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REGULAR ARTICLE

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

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Abstract
Aims Changes in root functional traits reveal important nutrient acquisition strategies, with well documented patterns in root trait expression within complex communities or along gradients of singular nutrients. In this field study, we investigate intra-root functional trait expression with six soil macro- and micro-nutrients in *Theobroma cacao* agroforestry systems.
Methods Using image, chemical, and spatial analysis, the fine root distribution, architecture, and morphology of *T. cacao* were compared to localized soil nutrients on two-dimensional soil profiles with conspecific and heterospecific neighbours.
Results Fine-scale variation in soil nutrients was observed within the range of *T. cacao* root systems. Higher NH_4^+ and Ca^{2+} was associated with greater root length and biomass densities, coupled with greater investment to individual roots, expressed as increased fine root tissue density and diameter and lower specific root length. Conversely, NO_3^- had the opposite effect. Overall, roots tended towards higher acquisitive trait values when next to a shade tree.
Conclusions Plants generally employ several concomitant and at times opposing strategies for nutrient acquisition in heterogeneous soils. We show that fine-scale root plasticity is highly linked to localized nutrient-specific and neighbour-specific effects, driving patterns of nutrient acquisition in agroforestry systems.

Keywords Functional traits · Intercropping · Nutrient distribution · Root foraging · Root system · *Theobroma cacao*

Abbreviations

A:T	Ratio of absorptive to transport fine root length
D	Average root diameter
FRLD	Fine root length density
FRBD	Fine root biomass density
RTD	Fine root tissue density
SRL	Specific root length

Introduction

Agroforestry is a prime example of applied ecology: species combinations are, in principle, chosen to enhance niche complementarity and/or facilitation, and

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thus improve nutrient cycles. The success of these interactions largely depends on differences in plant root functions and/or spatial distributions that, when compared to monocultures, permit more complete acquisition of soil nutrients (Brooker et al. 2015; Cardinael et al. 2015a). However, plant nutrient acquisition patterns are not static, and phenotypic plasticity in root systems, from whole plant to lateral root scales, can transform belowground interactions with neighbouring plants (Li et al. 2006; Cahill et al. 2010). The extent of root scale phenotypic plasticity and subsequent root foraging success is highly species-specific (Blair and Perfecto 2004; Malamy 2005; Chen et al. 2018). Yet, in humid tropical agroforestry systems, where plant nutrient demand is high and supply is constrained, little is known on root system foraging patterns under extremely complex conditions.

Plants can benefit from localized areas of high nutrient availability in soil, i.e. soil nutrient hotspots (Chen et al. 2018), by modifying their root systems via signalling mechanisms in roots that encounter elevated concentrations of nutrients (Forde and Lorenzo 2001; Malamy 2005). Although typically at the plant scale there is relatively higher allocation of biomass to roots in nutrient-poor environments (Wright et al. 2011), in heterogeneous but nutrient-limited soil environments there is generally greater allocation of root biomass to locations in soil where nutrients are more abundant (Drew 1975; Hutchings and de Kroon 1994; Hodge 2004). Additionally, studies on root morphological traits across soil nutrient gradients indicate higher investment to fine root organs given increased soil nutrients, with construction of longer-lived roots characterized by thicker diameter (D), higher root tissue density (RTD), and lower specific root length (SRL) (Ostonen et al. 2007). Alternatively, the reverse has been observed where roots grow more rapidly with higher turnover to exploit nutrient-rich soil and, thus, show increased absorptive area per unit of biomass (e.g., higher SRL), while in nutrient-poor soil roots develop morphologies that limit nutrient losses (e.g., thicker D) (Fort et al. 2016). In sum, there is evidence that plants generally employ several concomitant and at times opposing strategies to increase the nutrient acquisition in heterogeneous soils by altering root initiation and growth and patterns of root morphology. Plastic responses can be nutrient specific, presumably influenced by the mobility of the nutrient in the soil matrix, the signalling and uptake pathways employed by roots, and the capacity to translocate the nutrient within the plant (Drew 1975; Mou et al. 1995; López-Bucio et al. 2003; Hodge 2004), and are further contingent on the overall nutrient status of the plant and localized distribution of nutrients within the range of the root system (López-Bucio et al. 2003; de Kroon et al. 2009).

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

Plasticity of root systems in agroforestry systems can increase crop access to heterogeneous nutrient availability in soil but can also mitigate competitive effects from neighbouring trees (McGrath et al. 2001; Li et al. 2006; Isaac et al. 2014, 2017; Cardinael et al. 2015b). This is particularly important when there are few external nutrient inputs, which is generally the case for the tropical tree crop *Theobroma cacao* L. – the focal species in our study – that is commonly grown under the canopy of larger heterospecific neighbour trees (i.e., shade trees) on smallholder farms. While trees with more complementary root distributions can be preferentially planted with crops (i.e., tree species with deeper rooting profiles), typically there will be overlap of root systems in upper soil layers where nutrients are most abundant (Isaac et al. 2014; Borden et al. 2017b). To this end, we sought to capture two-dimensional distributions of cocoa root systems (rather than vertical zonation only) to account for more nuanced root allocation patterns (e.g., Sudmeyer et al. (2004), Li et al. (2006), and Laclau et al. (2013)).

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

Materials and methods

Study site and species combinations

The study was carried out in South Formangso, Ashanti Region, Ghana (6°36 N, 0°58 W) at a cocoa research station managed by the Forestry Research Institute of Ghana. The 2-ha site is situated on previously secondary forest that was cleared for cultivation and was left to fallow until the cocoa agroforestry system was established in 2001. *T. cacao* hybrid planting stock from the Cocoa Research Institute of Ghana was planted at a spacing of 3 × 3 m and, in agroforestry treatments, shade trees were planted in replacement of *T. cacao* at 12 × 12 m spacing. No fertilizer had been applied to the research site prior to the study. Soils are Acrisols with bulk density of 1.22 ± 0.02 g cm^{-3} and soil pH ranging from 6.2 ± 0.1 near the soil surface to 4.9 ± 0.0 near 60 cm depth. The site is in a moist semi-deciduous forest zone with mean annual rainfall of 1528 mm and mean annual temperature of 26.2 °C. Sampling was completed in the on-set of the rainy season, during *T. cacao* flowering and cocoa pod production and, thus, when nutrient demands were high (van Vliet and Giller 2017).

Study *T. cacao* trees (DBH = 14.6 ± 1.1 cm; mean ± SE) were selected from pre-established blocks of species combinations at the site, providing three replications of each species combination. The two shade tree species used in this study, *Terminalia ivorensis* Chev. (DBH = 58.8 ± 3.8 cm) and *Entandrophragma angolense* (Welw.) C. DC. (DBH = 19.9 ± 1.4 cm), are commonly used in this region to provide upper canopy shade (< 25% shade) for *T. cacao* cultivation. *T. ivorensis* is a fast-growing, early successional tree species and was the larger of the two heterospecific neighbour species. This species is characterized by many shallow lateral roots and has been shown to affect fine root length density (FRLD) of *Coffea arabica* L. (van Kanten et al. 2005) and was assumed to have strong belowground competitive effects due to high SRL (34.7 ± 9.3 m g^{-1}; $n = 30$). Slower-growing, late-successional *E. angolense* is perceived by farmers to be deeper rooted and had lower SRL (29.7 ± 6.2 m g^{-1}; $n = 30$; measured from the study site; data not shown).

T. cacao and neighbour soil interfaces: sampling on soil trenches

Nine soil trenches 1 m wide and at least 60 cm deep were manually excavated (three trenches per species combination). The exposed soil 'interfaces' in the trenches were perpendicular to transects connecting *T. cacao* with another *T. cacao*, or *T. cacao* with a shade tree, and located halfway between the trees' stems (i.e., 1.5 m from each stem) (Fig. 1). The location and size of the soil interfaces were selected to represent an area occupied by an individual *T. cacao* root system and with limited root system interactions from non-study *T. cacao* trees (Isaac and Anglaaere 2013; Borden et al. 2017a), while sampling scale and intensity was first assessed from preliminary soil profiles that were tested for soil nutrients (data not shown; Soils Institute of Ghana, Kumasi, Ghana). In each of the present study's soil interfaces, 40 soil cores (5 cm diameter; 100 cm^3 volume) were taken horizontally and in a stratified random sampling scheme. Samples were taken such that the soil core was centred at 2.5, 7.5, 15, 27.5 cm depths (i.e., y direction) to capture the dominant rooting zone of *T. cacao* (i.e., to 30 cm) and centred at 57.5 cm depth to capture root strategies in deeper soils. This vertical sampling scheme was repeated every 20 cm intervals (at 0, 20, 40, 60, 80, 100 cm) across the length of the trench (i.e., x direction) followed by 10 additional samples taken at random, non-sampled locations in the soil interface, recorded

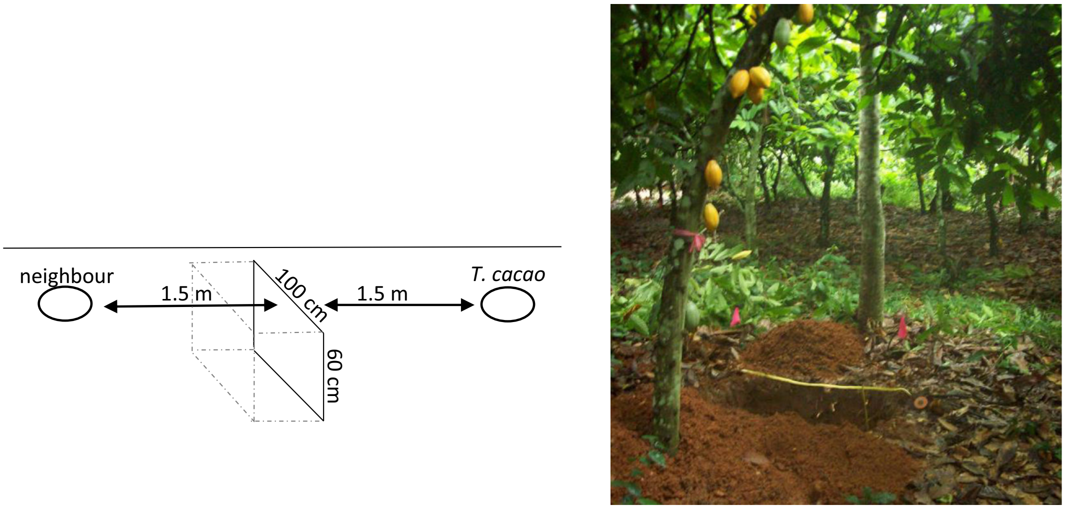

Fig. 1 Soil interfaces ($n = 9$) used in this study. Left panel: Schematic depicting the location of a soil interface between a *T. cacao* tree and a heterospecific or conspecific neighbour tree. Right panel: An excavated soil interface situated between a *T. cacao* tree (foreground) and a shade tree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

using an x, y coordinate systems. Thus, in sum, samples were taken from five depths at six horizontal locations and an additional 10 randomly located on each of the nine interfaces for a total of 360 samples. In the lab, samples were gently homogenized by hand and then divided into two approximately equal volumes of soil, with half of each sample (~50 cm^3) used for fine root analysis and the other half used for soil chemical analysis. Samples were stored in polyethylene bags and frozen until further processing.

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 were 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 (≤ 2 mm) were then scanned using a flatbed scanner (STD4800; Regent Instruments Inc., Canada) at 600 dpi. From these images, average fine root diameter, 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 h of drying at 65 °C. These data were used to calculate six root traits that characterized the root density in each soil sample: fine root length density (FRLD; cm cm^{-3}) and fine root biomass density (FRBD; mg cm^{-3}) of an individual *T. cacao*, and the morphology of the roots in each 100 cm^3 sample: specific root length (SRL; m g^{-1}), root tissue density (RTD = [dry root mass/ fresh root volume]; 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 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 ± 0.07% (± 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 (FRBD$_{\text{shade}}$; mg cm^{-3}) in each sample.

Soil chemical analysis

From each soil sample, available NO_3^- an NH_4^+ were extracted from field moist soils in KCl solution, filtered through Fisher P8 filter paper, and measured using a spectrophotometer flow injection analyzer (QuikChem 8500, Lachat Instruments, USA). The remaining soils from each sample were air-dried for 2 weeks and sieved through 2 mm mesh. From these soils, available PO_4^- was extracted in a 1:10 soil to Bray's 1 solution, filtered through Fisher P5 filter paper, and measured using a spectrophotometer. Air-dried soil was further ground in a ball mill (Retsch Ltd., Germany). From these soils, exchangeable K^+, Mg^{2+}, and Ca^{2+} were extracted with ammonium acetate (NH_4OAc), filtered through Fisher P8 filter paper, and analyzed using an atomic absorption spectrometer (AAnalyst 200, PerkinElmer, USA). Soil chemical analyses were carried out at the University of Toronto Scarborough, Toronto, Canada.

Statistical analysis

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

We examined the directional relationships between *T. cacao* fine root distribution, architectural, and morphological traits with localized soil nutrient availability, focusing on data within the dominant rooting zone of *T. cacao* (0 to 30 cm depth). To do so, linear mixed models (LMMs) for each root trait in each species combination were fit with sampling depth assigned as fixed variable and soil interface assigned as a random factor. As the fine roots of mature *T. cacao* grow as dense root mat in the top soil (Nygren et al. 2013), we assumed that soil cores taken within the top 30 cm of each interface were independent observations without spatial autocorrelation after depth was included as a fixed term. All measured soil nutrients were included in the LMMs as fixed variables to evaluate how a change in availability of each nutrient within 100 cm^3 soil volumes is related to variation in root traits while accounting for variation of the other measured soil nutrients under field conditions. For *T. cacao* in mixture, $FRBD_{shade}$ was also included as a fixed variable. To estimate the amount of variation in *T. cacao* root traits explained by all fixed variables, the 'fixed effects r^2' was calculated using the 'r2beta' function (with method 'nsj') in the '*r2glmm*' package (Nakagawa and Schielzeth 2013). This procedure also allowed us to estimate partial r^2 of each fixed variable. For parametric analyses, residuals were tested for normality using the Shapiro Wilk test. To meet parametric assumptions, root and soil data were $\log_{10}$ transformed. The level of significance was at $p < 0.05$.

Results

Soil nutrients: distribution and variation

Within the dominant lateral rooting zone of an individual root system (i.e., to 30 cm depth) there was large variation in soil nutrients (Table 1). Soil NO_3^- and K^+ could vary by two orders of magnitude, showing a large range and large CV, except for soil K^+ in *T. cacao*-*E. angolense* mixture. There were some particularly high concentrations of soil NO_3^- in monoculture (max: 82.0 mg g^{-1}), which was concentrated in surface soils (Fig. 2). Soil K^+ was highest in monoculture, particularly when compared to the *T. cacao*-*T. ivorensis* mixture (Table 1; Fig. 2). Soil NH_4^+, Ca^{2+}, and Mg^{2+} also showed high variability, while soil PO_4^- was the least variable with the lowest CV (6 to 47%) (Table 1). Overall, both mixtures had higher soil NH_4^+ than monoculture and soil PO_4^- was highest in *T. cacao*-*T. ivorensis* mixture (Fig. 2). Soil nutrients generally decreased with

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 and the coefficient of variation (%) of 100 cm^3 samples from nine soil interfaces ($n = 32$ per interface)

Soil interface		NO_3^- mg g^{-1}	NH_4^+ mg g^{-1}	PO_4^- mg g^{-1}	K^+ cmol(+) kg^{-1}	Ca^{2+} cmol(+) kg^{-1}	Mg^{2+} cmol(+) kg^{-1}
T. cacao 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%)
T. cacao- E. angolense 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%)
T. cacao- T. ivorensis 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%)

depth, although soil K^+ was more evenly distributed vertically in the soil profiles (Fig. 2).

T. cacao fine roots: distribution and variation

As with soil nutrients, *T. cacao* vertical distributions of fine roots were concentrated near the soil surface and decreased with depth (Figs. 3 and 4). Over 90% of *T. cacao* fine roots were in the top 30 cm of soil regardless of neighbour species. *T. cacao* roots next to *E. angolense* tended to be concentrated in shallow soils with 71% of both fine root length and biomass located in the top 10 cm of soil (Fig. 4). For *T. cacao* with conspecifics, 67% of fine root length and 64% of fine root biomass were in surface soil (top 10 cm). When next to *T. ivorensis*, there was 70% of fine root length but only 59% of fine root biomass in the top 10 cm, with more vertically dispersed fine root biomass between 10 and 30 cm. Vertically, the two shade tree species also showed decreasing densities of roots within the top 60 cm of soil, but *T. ivorensis* showed a higher concentration of fine root biomass in surface soil (top 10 cm) compared to *E. angolense* that had more evenly distributed fine root biomass within the soil interfaces (Figs. 3 and 4).

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

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

Significant directional effects of each nutrient on root traits were consistent regardless of species combination (Table 2). Soil NH_4^+ and Ca^{2+} had a generally positive effect on *T. cacao* fine root densities (FRLD and FRBD) and investment at the root scale,

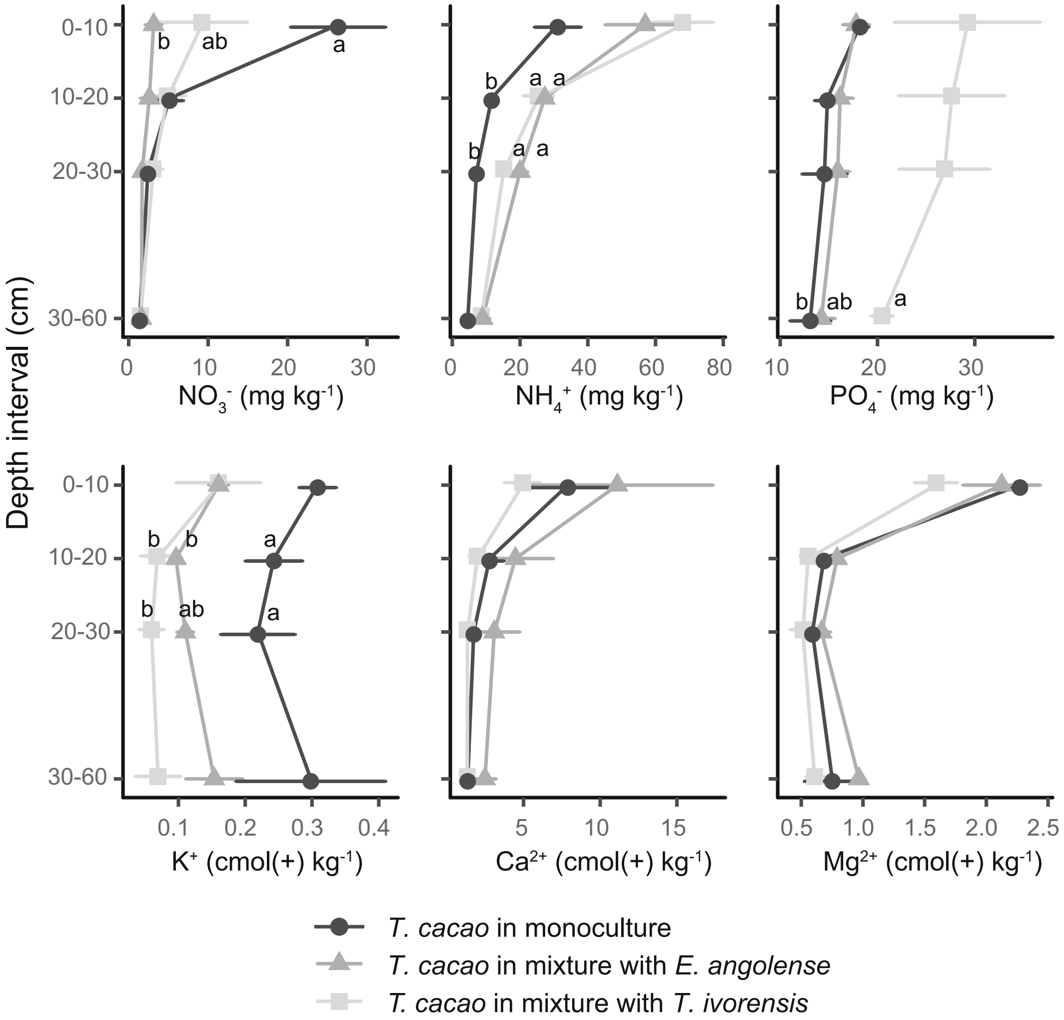

Fig. 2 Soil attributes with depth at a distance of 1.5 m from *T. cacao* stems (mean ± SE; $n = 3$). Same letters are non-significant differences for individual *T. cacao* among treatments at same depth when there was a significant treatment effect (ANOVA)

expressed as positive coefficients for D and negative coefficients for SRL and A:T in LMMs (Table 2; Table S1). However, opposite trends were observed for soil NO_3^- and K^+, particularly for *T. cacao* in monoculture and *T. cacao* next to *T. ivorensis*. Soil PO_4^- was limited as a predictor variable in root trait variation with the exception of A:T for *T. cacao* in mixture with *E. angolense*. Soil Mg^{2+} generally had a negative effect on localized investment to roots for *T. cacao* in mixture with *T. ivorensis*, with a significant negative D coefficient ($p = 0.04$) (Table 2; Table S1).

Depth, soil nutrients, and $FRBD_{shade}$ together explained similar proportion of variation in FRLD and FRBD of *T. cacao* in monoculture and *T. cacao* in mixture with *E. angolense* (fixed effects $r^2 = 0.52$ to 0.65) as well as FRLD for *T. cacao* in mixture with *T. ivorensis* ($r^2 = 0.61$) (Table 2). However, these same variables were less effective in explaining variation in FRBD of *T. cacao* next to *T. ivorensis* ($r^2 = 0.29$). In most cases, variation in root densities (FRLD and FRBD) was better explained by the fixed variables (depth, nutrients, $FRBD_{shade}$) than was the variation in root architecture (A:T; $r^2 = 0.07$ to 0.19) or morphology (SRL and D; $r^2 = 0.09$ to 0.22), except for a notably high fixed effects r^2 for RTD ($r^2 =$ 0.30 to 0.55). For *T. cacao* in mixture with

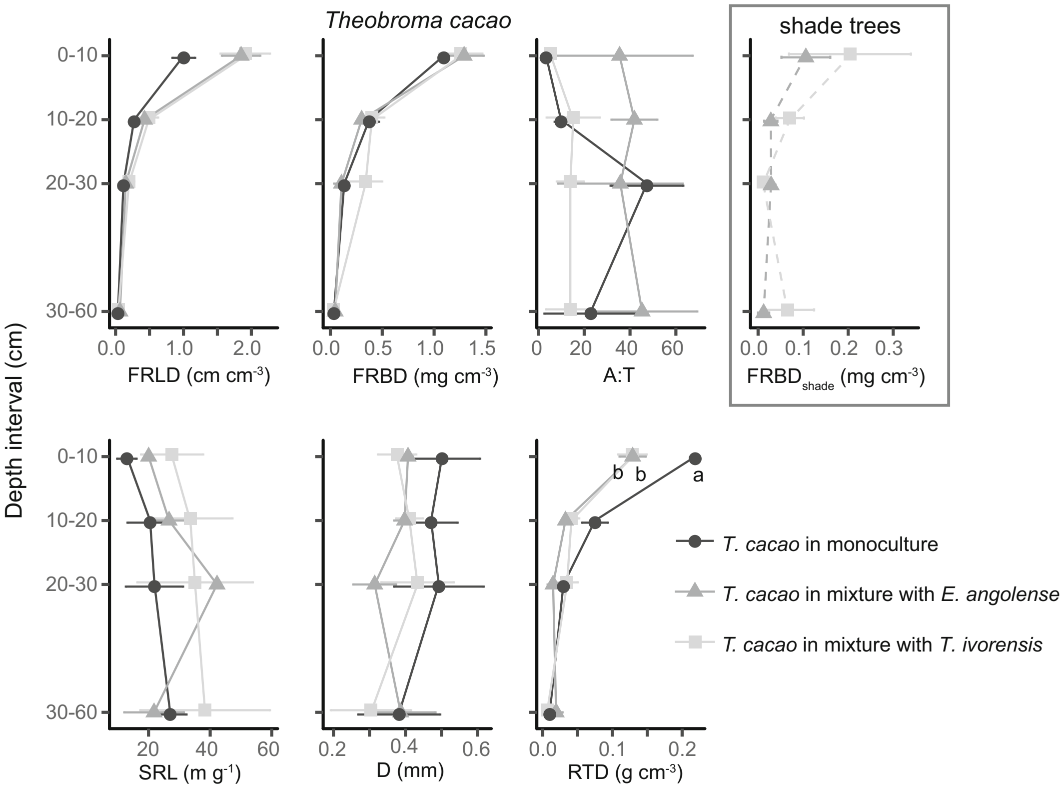

Fig. 3 Vertical distribution of fine root density (FRLD and FRBD), architecture (A:T), and morphology (SRL, D, RTD) of an individual *T. cacao* tree at a distance of 1.5 m from stems (mean ± SE, $n = 3$). Also shown is vertical distribution of fine root biomass shade trees (FRBD$_{shade}$). Same letters are non-significant differences for individual *T. cacao* among treatments at same depth when there was a significant treatment effect (ANOVA)

E. angolense, variation in root traits was mainly explained by differences in depth (partial $r^2 = 0.08$ to 0.21) while the effects of localized nutrient variation at similar depths were weakly related to variation in root traits. In contrast, variation in nutrients were just as, or more important than depth in explaining variation in root traits of *T. cacao* in monoculture and *T. cacao* in mixture with *T. ivorensis* (Table 2). We did not observe significant effects of FRBD$_{shade}$ on *T. cacao* fine root densities in localized soil volumes, which would indicate root avoidance, but only non-significant negative coefficients of FRBD$_{shade}$ of both shade tree species with *T. cacao* FRBD and FRLD. We did not observe localized impact of FRBD$_{shade}$ on the fine root morphology of *T. cacao*, but there was a marginally significant positive effect observed for SRL with FRBD$_{shade}$ of *T. ivorensis* ($p = 0.07$) and a marginally significant negative effect for RTD ($p = 0.07$) (Table 2; Table S1).

Discussion

Intra-root system foraging strategies for specific nutrients

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

Foraging strategies realized through root system architectural and morphological plasticity can be

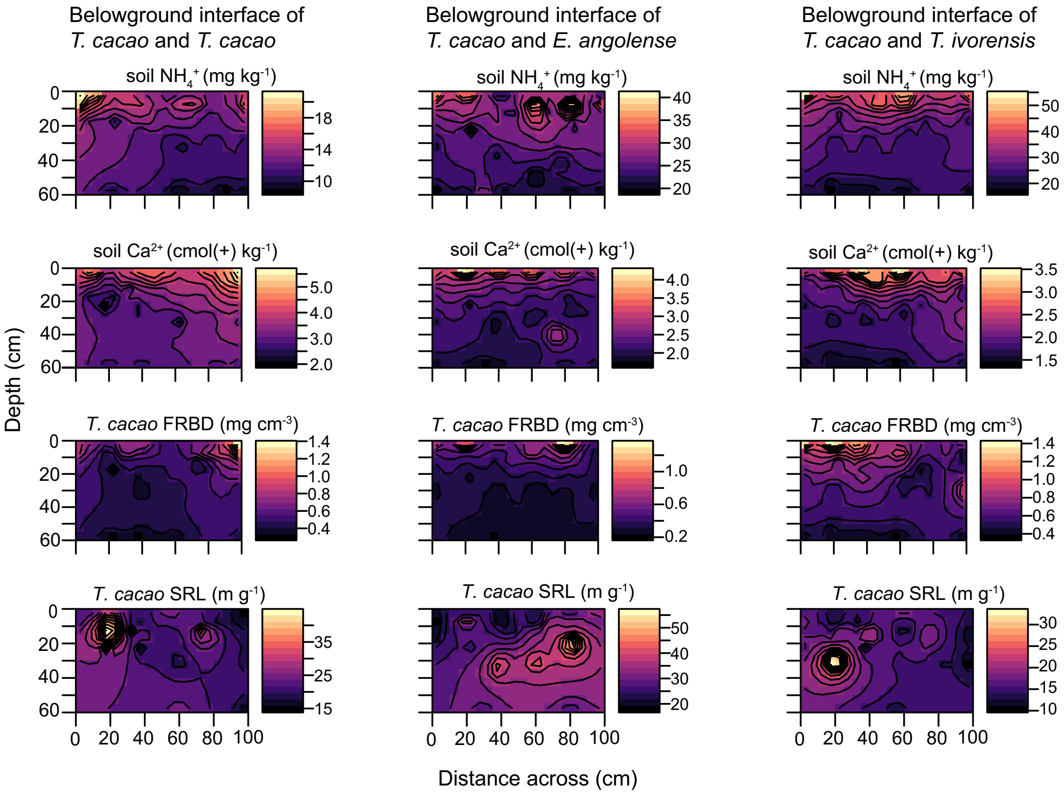

Fig. 4 Examples of interpolated interface maps depicting the distribution of soil nutrients (e.g., NH_4^+ and Ca^{2+}), shade tree fine roots ($FRBD_{shade}$), and *T. cacao* fine roots (e.g., FRLD and SRL) in three soil interfaces between two *T. cacao* (left column), *T. cacao* and *E. angolense* (middle column), and *T. cacao* and *T. ivorensis* (right column)

nutrient-specific (Drew 1975; Hodge 2004). For soil NH_4^+ and Ca^{2+}, our first hypothesis was consistently supported: within the scale of individual root systems, locations with higher soil nutrient availability were associated with higher density of fine roots (i.e., higher FRLD and FRBD). This trend was coupled with greater investment to root tissue (expressed as lower SRL and higher D and RTD), which was in support of our second hypothesis. However, inconsistent and/or opposite effects were found for soil NO_3^-, and to a lesser extent K^+, and Mg^{2+}: patterns were generally neutral or, in some cases, higher localized concentrations of these nutrients in soil were associated with reduced density of roots (lower FRLD and FRBD) and 'less expensive' roots (higher A:T and SRL; lower D and RTD). In the case of the more mobile soil nutrients: NO_3^- and Mg^{2+} (Gransee and Führs 2013), it may be more economical for plants to increase uptake with short-lived, younger roots (Blair and Perfecto 2004). Additionally, however, these negative associations between fine root density and nutrients were found when there was distinctly higher availability of the nutrient compared to other species combinations. Thus, we speculate over-supply in nutrients favours reduced root allocation; this explanation seems likely for soil K^+ in *T. cacao* monocultures.

The higher proportion of thinner absorptive root length that was associated with increased availability of PO_4^-, specifically for *T. cacao* when in mixture with *E. angolense*, would permit higher precision foraging for this relatively immobile nutrient (Hodge 2004; Hinsinger et al. 2011). Otherwise, however, root trait variation was generally unrelated to localized variation in soil PO_4^-. McGrath et al. (2001) reported increased proliferation of fine roots of *T. grandifolium* into soil cores that were artificially enriched with PO_4^-. However, PO_4^- gradients under natural conditions may occur predominantly at smaller scales (e.g., gradients of 1 mm or less within the rhizosphere) (Hinsinger et al. 2011). As rhizosphere soil was mixed in with bulk soil within 5 cm diameter soil cores, our sampling

Table 2 Coefficients from LMMs of *T. cacao* fine root traits with depth and soil nutrients as fixed effects and soil interface as a random effect

Root trait	Intercept	Depth (cm)	$\log NO_3^-$	$\log NH_4^+$	$\log PO_4^-$	$\log K^+$	$\log Ca^{2+}$	$\log Mg^{2+}$	$FRBD_{shade}$	'Fixed effects r^2'
T. cacao in monoculture										
logFRLD	−0.43	**−0.02** (0.06)	−0.16 (0.01)	0.16 (0.01)	−0.26 (0.00)	**−0.21** (0.03)	**0.75** (0.08)	0.24 (0.00)	–	0.55
logFRBD	−1.41	**−0.03** (0.05)	**−0.46** (0.05)	**0.48** (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	**1.82**	0.00 (0.00)	**0.32** (0.05)	**−0.39** (0.05)	−0.32 (0.01)	0.10 (0.01)	−0.17 (0.01)	−0.29 (0.01)	–	0.22
logD	**−0.45**	0.00 (0.00)	−0.04 (0.00)	**0.14** (0.03)	−0.09 (0.00)	0.00 (0.00)	0.11 (0.01)	−0.02 (0.00)	–	0.09
logRTD	**−2.13**	**−0.03** (0.04)	**−0.48** (0.05)	**0.48** (0.03)	0.44 (0.01)	−0.22 (0.02)	0.73 (0.05)	0.55 (0.02)	–	0.52
T. cacao in mixture with *E. angolense*										
logFRLD	−2.12	**−0.03** (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	**−0.05** (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	**−6.58**	**0.03** (0.05)	**−0.35** (0.06)	0.46 (0.04)	**4.46** (0.06)	−1.13 (0.04)	−0.03 (0.00)	0.76 (0.01)	−0.37 (0.01)	0.19
logSRL	1.03	**0.02** (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	**−0.01** (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	**−1.54**	−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
T. cacao in mixture with *T. ivorensis*										
logFRLD	−0.97	**−0.02** (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	**−2.50**	−0.01 (0.00)	−0.02 (0.00)	**1.00** (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	**2.56**	−0.01 (0.00)	0.07 (0.01)	**−0.68** (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	**−0.84**	0.00 (0.00)	−0.04 (0.01)	**0.22** (0.04)	0.09 (0.01)	0.01 (0.00)	−0.07 (0.00)	**−0.25** (0.04)	−0.05 (0.02)	0.12
logRTD	**−2.58**	−0.02 (0.02)	−0.02 (0.00)	**0.86** (0.06)	0.01 (0.00)	−0.08 (0.00)	−0.04 (0.00)	−0.04 (0.00)	−0.24 (0.05)	0.30

Significant ($p < 0.05$) coefficients are in bold. Partial r^2 are reported in parentheses. Complete LMM results are reported in Table S1 design likely limited our ability to detect foraging for this nutrient, a conclusion also supported by the relatively limited variation in PO_4^- in this present study. More importantly, other root characteristics, which were not measured in our study, may better capture acquisition strategies, such as root hair abundance or mycorrhizal associations (Hodge 2004; Chen et al. 2018). More generally, the use of a 2 mm diameter cut off for measuring fine root SRL, D, and RTD may have obscured some relationships between the fine roots predominantly responsible for nutrient acquisition and soil nutrients (Freschet and Roumet 2017). Increased precision in the delineation of absorptive roots, as well as the addition of root hairs and mycorrhizal associations into root functional trait research is critical for advancing assessment of root-soil patterns.

How is root foraging modified by shade trees?

Relationships between soil nutrients and fine root densities and morphology of *T. cacao* differed among species combinations. Such responses of fine roots to localized sources of nutrients are expected to be driven by differential nutrient demands of the plant (Forde and Lorenzo 2001). Previous research has shown the nutrient status of *T. cacao* to be modified by interactions with neighbouring shade trees (Isaac et al. 2007). We found that significant trends between root traits and soil nutrients were most pronounced for *T. cacao* in monoculture, and specifically for N (both NO_3^- and NH_4^+) and Ca, suggesting these may be co-limiting nutrients in the sole-cropping system. Patterns differed markedly in soil interfaces near shade trees. No dominant nutrient emerged for *T. cacao* next to *E. angolense*, while available NH_4^+ best explained root patterns for *T. cacao* next to *T. ivorensis*, suggesting a *T. cacao* response to N limitation within this species combination.

Differential tree root distribution and activity can contribute to belowground complementarity in tree-based agroecosystems (Brooker et al. 2015; Borden et al. 2017b). While fine roots of *T. cacao* below 60 cm can contribute to improved complementarity and total soil resource acquisition (Abou Rajab et al. 2018), we focus on the extensive lateral roots of *T. cacao* that are at highest concentration in the top 30 cm of soil (Nygren et al. 2013; Isaac et al. 2014; Borden et al. 2017a). Within this dominant rooting zone, previous studies have shown vertical stratification in *T. cacao* root distribution and activity with neighbouring shade trees (Moser et al. 2010; Isaac et al. 2014; Abou Rajab et al. 2018). The present study found some evidence that the species of shade tree controls developmental plasticity in *T. cacao*. We found that *T. cacao* next to *T. ivorensis* had more evenly distributed fine roots in the upper 30 cm of soil suggesting greater complementarity belowground, while *T. cacao* roots next to *E. angolense* were more concentrated near the surface and variation in root traits showed a stronger vertical trend. Significant effects of $FRBD_{shade}$ on localized distribution, architecture, and morphology of *T. cacao* roots were not detected in this study, but it is intriguing and worth noting the marginally significant directional trends of *T. cacao* root traits in relation to fine root density of the fast-growing *T. ivorensis*, that would suggest *T. cacao* fine roots have more acquisitive root morphology when in localized competition with roots of *T. ivorensis*. However, to elucidate root-root responses, more empirical evidence is needed of root trait response when there are higher densities of neighbouring tree roots to presumably increase the effects of neighbour root activity. Root-root interactions between conspecific and heterospecific neighbouring plants can be complex (Mommer et al. 2016) and we speculate that the strength of competitive (e.g., resource depletion) and facilitative effects (e.g., organic deposits) from root activity at localized scales is likely to depend on species combination and merits further investigation.

In low-input agroforests, nutrient cycling is a significant component of nutrient delivery and shade tree leaves can constitute a substantial proportion of litterfall in shaded cocoa agroecosystems (perhaps a third to a half of total litter inputs (van Vliet and Giller 2017)). Leaf litter from fast-growing species such as *T. ivorensis* is commonly associated with higher rates of decomposition (Cornwell et al. 2008) and, along with its extensive canopy, is likely an important determinant of nutrient dynamics and distribution. Belowground, variation in root traits, such as higher SRL and lower RTD, has been associated with shorter root lifespan and faster root turnover and decomposition (Freschet and Roumet 2017). In the present study, *T. cacao* fine roots had lower RTD when in mixture with a shade tree compared to when in monoculture, suggesting more rapid nutrient cycling. More acquisitive root traits in mixture compared to monoculture have been reported in other tree-based ecosystems (e.g., Bolte and Villanueva (2006) and Duan et al. (2017)) and in *T. cacao* specifically

(Abou Rajab et al. 2018). The impacts of farm- and ecosystem-scale processes on the plastic responses of root morphology in agroforests deserve further research attention, particularly as this will be critical to the precision of nutrient management on farms.

Conclusions

Our results support the conclusion that soil nutrient heterogeneity occurs at scales relevant to individual trees in a tropical low-input agroforest. We carried out one of the first studies on multiple soil macro- and micro-nutrient effects on root functional trait expression within a species in naturally heterogeneous soils. Root system phenotypic plasticity was expressed as variation in the distribution of fine roots (FRLD and FRBD), fine root architecture (A:T), and morphology (SRL, D, and RTD). By relating root traits to soil nutrient availability on two-dimensional soil interfaces, we found that fine root trait expression had nutrient-specific relationships at localized scales (100 cm^3) within the dominant rooting zone of individual *T. cacao*. At the plant scale, intraspecific root traits shifted towards nutrient-acquiring morphology when next to a shade tree. Taken together, these results indicate that modelling of the fine root system architecture and nutrient acquisition patterns in agroforests must consider species interactions to capture the full scope of root trait expression. Measuring drivers of this root trait variability is critical to improve our understanding of the root-soil continuum in agroforestry systems and for the development of ecologically-informed agricultural practices.

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