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REGULAR ARTICLE

Ground penetrating radar (GPR) detects fine roots of agricultural crops in the field

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Abstract
Aim Ground penetrating radar (GPR) as a non-invasive technique is widely used in coarse root detection. However, the applicability of the technique to detect fine roots of agricultural crops is unknown. The objective of this study was to assess the feasibility of utilizing GPR to detect fine roots in the field.
Methods This study was conducted in four locations with different soil types and soil moisture conditions in Texas. Several varieties of winter wheat and energy cane were scanned with GPR (1600 MHz). Soil cores were collected immediately after scanning to measure root parameters. Using an image analysis software, four pixel indices with or without intensity threshold were used to assess the relationships between GPR signal and root parameters.
Results There were significant relations between GPR indices and root parameters depending on soil conditions. The accuracy of root estimation was higher in wet clay soils than in dry sandy soils. Estimated root parameters from GPR had lower variation than measured roots. Average GPR pixel intensity without intensity threshold may be better to reflect root information than pixel indices with intensity threshold.
Conclusions This study demonstrates that GPR has the potential to predict bulk root biomass and diameter in winter wheat and energy cane.

Keywords Ground penetrating radar · Fine roots · Average pixel intensity · *Triticum aestivum* · *Saccharum*

Abbreviations

GPR	Ground penetrating radar
NRMSE	Normalized root mean square error
GSSI	Geophysical Survey Systems, Inc.

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Introduction

Plant roots play numerous critical roles in soil resource (water, nutrients, agrochemicals) acquisition, mechanical support, resource (water, carbohydrates, nutrients) storage, mediating interactions with pathogenic and beneficial organisms in the rhizosphere, and biogeochemical cycling in terrestrial ecosystems. The ability to detect and quantify root systems is crucial not only in providing insight into these belowground processes, but also for resource management, which ultimately determines agricultural productivity (Zhang et al. 2009; Sharma et al. 2014; Liu et al. 2015). This capability is very important in crop improvement programs (breeding) aimed at improving productivity as well as resource use efficiency. Hence, breeding for optimal root architecture has become a key strategy for improving productivity by enabling plants to utilize water and nutrients more efficiently (Kell 2011). Genetic improvement of root architecture requires identification of key root traits that allow plants to capture, and utilize soil resources more efficiently as well as adapt and thrive in different environments (Paez-Garcia et al. 2015). This calls for efficient root phenotyping methods to establish the genotype-to-phenotype linkages under varying scenarios of the genotype-by-environment (G × E) interactions (Trachsel et al. 2011; Pauli et al. 2016).

Traditional root observation includes excavation and sampling with soil cores or augers. Although relatively accurate, these methods are time/labor-intensive, destructive and point-based. More and more researchers are looking for improved high-throughput root measurement methods (Clark et al. 2011; Trachsel et al. 2011; York and Lynch 2015; Thomas et al. 2016), many of these are still destructive and cannot be repeated over time. Other methods such as mini-rhizotron and X-ray techniques are either point-based or time consuming (Majdi 1996; Mooney et al. 2012; Liu et al. 2016a). Electrical impedance tomography is a non-invasive tool to image root distribution, but it suffers from an inherent ambiguity of effective conductivity and can be difficult to apply in the field (Urban et al. 2011; Weigand and Kemna 2017). Ground penetrating radar (GPR) is an electromagnetic (EM) technique that has been used extensively to detect and measure coarse roots nondestructively (Hruška et al. 1999; Butnor et al. 2001; Butnor et al. 2003; Guo et al. 2013a). A GPR antenna is composed of a transmitter that propagates electromagnetic waves into a medium, in this case field soil, and a receiver that detects energy reflected back to the surface (Daniels 2004). When the wave encounters a reflective surface (such as a root) a portion of the energy waves will be reflected, absorbed or scattered (al Hagrey 2007). Some of the energy reflected to the soil surface will be detected by the receiver yielding data on the two-way travel time with amplitude of the reflected waves. Commercially available GPR equipment is used for a variety of geophysical applications (e.g. civil engineering, geology, archeology, forensics), but not specially designed for root detection applications per se, requiring some degree of customized data analysis. A comprehensive review of GPR principles, approaches to root quantification and strategies for data collection in the field was presented by Guo et al. (2013a). GPR has been used for rapid, noninvasive detection of coarse tree roots (>2 mm diameter) (Hruška et al. 1999; Butnor et al. 2001; Butnor et al. 2003; Cui et al. 2011; Guo et al. 2013a) under both controlled and field conditions. Delgado et al. (2017) successfully quantified storage root bulking rate of cassava under field conditions, though fine roots were assumed to be below the size threshold for detection. Given the critical role that fine roots play in water and nutrient uptake, it would be beneficial if non-destructive technologies such as GPR could be used to quantify their dynamics in diverse soils and management systems (Bodner et al. 2014). In a study to nondestructively measure wheat roots in the field, Thompson (2014) was able to predict root presence and absence, but could not quantify fine root biomass in wheat 60 days after crop harvest. Hence, root decomposition and changes in plant/soil dielectric properties during this period may have contributed to the inability to adequately quantify fine root biomass. Obtaining GPR scans at the same time as destructive sampling should improve assessment and prediction accuracy. Continuous root growth and dieback (root turnover) is a common trait of fine roots in many species and constitutes a major component of belowground carbon cycle (Lukac 2012; Swinnen et al. 1995). High throughput, nondestructive root detection methods that can be deployed at frequent intervals will likely result in a better picture, not only of standing root biomass, but also the temporal changes associated with belowground carbon cycling.

To detect plant roots with GPR, there needs to be differences in electromagnetic (EM) properties (dielectric permittivity, electrical conductivity and magnetic permeability) between roots and the soil (al Hagrey 2007). The larger the contrast between root and the soil EM properties, the greater the amplitude of the reflected EM waves and ease of detecting buried roots. GPR resolution is defined as the smallest object that can be individually sensed from other objects. If root is larger than this threshold, it can be singularly detected. Conversely, very fine roots may not reflect sufficient EM energy to allow unambiguous detection using GPR. For this reason, it is generally believed that GPR may not be able to unequivocally detect plant fine roots (< 2 mm in diameter) (Pierret et al. 2005; Pauli et al. 2016). Though this perception is based on both field experience and the general understanding of the resolution-frequency trade-offs inherent to GPR signals, theoretically GPR should have the potential to measure crop roots as long as there are sufficiently large differences particularly in soil dielectric permittivity between root and the soil (Wielopolski et al. 2000). Compared with trees, annual agricultural crops such as wheat growing in high plant density may have more concentrated fine roots per unit soil volume. Hence, absolute crop root biomass density (root mass within a given soil volume) of annual crops and tress may be similar (Butnor et al. 2003 and Liu et al. 2015). Therefore, if GPR can detect tree root biomass density, then it may also capture crop root biomass density, given their similar density level. Secondly, crop roots are able to absorb and concentrate both soil water and dissolved minerals (e.g. N, P, K) associated with plant nutrition which in aggregate, may lead to detectable changes in bulk soil dielectric properties directly under the crop. This change could be detected using GPR as recently proposed by Liu et al. (2016a) in a review of the potential application of GPR for crop fine root detections. Therefore, it is conceivable that the presence of crop roots can influence soil dielectric permittivity at a bulk soil level thereby facilitating GPR root detection. Under the normal field conditions, fine root xylem vessels maintain a continuous water column as part of the water potential and transpiration-driven soil-plant- atmosphere continuum (SPAC) that transports water from the soil, through the plant and then to the atmosphere. Also, water has a higher dielectric constant than other soil-borne materials. Due to osmotic forces, crop roots may have a higher water content than the bulk soil (Carminati et al. 2010), further accentuating the contrast in dielectric properties between crop roots and bulk soil. This should be particularly true if crops grow in a sandy soil with low soil moisture which is normally considered as an ideal soil condition for root detection with GPR (Doolittle and Collins 1995; Butnor et al. 2001). Gormally et al. (2011) reported that it is possible to use GPR to detect roots under high soil water conditions. They suggest that the presence of high soil water leads to signal attenuation reducing the amplitude of reflected signal, but data can still be obtained from wet soils despite some reduction in depth penetration and resolution. Therefore, it is important to characterize the impact of soil conditions especially soil moisture content on the practicality for root detection with GPR.

GPR can capture different belowground features beyond root characteristics, such as soil moisture and soil clay content. Regression analysis is a common way to correlate GPR data with root characteristics. Generally, there are two approaches to extract indicators to represent and quantify GPR signal: reflection strength indices from GPR radargrams (such as pixel number and intensity) based on image analysis and waveform parameters from reflected signals (such as two-way time and reflection amplitude) (Butnor et al. 2012; Guo et al. 2013a). Because most crop fine roots are smaller than the minimum detectable object size that can be singularly resolved with GPR (Liu et al. 2016a), GPR signal may not respond strongly (different from the case of coarse root detection) to form a discreet hyperbolic reflection. The reflection waveform indices such as time interval index require roots being traversed by GPR antenna at 90^0, having a level orientation and being without other nearby roots (Barton and Montagu 2004). This condition is typically difficult to meet under most field conditions. Therefore, a focus on the reflection strength indices offers greater promise for fine root detection. Typically, the number of pixels within a threshold range (defined by pixel intensity) in an image converted from radargram is related to excavated root parameters (Butnor et al. 2003; Butnor et al. 2012; Borden et al. 2014; Butnor et al. 2016). Using the number of pixels within a pre-set intensity range to estimate roots may cause some bias, as it is difficult to visually recognize the root related signal in the image to delineate fine roots. It is better to compare with several different pixel related indices. For example, average pixel intensity defined as the sum of all the pixel grey level values of an object divided by the total number of pixels may have advantage as it blindly identifies fine root cluster without an intensity threshold as long as GPR captures the root information, which was used by Cox et al. (2005).

In this study, we hypothesized that proper signal index selection would allow GPR to adequately capture crop root-related signals under ideal soil conditions. Specific objectives of the study were: 1) to evaluate the feasibility of using GPR for fine root detection in differing soils; 2) to compare different pixel indices based on reflection strength; and 3) to differentiate root traits from different crop varieties using GPR. It is anticipated that the results will help determine the extent to which GPR technology can be a vital component of root phenotyping studies in the future.

Materials and methods

Study sites

The experiment was conducted at four locations in Texas: the Texas A&M AgriLife Research and Extension Centers at Amarillo (35°52′N, 101°58′W; elevation 1114 m), Uvalde (29°13′N, 99°45′W; elevation 283 m) and Weslaco (26°10′N, 97°56′W; elevation 22 m), and a farmer's field at Dilley (28°49′N, 99°06′W; elevation 193 m). The Amarillo site is in the Texas High Plains and has a silty clay loam soil (fine, mixed, thermic Torrertic Paleustoll). The Uvalde site is in the Texas Winter Garden Region (a major agricultural producer of winter vegetables) and has a clay soil type (Hyperthermic Aridic Calciustol). The soil type at Weslaco site located in South Texas is sandy clay loam (fine-loamy, mixed, hyperthermic Typic Calciustolls). The Dilley site was located in the Winter Garden Region with a sandy loam soil (fine-sandy, mixed, hyperthermic) at Dilley in Frio County, TX. Soil properties including texture and relative soil moisture (the ratio of measured soil water content divided by field capacity) as well as experiment treatments at the four locations were listed in Table 1. Winter wheat was planted at Amarillo, Uvalde and Dilley. Energy cane (*Saccharum spp*) was planted at Weslaco. A preliminary analysis of root size classes indicated that winter wheat and energy cane both have fine (0.5–2.0 mm diameter) and very fine roots (< 0.5 mm diameter) (data not shown).

Experimental design

At Amarillo, ten winter wheat (*Triticum aestivum* L) varieties ('Duster', 'Fannin', 'Fuller', 'Gallagher', 'TAM 112', 'TAM 114', 'TAM 304', 'TAM 305', 'TAM 401', 'WB Cedar') were grown both in irrigated and non-irrigated (dryland) field. There were six replications for each variety in the irrigated field and three replications in the non-irrigated field. Each replication of wheat variety was grown in a plot with an area of 4.6 m × 1.5 m (6.9 m^2). All the plots including the following experiments were arranged in a completely randomized design. Winter wheat was planted on November 28, 2016 at a seeding rate of 40 g/plot in both locations. Row spacing was 19 cm.

Two winter wheat varieties (TAM304 and TAM305) with six replications of each were planted at Uvalde in an irrigated field. Crop planting was same as the Amarillo site.

A local winter wheat variety "Greer" was planted at Dilley on November 10, 2016 in an irrigated field. The irrigated field is equipped with a center pivot irrigation system. Row spacing was 20 cm. Weeds were controlled following standard area recommendations to exclude any potential effects on the GPR signal at all four locations.

Seven energy cane varieties ('Ho02–113', 'TCP10–4928', 'TUS10–2', 'TUS10–23', 'TUS10–8', 'TUS11–58', 'TUS11–62') were planted in twin rows (30 cm apart) on raised beds (2.02 m wide) with subsurface drip irrigation tubes (15 Mil-Typhoon Thinwall Dripline with 0.45 m emitter spacing, 1 $L \cdot h^{-1}$ flow rate; NETAFIM™ USA, Fresno, CA) installed at 15 cm depth below the soil surface. There were six replications for each variety for a total of 42 plots. Each plot has an area of 6.1 m × 1.9 m (11.4 m^2).. All energy cane was planted on March 27, 2015 under greenhouse conditions and transplanted to the field on November 11, 2015.

Table 1 Soil texture and moisture at 0–15 cm and field crops at the four Texas locations

Location	Soil texture			Relative soil moisture (%)	Crop	Treatment
	Sand (%)	Silt (%)	Clay (%)			
Amarillo	39	32	29	94% (76%)	Winter wheat	10 varieties and 2 water supplies
Uvalde	12	31	57	91%	Winter wheat	2 varieties
Dilley	79	12	9	54%	Winter wheat	1 variety
Weslaco	43	12	45	41%	Energy cane	7 varieties

The value in parentheses is from the non-irrigated field at Amarillo. Relative soil moisture is defined as the ratio of measured soil moisture divided by field capacity

GPR equipment and field procedures

The Subsurface Interface Radar (SIR) System-4000, manufactured by Geophysical Survey Systems, Inc. (GSSI, Nashua, NH, USA) with a 1600 MHz antenna was used in this study. The velocity of wave propagation for the surface soil layer was estimated by burying (with minimally disturbed soil) a metal object at a depth of 15 cm at the four locations. Soil dielectric constant was determined according to the relation between velocity and soil dielectric permittivity (al Hagrey 2007). Soil dielectric constant was then used to calibrate soil depth after data collection. Table 2 lists the parameter settings for GPR scanning suggested by the manufacturer. All data were collected with identical parameters.

Before scanning, the soil surface at each measurement transect was cleared of all plant debris/l and leveled to avoid interference with GPR signals. In each measurement transect, GPR antenna was moved at a steady speed over a 3-m distance parallel to plant rows and between the two middle rows in each of the plots at Amarillo and Uvalde. We manually flagged each meter as the antenna was moved along the transect, yielding one radargram per plot. We scanned 59 plots (6 replications for 9 varieties; 5 replications for TAM401 because one replication was deleted due to poor emergence and final stands) in the irrigated field and 20 plots (2 replications for 10 varieties) in the non-irrigated field on March 6, 2017 at Amarillo. All 12 plots (6 replications for 2 varieties) were scanned on February 10, 2017 at Uvalde. At Weslaco, energy cane plots ($n = 33$; 7 varieties) were scanned on March 31, 2017 by sliding the GPR unit along the side of each raised bed as described above for wheat. Finally, we randomly selected 28 sampling locations in the winter wheat field at Dilley on April 7, 2017 and scanned the soil using the GPR unit the same day. Scanning was conducted in adjacent rows. At both Weslaco and Dilley, a 1-m long segment of soil was scanned in each plot.

At each sampling location, immediately following the GPR scans, one soil core (5 cm diameter and 15 cm depth) was collected under the middle of the scanning path in each of the plots or sites. The soil cores containing roots were stored in a freezer (−20 °C) until processing. Root samples were separated from soil using a 0.15 mm sieve by washing and rinsing with water. Washed root samples were stored in plastic bottles with a 20% of ethanol solution and later the cleaned roots were scanned on a flatbed scanner and root diameter was analyzed with WinRhizo software ver. 2003b (Reagent Instruments Inc., Quebec, Canada). After scanning, roots were oven-dried until constant mass (75 °C for 72 h) and root dry mass was recorded. Additionally, 5–6 soil cores were collected at each location of Amarillo, Uvalde and Weslaco to measure soil moisture when we scanned the field. In Dilley, one soil core (2.5 cm diameter and 15 cm depth) was collected for soil moisture measurement in the immediate vicinity of each of the core sampling locations for root measurement. At Uvalde, we also used GPR to scan another irrigated field which had similar soil moisture with the experimental plots and took 20 randomly selected soil cores to examine the effects of soil moisture on GPR signal. Soil was then oven-dried at 120 °C for 24 h.

Table 2 Control settings used on SIR-4000

Parameter	Setting/Value
Collection mode	Time
Scans/s	48
Sample/scan	512
Scans/mark	Zero
Soil dielectric constant	8
Time range	15
Position	Auto
Soil type	Custom

Post-collection data processing

Figure 1 shows a raw GPR radargram and the steps to extract signal indices. Data processing followed the methods of Butnor et al. (2003), but we used raw data without any additional collection gain. Use of the raw GPR data avoids the complication of having different collection gain settings optimized for each site. Radar profile normalization, background removal and Hilbert transformation were processed with Radan 7 (GSSI, Nashua, NH, USA). All the profiles were converted to bitmap image files (*.bmp) and then analyzed with SigmaScan Pro 6.0 (Systat Software Inc., San Jose, CA, USA). To better compare the GPR signal against the measured root values (from the soil cores), radar profiles were sectioned with the most signal concentrated upper soil layer (0–15 cm) for further analysis. An intensity range of 140–200 was used to delineate the roots. Pixel number, intensity and total intensity within this threshold range were extracted. Average pixel intensity without threshold limit (0–255) was also used to compare with the different indices.

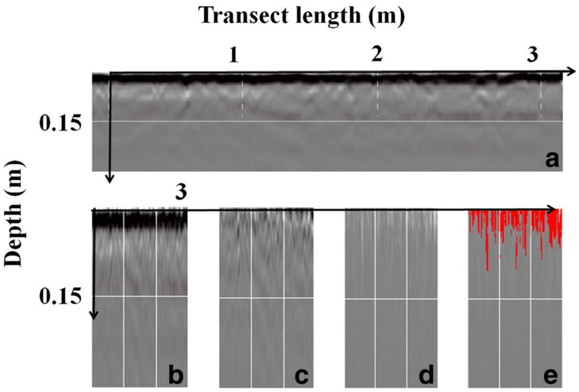

Fig. 1 An example of GPR radargrams (signals were concentrated in the 0–30 cm soil layer, but only the signals from 0 to 15 cm were used to compare with measured root) collected from winter wheat in the irrigated field at Amarillo. **a** Raw data from an SIR4000 GPR system with 1600 MHz antenna; **b**-**d** the same radargram processed in Radan 7 software after using distance normalization, background removal and Kirchoff migration to trace their sources; **e** the same radargram converted to 8-bit gray scale image, with the highlighted area in red color indicating pixels within the threshold range (intensity from 140 to 200) extracted with SigmaScan Pro 6.0 software. Vertical white lines indicate marks added when scanning the field and horizontal white lines indicate soil depth

Statistical analysis

Data analysis was performed with SPSS (version 16.0; SPSS, 2007). Before analysis, all variables were checked for distribution normality using the Kolmogorov–Smirnov test and variance homogeneity using Levene's test. Regression analysis was conducted using ordinary least squares regression, including uncertainty analysis. Analysis of variance with least significance difference was used to examine the significance among different varieties. Normalized root mean square error (NRMSE, root mean square error divided by range or average of measured root parameters) was used to compare different regression relationships of the different soils. Means and standard errors in bar graphs were calculated using the means procedure in SPSS.

Results

Relations between GPR signal and measured root characters

To determine if GPR could accurately estimate or predict fine root characteristics, we correlated (linear regression) the four pixel indices (pixel intensity, pixel number, total intensity and average intensity) with two root parameters (root diameter and biomass) (Fig. 2). The results show that there were significant relations between GPR signal and root characters at the four locations, even though the relations varied with different pixel indices and root characters (Table 3). All four pixel indices were significantly related to the two root characters with the coefficients of determination ranging from 0.084 to 0.18 in the irrigated field at Amarillo (Table 3). Among these relations, average intensity had higher correlation coefficients than the other three pixel indices. GPR signals were more related to root biomass than root diameter in the irrigated field at Amarillo. However, there were no significant relations between root traits and GPR signal in the non-irrigated field at Amarillo indicating lower soil moisture conditions may affect the relationship of root to GPR signal. Some negative relations between root biomass and GPR signal were also noted.

The relations between pixel indices and root characters also varied at the different locations. Only pixel intensity and average intensity were highly correlated with root biomass at Uvalde. No significant relation was found at Dilley. At Weslaco, pixel number and total intensity were significantly related to root diameter and average intensity was significantly related to root biomass. These different results imply that soil texture between sites affected root detection with GPR. However, there were some similar trends across the four locations: 1) root traits of wheat tend to be detected in wet soil conditions (Tables 1 and 3); 2) average intensity showed higher potential to reflect root information especially for root biomass; and 3) there were some negative relations detected in dry soil conditions (Tables 1 and 3).

Relationships between root traits and GPR indices for pooled data are illustrate in Fig. 2. Overall, root diameter and root biomass were best estimated by average intensity (Fig. 2g and h) further indicating the advantage of using average intensity instead of the three other indices. The best detection using GPR for root diameter and biomass was 56% and 39%, respectively.

Root character estimation

Based on the afore-mentioned relations, we selected the most significant pixel indices (highest R^2) at the same location with the same water supply to estimate the corresponding root parameters namely average intensity vs. root biomass in irrigated field at Amarillo, average

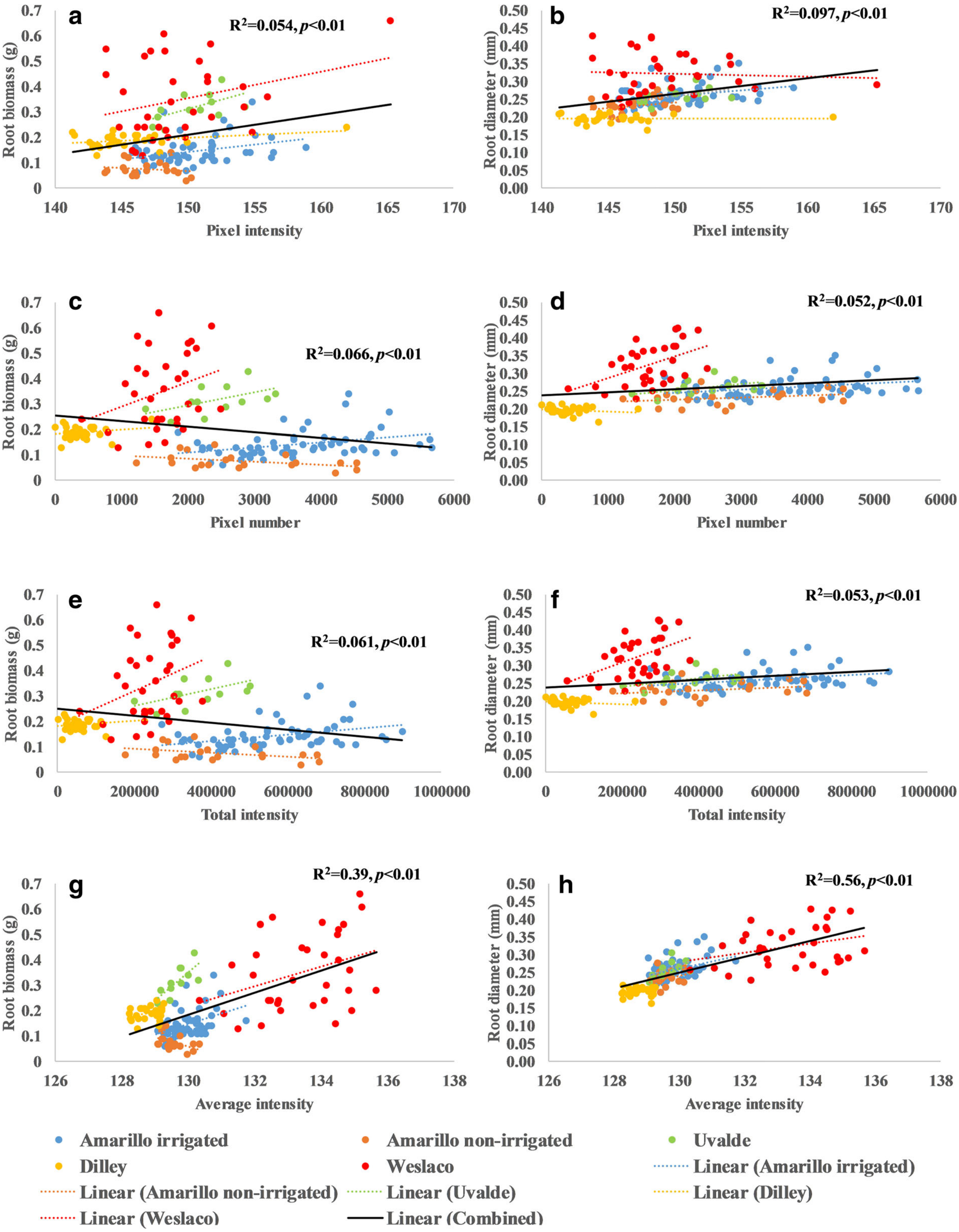

Fig. 2 Relationships between GPR signal and root traits (statistics are for combined regression)

Table 3 Regression coefficients (R^2) of soil-core measured root traits related to GPR signal indices

Location and sample size	Pixel index	Root diameter	Root biomass
Amarillo-irrigated *n* = 59	Intensity	0.10*	0.11**
	Pixel number	0.084*	0.12**
	Total intensity	0.090*	0.13**
	Average intensity	0.16* *	0.18**
Amarillo-non-irrigated *n* = 20	Intensity	0.063	*0.021*
	Pixel number	0.087	*0.16*
	Total intensity	0.088	*0.16*
	Average intensity	0.011	*0.20*
Uvalde *n* = 12	Intensity	0.19	0.34*
	Pixel number	0.21	0.27
	Total intensity	0.21	0.28
	Average intensity	0.30	0.54**
Dilley *n* = 28	Intensity	*0.0011*	0.11
	Pixel number	*0.039*	0.035
	Total intensity	*0.031*	0.044
	Average intensity	0.0034	0.074
Weslaco n = 33	Intensity	*0.0037*	0.087
	Pixel number	0.24**	0.084
	Total intensity	0.22*	0.095
	Average intensity	0.093	0.12*

Number in italic means negative relationships. "*" and "**" mean significance at $P = 0.05$ and $P = 0.01$ levels, respectively

intensity vs. root biomass at Uvalde and pixel number vs. root diameter at Weslaco. The three corresponding regression equations are: $y = 0.0411\times\text{-}5.1912$; $y = 0.1097\times\text{-}13.915$ and $y = 0.00006\times + 0.2252$; where y is root parameter and x is pixel index.

Figure 3 shows the estimated root data based on the three regression equations. There were seven varieties with less than 6% differences between estimated and measured roots. For certain varieties such as TAM114 and TAM401, estimated and measured root values were very close (around 1% difference) in the irrigated field at Amarillo. Conversely, the predictions are poor for some wheat varieties like Duster, Fannin and TAM112. Likewise, energy cane root traits can be over- or underestimated depending on different varieties with the differences between estimated and measured roots varying from 2% to 17% at Weslaco. However, discrepancies between predicted (GPR detected) and measured (soil core) root parameters were not statistically significant, except for genotype TUS10–2 (Fig. 3, Weslaco panel). It is interesting to note the lower variation (standard error) in the estimated roots (GPR detected) compared with measured roots from soil cores.

Analysis of variance was used to evaluate the difference among different varieties under the same treatment (Table 4). Due to the larger variation for wheat varieties, there was no significant difference found either from the measured root or estimated root. However, predicted root seems to have lower variability between or within varieties, hence increasing the significance potential (smaller P values). In contrast to wheat roots, there were significant differences among the energy cane varieties for the measured root parameters but not from the corresponding predicted values.

Comparison of different regression relations in different soils

Due to the different sample sizes and root characteristics, NRMSE values calculated from the range and average data of the measured roots were used to compare different regression parameters at the different locations (Table 5). The lowest NRMSE was found in the irrigated field at Amarillo indicating that the soil conditions there were the most suitable condition to use GPR to detect crop roots. The NRMSE was relatively large at

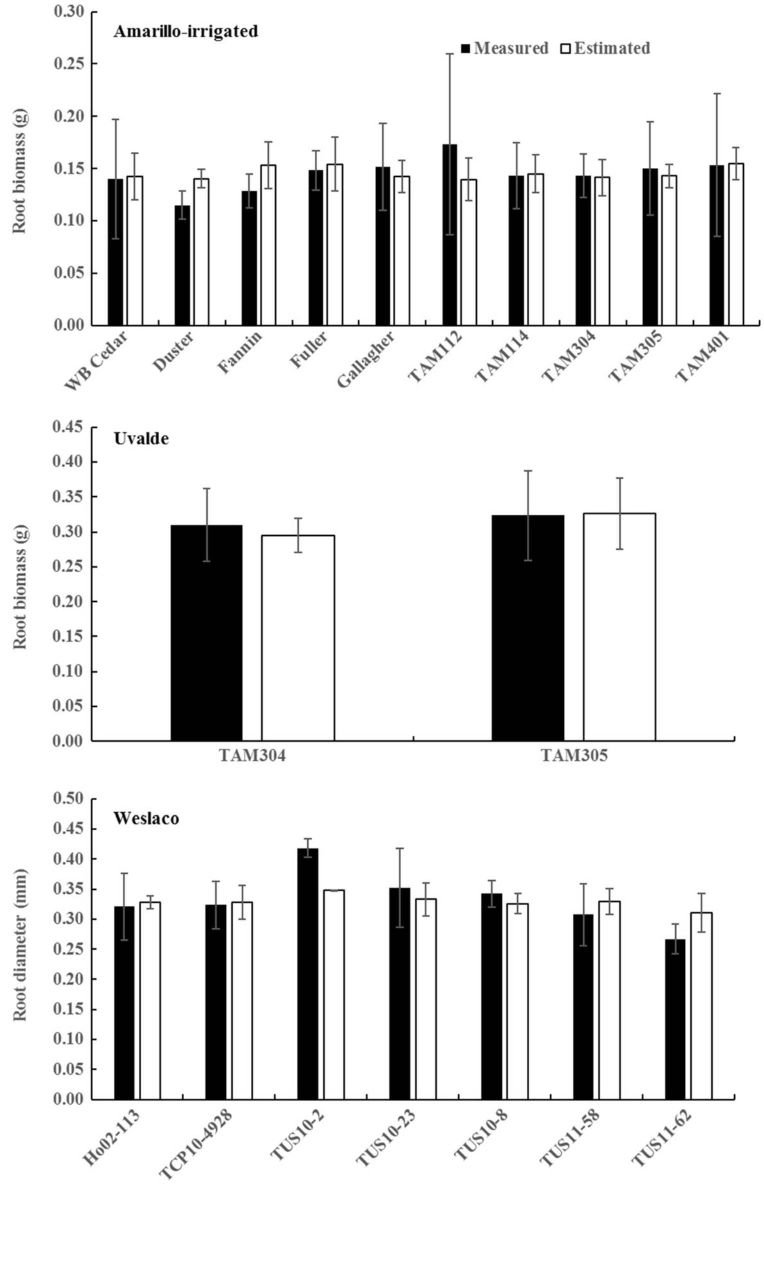

Fig. 3 Comparison of core-measured and GPR-estimated root parameters depicting the most significant relations for wheat (Amarillo, Uvalde) and sugar cane (Weslaco) varieties

Weslaco which may be a reason why estimated root traits did not differ significantly among varieties (Table 4). These results indicate that soil moisture content at the time of scanning can play a greater role than soil texture in the ability of GPR to detect fine and very fine roots under field conditions. Wet soil appears to be more suitable for fine root detection using GPR. Because we did not conduct a complete combination experiment with soil type and soil moisture, hereafter we will not discuss or compare their different roles in affecting root detection.

Effects of soil moisture on GPR signal

To explore the potential effects of soil moisture on root detection using GPR, we analyzed the relation between soil moisture and GPR signal at Uvalde and Dilley (Fig. 4). We used average intensity to correlate with soil moisture because three other pixel indices were expected to detect root with threshold. Results indicate that soil moisture content (perhaps mediated by soil texture) can significantly influence the sensitivity of GPR signals. Average pixel intensities were low (range 128–129) but

Table 4 Analysis of variance for soil-core measured and GPR-estimated root

Location	Source	Sum of squares	df	Mean square	F	P
Amarillo-irrigated	Between groups	0.012 (0.002)	9	0.001 (0.0002)	0.458 (0.53)	0.896 (0.846)
	Within groups	0.149 (0.019)	49	0.003 (0.0004)		
	Total	0.161 (0.01)	58			
Uvalde	Between groups	0.001 (0.003)	1	0.001 (0.003)	0.156 (1.853)	0.701 (0.203)
	Within groups	0.034 (0.016)	10	0.003 (0.002)		
	Total	0.035 (0.019)	11			
Weslaco	Between groups	0.041 (0.003)	6	0.007 (0.001)	2.868 (1.069)	0.028 (0.406)
	Within groups	0.062 (0.013)	26	0.002 (0.001)		
	Total	0.103 (0.016)	32			

Values in the parenthesis are from estimated root

more responsive ($R^2 = 0.54$; $p < 0.01$) to soil moisture changes under the relatively dry soil condition at Dilley (range from 0.05 to 0.09 g/g) (Fig. 4). At Uvalde on the other hand, average pixel intensities were high (range 130–136) but less responsive ($R^2 = 0.11$; $p > 0.05$) to the relatively moist but narrow soil moisture conditions (range from 0.18 to 0.23 g/g).

Discussion

Fine root detection

Although GPR has been used for non-invasive detection of coarse roots (Hruška et al. 1999; Butnor et al. 2003; Butnor et al. 2005; Cui et al. 2011; Borden et al. 2014; Moore and Ryder 2015; Butnor et al. 2016; Yeung et al. 2016), few studies have explored the feasibility of using this technology for fine root detection under field conditions (Butnor et al. 2012; Guo et al. 2013a) despite the crucial role of such roots in soil resource acquisition, and as regulators of biogeochemical cycles in diverse ecosystems. The results in this study revealed the significant relationships between GPR signal and root parameters of two crop species under different soil conditions (Table 3 and Fig. 2). There were few instances where root diameter and biomass were simultaneously significantly estimated in all conditions. By averaging root diameter in a bulk soil, it was possible for GPR to detect fine roots with reasonable certainty, in contrast to coarse roots, which can be deteced individually. Root biomass was more readily detected in wet than in dry soil conditions and this may be related to the fact that root biomass detection using GPR is affected by the large range of root water content in non-irrigated or dryland conditions (Guo et al. 2013b). Root water content typically does not vary as greatly in wet conditions, thus a better regression between GPR signal and root biomass can be expected with the narrower range of root water content (Guo et al. 2013b). There may be some interactions among the different root characters (York 2014) such that different root characters respond differently to the changes in soil conditions, which may have confounded the GPR signals.

Our data also suggest that the estimation based on the relation between GPR signal and measured root characters from soil cores varied under different soil conditions (Table 3 and Fig. 2). Previous studies using GPR to detect roots were primarily conducted in sand-dominated soils (Guo et al. 2013a; Liu et al. 2016a).

Table 5 Normalized root mean square error (NRMSE) values calculated from range and average of measured roots at the different test locations

Location	Soil texture/moisture	Root parameter	NRMSE (range)	NRMSE (average)
Amarillo	Silt clay loam and wet	Root biomass	0.0418	0.0814
Uvalde	Clay and wet	Root biomass	0.185	0.117
Weslaco	Sandy clay loam and dry	Root diameter	0.246	0.152

'range' indicates the difference between maximum and minimum values; 'average' indicates the mean value of all the measurements

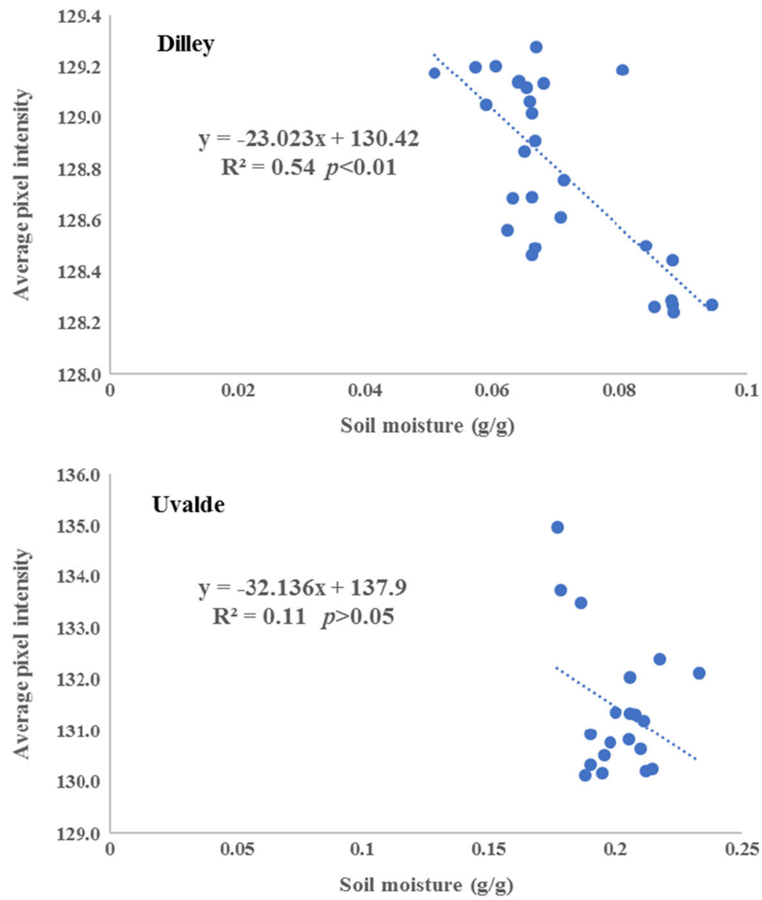

Fig. 4 Relationship between GPR signal and soil moisture measured at Dilley (low soil moisture) and Uvalde (high soil moisture)

GPR is generally considered not suitable for use in high clay soils because clay particles absorb, reflect and scatter electromagnetic energy, resulting in significant interference with GPR signal (Benedetto and Tosti 2013). However, our data suggest stronger relations between GPR signal and fine root characters in wet clay soil than those in dry sandy soils. There are two potential reasons that could explain the contrasting results. First, agricultural crops are usually grown with high plant density in the field conditions and roots normally concentrate in the upper soil layers (Xue et al. 2003; Wu et al. 1999). Soil moisture depletion under such conditions is rapid especially near the surface soil layers (0–40 cm) (Yadav et al. 2009; Dong et al. 2010; Zhang et al. 2011) and also at the early growing stages. Soil moisture near root in the surface soil layer may change very quickly compared with the remaining bulk soil. Consequently, the soil area near the upper roots may provide a drier environment (a relative dry area) for fine root detection because of the quick water uptake by roots under wet soil conditions (Oswald et al. 2008). Root water content can be kept at a high or normal level corresponding to a high soil dielectric permittivity which may differ from the relatively dry area, because more roots per unit soil volume (for agricultural crops) may increase the soil water uptake in the surrounding area, resulting in increased soil dielectric permittivity contrasts between roots and a relatively dry area. Therefore, this study shows that GPR can capture the fine root information. Hirano et al. (2009) reported that roots with higher water content could be more readily detected than those with lower water content. Furthermore, water content in the rhizosphere could still be higher than that of the surrounding bulk soil after 60 h of irrigation (Carminati et al. 2010). In this case, GPR signal may capture the sum of both the root and rhizosphere characteristics. The thickness (in diameter) of rhizosphere surrounding one root may be large enough such as 3.8 mm in lupin (*Lupinus albus*) (Carminati et al. 2010) to be detected by GPR in wet conditions even though we do not know the thickness of wheat rhizosphere in this study. Since the root rhizosphere is a dynamic environment and its size hard to measure in the field (York et al. 2016), additional information on rhizosphere properties is needed to fully understand how GPR fine root detection might be affected.

Secondly, the results also indicate that soil moisture variation may play a vital role in fine root detection using GPR in this study (Fig. 4). In relatively dry soil conditions, the soil may have been exposed to some intensive dry and wet cycles which would produce more soil pores (Peng and Horn 2007; Liu et al. 2016b). The pore distribution of soil controls the maximum value of the soil moisture variation (Vereecken et al. 2007) which may mask GPR signal in relatively dry soil. Soil moisture will scatter GPR signal (Benedetto 2010; Butnor 2011) as there was also a significant relation between the GPR signal and soil moisture found in the dry sandy loam soil in this study. Therefore, we could find better estimation in moist conditions (less soil moisture variation) rather than in dry conditions (more soil moisture variation). This reason may also partially explain why there were sometimes negative relations between GPR signal and root characters found in the dry conditions of this study (e.g., case of root mass in the Amarillo-non-irrigated plots in Table 3). Alternatively, root water content may be kept at a lower level with long-term dryness, which may result in the negative relations (Guo et al. 2013b). Root water always has a broad range of variation in sandy soil (Guo et al. 2013b), so the root character that was strongly influenced by root water may impact GPR signal. Root traits of energy cane seemed to be more easily detected than wheat in dry conditions, possibly because of their relatively larger root diameters. The geophysical mechanisms underlying GPR fine root detection clearly warrant further investigation.

In addition to unambiguous fine root detection in general, the results also indicate that GPR has the potential to resolve differences in root traits among wheat varieties (Fig. 3). Although it is evident that more information is needed to better understand how rhizosphere factors interact with GPR signals, the current results clearly demonstrate the feasibility of GPR as a remote-sensing tool for detecting fine roots and perhaps for phenotyping studies (Pauli et al. 2016). We propose that, instead of waiting for a "perfect" tool, our knowledge of the unseen roots advance more quickly if we choose to start using presently available tools such as GPR. The variation in GPR-estimated roots was smaller than that from measured root (Fig. 3 and Table 4), which concurs with Butnor et al. (2003), who also reported the smaller variation from predicted roots than field measured root under the same treatment. This may be attributed to the benefits of the larger area-averaging in GPR-based root estimation as compared to the point-based results in measured roots from soil cores. Different from wheat results in wet conditions, the significant difference in root characters of different energy cane varieties was not detected by GPR, perhaps because GPR signal was masked by the variation of soil moisture in the dry sandy soil.

As we expected in the introduction section, the results of this study also show the advantage of using average pixel intensity over pixel number to quantify fine roots (Table 3 and Fig. 2). However, this approach also depends on root traits and soil conditions. Future work on fine root detection may also need to consider more than one pixel index.

Implications for breeding

Identifying root characters that are responsible for drought tolerance is a key research objective for crop physiologists and breeders. Traditionally, an extensive root system is considered positive to improve crop drought tolerance. The paradigm is shifting as more studies assert that a reasonable root distribution and function is more helpful (Zhang et al. 2009; Wasson et al. 2012; Liu et al. 2015). Root traits, particularly those that promote deeper root systems, may also play a critical role in improving water uptake in deeper soil layers under water-limited environments (Blum 2005; Wasson et al. 2012) because root traits that enhance deeper water uptake include longer roots and greater branching with longer dense root hairs exhibiting decreased radial and axial resistance to water movement from the soil to the shoots (Wasson et al. 2012). Overall, root structure and function should be emphasized at the same time for a more complete assessment of a root system. In this study, we found that GPR can detect crop fine roots. Since GPR as a noninvasive technique, deployment should benefit crop root phenotyping for crop breeding allowing the measurement of hundreds or thousands of plots in a short time. We also found that crop above-ground growth was highly associated with GPR signal compared with the measured root (data not shown). We posit that GPR signal includes more than root information such as that of root water content and/or nutrient uptake. In this case, GPR will provide more valuable root-related information and assist in improving a root targeted breeding process.

Future work

The estimation accuracy of GPR-based root detection will need to be improved considering additional soil factors, since soil texture, soil moisture, soil organic matter, soil compaction and soil salt content etc. can significantly affect the GPR signal (Shih and Doolittle 1984; Raper et al. 1990; al Hagrey and Müller 2000; Benedetto 2010; Benedetto and Tosti 2013). Inclusion of these parameters in multiple regression models may improve the coefficient of determination and constrain more of the variation in the data. It will also be important to consider root water changes especially in dry sandy soils as it particularly affects estimation accuracy with signal-strength related indices (Guo et al. 2013b). Overall, a better understanding of the geophysical mechanism of fine root detection under particular soil dielectric conditions warrants further study.

In the future, we need to identify root information at different soil depths. Deep roots are as important as shallow roots for crop drought tolerance in water-limited regions. Another aspect of concern is about root growth angle. A narrow root growth angle is expected to reach deep soil layer (Wasson et al. 2012). By detecting the root biomass horizontal distribution, we may be able to acquire information on root growth angle indirectly.

To extend the usefulness of GPR for crop root detection, we should also try using it in other types of root systems such as the taproot system. Recently GPR was tested in the estimation of below-stump root biomass in trees (Butnor et al. 2016). Likewise, we may find a way to study crop taproot with a special borehole radar which can be inserted into soil allowing the investigation of vertical oriented targets (Butnor et al. 2012).

Conclusions

Even though GPR is widely used in coarse root estimation, detection of fine roots has previously been considered challenging and was not explored in depth. This is the first report to show that GPR can measure fine root characteristics at a bulk soil level. Average pixel intensity without a threshold limit showed higher potential to quantify root characters than pixel indices with a threshold range. Root diameter and root biomass could be detected by GPR in different soils. The relationships between GPR signal and root characters varied but generally stronger in moist soils than in dry soils. Soil moisture heterogeneity in dry soils may result in negative effects on GPR signal which will affect root detection. GPR-based estimates of root parameters had lower variation than core-based measured root results which may allow fine-scale differences between varieties to be revealed. There is great potential for using GPR to phenotype crop roots and it could become invaluable for guiding crop breeding decisions. Further work is needed to improve the root estimation and understand the mechanisms of detecting fine root using GPR as well as exploring more applications for different root traits of diverse agricultural root production systems.

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