

This is a post-peer-review, pre-copyedit version of an article published in *Agronomy for Sustainable Development*. The final authenticated version is available online at: <http://dx.doi.org/10.1007/s13593-019-0567-1>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

**Management strategies differentially affect root functional trait expression in cocoa agroforestry systems**

Kira A. Borden<sup>a,b</sup> and Marney E. Isaac<sup>\*a,b</sup>

<sup>a</sup>Department of Geography & Planning, University of Toronto, Canada

<sup>b</sup>Department of Physical & Environmental Sciences and Centre for Critical Development Studies, University of Toronto Scarborough, Canada

\*Corresponding author: [marney.isaac@utoronto.ca](mailto:marney.isaac@utoronto.ca); Department of Physical & Environmental Sciences, University of Toronto Scarborough, Toronto, ON M1C 1A4, Canada

33 **Abstract**

34 In tree-based agroecosystems, environmental heterogeneity can be high and resource acquisition  
35 strategies among individual plants can be expected to vary. This poses a challenge for appropriate nutrient  
36 prescriptions. We used a novel trait-based approach to measure *in-situ* fine root phenotypic response of  
37 cocoa (*Theobroma cacao*) to fertilization. Two levels of NPK fertilizer were applied to cocoa in  
38 agroforestry, with shade trees *Entandrophragma angolense* or *Terminalia ivorensis*, or in monoculture.  
39 Four months following fertilization, cocoa fine roots were extracted from ingrowth cores and analyzed for  
40 a suite of traits positively associated with resource acquisition: fine root production, ratio of absorptive to  
41 transport roots, specific root length, specific root area, specific root tip abundance, and root nitrogen  
42 content, as well as traits positively associated with longer-lived root organs (resource conservative): root  
43 tissue density, average root diameter, and root carbon to nitrogen ratio. In surface soils (0 to 10 cm),  
44 fertilization largely stimulated roots to express resource conservative strategies compared to unfertilized  
45 cocoa roots (up to 70% mean percent difference among all root traits) but with limited measurable  
46 differences in root trait response between the two fertilization levels. In subsurface soils (10 to 20 cm),  
47 however, inconsistent cocoa root responses to fertilization and shade trees suggested increasing  
48 complexity in nutrient acquisition strategies with soil depth. At both depths, we detected coordination  
49 among cocoa root trait variation including a resource acquisition to conservation axis explaining ~45% of  
50 total trait variation, yet an individual cocoa plant's position on these coordinated trait axes was  
51 predominately affected by the shade tree rather than fertilization level. This study provides some of the  
52 first insights into intraspecific root functional trait expression to fertilization in multispecies agriculture.  
53 We show that a trait-based approach can be used to improve diagnostics and prescriptions of nutrient  
54 amendments in agroforestry systems.

55

56 **Keywords**

57 agroforestry; functional traits; Ghana; intraspecific trait variation; nutrient management; root economics  
58 spectrum; root foraging; root traits; *Theobroma cacao*

59

60 **1. Introduction**

61 In tropical agroecosystems, nutrient amendments are often necessary for sustaining crop  
62 production, but nutrients from fertilizers that are not acquired by plants can be a source of non-point  
63 pollution as well as an inefficiency in resource use for a farmer (Vitousek et al. 2009). Therefore, it is  
64 imperative that nutrient management strategies simultaneously support crop productivity and limit  
65 nutrient losses to the wider environment. At the same time, increasing or maintaining higher levels of

66 biodiversity on farms is a critical management strategy employed by farmers to, in part, improve overall  
67 nutrient levels and nutrient cycling efficiencies on farms (Malézieux et al. 2009). However, even in  
68 biodiverse agroecosystems, nutrient amendments can be needed to offset the losses from harvested  
69 material. In tree-based agroecosystems in the tropics – such as in cocoa (*Theobroma cacao* L.)  
70 agroforests, the focus of the present study (Fig. 1) – environmental heterogeneity can be high and, thus,  
71 resource acquisition strategies among individual plants of the same species at the same site can be  
72 expected to vary, which poses a challenge for appropriate nutrient prescriptions.

73         Assessing root functional trait response to various management strategies may serve as important  
74 proxy to plants' capacity to adjust to management interventions, such as fertilization or planned species  
75 diversity. Indeed, systematic intraspecific trait variation in roots has been observed along fertilized  
76 nutrient gradients (Mou et al. 2013; Eissenstat et al. 2015; Wang et al. 2016, 2017; Chen et al. 2017). For  
77 example, plants will construct longer-lived root organs where soil is enriched with nutrients. This trend  
78 can be empirically assessed using functional root traits that are known to be positively related to root  
79 longevity: diameter (D; mm) (Eissenstat et al. 2015; Yan et al. 2017) and the ratio of carbon to nitrogen  
80 (C:N<sub>root</sub>) (Chen et al. 2017), which are root traits typically defined as resource conserving. At the same  
81 time, greater investment to fine root tissues also results in a decrease in root trait values typically defined  
82 as resource acquiring. These traits are generally positively related with nutrient uptake rates and root  
83 turnover: specific root length (SRL; m g<sup>-1</sup>), specific root tip abundance (SRTA; tips g<sup>-1</sup> or comparable trait  
84 describing branching intensity) (Ostonen et al. 2007; Eissenstat et al. 2015; Chen et al. 2017), as well as  
85 the nitrogen content of roots (N<sub>root</sub>), which is associated metabolic processes in the root (i.e., nutrient  
86 uptake) (Freschet and Roumet 2017). Other studies, however, have shown opposite trends with soil  
87 resource availability (Fort et al. 2016; Bordron et al. 2018) and newly emerging research has revealed  
88 functional specialization of roots for acquiring specific nutrients of different mobilities at different depths  
89 (Bordron et al. 2018). Under naturally heterogeneous soil nutrient environments, cocoa intra-root system  
90 trait variation in relation to soil nutrients has been shown to be nutrient specific (Borden et al. in press)  
91 but also dependent on interactions with con- or hetero-specific neighbor trees (Isaac et al. 2014), thus  
92 leading to further questions on the interactive nature of soil amendments and species combination on root  
93 trait expression in tree-based agroecosystems.

94         Inherent genotypic constraints in root phenotypic plasticity will limit root response to nutrients  
95 and consequently the extent and direction of root trait response to increased nutrient availability  
96 (Eissenstat et al. 2015). At these phenotypic limits, the capacity for plants to match uptake of nutrients  
97 with increasingly elevated nutrients in soil is expected to be constrained. Furthermore, it is unlikely that  
98 these root trait responses occur independently from one another and, thus, understanding the covariation

99 among multiple traits measured on the same individual may describe critical trade-offs in plant growth  
100 and construction. An illustrative example is the ‘root economics spectrum’, which places individuals that  
101 express more resource conserving strategies opposite to individuals that express more resource acquiring  
102 strategies (Isaac et al. 2017). Analyses of root traits and coordinated root trait variation have characterized  
103 resource acquisition strategies across soil nutrient availability gradients (Weemstra et al. 2017) and,  
104 importantly, trade-offs in resource acquiring versus conserving strategies have been reported among  
105 individuals of the same species (e.g., in *Cunninghamia lanceolata* (Wang et al. 2016) and in a crop  
106 species *Coffea arabica* (Isaac et al. 2017)). However, additional spectra or dimensions of coordinated trait  
107 variation in roots have also been found (Kong et al. 2014; Prieto et al. 2015; Liese et al. 2017) likely due  
108 to the complex role of roots in soil, which can vary, for example, with soil depth (Yan et al. 2017;  
109 Bordron et al. 2018) and with interactions with neighboring plants (Isaac et al. 2017).

110 Farm-scale heterogeneity can be largely controlled by management, such as species composition,  
111 which in turn can affect how roots respond to fertilization. For example, McGrath et al. (2001) reported  
112 that roots of *Theobroma grandiflorum* cultivated on P-limited soils in the Amazon preferentially grew in  
113 P-fertilized soil but that this growth response was affected by heterospecific neighboring trees. Wang et  
114 al. (2016) reported that coordinated root strategies in *C. lanceolata* were modified by nutrient  
115 amendments as well as thinning and pruning practices. However, other than root placement and growth, it  
116 is unknown how combined management effects of fertilization and species composition can modify root  
117 intraspecific trait variation in root morphology and chemical traits, particularly in the humid tropics, nor  
118 how these effects are reflected in coordinated resource acquisition strategies.

119 Our study has the following objectives: (1) to determine the extent and direction of intraspecific  
120 root trait response to fertilization, and (2) to assess if interspecific interactions (i.e. species combination)  
121 are important in modifying intraspecific root trait response. We also aim to determine (3) the overall  
122 resource acquisition strategies among individual cocoa at the same site assessed by coordinated root trait  
123 spectra and (4) the importance of management (species combination and fertilization) in controlling the  
124 expression of these strategies. We hypothesize that fine roots will preferentially grow into fertilized soil  
125 and that these fine roots will have more conservative morphology, but the extent of trait and coordinated  
126 trait responses will be modified by species combination due interspecific effects on nutrient demands. We  
127 carried out a manipulative fertilization experiment to measure root traits on similar-aged roots in a  
128 tropical agroforest featuring same-age and same-genotype stand of cocoa. The roots collected in this  
129 study environment were assumed to be responding directly to fertilization, but within limits of the studied  
130 genotype and environmental conditions invoked by species combination.

131

## 132 2. Materials and methods

### 133 2.1 Site description

134 This study was conducted on a research site (6°36'37"N 0°58'10"W) consisting of even-aged, 15-  
135 year-old cocoa (diameter at breast height (DBH) =  $12.4 \pm 2.8$  cm; height =  $6.1 \pm 1.1$  m) at a density of  
136 1,111 trees ha<sup>-1</sup> in monoculture or interspersed with 15-year-old shade trees that are commonly selected  
137 by farmers in the region: *T. ivorensis* (as fast-growing pioneer species) (DBH =  $58.8 \pm 3.8$  cm; mean  $\pm$   
138 SE) and *E. angolense* (a slower growing hardwood species) (DBH =  $19.9 \pm 1.4$  cm) that were planted at a  
139 density of 68 trees ha<sup>-1</sup>. The site consists of Acrisol soils with a sandy clay loam texture and a bulk  
140 density of  $1.22 \pm 0.16$  g cm<sup>-3</sup> ( $\pm$  SD;  $n = 9$ ) between 0 and 10 cm and  $1.47 \pm 0.14$  g cm<sup>-3</sup> between 10 and  
141 20 cm soil depth, determined using a metal corer of known volume and calculating the soil moisture  
142 content of soils after oven drying for 48 hours at 105 °C. The experiment occurred over the latter half of  
143 the rainy season (July through October), which coincided with the second peak of cocoa pod production,  
144 and roots were collected prior to the dry season. No fertilizer had been applied at the site prior to the  
145 experiment.

146

### 147 2.2 Experimental design

148 The experiment was established as a split-plot design with triplicate replication for each treatment  
149 combination. The main plot factor was cocoa in three species combinations: cocoa in monoculture, cocoa  
150 in mixture with *E. angolense*, and cocoa in mixture with *T. ivorensis*. Within each species combination,  
151 fertilizer was applied at two levels plus control plots with no fertilizer added. Thus, in total, twenty-seven  
152 cocoa trees were selected with the requirement that trees appeared healthy and structurally representative  
153 for trees at the site. To measure cocoa root response to mineral nutrients, opposed to an overall tree  
154 nutrient status response from widespread fertilization, fertilization plots of dimensions 2 × 1 m were  
155 established between the selected cocoa and neighbor tree, with center of each plot located 1.5 m from the  
156 study tree and neighbor tree and oriented perpendicularly to the transect between the stems. After  
157 installing root ingrowth cores (see section 2.3), multi-nutrient fertilizer (15-15-15 NPK granular fertilizer;  
158 15% N (6.5% NO<sub>3</sub><sup>-</sup>-N and 8.5% NH<sub>4</sub><sup>+</sup>-N) + 15% P<sub>2</sub>O<sub>5</sub> + 15% K<sub>2</sub>O + 2% MgO + 0.1% Zn) was broadcast  
159 applied at i) moderate fertilization (187.5 kg ha<sup>-1</sup>) or ii) high fertilization (375 kg ha<sup>-1</sup>). Fertilizer levels  
160 were characterized by recommended fertilizer dosages for this region (Isaac et al. 2007; van Vliet and  
161 Giller 2017). High fertilizer delivered an influx of nutrients representing 49, 51, 14, and 2% increases  
162 above available N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>), available P, exchangeable K, and exchangeable Mg in native soil,  
163 respectively (based on soil nutrient availability measured near the time of fertilization). Plots of the same  
164 dimensions were established in each of the three species combinations to measure fine roots that grew in

165 native soil (i.e., trees without fertilization plots). Surface roots (0 to 10 cm depth) and subsurface roots  
166 (10 to 20 cm depth) were analyzed separately due to expected differences in surface applied-fertilizer  
167 with depth, driven by nutrient mobilities in soil and nutrient interception, particularly by roots in the top  
168 10 cm of soil where cocoa fine root density is distinctly highest (Isaac et al. 2014; Borden et al. in press).

169

### 170 *2.3 Root ingrowth cores*

171 In each fertilization plot, two ingrowth cores (mesh size 2 mm) were deployed prior to application  
172 of fertilizer. Soil and roots were removed using a 7-cm diameter auger, incrementally to avoid  
173 compaction of soil, to 20 cm depth. Roots were removed, and then root-free soil was replaced into  
174 ingrowth bags that lined the soil cores. Soil was replaced by depth increments to emulate initial soil  
175 conditions. Leaf litter was removed for fertilizer application but replaced for the duration of the  
176 experiment. After four months, ingrowth cores were removed by digging into soil around the bags and  
177 cutting roots at the mesh interface. Samples were stored in polyethylene bags and frozen until processing.  
178 Samples were divided into depth intervals of 0 to 10 cm (for ‘surface’ roots) and 10 to 20 cm (for  
179 ‘subsurface’ roots).

180

### 181 *2.4 Fine root traits*

182 Fine roots (< 2 mm diameter) of cocoa were extracted from soil samples with forceps after  
183 handwashing over a sieve. These roots were then placed in petri dish of reverse osmosis water to loosen  
184 and remove soil particles from roots. Cocoa roots were visually identified by color, texture, and  
185 morphology using a stereoscopic microscope. Non-cocoa roots (i.e., shade tree roots and weed roots) had  
186 distinctive visual characteristics, particularly third order and higher fine roots which were used to trace to  
187 lower order roots, and led to high confidence in identifying cocoa roots. We identified and removed any  
188 non-cocoa roots from our samples and subsequent analysis in order to focus on intraspecific root trait  
189 variation of, exclusively, cocoa, which we assumed to originate from the individual cocoa tree adjacent to  
190 each fertilization plot. In monoculture, we assumed that fine roots in ingrowth cores located between two  
191 cocoa trees captured fine roots of two cocoa trees equally and thus we applied a correction factor of 0.5 to  
192 fine root biomass to derive comparable values on a tree basis across the species combination treatments.  
193 Cleaned roots were scanned using a flatbed scanner (STD4800; Regent Instruments Inc., Canada) at 800  
194 dpi. Images were analyzed in WinRhizo (2009; Regent Instruments, Canada) to quantify fine root length,  
195 average diameter, and number of tips. Dry weight biomass was determined after 48 hours of drying at 65  
196 °C. Carbon and nitrogen contents of roots were determined using combustion analysis on a CN analyzer  
197 (C:N 628, LECO Instruments, Canada). From these data we could calculate fine root production (FRP;

198 mg cm<sup>-3</sup> 4-mo<sup>-1</sup>), specific root length (SRL; m g<sup>-1</sup>), specific root area (SRA; cm<sup>2</sup> g<sup>-1</sup>), specific root tip  
199 abundance (STRA; tips g<sup>-1</sup>), and the nitrogen content in root tissues (N<sub>root</sub>) to capture root traits positively  
200 related with soil resource acquisition.. We also calculated the ratio of absorptive root length to transport  
201 root length (A:T) (i.e., relative amount of ephemeral fine roots that are predominantly responsible for  
202 nutrient uptake) by using a diameter cut off that captured most of the first three orders (Roumet et al.  
203 2016) based on cocoa root data from this site: fine roots of cocoa below a cut-off of 0.50 mm generally  
204 did not exhibit secondary growth and represented 85.2 ± 0.07% (± SD; n = 30) of absorptive (root orders  
205 one to three) length. To characterize conservative acquisition strategies that are positively related with  
206 root longevity, we calculated root tissue density (RTD; g cm<sup>-3</sup>), average fine root diameter (D; mm), and  
207 the carbon to nitrogen ratio (C:N<sub>root</sub>). Traits were selected *a priori* based on previously described patterns  
208 in root resource acquisition strategies (Freschet and Roumet 2017).

209

## 210 2.5 Statistical analysis

211 All statistical analyses were performed in R v. 3.2.4 (R Foundation for Statistical Computing,  
212 Austria). The trait values of roots removed from ingrowth cores in the same fertilization plot were  
213 averaged and used in all subsequent analyses: i.e., trait values representative of an individual cocoa.  
214 Analyses were completed separately for roots sampled from the two depth intervals (i.e., surface and  
215 subsurface roots). To test for a response of fine roots to nutrient additions, we ran unpaired two-sample *t*-  
216 tests of the fine root trait values of cocoa in fertilization plots compared to non-fertilized plots. The trait  
217 response was quantified as the percent difference between root trait values in the fertilization plots from  
218 mean trait values of the non-fertilized plots (similar to the response ratio calculated in Ostonen et al.  
219 (2007)). To describe coordinated root trait variation (i.e., resource acquisition strategies), principal  
220 component analysis (PCA) in the ‘*ade4*’ package was used to identify axes that best represent the  
221 combined variance of all measured root traits of individual cocoa. All variables were centered and scaled  
222 to unit variance prior to analysis. Principle component scores were used to evaluate the extent to which  
223 management (i.e., species combination and fertilization) influenced the relative position of individual  
224 cocoa on the dominant axes. Two-way ANOVA was used to test if there were differences in the overall  
225 position of cocoa on these axes among species combination, fertilization level, or if there were interaction  
226 effects. Prior to parametric tests, data were tested for normality of residuals using the Shapiro-Wilk test,  
227 and equality of variance among groups was tested using the Bartlett test. Root trait data were log<sub>10</sub>  
228 transformed when required to improve residual normality and reduce heteroscedasticity. The level of  
229 significance was  $p < 0.05$  and marginal significant response considered at  $p < 0.1$ .

230

### 231 3. Results and discussion

#### 232 3.1 Extent and direction of intraspecific root trait shifts following fertilization

233 More cocoa fine roots grew into fertilized plots within a resource constrained agroecosystem (Fig.  
234 2): FRP in the top 10 cm was  $24.3 \pm 4.73 \text{ g m}^{-2} \text{ 4-mo}^{-1}$  (mean  $\pm$  S.E.) with no fertilization and  $33.4 \pm 6.7$   
235 and  $34.5 \pm 5.2 \text{ g m}^{-2} \text{ 4-mo}^{-1}$  after moderate and high fertilization, respectively. At the same time, newly  
236 grown surface roots had more conservative morphology in fertilized plots. Specifically, fertilization  
237 generally lowered acquisitive trait values: A:T, SRTA, SRL, and SRA, and stimulated conservative trait  
238 values: D and C:N<sub>root</sub> in surface roots (Fig. 2). Directional trends in root morphological variation in this  
239 experiment are similar to those reported in long-term field fertilization and controlled laboratory  
240 experiments (Ostonen et al. 2007; Kramer-Walter and Laughlin 2017). These findings support our first  
241 hypothesis and suggests that where nutrients become more abundant, plants will invest in longer-lived  
242 root tissue. Overall, trait shifts in C:N<sub>root</sub> were minimal but positive (0 to 10% increase) as hypothesized.  
243 There were also small but inconsistent trait shifts in N<sub>root</sub> and RTD (Fig. 2). The magnitude in shifts of  
244 root trait values was most pronounced for FRP, A:T, and SRTA, which parallel results from other studies  
245 that show architectural traits and root branching to be highly responsive to soil environment (Kong et al.  
246 2014; Liese et al. 2017).

247 Observed trait shifts in surface roots within each species combination were generally consistent  
248 regardless of fertilization level. Shifts in root trait values in response to increased soil nutrients are  
249 presumably nonlinear and limited in extent. Wang et al. (2017) reported a hump-shaped curve of root  
250 morphological and hydraulic trait values in *Pinus tablifformus* seedlings in response to a gradient of N  
251 levels. Similarly, Einsmann et al. (1999) found rooting densities across multiple species to peak at  
252 intermediate fertilization levels and be suppressed at high levels. Therefore, the fertilizer dosages used in  
253 this study may characterize a limit of root trait response for cocoa in this environment. With a larger pulse  
254 of nutrients, the potential gains from variation in morphology to acquire nutrients could be less  
255 economical for the plant. Based on pre-experimental measurements of root length density, cocoa fine  
256 roots had reached 46% in surface (0 to 10 cm) and 100% in subsurface (10 to 20 cm) root length density  
257 at the end of the four-month experiment (surface: 0.73 of 1.59 cm cm<sup>-3</sup>; subsurface: 0.41 of 0.40 cm cm<sup>-3</sup>).  
258 Over a longer period of time, more fine roots would be expected to grow into surface soils and increase  
259 root access to soil nutrients. However, given our root trait findings in these agroforests, when fertilizer  
260 additions are beyond the nutrient demands of the crop and/or the capacity of the crop roots to acquire  
261 those nutrients, there is likely to be a reduction in overall nutrient use efficiency and a potential risk of  
262 nutrient loss from the farm. Tracking root trait response to nutrient additions in agroforestry systems can  
263 enhance farm-level diagnostics and nutrient regime prescriptions for nutrient efficient agroforests. As we

264 show in this study, assessing short term interactive effects on crop performance via functional trait  
265 expression and coordinated trait variation provides critical insights into plant-soil environment feedbacks.  
266 When baseline soil nutrient levels are elevated, such as in high-input cultivation systems, differential  
267 response of roots to patches of fertilizer may occur when investment to longer-lived root organs is no  
268 longer efficient for the crop. Undoubtedly, applying this trait-based approach to assess long-term  
269 interactive effects, particularly on farms with repeated fertilization and with strong intra- and inter-annual  
270 variation (Muñoz and Beer 2001; van Vliet and Giller 2017), will also elucidate key mechanisms  
271 underpinning crop performance and therefore requires further extended studies.

272 Structural and functional complexity in tree-based agroecosystems can influence resource  
273 availability. We found that cocoa fine root response to fertilization was mediated by species combination.  
274 For surface roots of cocoa in monoculture, the hypothesized shifts in root trait values were generally  
275 observed. Notably, cocoa roots had significantly or marginally significantly lower SRA, SRL, and SRTA  
276 after high fertilization, with 20, 28, and 43% decreases in these trait values, respectively (Fig. 2). Shifts in  
277 root trait values for cocoa in mixture with *T. ivorensis* also support our hypothesis, but only for chemical  
278 traits were these significant: C:N<sub>root</sub> showed a marginally significant increase of 5% under high  
279 fertilization and a marginally significant decrease of 9% in N<sub>root</sub> after moderate fertilization (Fig. 2). There  
280 were no significant shifts in trait values observed for surface roots of cocoa in mixture with *E. angolense*.  
281 However, the direction of percent changes in trait values did follow our hypothesized response to  
282 fertilization, including a 25% or greater decrease in mean trait values of A:T, SRTA, SRL, and  
283 corresponding increase in D (except D at moderate fertilization, which increased by 14%) with a large  
284 increase in FRP (62 and 157% increases after moderate and high fertilization, respectively) (Fig. 2). We  
285 speculate that cocoa in mixture with *E. angolense* was not as constrained by nutrient limitation(s)  
286 compared to cocoa in the other species combinations. This assertion was also suggested in another study  
287 at the same site, where cocoa root traits in mixture with *E. angolense* also exhibited limited relationships  
288 with nutrients under field conditions (Borden et al. in press).

289 More consistent trait shifts occurred in surface roots compared to subsurface roots, which  
290 presumably was, in part, due to more direct nutrient interception by shallower roots. Given that fertilizers  
291 are typically applied to the surface of soils, a pronounced effect in roots nearest the surface is expected,  
292 especially with shallow rooted species such as cocoa. However, the response pattern in subsurface roots is  
293 intriguing as trait shifts were directionally inconsistent between fertilization levels as well as being largely  
294 dependent on neighbor species. In subsurface roots, the expected shifts in trait values were observed for  
295 cocoa in mixture with *E. angolense* but only following high fertilization, and only significantly for A:T  
296 (40% decrease) (Fig. 2). A similar pattern emerged for cocoa in monoculture but only at moderate

297 fertilization with a significant 14% increase in D, while after high fertilization the directions of trait value  
298 shifts were reversed towards higher acquisitive trait values, with a significantly 13% higher  $N_{\text{root}}$ . The  
299 largest trait response was in the substantial increase in FRP of subsurface roots of cocoa in mixture with  
300 *T. ivorensis* after moderate fertilization (242% increase). Following high fertilization, there was also large  
301 positive shifts in acquisition traits for cocoa subsurface roots when in mixture with *T. ivorensis*, where  
302 SRL increased by 42%, SRTA increased by 105%, and A:T by over 214% although these were not  
303 significant (Fig. 2). Findings from our study point to differential interactions with neighboring trees and  
304 nutrient availability with depth. Notably, acquisition traits of subsurface roots of cocoa in mixture with *T.*  
305 *ivorensis* increased dramatically following fertilization. This may indicate where root trait plasticity may  
306 be advantageous for cocoa when next to a shallow-rooted pioneer species. We found few shade tree roots  
307 after a four-month ingrowth experiment ( $\leq 0.01 \text{ cm cm}^{-3}$  in all fertilization treatments for both shade tree  
308 species) and thus the present study is limited to evaluate direct effects of shade root activity. However,  
309 previous studies have shown cocoa to have a plastic response to *T. ivorensis* within the top 30 cm of soil  
310 (Isaac et al. 2014). Some large shifts in trait values in subsurface roots countered the hypothesized  
311 response. In these cases, cocoa roots may be demonstrating a nutrient-specific response to more mobile  
312 nutrients such as  $\text{NO}_3^-$  (presumably from increased nutrient leaching) through preference for root traits  
313 that lead to faster nutrient uptake (i.e., acquisitive root traits). Little is known on ion-specific root  
314 functional trait response but, in another study, we observed divergent root trait variation in relation to the  
315 form of soil N; soil  $\text{NO}_3^-$  induced acquisitive root trait expression and soil  $\text{NH}_4^+$  induced conservative root  
316 trait expression (Borden et al. in press). In the present study, we were interested in the overall root  
317 response to NPK fertilizer, containing both forms of N, and consequently we cannot tease out nutrient- or  
318 ion-specific responses. Characterization of nutrient-specific relationships with root trait expression is an  
319 important avenue for future studies.

320

### 321 *3.2 Coordinated resource acquisition strategies in a multispecies agroecosystem*

322 In an economically important tree crop, cocoa, at the same site, we found the expected root trait  
323 trade-off, known as the root economics spectrum (Liese et al. 2017; Isaac et al. 2017). The dominant  
324 coordinate trait axis (PC1) that captures this trade-off, cocoa with root traits associated with greater  
325 resource uptake given lower biomass investment (i.e., acquisitive traits) were aligned in opposition to  
326 cocoa expression root traits that are associated with higher investment into longer-lived root tissues (i.e.,  
327 conservative traits). Specifically, this trade-off shows individual cocoa with higher specific root length,  
328 specific root tip abundance, and the ratio of absorptive to transport roots aligning in opposition to cocoa  
329 with thicker diameter, and this was observed in roots at both depths (Fig. 3). Although specific root area

330 was uncoordinated with PC1 in surface roots (ANOVA;  $p = 0.54$ ; Table 1) it was aligned with acquisitive  
331 traits on the same axis in subsurface roots ( $p < 0.01$ ). The first axis explained over 40% of fine root trait  
332 variation among individuals of cocoa regardless of depth interval. Recent research of *Coffea arabica*  
333 suggests that coordinated leaf trait variability may be weakened by fertilization (Martin et al. 2016), but  
334 this has not been tested for roots and very little is known on coordinated root traits among individuals of  
335 the same species at local scales (Isaac et al. 2017). In the present study, cocoa were grown in tropical  
336 nutrient-limited soils that had not been previously fertilized and, thus, local nutrient heterogeneity and  
337 subsequent variation in resource acquisition strategies can be expected.

338 Tree species composition will affect the quantity and quality of inputs (e.g., litter fall, cocoa  
339 husks residues, root inputs), which results in a complex delivery of bioavailable nutrients in the soil,  
340 resulting in altered nutrient status in plants (Isaac et al. 2007) and soil and in turn, the root response to  
341 nutrient influx is expected to vary. Indeed, we found that neighbor species controlled coordinated root  
342 trait syndromes in cocoa, suggesting that the abiotic and biotic conditions invoked by different species of  
343 trees can lead to variation in the overall resource acquisition strategies of cocoa. Overall, species  
344 composition, as compared to fertilization, more strongly controlled coordinated trait variation for cocoa  
345 and there were no significant interactive effects of species combination  $\times$  fertilization level on  
346 coordinated root trait syndromes at either depth (Table 2). In surface roots, cocoa next to shade trees  
347 tended towards higher PC1 values (more conservative on the root economics spectrum) compared to  
348 cocoa in monoculture, but this was non-significant ( $p = 0.286$ ) (Table 2; Fig. 3). Similarly, in surface  
349 roots, cocoa fine roots in fertilized plots tended to express more conservative strategies than fine roots in  
350 the non-fertilized plots, but this was non-significant (PC1 axis scores;  $p = 0.153$ ). In subsurface roots,  
351 there was a marginally significant species combination effect on PC1 ( $p = 0.053$ ) (Table 2), with  
352 subsurface roots of cocoa in mixture with *T. ivorensis* significantly higher than cocoa subsurface roots in  
353 mixture with *E. angolense* ( $p = 0.044$ ). Similarly, location of individual *C. arabica* along a root  
354 economics spectrum was reported to be partially explained by shade tree management practices (Isaac et  
355 al. 2017).

356 Unlike in leaf traits which often coordinate on a dominate leaf economics spectrum (including in  
357 crop plants (Martin et al. 2016; Isaac et al. 2017)), it is increasingly recognized that root resource  
358 acquisition strategies are multidimensional. In the present study, a second axis (PC2) showed a trade-off  
359 of cocoa with dense, N-rich roots in opposition to cocoa with less dense roots of higher C:N<sub>root</sub>. This  
360 secondary axis explained close to 30% of total variation (Fig. 3; Table 1). Interestingly, coordinated root  
361 trait syndromes described by PC2 were more influenced by management than the root economics  
362 spectrum described by PC1, which suggests an important trade-off in the chemical and structural

363 construction of fine roots in cocoa's overall acquisition strategy and response to management. Species  
364 composition had a significant effect on PC2 scores at both depth intervals (surface roots:  $p = 0.014$ ;  
365 subsurface roots:  $p < 0.001$ ) (Table 2; Fig. 2). Cocoa in monoculture tended to have denser, N-rich roots  
366 (higher RTD and  $N_{\text{root}}$ ) than when in mixture with *E. angolense* at both depths, shown by the significantly  
367 lower PC2 axis scores, (surface:  $p = 0.020$ ; subsurface:  $p < 0.001$ ), and also lower PC2 scores than cocoa  
368 roots in mixture with *T. ivorensis*, but significantly so only in subsurface roots ( $p < 0.001$ ) and not in  
369 surface roots ( $p = 0.469$ ). Interestingly, fertilization level had an effect in determining the coordinated  
370 resource acquisition strategies in subsurface roots ( $p = 0.049$ ), which it did not in surface roots. There was  
371 significantly lower PC2 axis scores for subsurface roots in high fertilization compared to moderate  
372 fertilization ( $p = 0.049$ ). Notably, for surface roots, FRP was uncoordinated with PC1 ( $p = 0.380$ ) and or  
373 PC2 ( $p = 0.076$ ), which higher values were characterized by denser, N-rich fine roots. Conversely, in  
374 subsurface roots, FRP was positively associated with PC1, showing alignment with conservative root  
375 traits ( $p < 0.01$ ) (Table 1). Broadly, these root trait syndromes seemingly characterize different strategies  
376 between root growth and placement versus morphological and physiological adjustments in relation to the  
377 soil environment. Notably, we show for the first time that the presence and activity of shade trees are  
378 critical in determining belowground response to fertilization as well as a tree crops' overall soil resource  
379 acquisition strategies. This is important for understanding crop function as well as interspecific  
380 interactions in multispecies agroecosystems.

381

#### 382 4. Conclusions

383 Systematic variation in plant functional traits are known to relate with abiotic and biotic  
384 conditions, but much less is known on the direct and indirect effects of management practices on the  
385 expression of crop traits (Martin and Isaac 2015; Isaac et al. 2017; Damour et al. 2018). Root functional  
386 trait data for tree crops in the humid tropics are scarce. Our study characterizes fine root response of  
387 cocoa to active management strategies employed by farmers in low-input tropical agroecosystems. The  
388 existence of coordinated root trait strategies (e.g., root economics spectrum) among individuals of the  
389 same species within a farm indicates that plant trait-environment patterns previously only predicted across  
390 larger scales and across species may also apply to plant-scale variation within a farm. Results from this  
391 study show that shifts in trait values and trait syndromes can be used as indicators of plant response to  
392 fertilizer application. For example, if root functional traits can be used to estimate foraging strategies of  
393 specific crops at specific sites, then farmers can more accurately apply fertilizer dosages catered to soil  
394 and management conditions. These more refined nutrient diagnostics and prescriptions are essential to  
395 maintain high nutrient use efficiency and minimize nutrient losses in tree-based agroecosystems.

396

397 **Acknowledgements**

398 We would like to thank Luke Anglaaere at CSIR-Forestry Research Institute of Ghana as well as  
399 Agyeman Kofi, Kirstie Cadger, and community members of South Formangso for assistance in the field.  
400 We thank Serra Buchanan and Stephanie Gagliardi at University of Toronto Scarborough for assistance  
401 with laboratory work. We thank the anonymous reviewers who improved this article with their insightful  
402 comments.

403

404 **Funding information**

405 This study was funded by the Natural Sciences and Engineering Research Council of Canada and the  
406 Department of Geography & Planning, University of Toronto.

407

408 **Declaration on conflict of interest**

409 The authors declare that they have no conflict of interests.

410

411 **References**

- 412 Borden KA, Thomas SC, Isaac ME (in press) Variation in fine root traits reveals nutrient-specific  
413 acquisition strategies in agroforestry systems. *Plant Soil*. doi: 10.1007/s11104-019-04003-2
- 414 Bordron B, Robin A, Oliveira IR, Guillemot J, Laclau JP, Jourdan C, Nouvellon Y, Abreu-Junior CH,  
415 Trivelin PCO, Gonçalves JLM, Plassard C, Bouillet JP (2018) Fertilization increases the functional  
416 specialization of fine roots in deep soil layers for young *Eucalyptus grandis* trees. *For Ecol Manage*  
417 1–0. doi: 10.1016/j.foreco.2018.03.018
- 418 Chen GT, Tu LH, Peng Y, Hu HL, Hu TX, Xu ZF, Liu L, Tang Y (2017) Effect of nitrogen additions on  
419 root morphology and chemistry in a subtropical bamboo forest. *Plant Soil* 412:441–451. doi:  
420 10.1007/s11104-016-3074-z
- 421 Damour G, Navas ML, Garnier E (2018) A revised trait-based framework for agroecosystems including  
422 decision rules. *J Appl Ecol* 55:12–24. doi: 10.1111/1365-2664.12986
- 423 Einsmann JC, Jones RH, Pu M, Mitchell RJ (1999) Nutrient foraging traits in 10 co-occurring plant  
424 species of contrasting life forms. *J Ecol* 609–619. doi: 10.1046/j.1365-2745.1999.00376.x
- 425 Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT (2015) Linking root traits to nutrient  
426 foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytol* 208:114–124
- 427 Fort F, Cruz P, Lecloux E, de Oliveira LB, Stoia C, Theau J-P, Jouany C (2016) Grassland root functional  
428 parameters vary according to a community-level resource acquisition-conservation trade-off. *J Veg*

429 Sci 27:749–758. doi: 10.1111/jvs.12405  
430 Freschet G, Roumet C (2017) Sampling roots to capture plant and soil functions. *Funct Ecol* 31:1506–  
431 1518. doi: 10.1111/ijlh.12426  
432 Isaac ME, Anglaaere LCN, Borden K, Adu-Bredu S (2014) Intraspecific root plasticity in agroforestry  
433 systems across edaphic conditions. *Agric Ecosyst Environ* 185:16–23. doi:  
434 10.1016/j.agee.2013.12.004  
435 Isaac ME, Martin AR, de Melo Virginio Filho E, Rapidel B, Rouspard O, Van den Meersche K (2017)  
436 Intraspecific trait variation and coordination: root and leaf economics spectra in coffee across  
437 environmental gradients. *Front Plant Sci* 8:1–13. doi: 10.3389/fpls.2017.01196  
438 Isaac ME, Ulzen-Appiah F, Timmer VR, Quashie-Sam SJ (2007) Early growth and nutritional response to  
439 resource competition in cocoa-shade intercropped systems. *Plant Soil* 298:243–254. doi:  
440 10.1007/s11104-007-9362-x  
441 Kong D, Ma C, Zhang Q, Li Le, Chen X, Zeng H, Guo D (2014) Leading dimensions in absorptive root  
442 trait variation across 96 subtropical forest species. *New Phytol* 203:863–872. doi:  
443 10.1111/nph.12842  
444 Kramer-Walter KR, Laughlin DC (2017) Root nutrient concentration and biomass allocation are more  
445 plastic than morphological traits in response to nutrient limitation. *Plant Soil* 1–12. doi:  
446 10.1007/s11104-017-3234-9  
447 Liese R, Alings K, Meier IC (2017) Root branching is a leading root trait of the plant economics spectrum  
448 in temperate trees. *Front Plant Sci* 8:1–12. doi: 10.3389/fpls.2017.00315  
449 Malézieux E, Crozat Y, Dupraz C, Laurans M, Makowski D, Ozier-Lafontaine H (2009) Mixing plant  
450 species in cropping systems: concepts, tools and models. A review. *Agron Sustain Dev* 29:43–62.  
451 doi: 10.1051/agro:2007057  
452 Martin AR, Isaac ME (2015) Functional traits in agroecology: a blueprint for research. *J Appl Ecol*  
453 52:1425–1435. doi: 10.1111/1365-2664.12526  
454 Martin AR, Rapidel B, Rouspard O, Van den Meersche K, de Melo Virginio Filho E, Barrios M, Isaac  
455 ME (2016) Intraspecific trait variation across multiple scales: the leaf economics spectrum in coffee.  
456 *Funct Ecol* 1–9. doi: 10.1111/1365-2435.12790  
457 McGrath D, Duryea M, Cropper W (2001) Soil phosphorus availability and fine root proliferation in  
458 Amazonian agroforests 6 years following forest conversion. *Agric Ecosyst Environ* 83:271–284  
459 Muñoz F, Beer J (2001) Fine root dynamics of shaded cacao plantations in Costa Rica. *Agrofor Syst*  
460 51:119–130. doi: 10.1023/A:1010651203815  
461 Mou P, Jones RH, Tan Z, Bao Z, Chen H (2013) Morphological and physiological plasticity of plant roots

462 when nutrients are both spatially and temporally heterogeneous. *Plant Soil* 364:373–384. doi:  
463 10.1007/s11104-012-1336-y

464 Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Löhmus K, Majdi H, Metcalfe D, Olsthoorn  
465 AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of  
466 environmental change. *Plant Biosyst* 141:426–442. doi: 10.1080/11263500701626069

467 Prieto I, Roumet C, Cardinael R, Dupraz C, Jourdan C, Kim JH, Maeght JL, Mao Z, Pierret A, Portillo N,  
468 Rounsard O, Thammahacksa C, Stokes A (2015) Root functional parameters along a land-use  
469 gradient: evidence of a community-level economics spectrum. *J Ecol* 103:361–373. doi:  
470 10.1111/1365-2745.12351

471 Roumet C, Birouste M, Picon-Cochard C, Ghestem M, Osman N, Vrignon-Brenas S, Cao KF, Stokes A  
472 (2016) Root structure-function relationships in 74 species: evidence of a root economics spectrum  
473 related to carbon economy. *New Phytol* 815–826. doi: 10.1111/nph.13828

474 van Vliet JA, Giller KE (2017) Mineral nutrition of cocoa: a review. *Adv Agron* 141:185–270. doi:  
475 10.1016/bs.agron.2016.10.017

476 Vitousek PM, Naylor R, Crews T, David MB, Drinkwater LE, Holland E, Johnes PJ, Katzenberger J,  
477 Martinelli LA, Matson PA, Nziguheba G, Ojima D, Palm CA, Robertson GP, Sanchez PA,  
478 Townsend AR, Zhang FS (2009) Nutrient imbalances in agricultural development. *Science*  
479 324:1519–1520. doi: 10.1126/science.1170261

480 Wang G, Liu F, Xue S (2017) Nitrogen addition enhanced water uptake by affecting fine root morphology  
481 and coarse root anatomy of Chinese pine seedlings. *Plant Soil*. doi: 10.1007/s11104-017-3283-0

482 Wang P, Diao F, Yin L, Huo C (2016) Absorptive roots trait plasticity explains the variation of root  
483 foraging strategies in *Cunninghamia lanceolata*. *Environ Exp Bot* 129:127–135. doi:  
484 10.1016/j.envexpbot.2016.01.001

485 Weemstra M, Sterck FJ, Visser EJW, Kuyper TW, Goudzwaard L, Mommer L (2017) Fine-root trait  
486 plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant*  
487 *Soil* 415:175–188. doi: 10.1007/s11104-016-3148-y

488 Yan G, Chen F, Zhang X, Wang J, Han S, Xing Y, Wang Q (2017) Spatial and temporal effects of  
489 nitrogen addition on root morphology and growth in a boreal forest. *Geoderma* 303:178–187. doi:  
490 10.1016/j.geoderma.2017.05.030

491

#### 492 **Statement of data availability**

493 Data available from the corresponding author upon request.

494

495 **Figures**

496

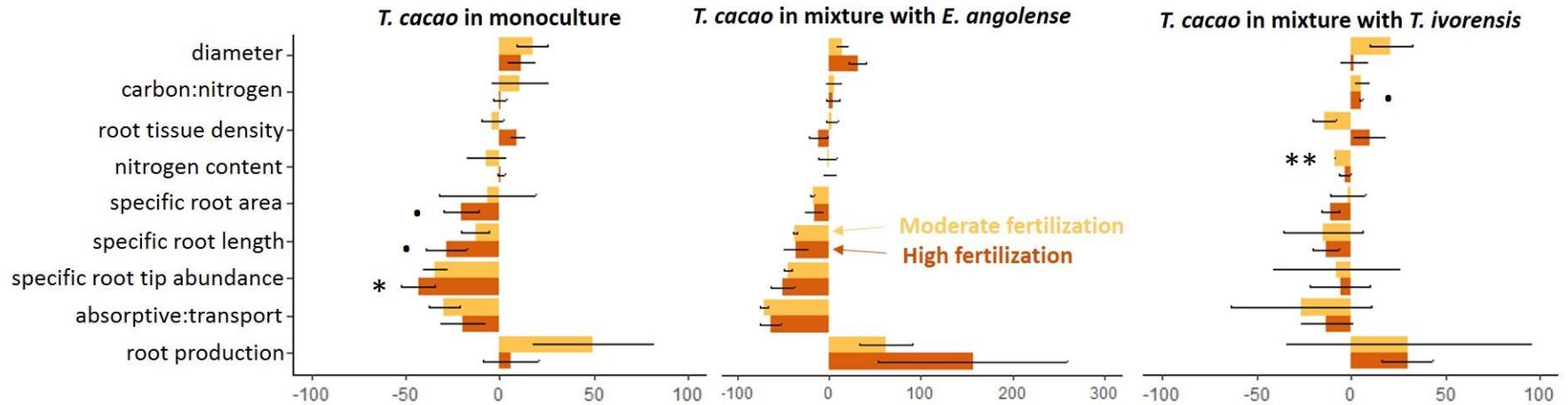


497

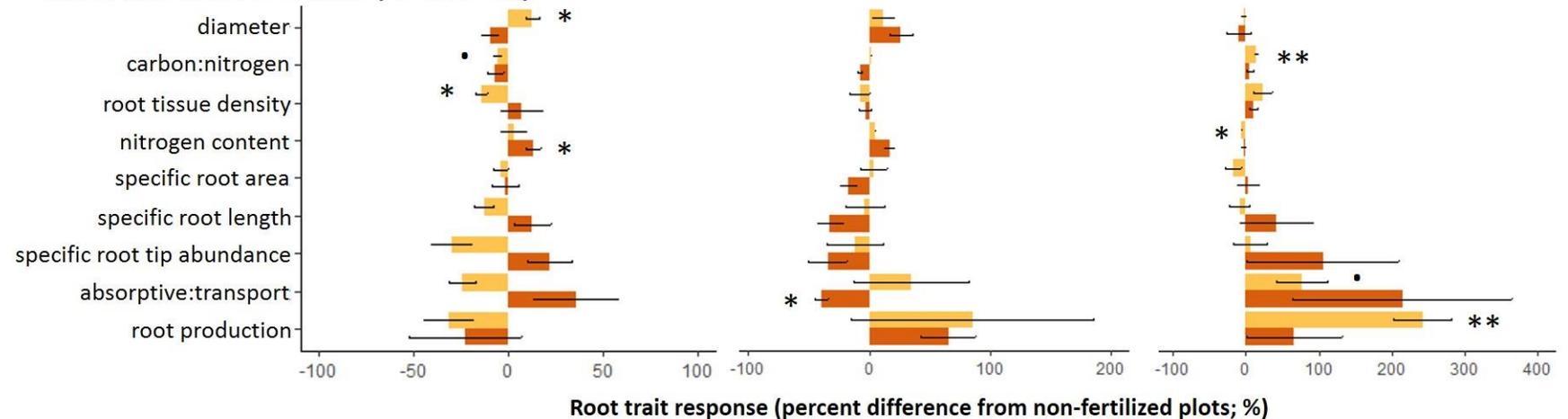
498 **Figure 1:** Cocoa agroecosystems in Ashanti Region, Ghana, showing cocoa growing in monoculture (left panel) and  
499 growing in agroforestry with a shade tree (*Terminalia ivorensis*).

500

**Surface cocoa root traits (0 to 10 cm)**



**Subsurface cocoa root traits (10 to 20 cm)**



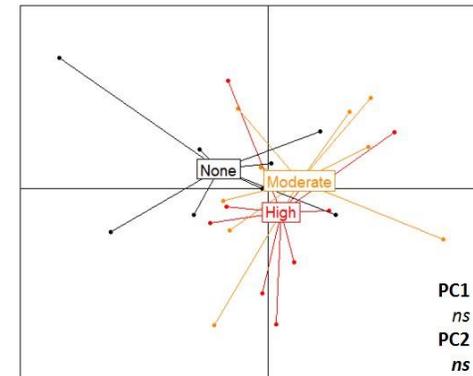
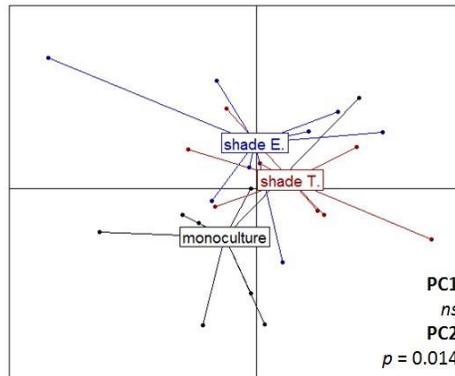
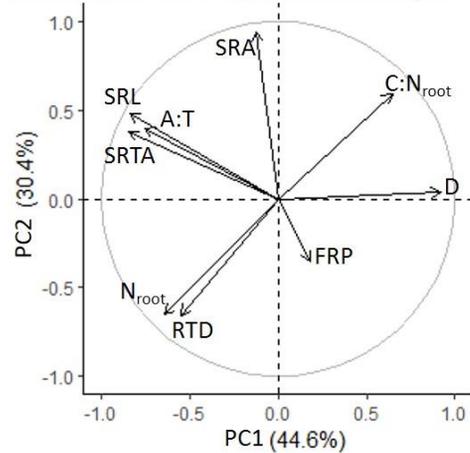
501

502 **Figure 2:** Root trait response to nutrient influx (mean ± SE percent difference from roots in non-fertilized soil) of cocoa in three different species combinations.

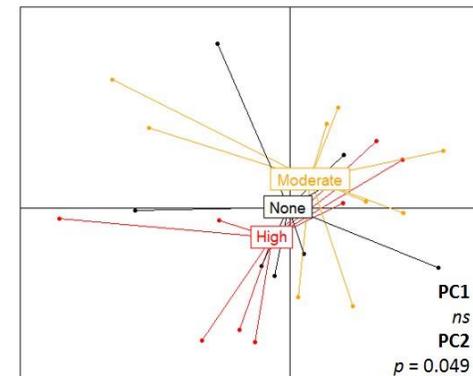
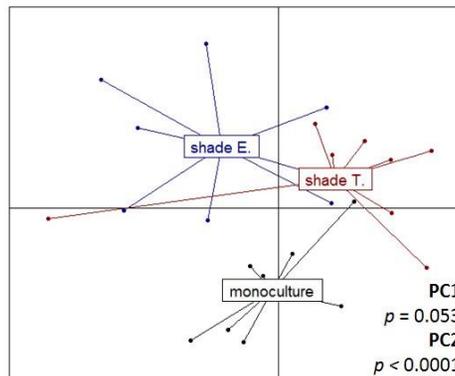
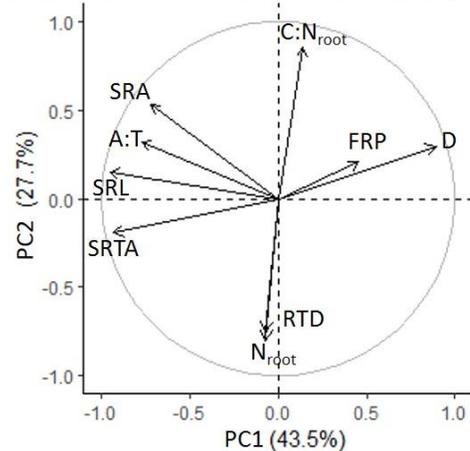
503 Top row shows data of surface roots and bottom row shows data from subsurface roots. Zero on the x axes signifies no difference in the root trait values from

504 non-fertilized plots. Results from *t*-tests between fertilized and non-fertilized groups shown (\*\*  $p < 0.01$ ; \*  $p < 0.05$ ;  $p > 0.1$ ).

Ordination of cocoa surface root traits (0 to 10 cm)



Ordination of cocoa subsurface root traits (10 to 20 cm)



505

506

507

508

509

**Figure 3:** Ordination of cocoa surface roots (top row) and subsurface roots (bottom row) from principal component analysis (left panels) and resulting biplots of axes scores grouped according to species composition (middle panels: cocoa in monoculture, cocoa in mixture with *E. angolense* (shade E.), or cocoa in mixture with *T. ivorensis* (shade T.)) and fertilization level (right panels). Results from two-way ANOVA are presented; there were no interactive effects of species composition and fertilization (Table 2).

510 **Tables**

511

512 **Table 1:** Trait loadings on the first two axes of principal component analyses of cocoa root traits: diameter (D), C:N  
 513 in root tissue (C:N<sub>root</sub>), root tissue density (RTD), N content of roots (N<sub>root</sub>), specific root - area (SRA), length (SRL),  
 514 and tip abundance (SRTA), absorptive:transport root length (A:T), fine root production (FRP). Significant  
 515 relationships between traits and axes are indicated in bold ( $p < 0.05$ ).

	PC1	PC2
Surface roots		
D	<b>0.46<sup>***</sup></b>	0.02
RTD	<b>-0.28<sup>**</sup></b>	<b>-0.40<sup>***</sup></b>
C:N <sub>root</sub>	<b>0.32<sup>***</sup></b>	<b>0.36<sup>**</sup></b>
N <sub>root</sub>	<b>-0.32<sup>***</sup></b>	<b>-0.39<sup>***</sup></b>
SRA	-0.06	<b>0.57<sup>***</sup></b>
SRL	<b>-0.42<sup>***</sup></b>	<b>0.29<sup>*</sup></b>
SRTA	<b>-0.42<sup>***</sup></b>	0.23
A:T	<b>-0.37<sup>***</sup></b>	<b>0.24<sup>*</sup></b>
FRP	0.09	-0.21
Subsurface roots		
D	<b>0.45<sup>***</sup></b>	0.19
RTD	-0.04	<b>-0.48<sup>***</sup></b>
C:N <sub>root</sub>	0.07	<b>0.54<sup>***</sup></b>
N <sub>root</sub>	-0.04	<b>-0.51<sup>***</sup></b>
SRA	<b>-0.36<sup>**</sup></b>	<b>0.34<sup>**</sup></b>
SRL	<b>-0.48<sup>***</sup></b>	0.09
SRTA	<b>-0.47<sup>***</sup></b>	-0.12
A:T	<b>-0.39<sup>***</sup></b>	0.20
FRP	<b>0.23<sup>*</sup></b>	0.13

516 <sup>\*\*\*</sup>  $p < 0.001$ ; <sup>\*\*</sup>  $p < 0.01$ ; <sup>\*</sup>  $p < 0.05$ 

517

518 **Table 2:** Results of two-way ANOVA of species combination and fertilization level on coordinated root strategies  
 519 of cocoa. Significant effects are indicated in bold ( $p < 0.05$ ).

Source	df	S.S.	F-value	p-value
Surface roots				
PC1				
Species combination	2	11.24	1.349	0.286
Fertilization level	2	17.49	2.100	0.153
Sp. comb. × Fert.	4	4.85	0.291	0.880
Residuals	17	70.82		
PC2				
Species combination	2	21.93	4.70	<b>0.024</b>
Fertilization level	2	6.10	1.31	0.296
Sp. comb. × Fert.	4	3.48	0.37	0.825
Residuals	17	39.63		
Subsurface roots				
PC1				
Species combination	2	22.36	3.51	0.053
Fertilization level	2	2.83	0.44	0.649
Sp. comb. × Fert.	4	22.38	1.75	0.185
Residuals	17	54.22		
PC2				
Species combination	2	39.17	22.40	<b>&lt;0.001</b>
Fertilization level	2	6.29	3.60	<b>0.049</b>
Sp. comb. × Fert.	4	4.60	1.32	0.304
Residuals	17	14.86		

520