A simple and cost-effective method for cable root detection and extension measurement in estuary wetland forests

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Abstract

This work presents the development of a low-cost method to measure the length cable roots of black mangrove (*Avicennia germinans*) trees to define the boundaries of central part of the anchoring root system (CPRS) without the need to fully expose root systems. The method was tested to locate and measure the length shallow woody root systems. An ultrasonic Doppler fetal monitor (UD) and a stock of steel rods (SR) were used to probe root locations with-out removing sediments from the surface, measure their length and estimate root-soil plate dimensions. The method was validated by comparing measurements with root lengths taken through direct measurement of excavated cable roots and from root-soil plate radii (exposed root-soil material when a tree tips over) of five up-rooted trees with stem diameters (D_{130}) ranging between 10-50 cm. The mean CPRS radius estimated with the use of the Doppler was directly correlated with tree stem diameter and was not significantly different from the root-soil plate mean radius measured from up-rooted trees or from CPRS approximated by digging trenches. Our method proved to be effective and reliable in following cable roots for large amounts of trees of both black and white mangrove trees. In a period of 40 days of work, three people were capable of measuring 648 roots belonging to 81 trees, out of which 37% were found grafted to other tree roots. This simple method can be helpful in following shallow root systems with minimal impact and help map root connection networks of grafted trees.

Key words: anchoring root system, woody cable roots, *Avicennia germinans*, fetal Doppler.

1. Introduction

The accessibility to below-ground biomass has limited our knowledge on structural-functional aspects of root systems, especially for large plants (Danjon et al., 2013). Most existing methodologies are destructive and either require the full excavation of root systems (Danjon et al., 2005; Smith et al., 2014), or pulling trees until up-rooted (Blackwell et al., 1990; Coutts, 1983; Crook and Ennos, 1998; Gasson and Cutler, 1990; Ray and Nicoll, 1998; Sapijanskas et al., 2014), an irreversible disturbance and destructive strategy that in many cases cannot be performed with species enlisted in the IUCN red list. Strategies to study roots *in situ* other than excavating the whole root system have been developed more recently, like rhizotrons, ground penetrating radar (GPR), and the use of medical instrumentation such as X-ray computed tomography (CT, Taylor et al. 1991; Perez et al. 1999; Butnor et al. 2001) and magnetic resonance imaging (MRI, Fang et al. 2012). Rhizotrons are structures with glass windows that allow the direct measurements of roots growing in the soil. (Taylor et al., 1991). Ground Penetrating radar technology is a fully non-destructive method that operates transmitting electromagnetic waves through the soil and records times of reflection to 3D images of the buried materials (Nadezhdina and Čermák, 2003; West, 2009). Finally, the use of medical instrumentation such as the CT and MRI allow for 3D reconstruction of fine root structure within intact core samples (Fang et al., 2012).

While rhizotrons are effective to estimate below ground biomass, root growth-rates and rhizosphere dynamics, they are unsuitable for mechanical stability studies because measurements can only be performed on root tissues that come in contact with the glass (Burke and Raynal, 1994; Taylor et al., 1991). On the other hand the CT, GPR and MRI point to a promising non-invasive methods for detailed studies on root structure of plants, nevertheless these are technologies of high economical costs (no less than USD 10,000 for GPR), and are still under development (Fang et al., 2012). To date, GPR has only been used to estimate stand level below ground biomass (Barton and Montagu, 2004; Butnor et al., 2003; Danjon et al., 2013), while CT and MRI can only be performed on soil cores extracted from the field and are highly sensitive to water content, making them inappropriate for wetland forested system studies (Butnor et al., 2001; Fang et al., 2012; Luo et al., 2008; Perez et al., 1999).

Studies on anchoring systems of large plants, to date, still relay in complete excavation of root systems to perform structural analysis through the use of terrestrial laser scanning (Danjon et al., 2013, 2005; Smith et al., 2014), or to pulling and up-rooting mechanisms to characterize the strength of root-soil plates (Blackwell et al., 1990; Coutts, 1983; Coutts et al., 1999; Cucchi et al., 2004). A trees root-soil plate, referring to the section of woody roots and soil that get exposed after mechanical failure of the stem, is the object of most studies dealing with tree mechanical stability and resistance to wind damage (Coutts, 1983). For standing stems, this region is known as the "central part of the anchoring root system", (hereafter CPRS) and represents the main area of plant anchorage (Coutts, 1983; Danjon et al., 2005; Stokes et al., 2005). While tree stability depends on root structure, the latter is influenced by soil structure; trees growing on deeper soils will have more vertical root growth than on shallow soils (Ray and Nicoll, 1998; Stokes et al., 2005) or at sites with a high water table that creates anoxic condition (Coutts, 1983; Keeley, 1988; Ray and Nicoll, 1998), thus limiting the development of deep roots. Wetland trees, like the black mangrove (*Avicennia germinans*), lack a tap root, or vertical sinker roots to increase anchorage, and root development is limited to the first 20 to 30 cm below ground surface (López-Portillo et al., 2005; McKee, 2001), thus, trees must compensate stability by growing longer horizontal woody roots. Still, the lack of a deep rooting system makes trees more vulnerable to windthrow (up-rooting due to wind forces), and it represents a particular risk in water-saturated soils (Coutts, 1983; Krause et al., 2014).

While our knowledge on mangrove wetlands has increased dramatically in the last few decades (Alongi, 2008, 2002; Field et al., 1998; Srikanth et al., 2015; Twilley, 1988), our understanding of their root system is limited to areal structures, biomass estimations and functional anatomy and physiology (Angeles et al., 2002; Brooks and Bell, 2005; Castañeda-Moya et al., 2011; Komiyama et al., 2000; Mendez-Alonzo et al., 2015; Ohira et al., 2012; Srikanth et al., 2015), while knowledge of the structure of the anchoring system becomes urgent to better understand and predict their mitigation effect on surges and ecosystem responses to environmental change (Srikanth et al., 2015). As previous studies on terrestrial forests show that the length of lateral roots, and thus the CPRS, increases with tree size (Smith et al., 2014), this study proposes a low-invasive method based on the application of the Doppler effect to detect and measure woody root lengths without digging trenches. The Doppler effect, referrers to the change in the frequency of a wave, for an observer moving relative to the source of the wave (Maulik, 2006). This principle was first described for light wave movements by Christian Doppler in 1842, and latter verified with sound waves in 1844 (Maulik, 2006) . Using this principle, a simple method was developed to measure the length of cable roots to approximate CPRS diameters of the species *A. germinans* with the use of a few steel rods (hereafter SR) and a portable ultrasonic fetal Doppler (hereafter UD).

The portable UD holds a transducer, a receiver and an amplifier; the transducer sends out an ultrasonic signal (a frequency higher than humans are capable of hearing), which travels through the surface it is in direct contact with. When the emitted high frequency waves encounter movements (i.e. the blood flowing in an artery or a heart beating), the waves bounce back modified by the frequency of the encountered waves, then the received frequency is further amplified into an audible signal (Maulik, 2006). The Doppler effect system can help in the detection of woody roots connected to a stem without digging trenches; if a sound wave is created on a given root by gently hitting on it with a SR, and the probe of the UD is located in the collar ring of a stem, the ultrasonic waves traveling from the UD through the stem and roots, will bounce with the waves generated by the SR and travel back to the UDs receiver, causing a positive signal in the UD, expressed as an audible sound and a frequency equal to that of the SR hitting on the root. The sensitivity of the UD is high enough to monitor the heartbeat of a five to7.6 cm long (8 to 12 weeks) human embryo (Papaioannou et al., 2010), and has been successfully employed to measure the heart rate of wrasse fish (*Notolabrus celidotus*) and small crab species with heart rates twice as high than a human heart rate at 13 weeks of development (Iftikar and Hickey, 2013; Iftikar et al., 2010; Papaioannou et al., 2010).

In this work shows the ability to effectively measure the length of horizontal woody roots and further approximate the size of the CPRS polygon with a major reduction on costs and time investment through the use of the UD. Our hypothesis is that the CPRS in wetland trees with cable root development, is mainly delimited by woody cable roots, thus the estimated CPRS radius will be similar to the radius of root-soil plates of uprooted trees of the same species. To test the accuracy of the developed method, data were compared between the measurements taken with the UD and 1) root-plate radius of uprooted trees found in the field; 2) lengths taken through the use of SRs without UD; and 3) through the excavation and direct measurements of roots. The potential applications of this method, for wetland forest woody root research, is discussed.

2. Methods

2.1 Study site

The method was developed between October and November 2015 and validated during the month of July 2016, in a mangrove ecosystem form the central Gulf Coast of Mexico, in the La Mancha Lagoon (19º35'N, 96º22'W). This region has an average annual precipitation between 1200 and 1500 mm and a mean annual temperature of 25° C, with minimum and maximum temperatures of 22° and 28° C in January in May, respectively (López-Portillo et al., 2005). The lagoon is surrounded by 300 ha of mangrove forest co-dominated by *Avicennia germinans* (black mangrove) *Rhizophora mangle* (red mangrove) and *Laguncularia racemosa* (white mangrove). Two main mangrove geomorphic habitats are recognized in the area: Mangrove-vegetated mudflats and interdistributary basins. The first is characterized by the accumulation of clay and loam sediments, and the latter is dominated by organic-rich sediments related to a marked fresh-water influence (Thom, 1967;

Vovides et al., 2014). Salinity within these sediments can range between 600 and 1200 mM NaCl (Vovides et al., 2014), while soil compaction in the area ranges between 1.13 and 4.8 kg cm^{-2} (Vovides et al. 2016), which means sediments are soft and easily penetrable.

2.2 Root length measurement with a portable Doppler

To approximate the CPRS polygon for the mangrove species *A. germinans*, 51 trees were selected, with stem diameters measured at 130 cm of height (D_{130}) ranging between 10 and 96 cm. A set of eight 1.20 m and 0.5 inch SRs and a portable SonoTrax fetal Doppler equipped with a 3Mhz waterproof probe (SonoTrax Basic, Edan Instruments GmbH, Hessen, Germany) were used to measure cable root lengths in eight cardinal directions. This UD is an economic portable instrument (approximately € 300 or USD $ 330), equipped with an LCD screen that allows visualization of the signal received frequency. (http://www.edan.com.cn/html/EN/products/OBGYN/UltrasonicDoppler/201203/20355.html, accessed on the 14th of August, 2016) .

A flagging was attached to each SR at distance of 30 cm from its bottom base to mark the maximum depth at which to probe the location of a given cable root, such depth was selected considering it is 10 cm deeper than the average root depth (10-20 cm) previously reported for the (McKee 2001; López-Portillo et al. 2005; Twilley and Rivera-Monroy 2009), and based in measurements performed during this study on recently up-rooted trees found in the study site. Further, the probe of the UD was protected with a gel band aid (Hydro Tac Gel-Plfaster, Gothaplast Verbandpflasterfabrik GmbH, Gotha, Germany). The gel band aid ensures maximum contact of the probe with the uneven surface of stem bark or tip of the SR, and proper transmission and reception of ultrasound waves. To follow a cable root, first the UD's probe was placed at the base of the tree collar, and the adjacent, exposed prop root was gently hit with a SR in its connection with the base of the stem, in the visible buttress area connecting to a root. The ultrasonic waves emitted by the UD are reflected by the waves caused by the SR on the root and are detected by the UD, which confirms the cable root belongs to the target tree by showing a heart symbol and a frequency number in the LCD screen. The UD is moved to the upper tip of the SR and a second SR is placed 5-10 cm from the first one, following the cable root, and further used to hit the root. When the UD detects the vibrations in the SR, a third SR is used to hit on the cable root to create vibrations while the UD is transferred to SR number two (See Fig. 1). The probe is passed consecutively from rod to rod to detect vibrations from the followed cable root, until the depth of 30 cm is surpassed and the root can no longer be located (Fig. 1). This procedure secures sufficient strength of the signal despite the increasing distance from the stem. Supplementary material video presents an animation of the methodology.

To relate the approximated CPRS radius to tree size, we measured the cable roots in eight cardinal directions for 51 trees with D_{130} ≥10 cm and tested the dependency of CPRS on D_{130} via a least square non-linear regression of the form:

$$CPRS = \frac{a * D_{130}}{b + D_{130}}$$

Where a and b are constants of regression. To achieve data normalization we computed the square root of CPRS. Further, to evaluate the limit of sensitivity of the UD, after 10 roots had been located and measured with the aid of the UD, the target root was evaluated by probing with a SR every 10 cm from the base of the stem leaving the UD based on the collar ring of the stem, until the UD no longer emitted a positive signal from the hitting.

2.3 Method validation

To validate the method, for five trees with D_{130} ranging between 10 and 50 cm, 1) eight roots were followed using only SRs (to test the possibility of measuring woody cable roots without the aid of the UD); 2) further, the roots were exposed down to 30 cm of depth to make direct measurements of the cable roots and compare them with the lengths measured with the UD to test for a 1:1 relationship; 3) the average lengths of cable roots per tree measured *via* UD, for four trees with D_{130} values equal to the D_{130} of four up-rooted trees found in the area, were compared to the radius mean radius of root-soil plates from the up-rooted trees.

2.4 Accuracy and limits of detection

The risk of false positives (i.e. hitting a neighbouring root other than the target root and receiving a positive signal in the UD) was evaluated in 40 roots belonging to five standing trees, SRs were left on the identified path of the target root, and an extra rod was used to hit the neighbouring zone on ten points around the target root, as close as one centimetre from the target root and as far as 15 cm. If a neighbouring rot was located, the SR was used to hit on it, and positive signals in the UD (located on a SR standing on the target root) were quantified. Afterwards, if positive signal were detected, roots were exposed in the point of intersection with the target root for visual inspection.

2.4 Statistical analyses

We used a Wilcoxon test to asses differences between methods used to measure cable root lengths, and compared data of each technique with a linear regression, to asses a 1:1 relationship method. The dependency of CPRS radius and tree size was evaluated regressing mean CPSR radius against D_{130}, via least squares non-linear regression after computing the square root of the response variable in order to achieve normality, which was evaluated using a Shapiro-Wilk test. Data analyses were performed using the R software for statistical computing (R Core Team, 2016), particularly "stats" package was used for most data analysis, and the "nlstools" package for the non-linear regression and model diagnostic (Baty et al., 2015; R Core Team, 2016).

3. Results

3.1 Method validation

The attempt to measure lengths using only the SRs was unsuccessful; for 80% of the cases (34/ 40) roots could not be followed with certainty for distances greater than 30 cm from stem and depths greater than 10 cm. As distance from stem increases, more roots are crossing each other, when probing with the SR and hitting on a hard surface it is impossible to know if the target root is being followed unless a trench is excavated for visual confirmation, or a positive signal is received (i.e. using a UD). On the other hand, the Wilcoxon test shows no statistical between measured lengths by UD and by excavating trenches (W= p=0.97, n= 40), while a relationship close to 1:1 (r^2= 0.98, p< 0.001, n= 40) is observed when regressing the lengths of roots measured with the UD against lengths measured by excavating (Fig. 2a). Additionally, Fig. 2b shows that the average radius of the CPRS approximated by measuring root lengths in eight cardinal directions with the UD has a ratio close to 1:1

when compared to the average radius of root-soil plates from uprooted trees (r^2=0.98 p<0.01, n= 4).

Using the UD method, cable roots on eight cardinal directions were measured for a total of 81 trees, from which 30 trees (37%) were found to have roots grafted to neighbouring trees, and were therefore eliminated from further analyses. If we add up the trees used for analysis, from a total of 81 trees, 37% show root grafting.

To relate CPRS radius with tree size, for 51 non-grafted trees, a total of 408 cable roots were measured with root lengths ranging between 0.01 and 7.9 m, and an average of 1.03 ± 0.27 m (mean ± se). The CPRS average radius per tree shows a mean of 0.98 ± 0.08 m, with minimum of 0.11 and a maximum of 2.7 m. The root square transformation of the CPRS helped to achieve a normal distribution (W=0.96, p=0.17), and further validation of the method is given by the positive relation found between the approximated CPRS radius and tree D_{130}. The model shows coefficients $a = 1.45 \pm 0.08$ $(p < 0.001)$ and, $b = 8.97 \pm 1.78$ $(p < 0.001,\ \mathrm{n} = 51)$, and explains 84% of the total variation in CPRS radius. Figure 3 shows this relationship and that the CPRS radius estimated by digging trenches and root-soil plates of up-rooted trees lay within the UD-data curve.

3.2 Accuracy and limits of detection

Out of 40 roots belonging to five trees, a total of 87 neighbouring roots were located between 5 and 20 cm from a target root and, were probed to evaluate false positive signals. The length of the target roots tested for neighbour-related false positives ranged between 0.30 and 2.5 m, corresponding to trees between 20 and 80 cm in diameter. From the 87 neighbouring roots probed, only two returned a false positive signal (2.3%). For the two false positives detected, excavation of neighbouring roots revealed that in one case two different roots were grafted, while in the second case, the roots were in direct contact. Additionally, we observed signal loss with increased distance between the location of the UD's probe and the SR, when leaving the UD on the stem; in 40% of 10 explored roots, the UD failed to receive a signal at distances greater than 40 cm, when roots were located at a depth smaller than 15 cm, nevertheless, at distances from stem base shorter than 40 cm, the depth of root location did not affect detection, since 100% of the attempts (40/40) gave positive signal in the UD, this probably due to the shallowness of root location, since no roots were found below 30 cm of depth

4. Discussion

Our results support the hypothesis that woody cable roots delimit a tree's CPRS. A confirmation of this is the fact that soil-plate radii are similar to the dimensions of approximated CPRS radii using the UD (Fig. 2 and Fig. 3), proving that the UD method is useful to approximate CPRS. The relationship found between CPRS and tree D_{130} are consistent with the relation described by Smith et al. (2014), who report an increase of tree volume with increasing root length, while a dependency between tree volume and D_{130} is acknowledged for (Pretzsch, 2009). As the CPRS represents the main anchoring zone of a tree, trees grow more, longer and stronger roots in the direction of wind, or towards directions of mechanical imbalance caused competition-related crown displacement (Bruce and Dunn, 2000; Danjon et al., 2005; Stokes et al., 1997), thus, this method can help develop studies to evaluate CPRS polygon asymmetry and responses to wind and neighbourhood competition (Vovides et al., 2016).

Unexpectedly, the UD method developed here was also useful to detect root grafting between neighbouring trees. When the wave transmission caused by hitting one root was followed until reaching a neighbouring tree, and a positive signal was returned by the UD in that second stem. When this occurred, the root was again carefully probed back to the starting stem, and a bigger root-area was searched and excavated for visual assessment, these due to the secondary growth caused during graft union formation (Bormann, 1966; De La Rue, 1934). Despite root grafting in mangroves seems to be acknowledged as common (Duke, 2001), to the extent of our knowledge, no research has been performed to evaluate the ecological significance (or frequency) of this phenomena in mangrove ecosystems, despite it could have ecological and functional implications in relation to water balance or resource sharing (Klein et al., 2016; Nadezhdina et al., 2012; Tarroux and DesRochers, 2011).

The use of the UD has helped to compare the direction of root displacement with neighbour presence and evaluate below ground facilitation strategies in trees (Vovides et al., 2016). Despite no detailed information on root thickness or diameter can be obtained with the UD, with this method connectivity networks of tree roots could be easily confirmed by locating root-grafts in a rapid and un-destructive manner. Such research would hold significant implications in understanding plant interactions at a landscape level (Deslippe et al., 2016; Fajardo and McIntire, 2010; Klein et al., 2016; McIntire and Fajardo, 2011), ranging from implications of root development on tree stability to ecological relevance of root grafting for carbon flux, water balance an ecosystem bio-complexity (Feller et al., 2010; Grimm et al., 2005; Nadezhdina, 1999; Nadezhdina et al., 2012).

Conflict of interest

The authors declare no conflict of interest. All contributions were discussed, approved and properly recognized by co-authors. The data presented here have not been published elsewhere.

Author contribution

A.V., B.M. and G.B. designed the method. B.M. and A.V. took field measurements to validate method. AV and UB analysed the data and wrote the paper. JLP and UB provided with ideas for method improvement and data analysis, and gave financial support and edited the paper.

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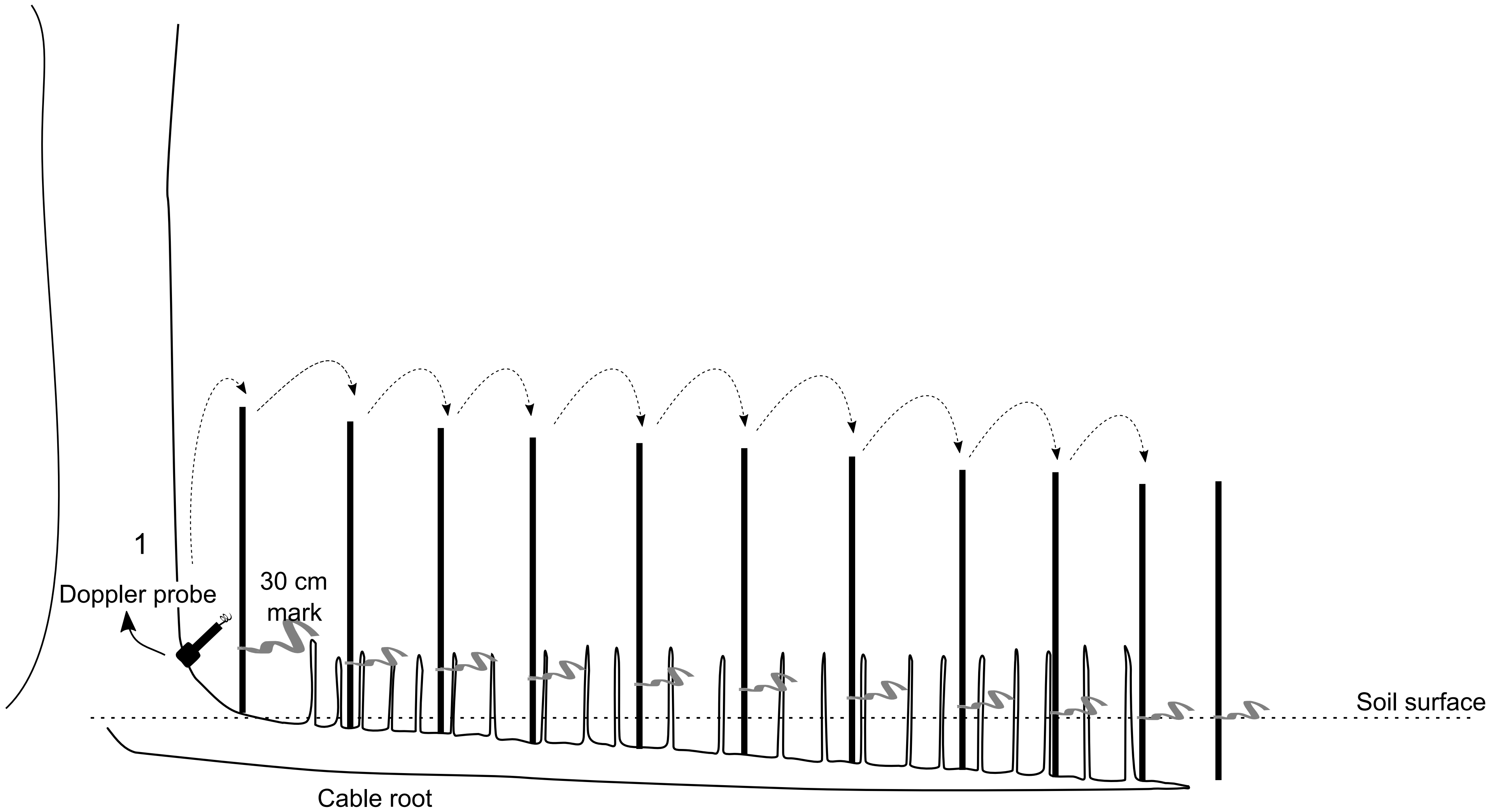
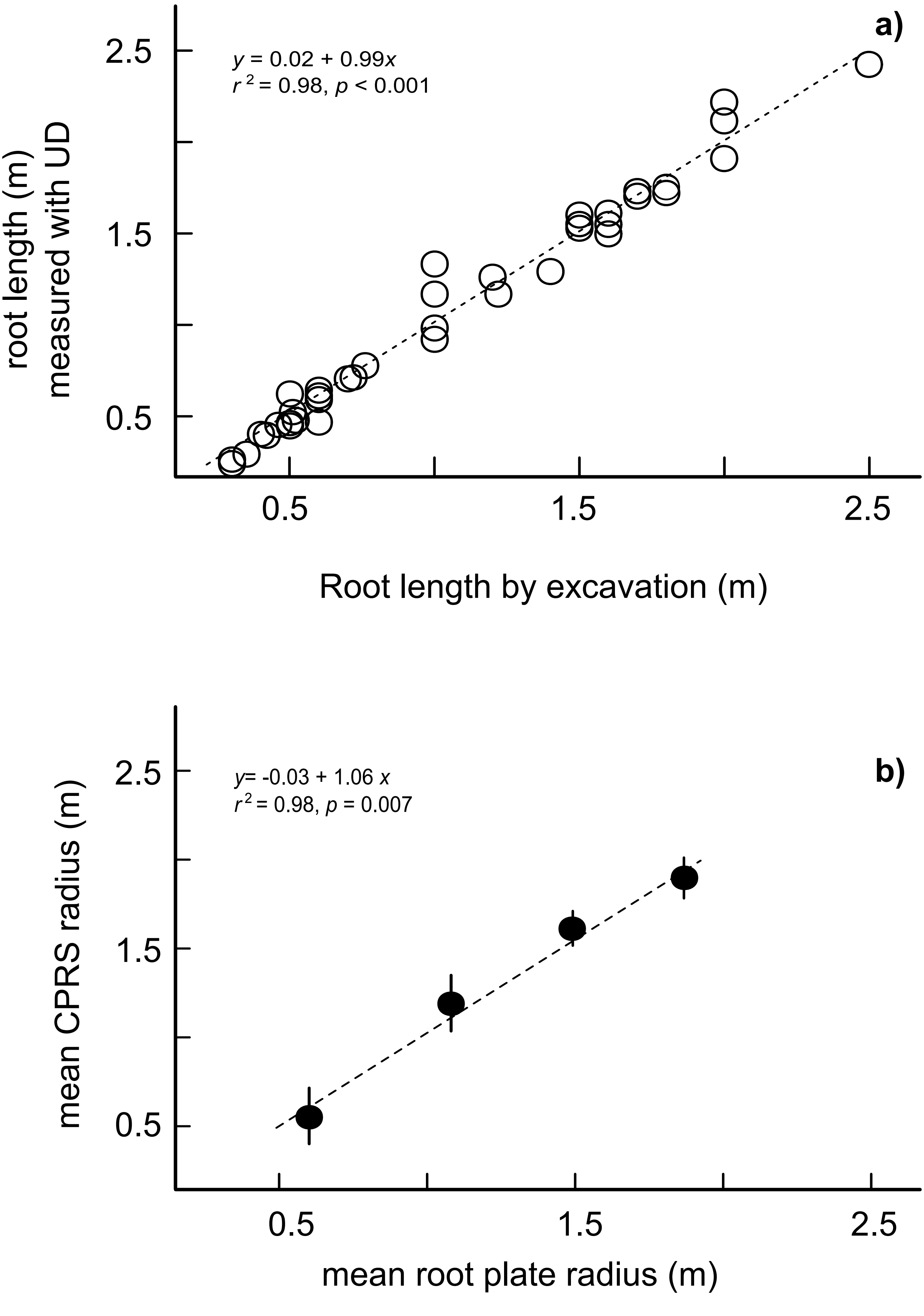
Legends to figures

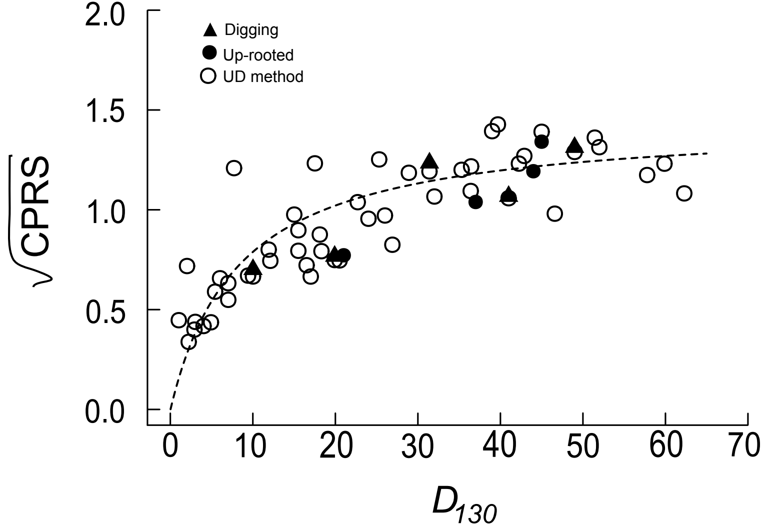
Figure 1. Graphic representation of root probing with the Doppler. SRs are used to follow the location of the cable root from base of a tree, placing the Doppler probe at the tree collar, and tapping the base of the cable root with SRs in a consecutive manner until the root reaches surpasses 30 cm of depth or is no longer detected.

Figure 2. Linear relation between **a)** root length measured with the UD and by excavating, and **b)** approximated CPRS radius mean root-soil plate radius of uprooted trees.

Figure 3. Least squares non-linear relation between the square root of the CPRS and tree D_{130} measured with the UD (open circles), by excavating (black triangles) and the mean radius of root-soil plates from uprooted trees (black circles).


1
Doppler probe
30 cm
mark
Soil surface
Cable root


a)
y = 0.02 + 0.99x
r 2 = 0.98, p < 0.001
root length (m)
measured with UD
2.5
1.5
0.5
0.5
1.5
2.5
Root length by excavation (m)
b)
y= -0.03 + 1.06 x
r2 = 0.98, p = 0.007
mean CPRS radius (m)
2.5
1.5
0.5
0.5
1.5
2.5
mean root plate radius (m)


Digging
Up-rooted
UD method
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
$\sqrt{CPRS}$
D_{130}