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Evaluation of the effects of elevated CO_2 concentrations on the growth of cassava storage roots by destructive harvests and ground penetrating radar scanning approaches

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Abstract

Cassava (*Manihot esculenta* Crantz) production will need to be improved to meet future food demands in Sub-Saharan Africa. The selection of high-yielding cassava cultivars requires a better understanding of storage root development. Additionally, since future production will happen under increasing atmospheric CO_2 concentrations ($[CO_2]$), cultivar selection should include responsiveness to elevated $[CO_2]$. Five farmer-preferred African cassava cultivars were grown for three and a half months in a Free Air CO_2 Enrichment experiment in central Illinois. Compared to ambient $[CO_2]$ (~400 ppm), cassava storage roots grown under elevated $[CO_2]$ (~600 ppm) had a higher biomass with some cultivars having lower storage root water content. The elevated $[CO_2]$ stimulation in storage root biomass ranged from 33% to 86% across the five cultivars tested documenting the importance of this trait in developing new cultivars. In addition to the destructive harvests to obtain storage root parameters, we explored ground penetrating radar as a nondestructive method to determine storage root growth across the growing season.

KEYWORDS
bulking rate, cassava storage roots, CO_2, elevated CO_2, ground penetrating radar, growth, storage carbohydrates

Abbreviations: CassFACE, Cassava Free Air CO_2 Enrichment; $[CO_2]$, carbon dioxide concentration; cv, cultivar; FACE, free air CO_2 enrichment; GPR, ground penetrating radar; R^2, coefficient of determination; RMSE, root mean squared error; TOY, time of the year; VBA, visual basic for applications; ε_r, relative dielectric permittivity.

Core Ideas:

Elevated $[CO_2]$ promoted a faster and higher biomass accumulation in cassava storage roots.

Elevated $[CO_2]$ reduced the water content in cassava storage roots.

Application of the ground penetrating radar (GPR) to monitor cassava storage root growth

Ursula M. Ruiz-Vera and Riley Balikian contributed equally to this work.

1 | INTRODUCTION

Cassava (*Manihot esculenta* Crantz.) is an important staple root crop for more than a billion people, and it is cultivated principally in countries located in tropical and subtropical regions (Chetty et al., 2013; El-Sharkawy, 2006). The cultivation of cassava as a staple food crop has experienced a rapid expansion in recent decades, with global production increasing by more than 60% between 2000 and 2013 (De Souza et al., 2017; Howeler, 2014). This is especially true in Africa, where more than 50% of the world's cassava is produced (Howeler, 2014). Despite this growth, the demand for cassava and other primary foodstuffs is expected to continue to rise as the global population continues to grow, with the largest increases projected for the African continent (Long et al., 2015; Tilman et al., 2011). For example, the population of Africa is expected to triple by the end of the century (United Nations, 2019). Food production will also be impacted in the 21st century by the changing climate due to the continuing increases in atmospheric CO_2 concentrations ($[CO_2]$). While cassava benefits from an enriched CO_2 environment due to its C3 photosynthetic metabolism and strong sink capacity from underground storage roots (Rosenthal et al., 2012; Ruiz-Vera et al., 2021), other factors resulting from human-induced climate change can negatively affects its productivity. Some of these negative effects of climate change include an overall increase in temperature as well as more frequent or severe drought and heat waves. These and other potentially adverse climatic conditions are predicted to increase in frequency in Sub-Saharan Africa (Serdeczny et al., 2017). In general, these climate change associated threats to cassava productivity are expected to be partially mediated by elevated $[CO_2]$ (IPCC, 2021). Thus, to develop climate change adapted cultivars, a quantitative understanding of cassava storage root development under elevated $[CO_2]$ is key. This information will enable breeding programs to select cultivars with more desirable traits for farmers, like bulking rate (i.e., the rate of change in storage root mass over time), early bulking (especially desirable in semi-arid areas), and high yield (e.g., Adjebeng-Danquah et al., 2016, 2020; Kamau et al., 2011).

Because there are no known morphological traits from the aerial parts of cassava that can be correlated quantitatively with root bulking (Kamau et al., 2011), the study of cassava root development in the field currently requires the excavation of the roots to measure root traits. This method—while valuable—is labour intensive, destructive, and requires a large number of replicates (e.g., Kengkanna et al., 2019; McGrail et al., 2020; Wasaya et al., 2018; York et al., 2018). Mini-rhizotrons have been used to phenotype roots in the field. This technique tracks root growth and other root characteristics by the use of cameras or scanners that are placed inside clear tubes inserted in the soil followed by time-consuming data analysis (e.g., Majdi, 1996; McGrail et al., 2020; York & Lobet, 2017). However, this method collects information from just one section of the roots and is thus not well suited for large storage roots and tubers for which only a small portion of the structure can be imaged. There is no established field technique for monitoring storage root development across a full growing season (e.g., Zhang et al., 2019).

Ground penetrating radar (GPR) is a geophysical technique that can be used to characterise subsurface materials and objects and thus might have potential application for the non-destructive monitoring of roots. Most of the studies that have used the GPR technique to image coarse roots have sought to locate the roots, measure their morphological traits, and predict their biomass (Barton & Montagu, 2004; Butnor et al., 2001, 2003; Guo et al., 2013; Simms et al., 2017; Zhang et al., 2019; Zhu et al., 2014). Two studies have tested GPR to measure cassava storage roots, obtaining estimated root bulking rate, root length, and root width (Delgado et al., 2017; Delgado et al., 2019). Despite those efforts, the use of GPR to monitor the growth of cassava storage roots requires further development and additional validation.

Different from other FACE studies in cassava that only report the final yield (Rosenthal et al., 2012; Ruiz-Vera et al., 2021), we evaluated the biomass accumulation of cassava storage roots at ambient or elevated $[CO_2]$ across different root developmental stages for over 3.5 months, during a period over which temperatures were permissive for cassava growth in the field. This study also uses GPR as a tool to measure cassava storage roots, expanding previous studies by non-destructively monitoring growth at multiple stages, followed by destructive harvests. We hypothesised that storage roots would grow more rapidly under elevated $[CO_2]$, which would be reflected in the higher biomass of these plants throughout the growing season.

2 | MATERIALS AND METHODS

2.1 | Plant material, field site and experimental design

Five African cassava (*Manihot esculenta*) cultivars were used for this study over 3.5-month growing seasons in 2017 and in 2018. The cultivars TME7, TMS98/0581 and TMS011412 were used in both growing seasons, whereas TME419 was only used in 2017 and was replaced by TMS30572 the following year because of the higher yield of the latter (Ruiz-Vera et al., 2021). These cultivars came from the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) and were imported to the University of Illinois under APHIS permit from the Swiss Federal Institute of Technology (Zurich, Switzerland). The plantlets were inspected for common viruses and bacteria before shipment to the University of Illinois at Urbana-Champaign (Illinois, USA). At the University of Illinois, cassava was propagated in vitro and grown in controlled conditions before transplantation into the field, as described in Ruiz-Vera et al. (2021).

This experiment had a split-split plot design and occupied space in the plots of the Cassava Free-Air CO_2 Enrichment experiment (CassFACE; at the SoyFACE facility in Urbana, IL, 40.04N, 88.23W). The CassFACE plots included four elevated $[CO_2]$ (~600 µmol mol^{-1}) and four ambient $[CO_2]$ (~400 µmol mol^{-1}) plots that were distributed randomly in blocks. Each block had one ambient and one elevated $[CO_2]$ plot. In 2018, one block was eliminated from the data due to severe flooding conditions (i.e., $n = 4$ in 2017 and $n = 3$ in 2018).

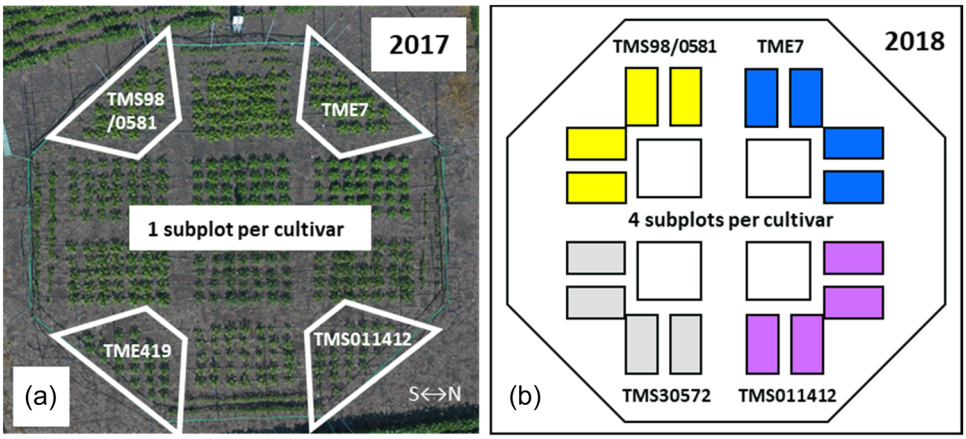

FIGURE 1 Subplot distribution inside [CO_2] plots during the two growing seasons. An aerial picture of an elevated [CO_2] plot during 2017 (a) and a diagram of a CO_2 plot in 2018 (b) are showing the location and number of subplots inside the plots. The subplots used in this experiment are indicated with white borders (a) (four subplots per CO_2 plot) and with coloured rectangles (blue, yellow, purple and grey) (b) (16 subplots per CO_2 plot). The name of the cassava cultivars used in this study are also indicated in the panels.

In 2017, there were four trapezoidal subplots per plot (one per cultivar), each one with nine 'inside' plants (plants that were not grown along the border of the subplot; Figure 1a). A higher number of subplots in 2018 allowed for more harvests through the season; consequently, each plot in 2018 had 16 rectangular subplots (four per cultivar). In each subplot, there were eight 'inside' plants (Figure 1b). The distribution of the subplots was the same between the two plots within a block, but subplot distribution was random among the blocks.

Field transplanting of cassava occurred from the 7th of June 2017 to the 9th of June 2017 and from the 28th of May 2018 to the 1st of June 2018. The soil was fertilised with 84 kg ha^{-1} of nitrogen before the transplanting, and no herbicides or pesticides were applied. In this experiment, N is applied to avoid its deficiency, being this amount in the middle range to maximise cassava yield (e.g., Howeler, 2014). However, it is important to point out that in the sub-Sahara Africa region, cassava receives very little or no fertilisation (e.g., Druilhe & Barreiro-Hurlé, 2012). The cassava transplants were planted with an approximately 0.7 m spacing between and within rows. A drip irrigation system was installed to ensure plants received at least the amount of water equivalent to 25 mm of rainfall per week. The irrigation system was rarely used in 2018 because of the abundant precipitation received that year (Figure S1b). A strong storm on the 10th of June 2018 caused damage to some plants, which were replaced with plants of the same age on the 14th of June. Daily precipitation values were obtained from the University of Illinois Willard Airport weather station (40.04N, 88.28W; Midwestern Regional Climate Centre; http://mrcc.isws.illinois.edu/CLIMATE/; Figure S1). More details about the CassFACE experiment and field management are in Ruiz-Vera et al. (2021) and more details about the FACE system are in Ort et al. (2006).

2.2 | Storage root data collection: GPR survey and destructive harvest

Direct root biomass information was collected from destructive harvests in all the cultivars used over the two growing seasons. However, only two cassava cultivars in each growing season, TME7 and TME419 in 2017 and TME7 and TMS30572 in 2018 were scanned with the GPR (Figure 1). In 2017, all the GPR and harvest measurements were done in the same subplots multiple times throughout the season. In 2018, a higher number of subplots were scanned, but each subplot was measured only once throughout the season, after which all of its plants were harvested.

For the GPR data collection, the equipment used was a 1000 MHz monostatic GPR antenna (Noggin® 1000, Sensors & Software Inc.) in a Flanagan/Drummer (fine-silty, mixed, mesic Typic Endoaquoll) soil (Figure 2a). An odometer wheel integrated into the triggering system of the GPR was used to collect a series of one-dimensional data (called traces) every 1 cm and to record the location of the stem of the plants. A series of traces combined into a single dataset is called a profile. A wooden frame (4 × 2 m) with a string guide was used to move the GPR along the profiles (Figure 2c).

In 2017, GPR profiles were collected in the 'Y-direction' on our grids with a spacing of 5 cm (Figure 2b). It was not possible to collect data where the plant stems emerged from the ground, which periodically created gaps of up to approximately 15 cm distance (Figure 2b). A 'test' set of GPR measurements were completed from the 19th of July 2017 to the 25th of July 2017, when only one inside plant per subplot was harvested as a reference for the size of the storage roots. The roots were still quite small at this time, and the GPR was not able to detect them. Thus, that 'test' set of data was not used for further analysis. The 1st and 2nd sets of GPR measurements were carried out from the 21st of August 2017 to the 24th of August 2017 and from the 18th of September 2017 to the 21st of

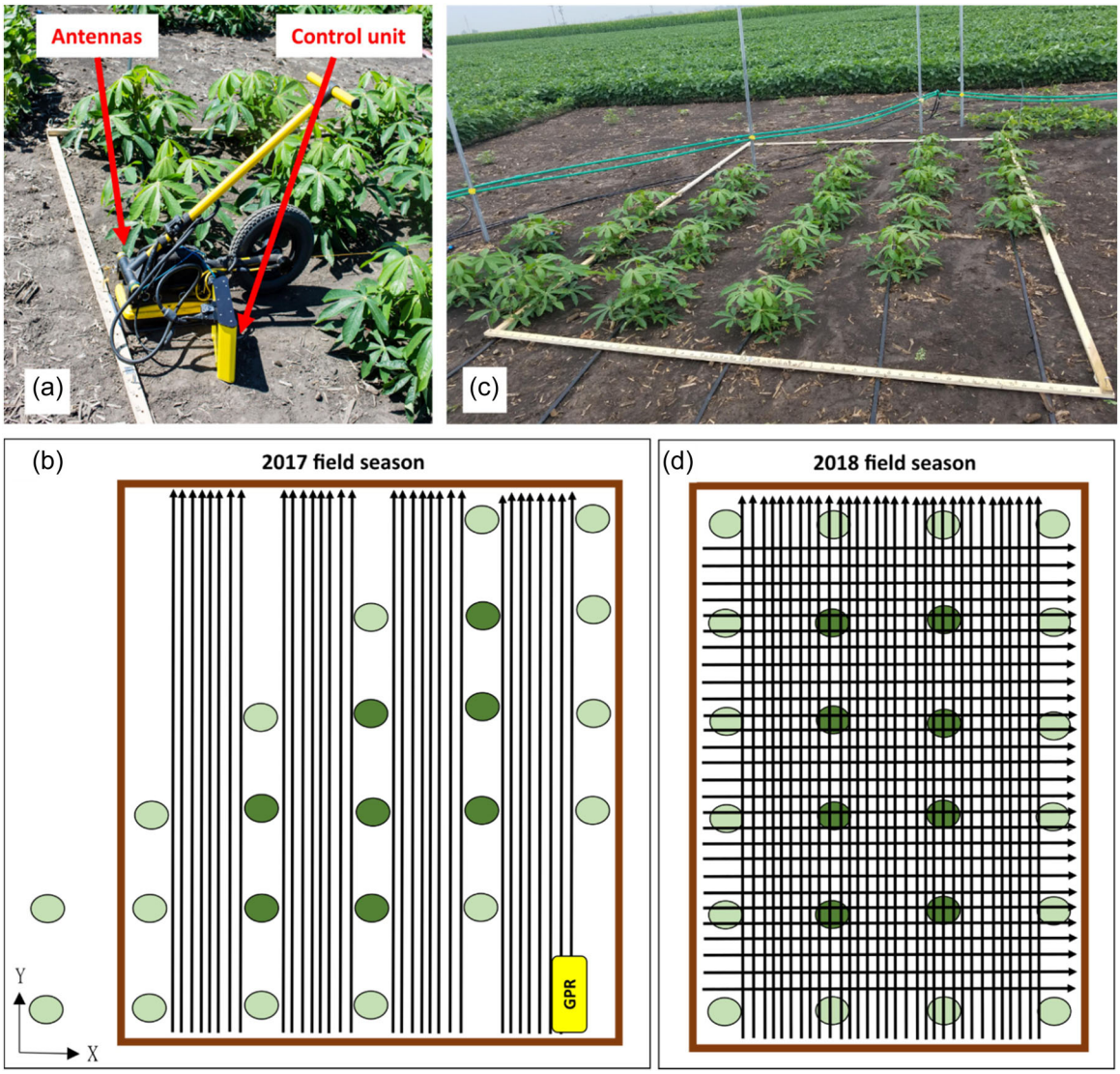

FIGURE 2 Field data collection design for the ground penetrating radar (GPR) Photo showing the GPR equipment (1000 MHz antenna, Noggin® 1000) used in this experiment (a). Photo showing the wooden frame inside a subplot (c). Diagrams are showing the GPR trajectory for the data collection during 2017 [Y-direction, (b)] and 2018 [Y- and X-directions, (d)]. The darker green circles indicate the location of the target plants, while the lighter green circles show the border plants which were not used in the study. [Color figure can be viewed at wileyonlinelibrary.com]

September 2017, avoiding rainfall events. Destructive harvests of all the cultivars were carried out on the same days (1st and 2nd harvests); for TME7 and TME419 the harvest was done immediately after the GPR measurements were finished. The fresh weights of storage roots were obtained from four inside plants per subplot and pictures of the top and side views of the roots with their geographic orientation were taken from cultivars TME7 and TME419 only after the 2nd set of GPR measurements.

In 2018, the whole root system was scanned after the above-ground biomass was cut off. GPR profiles were collected in two directions. The Y-direction profiles were collected at 5 cm spacing and those in the X-direction were collected at 10 cm spacing (Figure 2d). The eight 'inside' plants per subplot were used for all the measurements. The 1st harvest was on the 2nd of July 2018, but roots were too small to be detected by the GPR (mean of the fresh weight of the roots = 3.4 g; Figure 3; Figure S1b; Figure S2a). The rest of the field campaigns took place from the 23rd of July 2018 to the 26th of July 2018 (1st set of GPR measurements and 2nd harvest), from the 22nd of August 2018 to 30th of August 2018 (2nd set of GPR measurements and 3rd harvest), and from the 17th of September 2018 to the 19th of September 2018 (3rd set of GPR measurements and 4th harvest; Figure S1b), again avoiding rainfall events. Harvested storage roots from TME7 and TMS30572 cultivars were photographed from the top (Figure S2b–g) and sideways. In addition to measuring the fresh weight, storage roots were oven-dried to obtain their water content.

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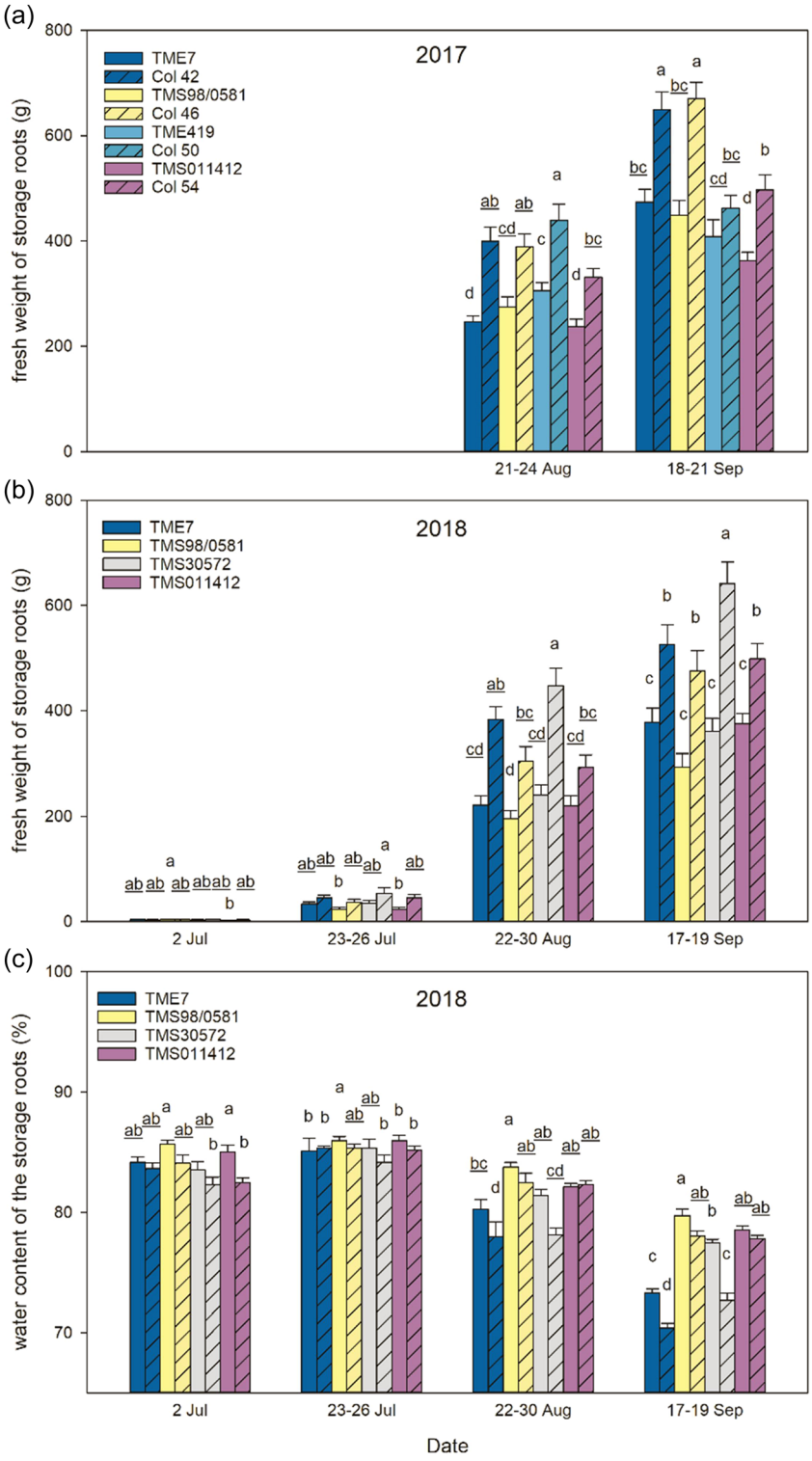

FIGURE 3 Fresh weight and water content of cassava storage roots. Average fresh weight of storage roots [g, (a) and (b)] and the water content of the storage roots [%, (c)] for five cultivars of cassava that grew at ambient [CO_2] (bars without a pattern) and elevated [CO_2] (bars with dashed lines) during the 2017 and 2018 growing seasons. Values are mean ± standard error (SE; $n = 4$ in 2017 and $n = 3$ in 2018). Treatments with different letters represent significant differences ($p \leq 0.1$). [Color figure can be viewed at wileyonlinelibrary.com]

2.3 | GPR data processing

The GPR data were processed using GPRSlice v.7 (Geophysical Archaeometry Laboratory) to obtain 2D profiles. The processing steps were the same for both years and were standardised across all GPR profiles. These steps included direct current shift correction, background subtraction, Kirchhoff migration, envelope creation using a Hilbert transform, and application of a custom depth-varying gain (Figure S3a–f). More details about these signal-processing methods can be found in Daniels (2004). Gain curves were created specifically for this study site and the target (cassava storage roots) to equalise the intensity of the signal in the first 0.5 m below the ground surface. After a satisfactory gain curve was tested across several profiles in different plots, this curve was used for all profiles across all the plots.

For the Kirchhoff migration, a propagation velocity was determined for each subplot and at each collection time through hyperbolic velocity fitting. These velocities, whose values were between 0.075 and 0.12 m/ns, were confirmed through field testing with buried metal targets near the plots at a known depth, location, and orientation.

In GPRSlice, the resulting cross-sectional profiles were interpolated horizontally for each subplot to create 2D depth slices every approximately 2.5 cm (Figure S4a,b). These depth slices were then interpolated vertically to create a 3D grid with approximately 1 cm resolution in all directions (Figure S4c). The 3D grid data were exported to Voxler (Golden Software LLC) where isosurfaces were created to separate regions with high and low intensity values. The higher intensity areas represent locations where larger amounts of radar energy were reflected back to the surface. Based on the known locations of the cassava stems (data recorded in the field using the odometer wheel), cubic regions of 0.4 m^3 were identified automatically using a VBA script in Voxler. This step eliminated most of the radar signal originating from non-root objects in our data. For example, this eliminated most high radar energy from desiccation cracks in the soil which were observed during the 2017 season. These 3D regions of interest were then increased or decreased in size according to the size of the roots, information that was obtained from pictures of the roots taken after harvest. Within these 3D regions of interest, a threshold signal intensity value for each root was identified that would separate signal (radar reflections from cassava roots) from surrounding noise. Voxler uses this signal intensity value to create an amorphous 3D region known as an isovolume encompassing only our target. The volumes of these high signal intensity regions were measured to obtain an apparent 'root' volume (m^3). This 3D reconstruction was carried out as an evaluation of the methodology in the 2nd set of GPR measurements in 2017 because data from the middle of the storage roots could not be collected since the stems were still in place (Figure 4a,b). However, detailed 3D reconstructions of cassava roots from the GPR data were carried out for all subplots in 2018 to enable a more thorough analysis of the GPR apparent 'root' volume data with respect to root traits identified from the destructive harvest.

2.4 | Statistical analysis for the harvest data

The mean value was calculated for the fresh weight and the water content of the storage roots per plot to evaluate the [CO_2] treatment's effect on the different cultivars. This analysis used a mixed model ANOVA (PROC MIXED, SAS System 9.4, SAS Institute) with repeated measurements (Table 1). The [CO_2], cultivar (cv), time of the year (TOY), and their interactions were the fixed effects. The repeated measurement factor was TOY. Block was the random effect. The degrees of freedom were calculated with the Kenward–Roger method, and pair-wise comparisons were done by the least square mean test (*t* test). The significance was determined a priori as $p \leq 0.1$ to avoid type II error due to the number of blocks.

2.5 | Volumetric data from the GPR surveys versus root traits

Analysis of the GPR volumetric data against root traits from the destructive harvests was performed using R Statistical Software (v4.0.4; R Core Team, 2021). The presence of outliers in the apparent 'root' volume data against the fresh weight of the roots was examined per cultivar and [CO_2] treatments by using the diagnostic plots of residuals, fitted values, Cook's distance, and leverage. Whenever it was needed, the robust regression method with the Huber and bisquare tests ('MASS' package in R) were used to detect outliers. Overall, 12% of the apparent 'root' volume data were excluded from all further analysis (Figure S5). Linear regression analyses for the apparent 'root' volume with and without the water content of the roots versus the fresh weight of the storage roots were done for the whole dataset and per cultivar and/or [CO_2] treatment (Figure 5; Figure S6). The quality of the linear models were assessed with the metrics of R-square (R^2) and root mean squared error (RMSE), which were obtained with the "fitting linear models" function (lm()) in R. The values for the *F* and *t* tests were also obtained from the linear models, $p \leq 0.05$ (Table 2).

3 | RESULTS

3.1 | Elevated [CO_2] increased cassava root biomass while decreasing their root's water content

In both growing seasons and in all five cultivars evaluated, the increase of [CO_2] resulted in higher storage root biomass (Figure 3a,b; Table 1). By the end of August 2017 (~75-day-old plants after field transplanting), TMS98/0581, TME419 and TMS011412 had increases in root biomass that were between 40% and 44%. Meanwhile, TME7 surpassed those increases with 62% more root biomass in plants at elevated [CO_2] compared to the ones that grew at ambient conditions. By mid-September 2017, the increases in root biomass were as follows: 37% for TME7, 49.5% for TMS98/0581, and 37% for TMS011412. In this last harvest, the storage root biomass in TME419 was not significantly different between the [CO_2] treatments.

During 2018, no significant differences in the fresh weight of the storage roots between the [CO_2] treatments were observed during the 1st and 2nd harvests (Figure 3b). The high variability of the data during the 2nd harvest (the range of the measured fresh weight of the roots was 4–171 g) might have contributed to there being no statistical difference in root biomass despite percentage increases higher than 34% in some instances. Increases in root biomass due to the elevated [CO_2] were apparent starting with the 3rd harvest (~88-day-old plants after transplanting and older; Figure 3b; Table 1). At the 3rd harvest, the fresh weight of the storage roots for the cultivars TME7, TMS98/0581 and TMS30572 were 73%, 57% and 86% higher at elevated [CO_2]. At the 4th harvest, the average increase in root biomass was 53% (from 33% to 78%) across all four cultivars. In

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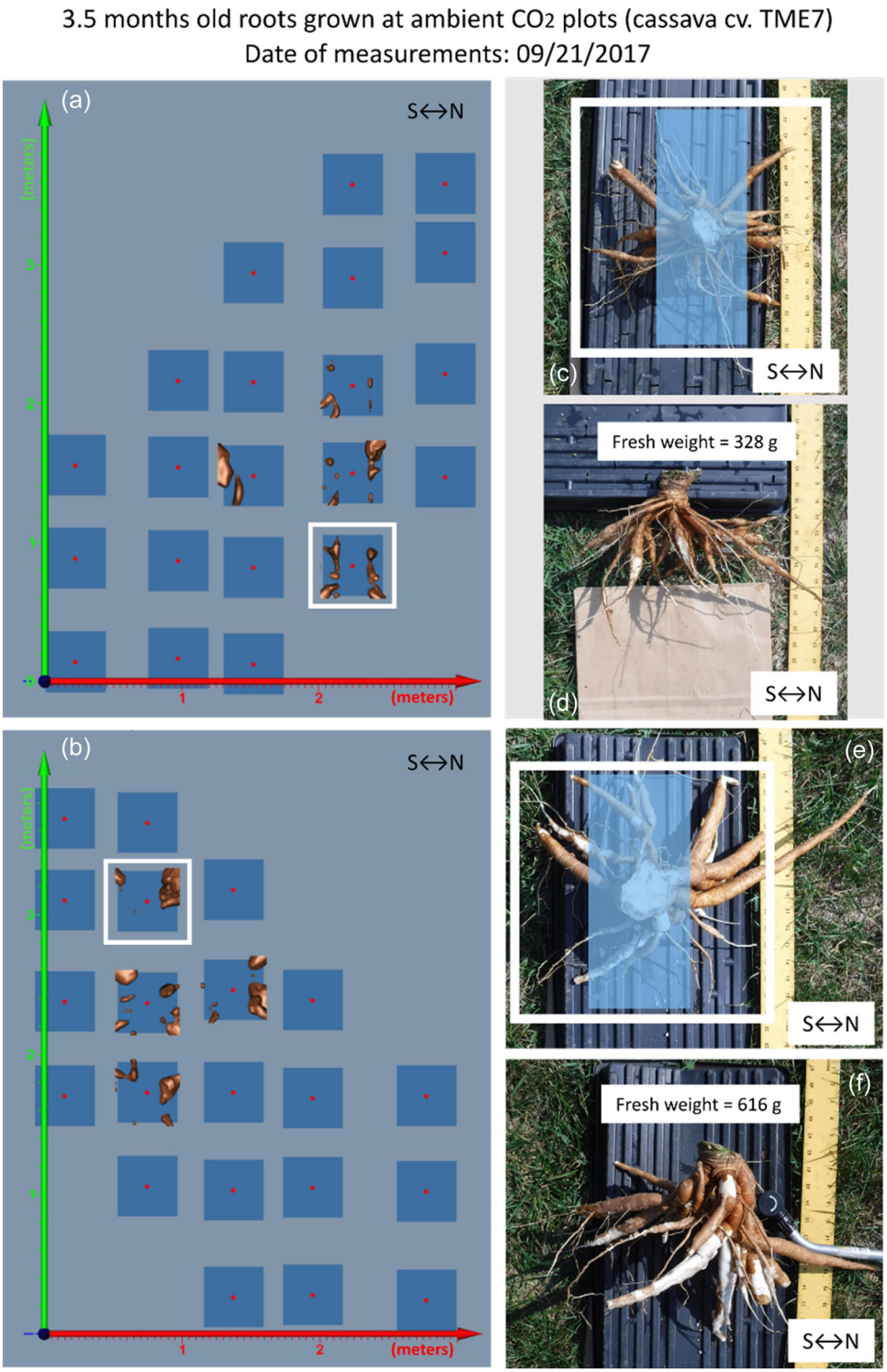

FIGURE 4 3D representation of the sides of the storage roots from the 2nd set of GPR measurements in the 2017 growing season. A total of 2 of the 16 experimental subplots are shown in (a) and (b), one in each panel. The location of the cassava stems is represented by red dots in the middle of the blue squares. The blue squares serve as a reference for the analysis region and have sides of around 40 cm in length. The 3D representations of storage roots are shown as brown solid shapes. The white squares indicate the roots for which pictures from the top and side are shown in (c)–(f). The images taken from above of the roots [(a) and (b)] have a light blue rectangle that corresponds to the area of the root that was not scanned by the GPR and a white square with sides of 40 cm as reference. [(d) and (e)] Pictures of the side view of the roots. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Statistical results for the harvest data

				Main effects						
Season	cv names	Parameters	Harvest	$[CO_2]$	cv	$[CO_2] \times cv$	TOY	TOY × $[CO_2]$	TOY × cv	TOY × $[CO_2] \times cv$
2017	TME7 and TME419	fresh weight (g)	1st	<0.001	ns	ns	–	–	–	–
			2nd	0.005	0.003	0.094	–	–	–	–
			season	<0.0001	0.097	ns	<0.0001	ns	<0.001	ns
2018	TME7, TMS98/0581, TMS30572, and TMS011412	fresh weight (g)	1st	ns	ns	ns	–	–	–	–
			2nd	ns	ns	ns	–	–	–	–
			3rd	0.009	0.84	ns	–	–	–	–
			4th	<0.0001	0.047	ns	–	–	–	–
			season	<0.0001	0.001	0.069	<0.0001	<0.0001	0.099	ns
		water content (%)	1st	0.063	ns	ns	–	–	–	–
			2nd	ns	ns	ns	–	–	–	–
			3rd	0.042	0.010	ns	–	–	–	–
			4th	0.001	<0.0001	ns	–	–	–	–
			season	0.051	<0.0001	ns	<0.0001	ns	0.002	ns

Note: Complete block analysis of variance (ANOVA) for the fresh weight (g), and the water content of storage roots (%). The data is from five cassava cultivars that were grown over two growing seasons. The fixed effects are: the CO_2 concentration ($[CO_2]$), the cassava cultivars (cv), the time of the year (TOY), and their interactions. The statistically significant differences ($p \leq 0.1$) and non-statistical significance (ns) are shown in the table.

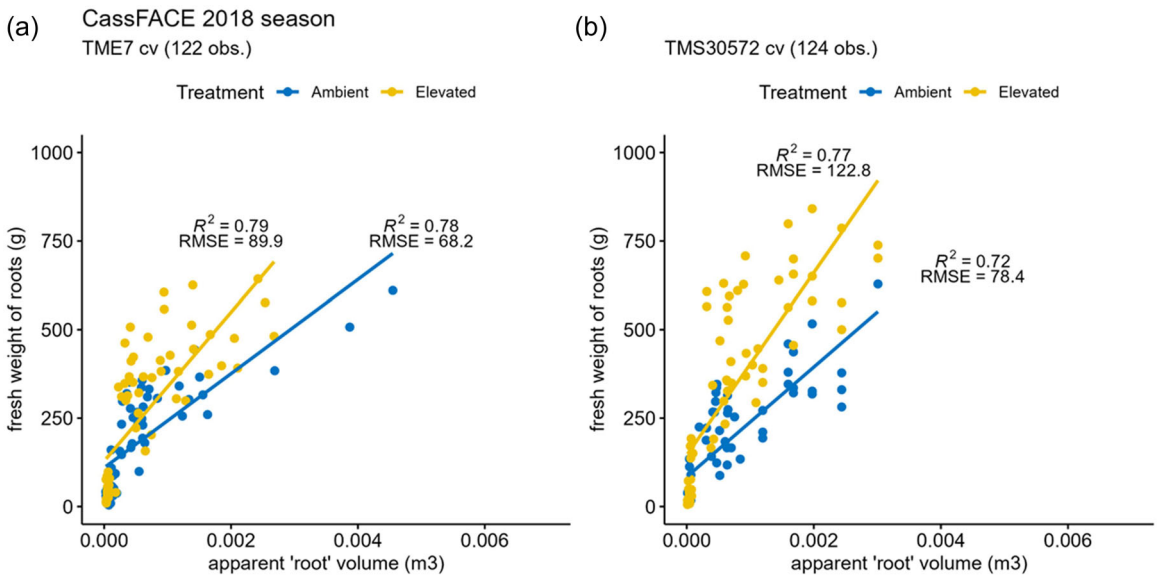

FIGURE 5 Linear regressions between the apparent 'root' volume and the fresh weight of storage roots, per $[CO_2]$ treatment and cultivar. (a) Shows the regressions for the cultivar TME7. (b) Shows the regressions for the cultivar TMS30572. Fitting lines in blue colour are for the ambient $[CO_2]$ treatment and in yellow are for the elevated $[CO_2]$ treatment. The statistic metrics of R-square (R^2) and root mean squared (RMSE) are indicated close to their, respectively, lines [Color figure can be viewed at wileyonlinelibrary.com]

2018, the water content in the roots was reduced under elevated $[CO_2]$ conditions during the last two measurements for the cultivars TME7 and TMS30572 (Figure 3c; Table 1). Additionally, only during the 1st harvest, storage roots in TMS011412 had lower water content under elevated $[CO_2]$ (~3% reduction).

3.2 | GPR was able to detect cassava storage roots in a dense silty clay loam soil

In 2017, the capabilities of the GPR to detect storage roots in a silty clay loam soil were tested. That year, the GPR scanned roots only from the

TABLE 2 Linear regression coefficients and parameters

Cultivar	$[CO_2]$	*y* = fresh weight of storage roots linear regression coefficients			R^2	RMSE (g)	*F*-statistic (*p* value)
		Intercept	Volume (m^3)	Water content (%)			
all	all	119.49	191276.39*	–	0.544	137.14	<0.001
all	ambient	98.55	142567.26*	–	0.626	89.17	<0.001
all	elevated	134.69	240038.72*	–	0.607	145.12	<0.001
TME7	ambient	1006.50	229.70*	–	0.781	68.17	<0.001
	elevated	1260.10	281.20*	–	0.789	89.89	<0.001
TMS30572	ambient	826.90	177.60*	–	0.717	78.40	<0.001
	elevated	1452.80	315.30*	–	0.772	122.75	<0.001
all	all	1569.83	108036.39*	−17.40*	0.660	119.16	<0.001
all	ambient	916.35	106466.13*	−9.78*	0.695	80.53	<0.001
all	elevated	1699.60	128377.47*	−18.77*	0.690	130.03	<0.001
TME7	ambient	1332.17	191.76*	−5.73*	0.782	67.95	<0.001
	elevated	1173.50	307.86	2.36*	0.811	87.57	<0.001
TMS30572	ambient	1275.99	152.51*	−6.57*	0.739	76.00	<0.001
	elevated	2580.20	211.00*	−18.90*	0.827	107.04	<0.001

Note: The linear regression analysis was performed between the apparent 'root' volume (volume) with or without the water content of the roots (water content) and the fresh weight of storage roots. The word 'all' in the Cultivar and $[CO_2]$ columns indicates that the data used is from all the cultivars and all the $[CO_2]$ treatments. The *t* test statistical significance is indicated by an asterisk (*) with a $p \leq 0.05$.

Abbreviation: RMSE, root mean squared error.

sides of the plants approximately 47, 77 and 105 days after planting. The storage roots measured at the earliest developmental stage had not yet expanded far enough to the sides of the plant, so it was difficult to differentiate between roots and noise. Root detection with GPR was evident for storage roots older than 2.5 months, but the 3D reconstruction of storage roots from images taken from the side was possible only for the last set of measurements when the larger side roots created a clearer radar signal (Figure S7). Figure 4a,b shows two examples of the 3D root reconstruction for relatively small and large storage roots that were harvested at around 3.5 months of field growth. Visual similarities between the 3D root reconstructions and the real roots were possible after processing the data (Figure 4a,b, 4c, 4e). To evaluate the data processing methodology and to obtain quantitative parameters from the GPR data that can be related to cassava storage root traits from the field, information from the middle of the roots was needed. Consequently, data collection over the entire root system by the GPR was done in 2018 by removing the aerial portions of the plants before the scans.

3.3 | Apparent 'root' volume estimations from the GPR data

To evaluate the relationship between the volume of the high signal intensity regions detected by the GPR (or apparent 'root' volume) and the fresh weight of storage roots, linear regression analyses were performed. A linear regression for the whole dataset, which included data from the two cultivars (TME7 and TMS30572) and $[CO_2]$ treatments, had an R^2 of 0.54 (Table 2). Relative to this R^2 value, the linear regressions done for each $[CO_2]$ treatment and for each $[CO_2]$ treatment per cultivar combination had higher R^2 (from 0.57 to 0.66; Table 2), increasing its value in a range of 5%–23%. When the water content of the storage roots was added as a predictor variable, the R^2 for each linear regression was 4%–21% higher (Table 2). The RMSE decreased in all the linear regressions that incorporated the water content of the roots in their model, decreasing from 85 to 154 g to a range of 78 to 130 g (Table 2). Linear regression of TME7 at elevated $[CO_2]$, when the water content of the roots was added as a predictor variable, showed that changes in the apparent 'root' volume as measured by GPR might not be associated with changes in the fresh weight of storage roots (Table 2).

4 | DISCUSSION

This study shows that cassava storage roots accumulated more biomass under elevated $[CO_2]$ conditions, which is translated into a faster bulking rate and that there is significant variability in this trait just among the five cultivars that we tested. Moreover, some cultivars had lower water content in the roots at the end of the season indicating that the effect of the elevated $[CO_2]$ can be both

cultivar- and time-dependent. In addition to harvest data obtained from destructive harvests, the GPR technique was shown to be able to image the roots of cassava in the field, especially in plants older than 2.5 months. A moderate correlation between the apparent 'root' volume data extracted from the GPR data and the fresh weight of storage roots was shown. These results suggest that the GPR may have potential to be used to monitor the growth of cassava roots in the field especially in older plants during their full 10-month-growing season in normal areas of cultivation. However, more precise and less labour-intensive ways to extract root parameters from the GPR data are needed, as well as a deeper understanding of the situations that impact the detectability of the roots.

4.1 | The effect of elevated [CO_2] on cassava storage roots

Previous studies evaluated the effects elevated [CO_2] on the growth of cassava storage roots (Cruz et al., 2014; Fernández et al., 2002; Forbes et al., 2020; Gleadow et al., 2009; Rosenthal et al., 2012; Ruiz-Vera et al., 2021). However, only two of them used FACE technology (Rosenthal et al., 2012; Ruiz-Vera et al., 2021), a technology which most realistically mimics projected future atmospheric [CO_2]. These two studies reported only the final yield of cassava after a 4-month-field season. Consequently, more detailed information about root development over time and growth for cassava under elevated [CO_2] in the field is needed to better understand the impacts of climate change in this staple crop. For that reason, one of the objectives of this study was to provide additional information about how cassava storage roots were impacted by high CO_2 atmospheric levels at different root developmental stages by measuring root biomass and the water content of the storage roots.

The timing of when root bulking starts is an important trait to classify cassava germplasm into early and late bulking categories, and this characterisation is essential to further develop varieties adapted to different environmental conditions (Wholey & Cock, 1974). The bulking in cassava usually begins in about 1-month-old plants (Kamau et al., 2011). In this study, roots of that age were very small, between 1 and 16 g, and the initiation of the bulking rate was not obvious (data from the 1st harvest of 2018; Figure 3b; Figure S2A). During the first month of the growing season, the heavy rains and a flood could have contributed to the slower root development. For example, flood conditions can increase leaf chlorosis and defoliation, while promoting the wilting of the plants and reducing leaf chlorophyll content (Dethvongsa et al., 2021), conditions that can affect physiological processes like photosynthesis and limit the growth of cassava. Moreover, respiration and nutrient uptake from the roots will be also limited under waterlogging conditions (Dethvongsa et al., 2021). By the 2nd harvest (~56-day-old plants from field transplanting), the storage roots were already expanding (Figure S2b,c), and there were not differences between their biomass at either ambient or elevated [CO_2] (Figure 3b), suggesting that elevated [CO_2] might not accelerate the initiation of the bulking in cassava storage roots. This may be due to initially weak sink strength or insufficient statistical power to detect elevated [CO_2] stimulation that was actually present (i.e., Type 1 error). A deeper study of the initiation of bulking is needed to resolve the cause.

To have more storage root biomass at elevated [CO_2] conditions during the two harvests in 2017 and the 3rd and 4th harvest on 2018 (Figure 3a,b), a faster accumulation of carbohydrates in the roots is required. This faster accumulation of carbohydrates will increase the rate of change in storage root biomass over time resulting in a faster bulking rate under elevated [CO_2] conditions. The amount of storage root biomass increase was cultivar dependent (Table 1; Figure 3b). Consequently, TME7 and TMS30572 had the highest increase in root biomass in approximately 75 to 88-day-old plants after field transplanting. Meanwhile, TMS011412 maintained a similar increase in biomass after the plants had approximately 75 days of growing on the field. TMS980581 had a larger increase in biomass during the last harvest on both growing season, when plants had more than approximately 103 days of growing on the field. TME419 was the only cultivar where an increase in storage root biomass was not detected in the last harvest. This may reflect some constraints in the growth of the storage roots or limitations in the supply of photoassimilates to the storage roots. In Ruiz-Vera et al., 2021; TME419 was the cultivar with the lowest root biomass after 4 months of growing in the field. Moreover, this cultivar was the shortest, had fewer leaves, and almost no branches. The fact that TME419 is in general a small cassava cultivar (small above and below ground biomass) helps to explain why the increase of elevated [CO_2] did not drive higher increases in root biomass later in the season. In general, the variable rate of growth for the cassava storage roots observed in this study highlights the importance of incorporating a diverse group of cassava cultivars in experiments looking to understand cassava response to different environmental conditions.

The reduction of the water content in the storage roots will allow a larger root biomass increase on a dry weight rather than on a fresh weight basis. However, larger increases were not always observed in the other FACE experiments that evaluated four additional cassava cultivars (Ruiz-Vera et al., 2021), suggesting that the influence of elevated [CO_2] on the water content of storage roots is cultivar dependent. In this study, the water content of the roots was evaluated in the four cassava cultivars grown in 2018. Out of those cultivars, two of them (TME7 and TMS30572) had reduced water content when plants were more than 2.5 months old (~75 days and older of growing in the field) and were grown under elevated [CO_2] conditions. Moreover, storage roots had less water in the last harvest (Figure 3c). Consequently, the reduction of the water content of storage roots due to elevated [CO_2] is also time dependent. The mechanism involved in the reduction of the water content of the roots at elevated [CO_2] is not clear but it might be related with some physiological changes observed in cassava under elevated [CO_2], like changes in carbon metabolism and water use efficiency (Rosenthal et al., 2012; Ruiz-Vera et al., 2021). High moisture content in cassava storage roots makes them more perishable (Girma et al., 2015; Omosuli et al., 2017). In the case of TME7 and TMS30572, the

reduction of the water content of the storage roots, together with the higher root biomass under elevated [CO_2], are promising changes for increasing cassava productivity while potentially reducing perishability and, ultimately, post-harvest loss.

4.2 | GPR technology testing to monitor cassava storage roots

GPR technology has been previously used to image cassava storage roots in the field (Delgado et al., 2017). In that study, empirical models that used the GPR data to predict storage root biomass, overestimated the biomass when roots where small (less than ~800 g fresh weight of storage roots) and in storage roots from plants with less than 5 months of growing in the field. In contrast to that study, we evaluated cassava storage roots that weighed less than 800 g from plants that were growing in the field as early as approximately 3.5 months. Additionally, our experiment investigated whether changes in the GPR signal were detected when plants grew under different [CO_2] levels and the potential utility of this technique for plants grown in a dense silty clay loam soil where the contrast is lower than for sandier, lighter soils (Martinez & Byrnes, 2001; Zajícová & Chuman, 2019).

Most of the previous GPR studies on storage roots (e.g., Delgado et al., 2017; Liu et al., 2018) obtained grayscale digital images from the GPR data to extract information related with the roots by following a method developed for trees (Butnor et al., 2003). This study used a 3D reconstruction approach, similar to the one used in Simms et al. (2017) and Delgado et al. (2019), to obtain volume data from regions of high radar signal. Then, linear regression analyses were done between the apparent 'root' volume data (obtained from the GPR) and the fresh weight of storage roots, indicating moderate correlations (Table 2).

Interestingly, the correlations were increased by up to 21% (R^2 increased) when the data from the water content of the storage roots was added as a predictor in the models. These results highlight the importance of knowing how different parameters of the composition of storage roots can influence their detection by the GPR. Water has a much higher relative dielectric permittivity (ε_r; the ability of a substance to hold an electrical charge) than most geologic materials (Cassidy, 2009; Cihlar & Ulaby, 1974). Consequently, the amount of water in a storage root may make it easier to detect using GPR. However, this could also mean that after a rain event, it would be harder to differentiate roots in the wet soil with GPR.

Because there were changes in the water content of the roots associated with the increase of [CO_2], the models also improved when they were performed separately for each [CO_2] treatment (Table 2). For example, the linear regression models in Figure 5 show that for the same volume value, the root biomass at elevated [CO_2] treatment was larger than at ambient [CO_2]. This means that the GPR signal response was lower in roots that grew at elevated [CO_2], which could be attributed in part to the reduction of water content in the storage roots under high [CO_2] conditions. Elevated [CO_2] can help to conserve soil water content due to its universal reduction in stomatal conductance, though this response varies throughout the soil profile and is affected by factors like the ambient and canopy temperature, the magnitude of drought events, and the leaf area of the plant for transpiration (Blumenthal et al., 2018; Grey et al., 2016). Consequently, retention of soil moisture by elevated [CO_2] conditions is more likely to be observed when cassava plants are small because they do not have a closed canopy, which increases the difficulty of storage root detection by the GPR. In this study, the apparent 'root' volume data obtained from roots less than 200 g of fresh weight seemed to be less accurate due the small volume changes observed (Figure 5). Despite that, our test was able to detect cassava storage roots at earlier developmental stages than a previous GPR study in cassava even in a dense silty clay loam soil. Finally, our results demonstrate that continuing to explore novel ways of processing and analyzing GPR data may lead to new insights, particularly by automating the extraction of meaningful root parameters from GPR data.

5 | CONCLUSION

Results from this study showed that the faster accumulation of root biomass, evidenced by higher storage root weight, under elevated [CO_2] conditions starts early in the development of the roots. Moreover, it was also observed that the water content of storage roots could be decreased under conditions of elevated [CO_2]. Because results varied depending on the cultivar and root developmental time, this study highlights the importance of screening a variety of cassava cultivars to understand which ones will be more adapted to future climate conditions. In addition to the growth monitoring of cassava storage roots by destructive harvests, this study tested the GPR technology as a possible non-destructive alternative to screen roots in the field. The results demonstrated how changes in the composition of the storage roots, like lower water content due to elevated [CO_2], affected the information received by GPR. Consequently, the utility of this technique in monitoring storage root growth requires more validation and a better understanding of its limitations.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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