to calculate fine root biomass density of neighbouring shade trees ( $\text{FRBD}_{\text{shade}}$ ;  $\text{mg cm}^{-3}$ ) in each sample.

208 *Soil chemical analysis*

209 From each soil sample, available  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were extracted from field moist soils in  
210 KCl solution, filtered through Fisher P8 filter paper, and measured using a spectrophotometer  
211 flow injection analyzer (QuikChem 8500, Lachat Instruments, USA). The remaining soils from  
212 each sample were air-dried for 2 weeks and sieved through 2 mm mesh. From these soils,  
213 available  $\text{PO}_4^-$  was extracted in a 1:10 soil to Bray's 1 solution, filtered through Fisher P5 filter  
214 paper, and measured using a spectrophotometer. Air-dried soil was further ground in a ball mill  
215 (Retsch Ltd., Germany). From these soils, exchangeable  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  were extracted with  
216 ammonium acetate ( $\text{NH}_4\text{OAc}$ ), filtered through Fisher P8 filter paper, and analyzed using an  
217 atomic absorption spectrometer (AAAnalyst 200, PerkinElmer, USA). Soil chemical analyses were  
218 carried out at the University of Toronto Scarborough, Toronto, Canada.

219

220 *Statistical analysis*

221 All statistical analyses were completed in R (version 3.2.4). We quantified and compared  
222 the *in situ* nutrient conditions within the scale of individual *T. cacao* root systems. The amount of  
223 variation in soil nutrients encountered by individual *T. cacao* root systems in the soil interfaces  
224 was assessed by the range and coefficient of variation (CV) of each soil nutrient. Overall soil  
225 nutrient levels in each species combination were described using the mean values calculated  
226 within 10 cm depth intervals on each interface, and differences of soil nutrient levels among  
227 treatments were tested using ANOVA and when significant this was followed by Tukey HSD.  
228 Next, we compared intra-root system variation of *T. cacao* with different neighbour species.  
229 Systematic variation in the vertical distribution, with data pooled into 10-cm intervals, of *T.*  
230 *cacao* fine root densities and morphology by species combination were assessed using ANOVA.  
231 Two-dimensional visual interpretations of root and soil variables in each 100-cm wide  $\times$  60-cm  
232 deep soil interface were produced using inverse distance weighting on a grid with cells of  $5 \times 5$   
233 cm, approximating soil core diameter, in the 'gstat' package and examples of these interfaces  
234 (one per species combination) were visualized using the 'rasterVis' package.

235 We examined the directional relationships between *T. cacao* fine root distribution,  
236 architectural, and morphological traits with localized soil nutrient availability, focusing on data  
237 within the dominant rooting zone of *T. cacao* (0 to 30 cm depth). To do so, linear mixed models  
238 (LMMs) for each root trait in each species combination were fit with sampling depth assigned as

239 fixed variable and soil interface assigned as a random factor. As the fine roots of mature *T. cacao*  
240 grow as dense root mat in the top soil (Nygren et al. 2013), we assumed that soil cores taken  
241 within the top 30 cm of each interface were independent observations without spatial  
242 autocorrelation after depth was included as a fixed term. All measured soil nutrients were  
243 included in the LMMs as fixed variables to evaluate how a change in availability of each nutrient  
244 within 100 cm<sup>3</sup> soil volumes is related to variation in root traits while accounting for variation of  
245 the other measured soil nutrients under field conditions. For *T. cacao* in mixture, FRBD<sub>shade</sub> was  
246 also included as a fixed variable. To estimate the amount of variation in *T. cacao* root traits  
247 explained by all fixed variables, the ‘fixed effects  $r^2$ ’ was calculated using the ‘r2beta’ function  
248 (with method ‘nsj’) in the ‘r2glmm’ package (Nakagawa and Schielzeth 2013). This procedure  
249 also allowed us to estimate partial  $r^2$  of each fixed variable. For parametric analyses, residuals  
250 were tested for normality using the Shapiro Wilk test. To meet parametric assumptions, root and  
251 soil data were log<sub>10</sub> transformed. The level of significance was at  $p < 0.05$ .

252

## 253 **Results**

### 254 *Soil nutrients: distribution and variation*

255 Within the dominant lateral rooting zone of an individual root system (i.e., to 30 cm  
256 depth) there was large variation in soil nutrients (Table 1). Soil NO<sub>3</sub><sup>-</sup> and K<sup>+</sup> could vary by two  
257 orders of magnitude, showing a large range and large CV, except for soil K<sup>+</sup> in *T. cacao-E.*  
258 *angolense* mixture. There were some particularly high concentrations of soil NO<sub>3</sub><sup>-</sup> in  
259 monoculture (max: 82.0 mg g<sup>-1</sup>), which was concentrated in surface soils (Fig. 2). Soil K<sup>+</sup> was  
260 highest in monoculture, particularly when compared to the *T. cacao-T. ivorensis* mixture (Table  
261 1; Fig. 2). Soil NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> also showed high variability, while soil PO<sub>4</sub><sup>-</sup> was the least  
262 variable with the lowest CV (6 to 47%) (Table 1). Overall, both mixtures had higher soil NH<sub>4</sub><sup>+</sup>  
263 than monoculture and soil PO<sub>4</sub><sup>-</sup> was highest in *T. cacao-T. ivorensis* mixture (Fig. 2). Soil  
264 nutrients generally decreased with depth, although soil K<sup>+</sup> was more evenly distributed vertically  
265 in the soil profiles (Fig. 2).

266

### 267 *T. cacao fine roots: distribution and variation*

268 As with soil nutrients, *T. cacao* vertical distributions of fine roots were concentrated near  
269 the soil surface and decreased with depth (Fig. 3; Fig. 4). Over 90% of *T. cacao* fine roots were

270 in the top 30 cm of soil regardless of neighbour species. *T. cacao* roots next to *E. angolense*  
271 tended to be concentrated in shallow soils with 71% of both fine root length and biomass located  
272 in the top 10 cm of soil (Fig. 4). For *T. cacao* with conspecifics, 67% of fine root length and 64%  
273 of fine root biomass were in surface soil (top 10 cm). When next to *T. ivorensis*, there was 70%  
274 of fine root length but only 59% of fine root biomass in the top 10 cm, with more vertically  
275 dispersed fine root biomass between 10 and 30 cm. Vertically, the two shade tree species also  
276 showed decreasing densities of roots within the top 60 cm of soil, but *T. ivorensis* showed a  
277 higher concentration of fine root biomass in surface soil (top 10 cm) compared to *E. angolense*  
278 that had more evenly distributed fine root biomass within the soil interfaces (Fig. 3; Fig. 4).

279 There were no significant differences in fine root densities for *T. cacao* on the vertical  
280 profile 1.5 m from a *T. cacao* stem (Fig. 3). However, notably in surface soils (0 to 10 cm),  
281 where root densities were highest, FRBD for *T. cacao* next to shade trees was 13 to 19% higher  
282 than in monoculture, though this was not significant:  $1.23 \pm 0.18 \text{ mg cm}^{-3}$  and  $1.30 \pm 0.17$  when  
283 next to *T. ivorensis* and *E. angolense*, respectively, and in monoculture:  $1.09 \pm 0.17 \text{ mg cm}^{-3}$ .  
284 Mean FRLD of individual *T. cacao* trees in surface soils was  $1.85 \pm 0.28$  and  $1.90 \pm 0.29 \text{ cm cm}^{-3}$   
285 <sup>3</sup> when next to *E. angolense* and *T. ivorensis*, respectively, which was 83 to 88% above that from  
286 a *T. cacao* tree in monoculture ( $1.01 \pm 0.28 \text{ cm cm}^{-3}$ ) (Fig. 3). Generally, *T. cacao* fine roots in  
287 monoculture expressed more conservative morphology with significantly lower RTD ( $p = 0.01$ )  
288 than when next to a shade tree (Fig. 3).

289  
290 *T. cacao* fine root distribution and morphology in relation to soil nutrients and shade tree roots

291 Significant directional effects of each nutrient on root traits were consistent regardless of  
292 species combination (Table 2). Soil  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$  had a generally positive effect on *T. cacao*  
293 fine root densities (FRLD and FRBD) and investment at the root scale, expressed as positive  
294 coefficients for D and negative coefficients for SRL and A:T in LMMs (Table 2; Table S1).  
295 However, opposite trends were observed for soil  $\text{NO}_3^-$  and  $\text{K}^+$ , particularly for *T. cacao* in  
296 monoculture and *T. cacao* next to *T. ivorensis*. Soil  $\text{PO}_4^-$  was limited as a predictor variable in  
297 root trait variation with the exception of A:T for *T. cacao* in mixture with *E. angolense*. Soil  
298  $\text{Mg}^{2+}$  generally had a negative effect on localized investment to roots for *T. cacao* in mixture  
299 with *T. ivorensis*, with a significant negative D coefficient ( $p = 0.04$ ) (Table 2; Table S1).

300 Depth, soil nutrients, and FRBD<sub>shade</sub> together explained similar proportion of variation in  
301 FRLD and FRBD of *T. cacao* in monoculture and *T. cacao* in mixture with *E. angolense* (fixed  
302 effects  $r^2 = 0.52$  to  $0.65$ ) as well as FRLD for *T. cacao* in mixture with *T. ivorensis* ( $r^2 = 0.61$ )  
303 (Table 2). However, these same variables were less effective in explaining variation in FRBD of  
304 *T. cacao* next to *T. ivorensis* ( $r^2 = 0.29$ ). In most cases, variation in root densities (FRLD and  
305 FRBD) was better explained by the fixed variables (depth, nutrients, FRBD<sub>shade</sub>) than was the  
306 variation in root architecture (A:T;  $r^2 = 0.07$  to  $0.19$ ) or morphology (SRL and D;  $r^2 = 0.09$  to  
307  $0.22$ ), except for a notably high fixed effects  $r^2$  for RTD ( $r^2 = 0.30$  to  $0.55$ ). For *T. cacao* in  
308 mixture with *E. angolense*, variation in root traits was mainly explained by differences in depth  
309 (partial  $r^2 = 0.08$  to  $0.21$ ) while the effects of localized nutrient variation at similar depths were  
310 weakly related to variation in root traits. In contrast, variation in nutrients were just as, or more  
311 important than depth in explaining variation in root traits of *T. cacao* in monoculture and *T.*  
312 *cacao* in mixture with *T. ivorensis* (Table 2). We did not observe significant effects of FRBD<sub>shade</sub>  
313 on *T. cacao* fine root densities in localized soil volumes, which would indicate root avoidance,  
314 but only non-significant negative coefficients of FRBD<sub>shade</sub> of both shade tree species with *T.*  
315 *cacao* FRBD and FRLD. We did not observe localized impact on fine root morphology, but there  
316 was a marginally significant positive effect observed for SRL with FRBD<sub>shade</sub> of *T. ivorensis* ( $p =$   
317  $0.07$ ) and a marginally significant negative effect for RTD ( $p = 0.07$ ) (Table 2; Table S1).

318

## 319 Discussion

### 320 *Intra-root system foraging strategies for specific nutrients*

321 In tropical ecosystems, tree roots are generally concentrated in the top 30 cm of soil  
322 (Jackson et al. 1996), reflecting rapid uptake of soil nutrients and nutrient deposition by leaf litter  
323 in this upper soil layer. Our study confirmed high densities of *T. cacao* fine roots in the  
324 uppermost mineral soil, which mirrored the vertical patterns in soil nutrient availability. We also  
325 found important soil nutrient variation that occurred laterally within the scale of individual root  
326 systems. Cumulatively, we show that fine roots of *T. cacao* were spatially coupled to  
327 heterogeneously distributed nutrients indicating active modular root development in the foraging  
328 of soil nutrients for this species.

329 Foraging strategies realized through root system architectural and morphological  
330 plasticity can be nutrient-specific (Drew 1975; Hodge 2004). For soil  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$ , our first

331 hypothesis was consistently supported: within the scale of individual root systems, locations with  
332 higher soil nutrient availability were associated with higher density of fine roots (i.e., higher  
333 FRLD and FRBD). This trend was coupled with greater investment to root tissue (expressed as  
334 lower SRL and higher D and RTD), which was in support of our second hypothesis. However,  
335 inconsistent and/or opposite effects were found for soil  $\text{NO}_3^-$ , and to a lesser extent  $\text{K}^+$ , and  $\text{Mg}^{2+}$ :  
336 patterns were generally neutral or, in some cases, higher localized concentrations of these  
337 nutrients in soil were associated with reduced density of roots (lower FRLD and FRBD) and  
338 'less expensive' roots (higher A:T and SRL; lower D and RTD). In the case of the more mobile  
339 soil nutrients:  $\text{NO}_3^-$  and  $\text{Mg}^{2+}$  (Gransee and Fühns 2013), it may be more economical for plants to  
340 increase uptake with short-lived, younger roots (Blair and Perfecto 2004). Additionally,  
341 however, these negative associations between fine root density and nutrients were found when  
342 there was distinctly higher availability of the nutrient compared to other species combinations.  
343 Thus, we speculate over-supply in nutrients favours reduced root allocation; this explanation  
344 seems likely for soil  $\text{K}^+$  in *T. cacao* monocultures.

345         The higher proportion of thinner absorptive root length that was associated with increased  
346 availability of  $\text{PO}_4^-$ , specifically for *T. cacao* when in mixture with *E. angolense*, would permit  
347 higher precision foraging for this relatively immobile nutrient (Hodge 2004; Hinsinger 2011).  
348 Otherwise, however, root trait variation was generally unrelated to localized variation in soil  
349  $\text{PO}_4^-$ . McGrath et al. (2001) reported increased proliferation of fine roots of *T. grandifolium* into  
350 soil cores that were artificially enriched with  $\text{PO}_4^-$ . However,  $\text{PO}_4^-$  gradients under natural  
351 conditions may occur predominantly at smaller scales (e.g., gradients of 1 mm or less within the  
352 rhizosphere) (Hinsinger 2011). As rhizosphere soil was mixed in with bulk soil within 5 cm  
353 diameter soil cores, our sampling design likely limited our ability to detect foraging for this  
354 nutrient, a conclusion also supported by the relatively limited variation in  $\text{PO}_4^-$  in this present  
355 study. More importantly, other root characteristics, which were not measured in our study, may  
356 better capture foraging strategies for, specifically available P, such as root hair abundance or  
357 mycorrhizal associations (Hodge 2004; Chen et al. 2018). More generally, the use of a 2 mm  
358 diameter cut off for measuring fine root SRL, D, and RTD may have obscured some  
359 relationships between the fine roots predominantly responsible for nutrient acquisition and soil  
360 nutrients (Freschet and Roumet 2017). Increased precision in the delineation of absorptive roots,

361 as well as the addition of root hairs and mycorrhizal associations into root functional trait  
362 research is critical for advancing assessment of root-soil patterns.

363

364 *How is root foraging modified by shade trees?*

365 Relationships between soil nutrients and fine root densities of *T. cacao* differed among  
366 species combinations. Likewise, the combination of species resulted in substantially altered  
367 relationships between soil nutrients and root architecture and morphology. Such fine root  
368 responses to localized sources of nutrients are expected to be driven by differential nutrient  
369 demands of the plant (Forde and Lorenzo 2001). Previous research has shown the nutrient status  
370 of *T. cacao* to be modified by interactions with neighbouring shade trees (Isaac et al. 2007). We  
371 found that significant trends between root traits and soil nutrients were most pronounced for *T.*  
372 *cacao* in monoculture, and specifically for N (both  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) and Ca, suggesting these  
373 may be co-limiting nutrients in the sole-cropping system. Patterns differed markedly in soil  
374 interfaces near shade trees. No dominant nutrient emerged for *T. cacao* next to *E. angolense*,  
375 while available  $\text{NH}_4^+$  best explained root patterns for *T. cacao* next to *T. ivorensis*, suggesting a  
376 *T. cacao* response to N limitation within this species combination.

377 Differential tree root distribution and activity can contribute to belowground  
378 complementarity in tree-based agroecosystems (Brooker et al. 2015; Borden et al. 2017b). While  
379 fine roots of *T. cacao* below 60 cm can contribute to improved complementarity and total soil  
380 resource acquisition (Abou Rajab 2018), we focus on the extensive lateral roots of *T. cacao* that  
381 are at highest concentration in the top 30 cm of soil (Nygren et al. 2013; Isaac et al. 2014;  
382 Borden et al. 2017a). Within this dominant rooting zone, previous studies have shown vertical  
383 stratification in *T. cacao* root distribution and activity with neighbouring shade trees (Moser et  
384 al. 2010; Isaac et al. 2014; Abou Rajab et al. 2018). The present study found some evidence that  
385 the species of shade tree controls developmental plasticity in *T. cacao*. We found that *T. cacao*  
386 next to *T. ivorensis* had more evenly distributed fine roots in the upper 30 cm of soil suggesting  
387 greater complementarity belowground, while *T. cacao* roots next to *E. angolense* were more  
388 concentrated near the surface and variation in root traits showed a stronger vertical trend.  
389 Significant effects of FRBD<sub>shade</sub> on localized distribution, architecture, and morphology of *T.*  
390 *cacao* roots were not detected in this study, but it is intriguing and worth noting the marginally  
391 significant directional trends of *T. cacao* root traits in relation to fine root density of the fast-

392 growing *T. ivorensis*, that would suggest *T. cacao* fine roots have more acquisitive root  
393 morphology when in localized competition with roots of *T. ivorensis*. However, to elucidate root-  
394 root responses, more empirical evidence is needed of root trait response when there are higher  
395 densities of neighbouring tree roots to presumably increase the effects of neighbour root activity.  
396 Root-root interactions between conspecific and heterospecific neighbouring plants can be  
397 complex (Mommer et al., 2016) and we speculate that the strength of competitive (e.g., resource  
398 depletion) and facilitative effects (e.g., organic deposits) from root activity at localized scales is  
399 likely to depend on species combination and merits further investigation.

400 In low-input agroforests, nutrient cycling is a significant component of nutrient delivery  
401 and shade tree leaves can constitute a substantial proportion of litterfall in shaded cocoa  
402 agroecosystems (perhaps a third to a half of total litter inputs (van Vliet and Giller 2017)). Leaf  
403 litter from fast-growing species such as *T. ivorensis* is commonly associated with higher rates of  
404 decomposition (Cornwell et al. 2008) and, along with its extensive canopy, is likely an important  
405 determinant of nutrient dynamics and distribution. Belowground, variation in root traits, such as  
406 higher SRL and lower RTD, has been associated with shorter root lifespan and faster root  
407 turnover and decomposition (Freschet and Roumet 2017). In the present study, *T. cacao* fine  
408 roots had lower RTD when in mixture with a shade tree compared to when in monoculture,  
409 suggesting more rapid nutrient cycling. More acquisitive root traits in mixture compared to  
410 monoculture have been reported in other tree-based ecosystems (e.g., Bolte and Villanueva  
411 (2006) and Duan et al. (2017)) and in *T. cacao* specifically (Abou Rajab et al. 2018). The  
412 impacts of farm- and ecosystem-scale processes on the plastic responses of root morphology in  
413 agroforests deserve further research attention, particularly as this will be critical to the precision  
414 of nutrient management on farms.

415

## 416 **Conclusions**

417 Our results support the conclusion that soil nutrient heterogeneity occurs at scales  
418 relevant to individual trees in a tropical low-input agroforest. We carried out one of the first  
419 studies on multiple soil macro- and micro-nutrient effects on root functional trait expression  
420 within a species in naturally heterogeneous soils. Root system phenotypic plasticity was  
421 expressed as variation in the distribution of fine roots (FRLD and FRBD), fine root architecture  
422 (A:T), and morphology (SRL, D, and RTD). By relating root traits to soil nutrient availability on

423 two-dimensional soil interfaces, we found that fine root trait expression had nutrient-specific  
424 relationships at localized scales (100 cm<sup>3</sup>) within the dominant rooting zone of individual *T.*  
425 *cacao*. At the plant scale, intraspecific root traits shifted towards nutrient-acquiring morphology  
426 when next to a shade tree. Taken together, these results indicate that modelling of the fine root  
427 system architecture and nutrient acquisition patterns in agroforests must consider species  
428 interactions to capture the full scope of root trait expression. Measuring drivers of this root trait  
429 variability is critical to improve our understanding of the root-soil continuum in agroforestry  
430 systems and for the development of ecologically-informed agricultural practices.

431

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441

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566 **Tables**

567

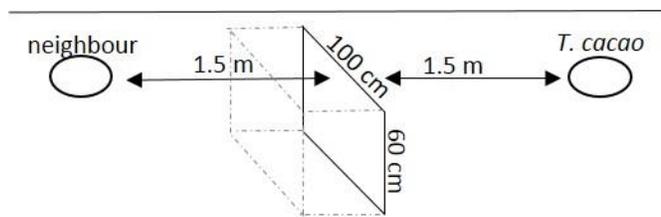
568 **Table 1:** Variation in soil nutrients in the lateral rooting zone (0 to 30 cm depth) of *T. cacao* reported as the minimum and maximum  
 569 and the coefficient of variation (%) of 100 cm<sup>3</sup> samples from nine soil interfaces (*n* = 32 per interface).

Soil interface	NO <sub>3</sub> <sup>-</sup> mg g <sup>-1</sup>	NH <sub>4</sub> <sup>+</sup> mg g <sup>-1</sup>	PO <sub>4</sub> <sup>-</sup> mg g <sup>-1</sup>	K <sup>+</sup> cmol(+) kg <sup>-1</sup>	Ca <sup>2+</sup> cmol(+) kg <sup>-1</sup>	Mg <sup>2+</sup> cmol(+) kg <sup>-1</sup>
<i>T. cacao</i>						
monoculture						
1	0.6 – 43.62 (140%)	4.7 – 100.9 (105%)	7.4 – 31.3 (42%)	0.01 – 0.91 (68%)	1.8 – 22.3 (84%)	0.4 – 3.5 (65%)
2	0.8 – 82.0 (111%)	2.7 – 133.4 (115%)	11.8 – 21.2 (13%)	0.06 – 1.76 (140%)	0.8 – 8.7 (78%)	0.3 – 5.2 (97%)
3	2.2 – 67.5 (97%)	5.8 – 39.9 (53%)	12.6 – 24.1 (15%)	0.02 – 0.40 (34%)	1.2 – 9.0 (60%)	0.3 – 4.0 (72%)
<i>T. cacao-</i>						
<i>E. angolense</i>						
mixture						
1	0.6 – 19.5 (132%)	4.6 – 104.3 (75%)	16.1 – 20.8 (6%)	0.05 – 0.25 (38%)	1.1 – 9.4 (75%)	0.4 – 3.0 (59%)
2	0.3 – 8.6 (127%)	2.6 – 148.2 (72%)	15.6 – 28.1 (14%)	0.07 – 0.45 (54%)	1.3 – 14.9 (89%)	0.5 – 5.5 (100%)
3	0.0 – 15.7 (144%)	9.6 – 102.0 (60%)	12.0 – 16.9 (9%)	0.07 – 0.27 (43%)	3.5 – 34.2 (64%)	0.6 – 3.5 (59%)
<i>T. cacao-</i>						
<i>T. ivorensis</i>						
mixture						
1	1.2 – 53.5 (106%)	12.9 – 178.5 (97%)	6.4 – 24.6 (40%)	0.01 – 0.25 (60%)	0.9 – 7.5 (71%)	0.3 – 2.7 (68%)
2	0.4 – 20.8 (108%)	7.3 – 117.4 (76%)	27.3 – 47.4 (13%)	0.01 – 0.16 (83%)	0.7 – 6.7 (74%)	0.3 – 3.1 (83%)
3	0.2 – 17.7 (256%)	7.2 – 126.9 (72%)	27.9 – 41.3 (11%)	0.04 – 1.91 (172%)	1.2 – 14.5 (76%)	0.4 – 8.2 (115%)

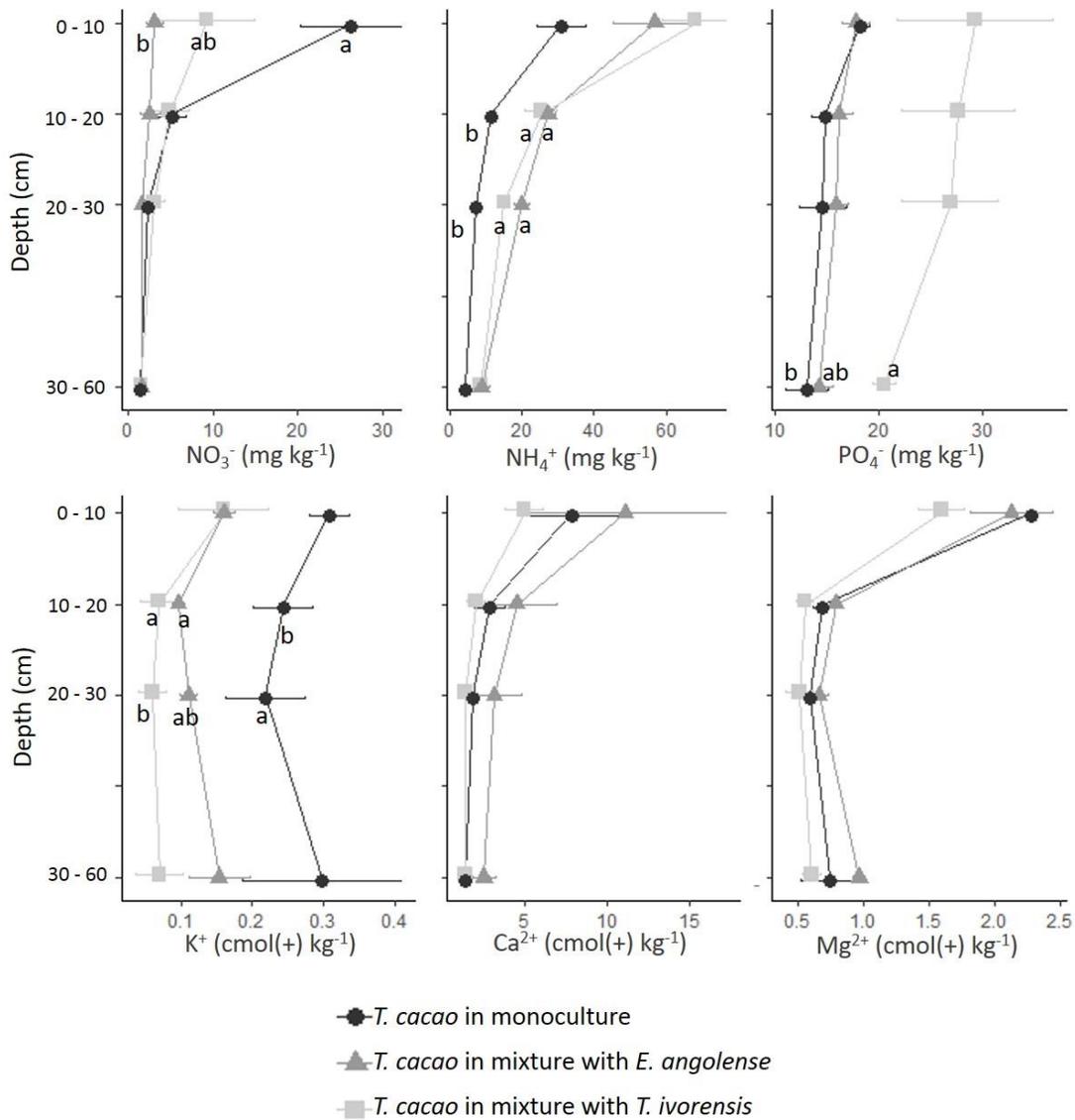
570 **Table 2:** Coefficients from LMMs of *T. cacao* fine root traits with depth and soil nutrients as fixed effects  
571 and soil interface as a random effect. Significant ( $p < 0.05$ ) coefficients are in bold. Partial  $r^2$  are reported in  
572 parentheses. Complete LMM results are reported in Table S1.

root trait	intercept	depth (cm)	logNO <sub>3</sub> <sup>-</sup>	logNH <sub>4</sub> <sup>+</sup>	logPO <sub>4</sub> <sup>-</sup>	logK <sup>+</sup>	logCa <sup>2+</sup>	logMg <sup>2+</sup>	FRBD <sub>shade</sub>	'fixed effects $r^2$ '
<i>T. cacao</i> in monoculture										
logFRLD	-0.43	<b>-0.02</b> (0.06)	-0.16 (0.01)	0.16 (0.01)	-0.26 (0.00)	<b>-0.21</b> (0.03)	<b>0.75</b> (0.08)	0.24 (0.00)	--	0.55
logFRBD	-1.41	<b>-0.03</b> (0.05)	<b>-0.46</b> (0.05)	<b>0.48</b> (0.03)	0.44 (0.01)	-0.22 (0.02)	0.68 (0.05)	0.54 (0.01)	--	0.52
logA:T	1.05	0.02 (0.02)	0.34 (0.02)	-0.36 (0.01)	-0.60 (0.01)	0.12 (0.00)	0.37 (0.01)	-0.60 (0.02)	--	0.14
logSRL	<b>1.82</b>	0.00 (0.00)	<b>0.32</b> (0.05)	<b>-0.39</b> (0.05)	-0.32 (0.01)	0.10 (0.01)	-0.17 (0.01)	-0.29 (0.01)	--	0.22
logD	<b>-0.45</b>	0.00 (0.00)	-0.04 (0.00)	<b>0.14</b> (0.03)	-0.09 (0.00)	0.00 (0.00)	0.11 (0.01)	-0.02 (0.00)	--	0.09
logRTD	<b>-2.13</b>	<b>-0.03</b> (0.04)	<b>-0.48</b> (0.05)	<b>0.48</b> (0.03)	0.44 (0.01)	-0.22 (0.02)	0.73 (0.05)	0.55 (0.02)	--	0.52
<i>T. cacao</i> in mixture with <i>E. angolense</i>										
logFRLD	-2.12	<b>-0.03</b> (0.21)	-0.06 (0.01)	0.06 (0.00)	1.64 (0.03)	0.03 (0.00)	0.38 (0.04)	0.07 (0.00)	-0.09 (0.00)	0.65
logFRBD	-1.62	<b>-0.05</b> (0.21)	0.07 (0.00)	0.13 (0.01)	0.73 (0.00)	-0.39 (0.01)	0.42 (0.03)	0.01 (0.00)	0.05 (0.00)	0.58
logA:T	<b>-6.58</b>	<b>0.03</b> (0.05)	<b>-0.35</b> (0.06)	0.46 (0.04)	<b>4.46</b> (0.06)	-1.13 (0.04)	-0.03 (0.00)	0.76 (0.01)	-0.37 (0.01)	0.19
logSRL	1.03	<b>0.02</b> (0.08)	-0.07 (0.01)	-0.16 (0.02)	0.26 (0.00)	-0.02 (0.00)	-0.09 (0.00)	0.44 (0.02)	-0.15 (0.01)	0.17
logD	-0.14	<b>-0.01</b> (0.19)	0.00 (0.00)	0.01 (0.00)	-0.07 (0.00)	0.06 (0.00)	-0.03 (0.00)	-0.21 (0.02)	0.02 (0.00)	0.21
logRTD	<b>-1.54</b>	-0.04 (0.15)	0.08 (0.01)	0.18 (0.01)	-0.03 (0.00)	-0.07 (0.00)	0.35 (0.02)	0.13 (0.00)	0.05 (0.00)	0.55
<i>T. cacao</i> in mixture with <i>T. ivorensis</i>										
logFRLD	-0.97	<b>-0.02</b> (0.08)	0.04 (0.00)	0.61 (0.05)	-0.24 (0.01)	-0.10 (0.00)	0.71 (0.03)	-0.32 (0.01)	-0.03 (0.00)	0.61
logFRBD	<b>-2.50</b>	-0.01 (0.00)	-0.02 (0.00)	<b>1.00</b> (0.05)	0.13 (0.00)	-0.10 (0.00)	0.78 (0.02)	-0.62 (0.02)	-0.20 (0.02)	0.29
logA:T	1.68	-0.01 (0.00)	0.06 (0.00)	-0.59 (0.02)	-0.09 (0.00)	0.00 (0.00)	-0.14 (0.00)	0.66 (0.02)	0.21 (0.02)	0.07
logSRL	<b>2.56</b>	-0.01 (0.00)	0.07 (0.01)	<b>-0.68</b> (0.04)	-0.23 (0.01)	0.05 (0.00)	0.30 (0.04)	0.30 (0.01)	0.19 (0.03)	0.10
logD	<b>-0.84</b>	0.00 (0.00)	-0.04 (0.01)	<b>0.22</b> (0.04)	0.09 (0.01)	0.01 (0.00)	-0.07 (0.00)	<b>-0.25</b> (0.04)	-0.05 (0.02)	0.12
logRTD	<b>-2.58</b>	-0.02 (0.02)	-0.02 (0.00)	<b>0.86</b> (0.06)	0.01 (0.00)	-0.08 (0.00)	-0.04 (0.00)	-0.04 (0.00)	-0.24 (0.05)	0.30

573

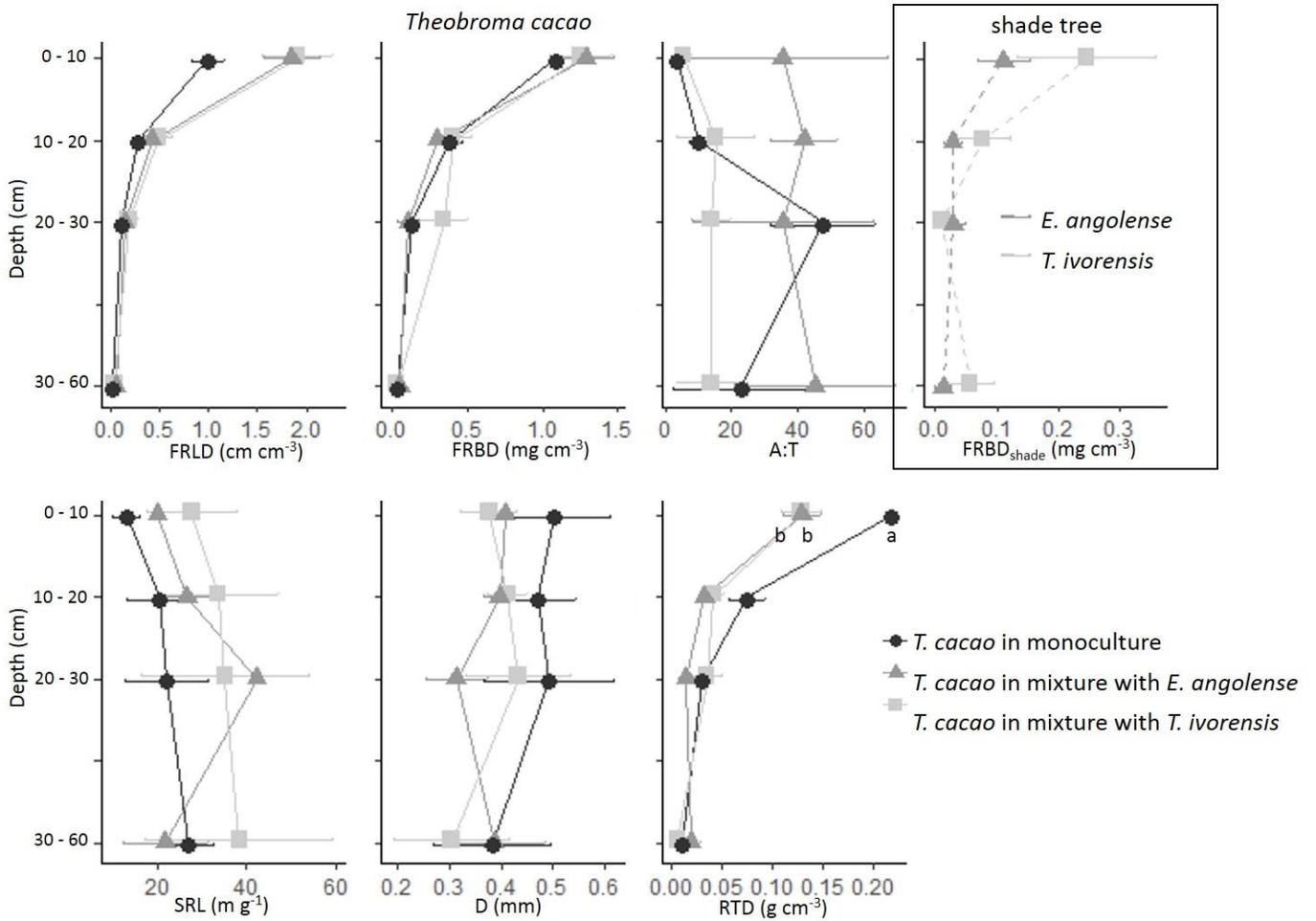


577 **Figure 1:** Soil interfaces ( $n = 9$ ) used in this study. Left panel: Schematic depicting the location of a soil  
578 interface between a *T. cacao* tree and a heterospecific or conspecific neighbour tree. Right panel: An  
579 excavated soil interface situated between a *T. cacao* tree (foreground) and a shade tree *Entandrophragma*  
580 *angolense* (background).



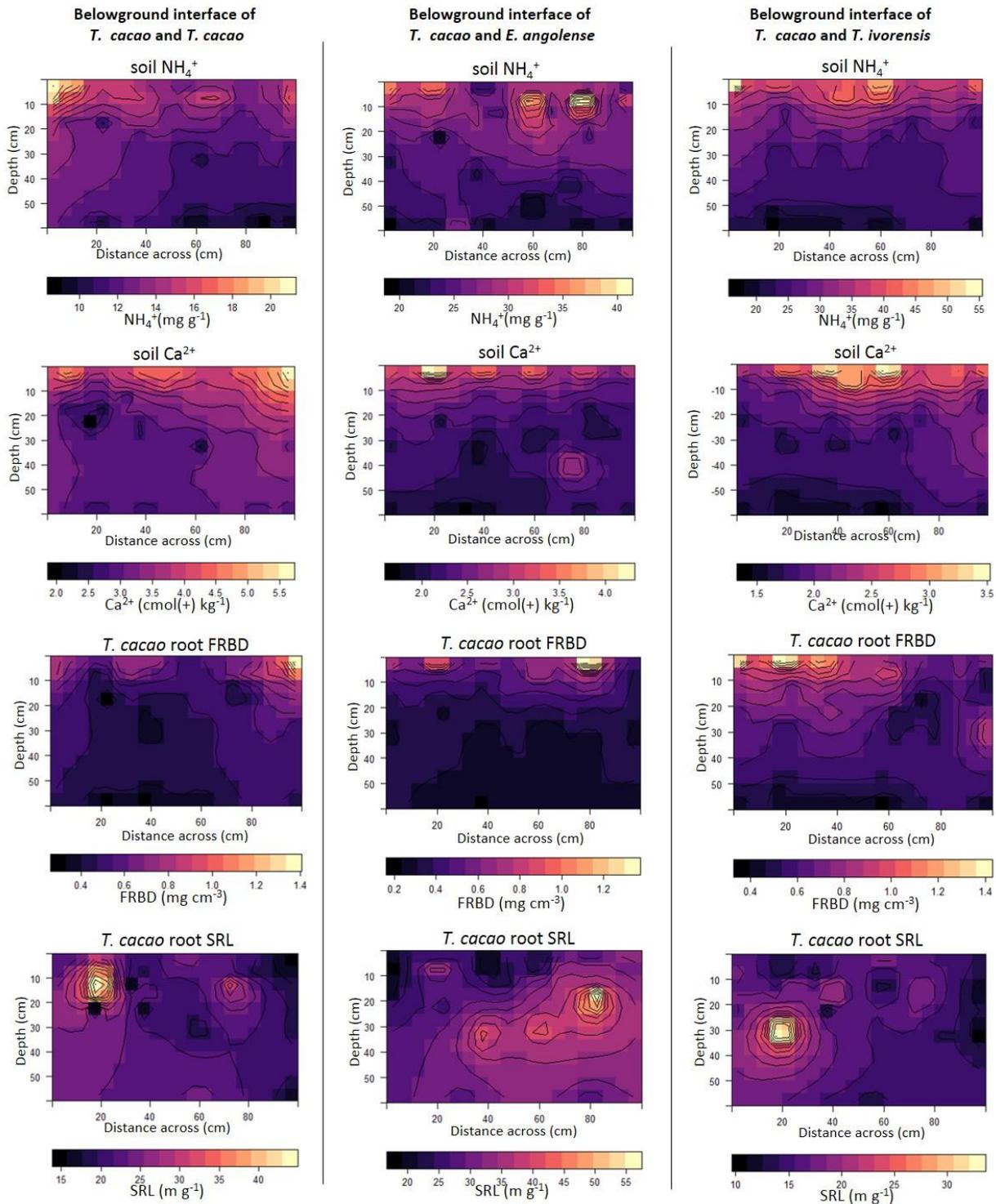
582

583 **Figure 2:** Soil attributes with depth 1.5 m distance from stems (mean  $\pm$  SE;  $n = 3$ ). Same letters are non-  
 584 significant differences for individual *T. cacao* among treatments at same depth when there was a significant  
 585 treatment effect (ANOVA).



586

587 **Figure 3:** Vertical distribution of fine root density (FRLD and FRBD), architecture (A:T), and morphology  
 588 (SRL, D, RTD) of an individual *T. cacao* tree 1.5 m distance from stems (mean  $\pm$  SE,  $n = 3$ ). Also shown is  
 589 vertical distribution of fine root biomass shade trees (FRBD<sub>shade</sub>). Same letters are non-significant  
 590 differences for individual *T. cacao* among treatments at same depth when there was a significant treatment  
 591 effect (ANOVA).



592

593 **Figure 4:** Examples of interpolated interface maps depicting the distribution of soil nutrients (e.g.,  $\text{NH}_4^+$   
 594 and  $\text{Ca}^{2+}$ ), shade tree fine roots (FRBD<sub>shade</sub>), and *T. cacao* fine roots (e.g., FRLD and SRL) in three soil  
 595 interfaces between (on the left) two *T. cacao*, (in the middle) *T. cacao* and *E. angolense*, and (on the right)  
 596 *T. cacao* and *T. ivorensis*.