

Root biomass variation of cocoa and implications for carbon stocks in agroforestry systems

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Abstract Cocoa (*Theobroma cacao* L.) root systems are typically assumed to contribute a small portion of carbon (C) to total C stocks in cocoa agroecosystems. Yet there are almost no direct measurements of cocoa coarse root biomass to support this assumption, presumably due to the difficulty of measuring coarse roots in situ and the risk to farmers' livelihoods. Instead, root biomass is commonly estimated using allometry based on forest data, which might not be accurate for perennial crops given their range of management conditions. In this study, we bridge conventional methods of quantifying coarse root biomass with non-destructive application of ground penetrating radar to estimate cocoa belowground biomass (BGB) and C stocks in an agroforestry system

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Department of Physical & Environmental Sciences and Centre for Critical Development Studies, University of Toronto Scarborough, Toronto, Canada in Ghana. BGB was measured for cocoa grown with shade trees (Entandrophragma angolense or Terminalia ivorensis) and in monoculture. BGB estimates showed good accuracy, with a relative root mean square error of 7% from excavated plants. It was estimated that 15-year-old cocoa hold approximately 6.0 kg C plant⁻¹ in coarse root biomass and have a root to shoot ratio of approximately 0.23. However, the results indicate that proportionally more biomass was allocated to roots for cocoa grown in mixture with shade trees. Plot scale estimates show that cocoa roots contributed 5.4–6.4 Mg C ha⁻¹, representing 8–16% of C stocks in all live tree biomass (cocoa + shade trees), depending on shade tree management. Our findings illustrate a promising approach for nondestructive BGB inventories of perennial crops. It is highlighted that although commonly used pan-tropical allometric equations may broadly function in estimating BGB for cocoa, this approach assumes proportional allocation between aboveground biomass and BGB, which may translate into inaccuracies in C stock inventories across diverse cocoa agroecosystems.

Keywords Carbon inventory \cdot Coarse root \cdot Ghana \cdot Ground penetrating radar \cdot *Theobroma cacao* \cdot Root to shoot ratio

Abbreviations

AGB_H Aboveground biomass measured from destructive harvesting

AGB_{SA}	Aboveground biomass calculated using a
	species-specific allometric equation
$BGB_{\rm H}$	Belowground biomass measured from
	destructive harvesting/excavation
BGB_{GA}	Belowground biomass calculated using a
	generalized allometric equation
BGB _{GPR}	Belowground biomass estimated from
	ground penetrating radar geo-imagery and

destructive sampling

Introduction

Cocoa (Theobroma cacao L.) cultivation occurs on 10 million ha of land globally (FAO 2013) and in regions where it is produced can be a dominant form of landuse (Norris et al. 2010; Schroth et al. 2015), thus deserving inclusion in national or sub-national carbon (C) inventories (Wade et al. 2010). Dedicated attention towards the role of cocoa agroecosystems in the C cycle is reflected by many recent C stock assessment studies in West Africa (Wade et al. 2010; Norgrove and Hauser 2013; Saj et al. 2013), Central and South America (Somarriba et al. 2013; Jacobi et al. 2014; Schroth et al. 2016), and Southeast Asia (Smiley and Kroschel 2008; Leuschner et al. 2013; Abou Rajab et al. 2016). As cocoa is often cultivated under shade trees, cocoa agroforestry systems are characterized by diverse vegetative structure, balancing the objectives of maintaining agricultural productivity with increasing C stocks in tropical landscapes (Vaast and Somarriba 2014). Given that tree species composition in agroforestry systems is a strong determinant of C stocks at the farm or plot scale (Dixon 1995; Montagnini and Nair 2004; Kirby and Potvin 2007; Jose 2009; Kessler et al. 2012), attention has been focused on contrasting the amount of C given a range of on-farm tree diversity in cocoa agroecosystems (Wade et al. 2010; Somarriba et al. 2013; Saj et al. 2013; Jacobi et al. 2014; Obeng and Aguilar 2015; Abou Rajab et al. 2016).

The largest contributors to C stocks in cocoa agroforestry systems are typically shade tree biomass and soil organic matter (Wade et al. 2010; Somarriba et al. 2013; Jacobi et al. 2014). However, the contribution from cocoa plants becomes increasingly dominant with higher densities of cocoa and/or thinning of shade trees (Wade et al. 2010; Saj et al.

2013). To quantify the biomass C in cocoa plants, a number of species-specific allometric equations for aboveground biomass (AGB) have been developed for cocoa (e.g., Andrade et al. 2008; Smiley and Kroschel 2008; Somarriba et al. 2013). However, studies that include estimates of belowground biomass (BGB) of cocoa almost entirely rely on allometric equations for tropical forests or a set proportion of BGB in relation to AGB (i.e., root to shoot (RS) ratios) (Table 1). Given the challenges of directly measuring root systems, these approaches are viable alternatives to destructive excavation. However, equations derived from forest data arguably have reduced accuracy in cultivated systems (Kuyah et al. 2012; Borden et al. 2014). Additionally, generalized allometry might be limited in capturing variation of biomass allocation. This is of interest when management and/or environment exhibit strong control over resource availability, inducing variable growing conditions for a cultivated species. The plasticity of cocoa coarse and fine root architecture and activity under different environmental and management conditions has been documented via soil profiles (Moser et al. 2010; Schwendenmann et al. 2010), isotope signatures (Schwendenmann et al. 2010; Isaac et al. 2014), and near-surface imaging using ground penetrating radar (GPR) (Isaac et al. 2014). However, to our knowledge, there are no studies that test for altered root biomass of productive cocoa in response to effects from shade trees.

In this study, we used a combination of GPR geoimagery with conventional sampling techniques (soil cores and excavation) to quantify coarse root biomass for cocoa in monoculture, cocoa in mixture with *Entandrophragma angolense* (Welw.) C. DC., and cocoa in mixture with *Terminalia ivorensis* A. Chev. The objectives of this study were (i) to determine the contribution of cocoa coarse roots to vegetative C stocks, (ii) to distinguish intraspecific variation of cocoa root biomass in three shade tree compositions that are commonly practiced in Ghana (Anglaaere et al. 2011), and (iii) to evaluate the accuracy of estimates, as this study represents a first-time application of GPR for BGB estimation within cocoa agroecosystems.

Table 1 Previously reported	coarse root bion	nass (BGB) carbon in cocoa agroece	osystems and methods of e	stimation based	on aboveground	l parameters	
Age classification ^a (years)	Country	Type of cocoa agroecosystem	Reported C fraction	BGB C (Mg	$C ha^{-1}$)	Density (trees/	plants ha^{-1})
				Total	Cocoa	Tree	Cocoa
Immature (< 8)	Cameroon	AFS1 AFS3	0.475	0.6	0.3	328	1728
Immature to young (5-20)	Bolivia	Monoculture	0.50	5	3.5^{b}	23.9 ± 14.8	625
Young (9–20)	Cameroon	AFS1 AFS3	0.475	12.5	1.0	258	1783
Young (6)	Indonesia	AFS2	0.47	3.4 ± 1.0	2.5 ± 0.8	325 ± 1.3	1030 ± 0.8
Young (8–17)	Bolivia	AFS1	0.50	11	4.2 ^b	296 ± 71.2	625
Young (13–16)	Bolivia	AFS3	0.50	17	1.8^{b}	699 ± 114	625
Young (18.1 ± 8.4)	Guatemala	AFS1 AFS3	0.50	6.6	2.4 ^b	222	604 ± 128
Young to mature (13-23)	Indonesia	Monoculture	Unreported	1.9	1.9	I	892 ± 128
Young to mature (13–23)	Indonesia	AFS2	Unreported	3.1	1.5	428	1047 ± 150
Young to mature (13-23)	Indonesia	AFS3	Unreported	8.0	2.1	357	1384
Mature (20.3 ± 6.7)	Nicaragua	AFS1 AFS3	0.50	6.9	2.4 ^b	293	562 ± 98
Mature (20.5 ± 6.5)	Honduras	AFS1 AFS3	0.50	9.4	2.6^{b}	225	583 ± 205
Mature (24.9 ± 14.5)	Costa Rica	AFS1 AFS3	0.50	9.3	2.1 ^b	480	591 ± 218
Mature (26.9 \pm 6.1)	Panama	AFS1 AFS3	0.50	11.5	1.8^{b}	477	588 ± 215
Mature (30.8 ± 20.2)	Guatemala	AFS3	0.50	13.4	2.1 ^b	209	335 ± 148
Mature (35)	Cameroon	AFS1	0.45	23.0	1.9	115	1362
Mature (21-40)	Cameroon	AFS1 AFS3	0.475	12.4	1.6	188	1527
Senescent (> 40)	Cameroon	AFS1 AFS3	0.475	12.4	1.6	147	1303
Age classification ^a (years)	BGB C (kg C plant Cocoa	⁻) Method of estimation	Equation			Referer	ICeS
Immature (< 8)	0.2	GA-GA	AGB = exp[-2.187 +	$0.916 \ln(\rho D^2 H)]^c$		Saj et al.	(2013)
			AGB = exp[-2.977 +	0.94 $\ln(\rho D^2 H)]^c$			
			BGB = exp[-1.0587 +	0.8836 ln(AGB)] ^d			
Immature to young (5-20)	5.6	SA-GA	$AGB = 10^{(-1.625+2.63\log)}$	D ₃₀))		Jacobi et	al. (2014)
			BGB = exp[-1.085 +	0.926 ln(AGB)] ^d			
Young (9–20)	1.3	GA-GA	AGB = exp[-2.187 +	$0.916 \ln(\rho D^2 H)]^c$		Saj et al.	(2013)
			$AGB = \exp[-2.977 +$	$0.94 \ln(\rho D^2 H)]^c$			
			BGB = exp[-1.0587 +	0.8836 ln(AGB)] ^d			
Young (6)	2.4	SA-GA/S	$AGB = 0.202 \times D^{2.112}$			Leuschne	x et al. (2013)
			BGB: combined soil pit (Mokany et al. 2006);	s > 1 m from stem excavations 1 m ra	and R:S ratio 0.235 Idius	10	
Young (8–17)	6.7	SA-GA	$AGB = 10^{(-1.625+2.63\log)}$	D ₃₀))		Jacobi et	al. (2014)
			BGB = exp[-1.085 +	0.926 ln(AGB)] ^d			
Young (13–16)	2.9	SA-GA	$AGB = 10^{(-1.625+2.63)}$	D ₃₀))		Jacobi et	al. (2014)
			BGB = exp[-1.085 +	0.926 ln(AGB)] ^d			

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Table 1 continued				
Age classification ^a (years)	BGB C (kg C plant ⁻¹) Cocoa	Method of estimation	Equation	References
Young (18.1 ± 8.4)	4.0	SA-GA	AGB = $exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = $exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Somarriba et al. (2013)
Young to mature (13-23)	2.1	SA-GA	AGB = -0.0376 + 0.133BA BGB = exp[-1.0587 + 0.8836 ln(AGB)] ^d	Abou Rajab et al. (2016)
Young to mature (13–23)	1.4	SA-GA	AGB = $-0.0376 + 0.133BA$ BGB = $\exp[-1.0587 + 0.8336 \ln(AGB)]^d$	Abou Rajab et al. (2016)
Young to mature (13-23)	1.5	SA-GA	AGB = $-0.0376 + 0.133BA$ BGB = $\exp[-1.0587 + 0.8336 \ln(AGB)]^d$	Abou Rajab et al. (2016)
Mature (20.3 \pm 6.7)	4.3	SA-GA	AGB = $\exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = $\exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Somarriba et al. (2013)
Mature (20.5 ± 6.5)	4.5	SA-GA	AGB = $\exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = $\exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Somarriba et al. (2013)
Mature (24.9 \pm 14.5)	3.6	SA-GA	AGB = $\exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = $\exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Somarriba et al. (2013)
Mature (26.9 ± 6.1)	3.1	SA-GA	$AGB = exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = exp[-1.0587 + 0.8836 ln(AGB)] ^d	Somarriba et al. (2013)
Mature (30.8 ± 20.2)	6.3	SA-GA	$AGB = exp[-1.684 + 2.158 \log(D_{30}) + 0.892 \log(H)]$ BGB = exp[-1.0587 + 0.8836 ln(AGB)] ^d	Somarriba et al. (2013)
Mature (35)	1.4	SA-SA	AGB = ρ CSA (L + 2.32 B) BGB: root:shoot ratio 0.15	Norgrove and Hauser (2013)
Mature (21-40)	1.3	GA-GA	AGB = $\exp[-2.187 + 0.916 \ln(\rho D^2 H)]^c$ AGB = $\exp[-2.977 + 0.94 \ln(\rho D^2 H)]^c$ BGB = $\exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Saj et al. (2013)
Senescent (> 40)	1.3	GA-GA	AGB = $\exp[-2.187 + 0.916 \ln(\rho D^2 H)]^c$ AGB = $\exp[-2.977 + 0.94 \ln(\rho D^2 H)]^c$ BGB = $\exp[-1.0587 + 0.8836 \ln(AGB)]^d$	Saj et al. (2013)
AFSI shade trees for timber or fi	ruit production, AFS2	N ₂ -fixing shade trees, AFS3 mul	ti-species agroforestry systems, GA-GA AGB from generalized a	lometric equation used in

3 ILOII **B**DA DUD, 3A-3A/3 101 equation allolleuric u III generalizeu a generalized autometric equation for BUB, 3A-UA AUB from coccoa-specific autometric equation u specific allometric equation used in coccoa-specific R:S ratio or with direct measurements of roots

^aAge classification follows Saj et al. (2013): immature plantations = under 8 years old; young plantations = 9-20 years, mature plantations = 21-40 years; senescent plantations = 41 years and older

^bCalculated using the methods in cited paper; excludes fine root biomass

°Chave et al. (2005)

^dCairns et al. (1997)

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Methods

Study site and study plants

The study was conducted at a cocoa research station, managed by the CSIR-Forestry Research Institute of Ghana, located in South Formangso, Ashanti Region, Ghana (6°36'N and 0°58'W). The site was previously secondary forest until it was cleared for food crop cultivation and left to fallow until the research plot was established in 2001. It is located within a moist semi-deciduous forest zone. The mean annual rainfall ranges from 1700 to 1850 mm with two maxima rainfall seasons from March to July and September to November. Soils are predominantly loam or sandy clay loam and are classified as Acrisols (Isaac et al. 2014).

Three shade tree treatments were used: (1) cocoa in monoculture, (2) cocoa in mixture with E. angolense $(DBH = 22.4 \pm 3.6 \text{ cm}; \text{ height} = 14.1 \pm 3.0; n =$ 5), and (3) cocoa in mixture with T. ivorensis (DBH =height = 21.6 ± 2.0 m; 52.8 ± 8.0 cm; n = 5). These shade tree species are used for timber and are often selected by farmers in this region (Anglaaere et al. 2011). The 15-year-old cocoa plants at this site $(DBH = 12.4 \pm 2.8 \text{ cm}; \text{height} = 6.1 \pm 1.1 \text{ m}; n =$ 45) are in regular spacing of 3 m \times 3 m and are all the same variety (hybrid cocoa from the Cocoa Research Institute of Ghana). In mixture, the shade trees were planted at the same time as cocoa and in replacement design of 12 m × 12 m spacing. Thus, cocoa plant density in monoculture is 1111 plants ha⁻¹ while in mixtures it is 1042 plants ha⁻¹. Management was consistent across all treatments and no fertilizer was applied to the site prior to this study. The coarse root systems of 15 individual cocoa plants were surveyed, which included five cocoa plants for each of the shade tree treatments. Plants were chosen at random from pre-established study blocks (one per block), although limited to where GPR survey was appropriate (e.g., relatively flat soil) and, when in mixture, cocoa plants that were 3 m from a shade tree.

Coarse root biomass estimation using GPR

After removal of leaf litter, grids $3.0 \text{ m} \times 3.0 \text{ m}$ were centred at the base of each surveyed cocoa, with consistent orientation to plant spacing, and assumed to be the unit soil area of a single cocoa plant (Bengough

et al. 2000). A GPR unit with a centre frequency of 1000 MHz with an attached odometer (Sensors & Software Inc., Canada) (Table S1) was used to collect geo-imagery data in straight orthogonal lines with transect spacing of 0.10 m, which produced 62 geoimages for each surveyed root system. Potential sources of signal interference, such as where understory crops were planted (e.g., cocoyam Xanthosoma sagittifolium (L.) Shott), were marked in the data. These locations were later reviewed to omit any signal response that could be falsely identified as cocoa root biomass. All geo-image data were processed following a data processing sequence that reduces background signal noise, compensates for signal attenuation with depth, and delineates possible root reflections (Guo et al. 2013; Borden et al. 2014). Geoimage processing was completed in EKKO software (Sensors & Software). Subsequently, thresholding of processed images was completed in ImageJ 1.48v (US National Institutes of Health, USA) to measure the number of pixels in a geo-image that were delineated as coarse root biomass (Table S2).

A calibration model was populated whereby root biomass was related to the corresponding radar response following a user-guided approach that included a range of radar responses (Butnor et al. 2015). The corresponding roots at each identified location were excavated, cut to 10 cm lengths (matched to GPR transect spacing), oven dried at 70 °C to constant mass, and then weighed for root mass (n = 30). The minimum detectable root mass was 4.9 g, identified by the y-intercept of the calibration relationship (Table S2; Fig. S1). This calibration relationship was applied to all geo-imaged root responses to estimate biomass and summed at the tree scale to estimate coarse root biomass. GPR signal attenuation occurred between 30 and 40 cm soil depth and, thus, we limited lateral root biomass estimation to the top 30 cm of soil. Cocoa have shallow lateral root systems primarily in the top 30 cm of soil, but some lateral root biomass might be located below 30 cm (Moser et al. 2010; Nygren et al. 2013; Isaac et al. 2014).

Sampling of coarse roots and whole plant excavations

To quantify coarse root biomass too small for GPR signal detection, a 10-cm diameter auger was used to

extract soil and roots to 30 cm depth (soil core volume = 2356 cm^3) at 5 random locations within the GPR survey area of each study plant. Coarse roots (> 0.2 cm diameter) were removed by hand from extracted soil cores. Cocoa roots were separated from the shade tree roots. The cocoa roots were identified by their distinctly dark reddish-brown colour. The mean small coarse root (0.2 cm to 1.3 cm diameter) biomass extracted from the soil cores was used to estimate the small root biomass for each surveyed root system.

As GPR-based estimates cannot discern roots by species, using the same soil cores as above, we calculated the biomass contribution of shade tree species to subtract from our estimates. To 30 cm sampling depth, *T. ivorensis* contributed 9% of the coarse root biomass in cocoa-*T. ivorensis* mixture and *E. angolensis* contributed less than 1% of the coarse root biomass in the cocoa-*E. angolensis* mixture and was assumed to be negligible.

A subsample of the study plants (n = 3) were destructively harvested to quantify both aboveground biomass $(ABG_{\rm H})$ and belowground biomass $(BGB_{\rm H})$. Cocoa coarse root systems were excavated for the purposes of (i) estimating taproot biomass that would be undetected by GPR [i.e., below-stem biomass (Butnor et al. 2015)] and (ii) evaluating the accuracy of the estimates. Square plots matching the area of the GPR survey grids were manually excavated to 30 cm. Tap roots were excavated completely (below 30 cm depth) and separated from lateral roots. The destructively sampled cocoa trees were stratified into stem, branch, and root organs, and the fresh weight of the various organs determined with a weighing scale. Samples of stem, branch, and root (n = 6) were weighed and oven dried to determine moisture contents, which were used to calculate the dry weight biomass of each biomass organ for each harvested cocoa plant.

Biomass allocation calculations

AGB of cocoa plants was estimated using a cocoaspecific allometric equation (Somarriba et al. 2013):

$$Log(AGB_{SA}) = (-1.684 + 2.158 \times Log(D_{30}) + 0.892 \times Log(H))$$
(1)

where AGB_{SA} is the species-specific allometric estimate of cocoa aboveground biomass, D_{30} is the diameter in cm of the stem at 30 cm above the ground and *H* is tree height in m, which was measured using a clinometer.

BGB of cocoa plants was estimated in two ways. One method was based on GPR data and calculated as:

$$BGB_{\rm GPR} = BGB_{\rm lateral} + BGB_{\rm taproot} \tag{2}$$

where BGB_{GPR} is the sum of lateral root biomass $(BGB_{lateral})$, which is the large coarse root biomass estimated using GPR and the small coarse root biomass estimated from soil cores, and $BGB_{taproot}$, which was calculated using the ratio of lateral roots to taproot biomass of the harvested cocoa plants. The second method involved application of the most commonly used allometric equation (Table 1) reported in Cairns et al. (1997) as:

$$BGB_{GA} = \exp[-1.0587 + 0.8836 \times \ln(AGB_{SA})]$$
(3)

where BGB_{GA} is the BGB estimated using generalized allometric equation for tropical forests and calculated using AGB_{SA} from Eq. 1. RS ratios of cocoa plants were calculated as the ratio of BGB_{GPR} to AGB_{SA} .

Biomass carbon calculations

To determine the C fraction of cocoa biomass, coarse roots were collected during excavation and stem samples were collected at 1.3 m above the ground using an increment borer (n = 3). Stem and root samples were stored in air-tight bags and frozen until they were later freeze-dried to constant weight. Samples were ground in ball-mill and analyzed for total C using a CN elemental analyzer (Thermo Flash 2000) at University of Toronto Scarborough, Canada. The C fraction was determined using protocol found in Thomas and Martin (2012) that includes C volatilized during oven drying.

An inventory of cocoa plant metrics, D_{30} and H, was carried out to calculate AGB_{SA} of cocoa at the study site (n = 45). Subsequently, BGB estimates of cocoa across the site were calculated using the RS ratios of surveyed cocoa plants, as well as generalized allometry (Eq. 3) for comparative purposes. C fractions from chemical analysis were applied to respective above- and belowground biomass to calculate the C content of cocoa plants. Plot scale estimates were determined as the products of the cocoa plant densities and C contents of cocoa plants, based on shade tree treatment. As the effects of distance from shade tree on cocoa BGB were not tested, it was assumed that cocoa plants 6 m from the shade trees (which was 50% of cocoa plants in mixture) were not affected by shade trees (Isaac et al. 2007a). Species-specific allometric equations, C fractions, and a shade tree density of 68 trees ha⁻¹ were used to estimate biomass (AGB + BGB) C of the shade trees *E. angolense* and *T. ivorensis* (Deans et al. 1996; Henry et al. 2011; Yeboah et al. 2014).

Statistical analysis

The relationship between coarse root biomass and radar signal response (i.e., calibration model) was assessed with Pearson's correlation coefficient and one-way analysis of variance (ANOVA) (Fig. S1). Relationships between aboveground plant metrics (DBH) and BGB estimates were described using linear regression. Differences in BGB and RS ratios among shade tree treatments were tested using ANOVA and, when significant, pairwise comparisons using Tukey's HSD test. Percent differences, root mean square error (RMSE), relative root mean square error (RRMSE), and coefficient of determination (r^2) were used to evaluate BGB estimator performance when comparing methodologies. Prior to parametric tests, data were tested for equality of variance using the Bartlett test and tested for normality of residuals using the Shapiro-Wilk test. Statistical analyses were completed in R (R Foundation for Statistical Computing, Austria) with the level of significance set at p < 0.05.

Results

Coarse root biomass estimation

Within this even-aged agroecosystem, aboveground plant size was related to amount of biomass belowground, with BGB_{GPR} positively correlating with the size of stem ($r^2 = 0.37$; $F_{1,13} = 7.54$; p = 0.02) (Fig. 1). This correlation between structural roots and stem size was driven by variation in large coarse roots. GPR detected between 4.6 and 13.7 kg plant⁻¹ in BGB and these values were significantly and positively correlated with *DBH* ($r^2 = 0.36$; $F_{1,13} = 7.45$; p = 0.02; data not shown) unlike the smaller (0.2–1.3 cm diameter) coarse root biomass measured from soil cores that did not correlate with *DBH* ($F_{1,12} = 3.89$; p > 0.05; data not shown). The ratio of excavated lateral to excavated taproot biomass was 3.3 ± 0.2 (n = 3), which was used to estimate the taproot biomass of each study tree, and thus taproots were found to comprise approximately 23% of *BGB*_{GPR}.

 BGB_{GPR} displayed good accuracy, with a mean percent difference of $12.0 \pm 7.8\%$ (n = 3), RMSE of 3.0 kg plant⁻¹, and RRMSE of 7.3% from $BGB_{\rm H}$ of matched plants. BGBGA was less accurate, indicated by a larger mean percent difference of $30.1 \pm 1.6\%$, RMSE of 7.5 kg plant⁻¹, and RRMSE of 18.3%. While there was a significant correlation between estimates from the two approaches $(r^2 = 0.44;$ $F_{1,13} = 10.10; p = 0.007$ (Fig. 2), some inconsistencies were observed. The RMSE between the two approaches was 5.6 kg $plant^{-1}$ (RRMSE of 7.0%). There was a tendency for BGB_{GA} to be overestimated when there was less than 16.4 kg $plant^{-1}$ and underestimated when there was more (Fig. 2), or in more tangible terms, when cocoa DBH was less than or more than 12 cm.

Biomass allocation

There was a preference for cocoa to allocate proportionally more biomass belowground when in mixture, although not significantly $(F_{2,12} = 2.39; p > 0.05)$. Cocoa in mixture with T. ivorensis had the highest RS ratio of 0.28 \pm 0.05 (\pm SE; n = 5), cocoa in mixture with *E. angolense* had a RS ratio of 0.23 ± 0.01 (n = 5), while cocoa in monoculture had the lowest RS ratio of 0.19 ± 0.02 (n = 5) (Table 2). Applying these treatment-specific RS ratios to AGB_{SA} across the study site $(55.4 \pm 5.2 \text{ kg plant}^{-1}; n = 45)$, BGB for 15-year-old cocoa was estimated to be $10.4 \pm$ 1.0 kg plant⁻¹ in monoculture, 12.9 ± 1.2 kg plant⁻¹ for cocoa in mixture with E. angolense, and 15.6 ± 1.5 kg plant⁻¹ for cocoa in mixture with T. ivorensis, in which cocoa in monoculture was significantly less than cocoa in mixture with T. ivorensis (p = 0.01) (Table 2). Using generalized allometry, BGB_{GA} of cocoa was 11.8 \pm 1.0 kg plant⁻¹ in all shade tree treatments and the biomass allocation patterns were



Fig. 1 Relationship between cocoa DBH (cm) and coarse root biomass (kg plant⁻¹) (*BGB*_{GPR} = 0.50 + 1.37 × *DBH*). Symbols represent cocoa grown in different shade tree treatments (filled square = cocoa in monoculture, filled circle = cocoa in mixture with *E. angolense*, filled triangle = cocoa in mixture with *T. ivorensis*). Excavated amounts (*BGB*_H) are indicated (cross), but are not included in the regressions

concentrated around a mean RS ratio of 0.21 ± 0.00 (\pm SE), ranging between 0.19 and 0.22 (Fig. 3).

Biomass carbon

Cocoa coarse root C fraction was 0.469 ± 0.005 (mean \pm SE; n = 3), with a volatile mass fraction of 0.036 ± 0.005 . The C fraction of cocoa stems was 0.463 ± 0.002 , with a volatile mass fraction of 0.051 ± 0.005 . BGB_{GPR} C for cocoa at this site amounted to 4.9, 6.1, and 7.3 kg C plant⁻¹ for cocoa in monoculture, cocoa in mixture with *E. angolense* and cocoa in mixture *T. ivorensis*, respectively (Table 2). BGB_{GA} C was consistently 5.6 kg C plant⁻¹ in all shade tree treatments.

Even though there was a higher density of cocoa planted in monoculture (1111 plants ha⁻¹) compared to in mixture (1046 plants ha⁻¹), plot scale estimates of cocoa BGB C were relatively similar for cocoa in monoculture (5.4 Mg C ha⁻¹) and cocoa in mixture with *E. angolense* (5.7 Mg C ha⁻¹), while cocoa BGB C in mixture with *T. ivorensis* (6.4 Mg C ha⁻¹) was approximately 15% higher than either system (Fig. 4). Using generalized allometry, cocoa *BGB*_{GA} C at the plot scale in both mixtures was estimated to be 5.9 Mg C ha⁻¹, which was proximate to estimates



Fig. 2 Relationship between BGB of individual cocoa plants (kg plant⁻¹) as estimated from GPR and destructive sampling $(BGB_{\rm GPR})$ and estimated using a generalized allometric equation $(BGB_{\rm GA})$. Linear regression is the solid line $(BGB_{\rm GA} = 7.07 + 0.57 \times BGB_{\rm GPR})$. Symbols represent cocoa grown in different shade tree treatments (filled square = cocoa in monoculture, filled circle = cocoa in mixture with *E. angolense*, filled triangle = cocoa in mixture with *T. ivorensis*)

based on BGB_{GPR} for cocoa in mixture with *E*. *angolense* but was an underestimate of 8% for cocoa in mixture with *T. ivorensis*. BGB_{GA} plot scale estimate for cocoa in monoculture (6.3 Mg C ha⁻¹) was 16% higher than the corresponding estimate based on BGB_{GPR} .

Total (AGB + BGB) live tree (cocoa + shade) C stocks were estimated to be 33.9, 41.9, and 83.7 Mg C ha⁻¹ for cocoa monoculture, cocoa-*E.* angolense mixture, and cocoa-*T. ivorensis* mixture, respectively (Fig. 4). Of these amounts, BGB (co-coa + shade) C stock amounted to 5.4, 7.2, and 17.8 Mg C ha⁻¹ in cocoa monoculture, cocoa-*E.* angolense mixture, and cocoa-*T. ivorensis* mixture, respectively (Fig. 4).

Discussion

Biomass C stocks in cocoa agroecosystems

Cocoa monoculture contained the least amount of biomass (AGB + BGB) C in plot scale estimates, representing less than half (41%) of the biomass C in

	Root:shoot (BGB _{GPR} :AGB _{SA})	Coarse root biomass (kg plant ⁻¹)	Carbon in coarse root (kg C plant ^{-1})
Cocoa average	0.23 ± 0.02	12.7 ± 1.2	6.0
By shade management			
Cocoa in monoculture	0.19 ± 0.02	10.4 ± 1.0^{a}	4.9
Cocoa in mixture with Entandrophragma angolense	0.23 ± 0.01	12.9 ± 1.2^{ab}	6.1
Cocoa in mixture with Terminalia ivorensis	0.28 ± 0.05	15.6 ± 1.5^{b}	7.3

Table 2 Root to shoot ratios (BGB_{GPR} : AGB_{SA} ; mean \pm SE) of 15-year-old cocoa plants. Cocoa coarse root biomass and carbon estimates are based on cocoa aboveground biomass^a estimated for the site and tissue-specific carbon fraction^b

Non-significant differences between treatments are indicated by same letters (Tukey HSD; p < 0.05)

There was no significant variation of root to shoot ratios among shade treatments (ANOVA; p > 0.05)

^a 55.4 \pm 5.2 kg plant⁻¹ (mean \pm SE; n = 45) calculated using cocoa-specific allometric equation from Somarriba et al. (2013)

 b 0.469 \pm 0.005 (mean \pm SE; n = 3) is the volatile-inclusive C fraction of cocoa coarse roots on an oven dry basis



Fig. 3 Root to shoot (RS) ratios calculated for cocoa plants across three different shade tree treatments. Root estimates are from GPR and destructive sampling $(BGB_{\rm GPR})$ and estimated using a generalized allometric equation $(BGB_{\rm GA})$. $AGB_{\rm SA}$ was based on species-specific allometric equation (Somarriba et al. 2013). The horizontal dashed line indicates a IPCC recommended RS ratio of 0.20. The RS ratios measured from one complete harvested cocoa plant per treatment are also shown (cross)

cocoa-*T. ivorensis* mixture. Furthermore, biomass C in cocoa monoculture was 23% below current average biomass C stocks on agricultural lands in Ghana (44 Mg C ha⁻¹) (Zomer et al. 2016). Cocoa-*T. ivorensis* mixture had the highest biomass C stocks (84 Mg C ha⁻¹). This C estimate was generally higher than reported biomass C in other cocoa agroforestry systems [Cameroon: 70 Mg C ha⁻¹ (Saj et al. 2013), Bolivia: 69 Mg C ha⁻¹ (Jacobi et al.

2014)], but was less than the 131 Mg C ha⁻¹ reported for traditional, high-shade, cocoa agroforestry systems in Ghana (Wade et al. 2010). *T. ivorensis* contributed 60% of the biomass C to the plot scale estimate, demonstrating the high C storage potential of this shade tree species. Biomass C in cocoa-*E. angolense* mixture was the lowest of the two mixtures. In this species combination, cocoa plants contributed the majority (77%) of biomass C. While individual *E. angolense* trees contained two to three times the amount of C than individual cocoa plants, more substantial C stocking advantages of cocoa-*E.*



Fig. 4 Plot scale estimates of biomass carbon (Mg C ha^{-1}). Carbon estimates are for cocoa plants and shade trees. Total biomass (cocoa + shade) is indicated by horizontal lines

angolense mixtures over cocoa monoculture might not be realized until at an older plantation age given slower growth of late-successional *E. angolense*.

The relative contribution of cocoa coarse roots to total biomass C stocks can largely depend on the density that cocoa is planted and the amount of BGB C of individual cocoa plants. Cocoa roots contributed 5.7 and 6.4 Mg C ha⁻¹ to cocoa-*E*. angolense and cocoa-*T*. ivorensis mixture, respectively, which was 13 and 8% of C in all live tree biomass (cocoa + shade). Our estimates were proximate to root C contributions (11-13%) reported in other agroforestry systems that had similar cocoa density (> 1000 plants ha^{-1}) (Leuschner et al. 2013) and root system size (> 5.0 C plant⁻¹) (Jacobi et al. 2014) (Table 1). In less intensively managed agroforestry systems, with fewer cocoa plants and greater density of shade trees, cocoa BGB may become less significant but perhaps not inconsequential for total C stocks. For example, in agroforests across Central America, the densities of cocoa were often half that reported in our study and densities of shade trees were three to four times greater (Somarriba et al. 2013). Yet, BGB C in cocoa plants (~ 3.6–6.3 kg C plant⁻¹) contributed approximately 2-6% of total biomass C in these agroforests (Table 1) (Somarriba et al. 2013). Differences reported among studies and among cocoa growing regions highlight the diversity in agroforestry systems and the challenge and the need to improve context-specific estimates of C stocks.

Toward accuracy of C accounting in agroforestry systems

The use of site- and species-specific allometric equations, when available, is recommended over generalized equations for higher accuracy in AGB estimates (Djomo et al. 2010). This recommendation is seemingly true, but less tested, for estimating BGB (Keller et al. 2001; Mokany et al. 2006; Kuyah et al. 2012). Kuyah et al. (2012) reported that pan-tropical BGB allometric equations underestimated root biomass in agroforestry systems by approximately 21 and 35% [using equations from Mokany et al. (2006) and Cairns et al. (1997), respectively]. This study indicates that generalized allometric equations may be limited in tracking allocation patterns of this perennial crop. More empirical evidence beyond the size range of the same-aged cocoa plants used in our study are required to respond to this concern. Yet, in many cases, destructive harvesting to populate specialized ratios and models might not be viable, particularly when complete harvesting can interfere with farm productivity. As we show here, estimates from GPR data paired with small coarse root sampling and taproot estimates (BGB_{GPR}) were more proximate than allometric estimates (BGB_{GA}) to excavated amounts of coarse root biomass (BGB_H). Non- and/or low-destructive sampling of root systems can aid in testing for variation in RS ratios or allometry among agroforestry management conditions. However, it is important to note that there are constraints on the appropriateness of this technology across variable cocoa growing sites, mainly when soils have high moisture or clay content that attenuate radar signal and/or reduce dielectric contrast at soil-root interfaces. Additional destructive sampling might also be required to correctly compensate for variable contributions of coarse root biomass from non-target tree species (e.g., shade trees).

The mean RS ratio of 0.23 was within the range of RS ratios previously reported for cocoa (0.22-0.28) (Moser et al. 2010; Leuschner et al. 2013; Abou Rajab et al. 2016), but was greater than the IPCC default RS ratio of 0.20 for this forest zone (International Panel for Climate Change 2006). Also of note, was the range of RS ratios (means 0.19-0.28) associated with different forms of shade tree treatments, for sameaged cocoa plants at the same site. Shade trees, particularly large fast-growing shade trees such as T. ivorensis, can modify light, humidity, soil moisture, and nutrient availability and cycling, all of which can, in turn, influence the nutrient status of cocoa plants (Isaac et al. 2007a, b) and plant response to environment (e.g., in root distribution) (Isaac et al. 2014), which may partially explain the differential allocation patterns observed in this study. Furthermore, although not tested in this study, agricultural interventions (e.g., pruning, harvesting, fertilizer application) may also affect growth and stature of tree crops, resulting in altered allometric trajectories than forest trees, which allometric equations are based (Kuyah et al. 2012).

Whether intraspecific variation in root-based C stocks are deemed important for inclusion in C inventories may depend on the scale of the study and/ or ecosystem service compensation program. In Ghana, average cocoa farm size is approximately 2 ha (Asare and Ræbild 2016) and clear gains in biomass C stocks at this scale would mainly be credited

to the presence of large shade trees. However, variation reported in this study for cocoa root biomass in agroforestry compared to monocrop cultivation, a difference of 1 Mg C ha⁻¹, represents a 12% improvement on recent gains of biomass C on agricultural land in Ghana (average increase of 8.1 Mg C ha⁻¹ between 2000 and 2010) (Zomer et al. 2016). Additional research is required to determine how representative the results from this study are to regional estimates and if other management factors, such as nutrient additions, are impactful on systematic variation of biomass allocation. Nonetheless, generalized allometry should be used cautiously when estimating BGB, for example, to avoid overestimating BGB of cocoa in monoculture, which in this study was by 16% or approximately 1 Mg C ha⁻¹. At regional or national scales, even small variation could lead to substantial differences in C stock estimation considering that cocoa cultivation occurs on 1.6 million ha of land in Ghana (FAO 2013). Improvement in biomass and C estimation may refine accounting of national or regional level C stocks, but could also contribute to development of compensation schemes for C sequestration that may help farmers maintain and/or convert to pro-environmental agricultural practices such as agroforestry.

Conclusions

Belowground biomass should be included in C stock assessments of cocoa agroecosystems. In agroforestry systems, the relative contribution of cocoa BGB to total C stocks is particularly important when cocoa are planted in high densities. In this study, a widely-used pan-tropical allometric equation was broadly functional in estimating BGB for the perennial crop species, but predicted near-static partitioning between above and belowground biomass and was subsequently less accurate than methods using GPR. There was evidence of higher allocation to BGB of cocoa in agroforestry, particularly when grown in mixture with a fast-growing early successional shade tree (Terminalia ivorensis). Conversely, cocoa in monoculture may have lower allocation to coarse roots and caution is suggested when estimating total C stocks to avoid overestimation. More research is required to determine the magnitude of variability of biomass allocation for this perennial crop at different ages and under different forms of management. When present, these differences in root biomass may result in consequential differences for large scale estimates of biomass C stocks.

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