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ORIGNAL ARTICLE

# Electrical capacitance estimates crop root traits best under dry conditions—a case study in cotton (*Gossypium hirsutum* L.)

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**Abstract**
*Purpose* Electrical capacitance (EC) is widely used to measure root traits especially in hydroponic and wet soil environments, but its feasibility was recently questioned due to the possible leakage of electrical current before entering the root system. To investigate whether the current can travel deeper in woody roots, EC was evaluated in cotton (*Gossypium hirsutum* L.) grown under different soil moisture conditions (two rounds of pot experiments, one PVC tube experiment and one field experiment).
*Methods* In these experiments, electrical capacitance of the root ($EC_{root}$) was recorded before and after severing roots, and immediately afterward, the roots were collected to measure root dry weight, surface area, length, and volume.
*Results* The results showed that $EC_{root}$ was significantly reduced after root cutting especially under dry conditions in the three experiments. The PVC tube experiment further demonstrated that root cutting did not significantly affect the changes in $EC_{root}$ measured at different soil layers under dry conditions possibly suggesting that the current could flow into the deep roots under these conditions. Moreover, incorporating root tissue density and soil moisture into the prediction model can improve the predictive accuracy of $EC_{root}$. In contrast, $EC_{root}$ was less affected by root cutting under wet soil conditions, indicating that it may not directly measure roots under these conditions.

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Huijie Gu and Liantao Liu contributed equally to this work.

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*Conclusions* $EC_{root}$ shows the potential to directly quantify root traits under dry conditions rather than wet conditions.

**Keywords** Electrical capacitance · Root dry biomass · Soil moisture · Root tissue density · Cotton

**Abbreviations**

| | |
|---|---|
| EC | Electrical capacitance |
| $EC_{root}$ | Electrical capacitance of the root |
| $EC_{soil}$ | Electrical capacitance of the soil |
| RTD | Root tissue density |

## Introduction

Crop root systems take up soil water and nutrients and anchor the plants. The importance of root research has been widely recognized in many areas including agriculture (Liu et al. 2016; Lynch 2013, 2019; Rabbi et al. 2021; Streda et al. 2020). Traditional root measurement methods (such as excavation, soil coring and trenching) can acquire detailed root information, but they are time-consuming, are labor-intensive and destroy the root structure associated with point-based information (Liu et al. 2016). While "shovelomics" (a method to phenotype root crown traits by digging and imaging roots) improves the efficiency of root measurements, it is still a destructive method and only provides information on surface roots (Trachsel et al. 2011). Very large sample sizes are needed to characterize root traits for breeding programs, making destructive assessments unfeasible. Nondestructive methods for detecting in situ root parameters have spurred interest among researchers in recent years (Cimpoiasu et al. 2020; Guo et al. 2013; Liu et al. 2016; Mancuso 2012; Streda et al. 2020) and are needed to advance comprehensive breeding programs.

Mancuso (2012) and Atkinson et al. (2019) systematically reviewed the progress of nondestructive root phenotyping methods, which centered on geophysical exploration technology (Cimpoiasu et al. 2020). Ground penetrating radar has been successfully used to detect coarse roots (Guo et al. 2013; Zajicova and Chuman 2019) and shows potential to measure crop root biomass (Liu et al. 2016, 2018). Electrical resistivity tomography indirectly characterizes root distribution by measuring the change in soil moisture (Cassiani et al. 2015; Mary et al. 2020) or maps the root system distribution when combined with the *mise a la masse* technique (the current is injected into a conductor and the voltage is measured at the ground surface or in the borehole) (Mary et al. 2018). The three-dimensional structure of the root system can be obtained by electrical magnetic induction, X-ray computed tomography, electrical impedance tomography, and spectral induced polarization approaches (Corona-Lopez et al. 2019; Mary et al. 2017; Metzner et al. 2015; Weigand and Kemna 2017, 2019). However, these geophysical methods are mostly tested and applied in controlled environments. With current technology, it is difficult to overcome the interference of soil heterogeneity when detecting root systems in the field (Peruzzo et al. 2020). In addition, these technologies require expensive equipment, which is problematic for the large-scale collection of agricultural root information in developing countries.

Among the nondestructive methods, electrical capacitance (EC) is a physical quantity that characterizes the ability of capacitors to hold electrical charge and, is part of bioimpedance technology (Cao et al. 2010; Dalton 1995; Mancuso 2012). EC correlates with root fresh mass, root dry mass, root length and root surface area (Chloupek 1972). Typically, a single frequency alternating current is applied to the stem through the electrodes (Chloupek 1972; Streda et al. 2020), and the current travels through the root system in the xylem to induce root cell membrane polarization (Repo et al. 2000). Root membranes act as dielectrics in the capacitor, in which root sap and soil solutions are similar to the capacitor plates; therefore, the root-soil system can be viewed as a capacitor (Dalton 1995; Ellis et al. 2013a). The capacitance is related to the surface area of the capacitor (Dalton 1995), so EC method may be used to estimate the root parameters such as root length, root surface area and root dry matter (Chloupek 1972; Streda et al. 2020). EC equipment is easy to operate and relatively inexpensive, and it has been used to measure crop roots in the field even in large breeding populations (Cseresnyés et al. 2018b, 2019, 2020a; Hermanska et al. 2015).

Chloupek (1972) was likely the first to report a significant linear relationship between root capacitance ($EC_{root}$) and root traits in various crops. Dalton (1995) proposed a conceptual model for measuring root systems by the EC method, where all of the roots serve as parallel cylindrical capacitors. Later, researchers thought that root capacitors were connected to growth medium such as soil capacitors in a series (Dietrich et al. 2012; Rajkai et al. 2005). After these initial studies, the EC method was used to estimate the plant root parameters in a variety of situations, including the response of canola $EC_{root}$ to high temperature and drought stress (Wu et al. 2017) and the effect of soil moisture content on $EC_{root}$ (Cseresnyés et al. 2018b, 2020a). Numerous other applications of the EC method to estimate root properties were detailed in a review by Streda et al. (2020). Correlations between $EC_{root}$ and root traits vary widely from 0.21 to 0.99 (Streda et al. 2020) depending on the application and other factors, suggesting that $EC_{root}$ may not always be feasible for measuring plant roots and that application-specific testing is required.

The acceptance of the EC method is not universal and has been questioned by some researchers (Dietrich et al. 2012, 2013; Urban et al. 2011). First, Dietrich et al. (2012) found that there were no significant changes in $EC_{root}$ when the roots of barley were excised in solution, and $EC_{root}$ was linearly correlated with the cross-sectional area of the root at the solution surface. They asserted that $EC_{root}$ assesses the cross section at the junction of the root and the medium instead of the root system itself. Indeed, this study was supported by the results of Peruzzo et al. (2020), who reported that electric current leaks out from the root-medium surface and the root system cannot be directly detected. Urban et al. (2011) also deduced that most of the current flows from the root system in the proximal parts of the root–soil interface to the soil. These findings were further verified by imaging current flow in roots when using an electrical method to detect roots (Peruzzo et al. 2020). The authors found that current cannot flow as deep as the full extent of the root system. Second, except for the root-soil capacitor in the Dalton model, where all of the roots serve as parallel cylindrical capacitors, the plant stem and root system also form a series circuit with the growth medium as the boundary (Cao et al. 2010; Dietrich et al. 2012, 2013). In the series circuit, the capacitance is mainly determined by the capacitor with a smaller value (Dietrich et al. 2012). The capacitance of the stem is less than that of the roots, so the value measured by the EC method is indicative of the interface between the stem and the substrate. However, the stem cross-sectional area of plants was positively correlated with root parameters (such as root length); therefore, the significant correlations between $EC_{root}$ and root traits reported previously are indeed indirect measurements of the root system.

Conversely, there is a body of evidence showing that the EC method can accurately estimate root traits (Cseresnyés et al. 2013a; Mary et al. 2018, 2020; Ozier-Lafontaine and Bajazet 2005). Ozier-Lafontaine and Bajazet (2005) observed that the progressively immersed root dry weight was positively correlated with $EC_{root}$ unlike the results from Dietrich et al. (2012). Cseresnyés et al. (2013a) found that after inoculation on soybean and maize roots with mycelia, the dry mass of the root system did not change significantly under drought conditions, but the capacitance of inoculated roots was significantly greater than that of non-inoculated roots. This result may be attributed to inoculation increasing the root-soil interface, which may indicate that current can travel from the root system to external mycelial networks thereby increasing EC. Mary et al. (2018) found that current can be detected in the grapevine root system at a depth of 30 cm. A recent study verified that roots are the main determinant of detected capacitance even in wet soil at field water capacity, although stem basal area is also highly influential (Cseresnyés et al. 2020b). While these studies assumed that electricity enters the root system, the results eventually confirmed that root systems can be directly measured by EC methods under certain conditions. Peruzzo et al. (2020) recently proposed that the transmission of current in the root system is dependent on two conditions: a) electrical longitudinal conductivity is significantly higher than radial conductivity; b) soil resistivity is higher than root resistivity. A woody species may have a higher longitudinal conductivity than radial conductivity in the roots compared with a grass species such as maize (*Zea mays* L.) (Aubrecht et al. 2006; Cermak et al. 2006; Ginsburg 1972), and drier soil can have higher resistivity than root resistivity (Hagrey and Petersen 2011); therefore, Peruzzo et al. (2020) reported that the current travels deeper through the root system in cotton (*Gossypium hirsutum* L.) than that in maize, especially when surface soils are dry.

This phenomenon drives us to hypothesize that EC may be used to assess root traits in woody species under drought conditions. It should be noted that Peruzzo et al. (2020) did not observe an electrical current signal at deep soil layers even though there were many roots present. As mentioned above, many publications report that wet soil conditions yield more consistent measurements of plant roots with EC since the impacts of wet soil on EC are minimized (by increasing the soil capacitance and decreasing its reciprocal in the series model) and the contact area between the ground electrode and soil is increased (Dalton 1995; Ellis et al 2013b; Rajkai et al. 2005). Furthermore, wet soil is sometimes an applicable condition for directly measuring roots (Cseresnyés et al. 2020b). However, presently, it is unknown how efficiently current moves through root systems under different soil water conditions, which necessitates a systematic study.

In addition to the traditional regression model used for predicting root traits using the EC method, a forward model (using factors to simulate the EC) can also help illustrate the importance of these different factors, although there has been little research on this in the literature. Ellis et al. (2013a) are likely the first to test the effect of root tissue density (root mass divided by root volume, RTD) on EC-based root measurements. This is relevant, as root suberization and lignification, as well as root morphological traits such as RTD, are altered under drought stress (Malavasi et al. 2016; Song et al. 2019; Zhou et al. 2020). Namely, an increase in RTD will lead to a decrease in the relative dielectric constant of roots (Guo et al. 2013; Paz et al. 2011). As the dielectric constant is directly proportional to EC, RTD should affect EC (Ellis et al. 2013a). Incorporating RTD into the simulation model could increase the EC prediction accuracy; however, this has not been applied in crop root measurements, particularly under dry conditions.

Since drought stress induces root suberization and lignification to reduce water loss (Malavasi et al. 2016; Song et al. 2019), we hypothesize that drought stress can also reduce electrical current leakage, particularly for woody species (Peruzzo et al. 2020). We also posit that including RTD can improve the forward simulation of EC under drought conditions. The specific objectives of this paper are to: (1) confirm that current enters the root system in a woody crop (cotton) under different soil water conditions; (2) determine if EC can estimate root traits under drought conditions; and (3) investigate whether RTD affects the estimation of root system parameters with the EC method.

## Materials and methods

### Study site

We conducted two rounds of plastic pot experiments (in 2019 and 2020), one PVC tube experiment (in 2020) and one field experiment (in 2020) at the Luancheng Agro-Eco-Experimental Station of the Chinese Academy of Sciences (37°53′N, 114°41′E, altitude: 50.1 m) located in Shijiazhuang, China. The soil texture is silty loam (Haplic luvisol according to FAO-WRB) in the top 20 cm soil layer, and the field water capacity is approximately 38% (V $V^{-1}$). The topsoil (0–20 cm) organic matter content, bulk density, total nitrogen, available nitrogen, available phosphorus and available potassium are 17 g $kg^{-1}$, 1.2–1.4 g $cm^{-3}$, 1.11 g $kg^{-1}$, 80 mg $kg^{-1}$, 21 mg $kg^{-1}$ and 120 mg $kg^{-1}$, respectively. The study site has a semiarid climate with an annual mean air temperature of 12.9 °C and a mean annual precipitation of 466 mm, with 70% falling from June to September. The cotton growth conditions and experimental design can be found in Table 1.

### Plastic pot experiments

For the first pot experiment, we designed a two-factorial experiment: cultivar and soil water. There were two soil water levels: wet and dry. Soil water contents were maintained at 0.10 V $V^{-1}$ (0.085 g $g^{-1}$) for the dry treatment and 0.28 V $V^{-1}$ (0.24 g $g^{-1}$) for the wet treatment. Five local cotton cultivars were selected: Lumiang28, Nongdamiang601, Jimian315, Nongdamian10 and Guoxin9. The different cultivars were used to provide a variety of root systems for analysis. Each treatment had three replications. In the first round of the pot experiment (Pot + Field topsoil), we used field topsoil as the culture medium to fill the plastic pots (30 cm in diameter, 30 cm in height). Each pot was filled with 24 kg of soil, and the initial gravimetric water content was 0.10 g $g^{-1}$. The soil bulk density was kept at 1.17 g $cm^{-3}$, and 4 L of water was added to each pot. The next day, six cotton

**Table 1** Cotton growing conditions and experimental treatments

| Experiments | Cotton cultivars | The growth substrate | Water treatment | Soil moisture content (g $g^{-1}$) when EC measured |
|---|---|---|---|---|
| Pot + Field topsoil | Lumiang8, Nongdamiang601, Jimian315, Nongdamian10 and Guoxin9 | Field topsoil | Dry | 0.04–0.07 |
| | | | Wet | 0.19–0.24 |
| Pot + Mixed topsoil | Lumiang8, Nongdamiang601, Jimian315, Nongdamian10 and Guoxin9 | Mixed topsoil | Dry | 0.04–0.10 |
| | | | Wet | 0.22–0.26 |
| Tube + Mixed topsoil | Nongdamian10 | Mixed topsoil | Dry | 0.04–0.06 |
| | | | Wet | 0.22–0.24 |
| Field | Lumiang28, Nongdamiang601, Jimian315, Nongdamian10, Guoxin9, Guoxin27 and Nongdamian23 | Field soil | Irrigated | 0.16–0.19 |
| | | | Rain-fed | 0.12–0.17 |

Field topsoil: the top 20 cm of soil in the field; Mixed topsoil: field topsoil mixed with pure sand in a ratio of 2:1; EC: electrical capacitance

seeds were sown in each pot to a depth of 3 cm, and the seedlings were thinned to one after emergence. A movable canopy was used to exclude rainfall. A 5TE soil moisture sensor (Decagon, USA) was used to measure the soil water content in each plot every two or three days, and then the required water was replenished to maintain the designed soil water content for different water treatments.

In the second round of pot experiments (Pot + Mixed topsoil), we conducted a second soil water × cultivar experiment but used surface field soil mixed with pure sand at a ratio of 2:1 to lower the water holding capacity of the medium. The initial gravimetric water content was 0.11 g $g^{-1}$. We used smaller plastic pots (23 cm in diameter, 22 cm in height) filled with 12 kg of the mixed soil and then irrigated each pot with 1.1 L of water containing 6 g of compound fertilizer and 4 g of urea. Soil water content was maintained at 0.28 V $V^{-1}$ for the wet treatment and 0.08 V $V^{-1}$ for the dry treatment with the same five tested cultivars. Each treatment had four replications. The pot arrangements, planting and management followed the first Pot + Field topsoil experiment.

## PVC tube experiment

We conducted an additional PVC tube (19.2 cm in diameter and 70 cm in height; Tube + Mixed topsoil) experiment to verify whether the applied current was detectable in the deep root system. In this experiment, we arranged two water treatments (0.3 V $V^{-1}$ for the wet treatment and 0.08 V $V^{-1}$ for the dry water treatment) for one cotton cultivar (Nongdamian10), with three replications for each treatment (total of 6 tubes). Each tube was filled with 32 kg of soil (same mixed soil as in the Pot + Mixed topsoil experiment), the bottom was sealed with plastic, and 3 L of water was added. Four seeds were sown and thinned to one after emergence. For the dry treatment, 0.5 L of water was applied when cotton leaves began to wilt, and for the wet treatment, 3 L of water was applied at the same time. The soil water content at harvest averaged by 0.22 g $g^{-1}$ under wet and 0.08 g $g^{-1}$ under dry treatments.

## Field experiment

In the field, we conducted a two-factorial experiment with water (rain-fed and irrigated) and cultivar as the factors. To increase the sample size and examine diverse root systems, seven cotton cultivars were selected, including the original five used in the Pot + Field topsoil experiment and additional cultivars: Guoxin27 and Nongdamian23. Each treatment was repeated three times, creating 42 (3 replications × 2 treatments × 7 cultivars) completely randomized plots with an area of 7.5 m × 5 m for each. Before planting, the field was well watered (approximately 100 mm). Then diammonium phosphate at 450 kg $ha^{-1}$ was broadcast and incorporated into the soil through tillage. One week later, the plots were planted at a seeding rate of 225,000 plants per hectare. After planting, the plots were covered with 0.015 mm thick plastic film to ensure good emergence. Irrigation water was transported by a plastic tube pipeline system with low-pressure equipped with a flow meter to record the irrigation amount. On June 10, 2020, 40 mm of water was applied to the irrigation treatment. After this irrigation, there was significant precipitation, making the difference in the mean soil water content between the two treatments marginal (0.18 g $g^{-1}$ vs. 0.15 g $g^{-1}$). Therefore, in most cases, we combined the rain-fed and irrigated treatments into a single moisture level best described as "moderate drought stress" compared with the dry (0.08–0.085 g $g^{-1}$) and wet treatments (0.22–0.26 g $g^{-1}$) in the Pot + Field topsoil and Tube + Mixed topsoil experiments. The fertilizer amount, pesticide control and field management followed local practices. Weeds were well controlled during the experimental period.

EC measurement

The $EC_{root}$ and soil electrical capacitance ($EC_{soil}$) were measured with a Passive Component LCR Meter (EXTECH LCR200, USA) in a parallel equivalent circuit at 100 Hz, 1 kHz, and 10 kHz AC frequencies with 1 V terminal voltage. The capacitance measurement dates for the Pot + Field topsoil, Pot + Mixed topsoil, Tube + Mixed topsoil and Field experiments were September 18, 2019, September 22, 2020, September 21, 2020, and July 6, 2020, respectively. The EC measurement process is presented in Fig. 1. For Pot + Field topsoil experiments, we measured the $EC_{root}$ before and after severing the root with a sharp knife (the knife was inserted at an angle of approximately 45 degrees from the horizontal plane and approximately 5 cm from the stem.). As cotton has a taproot system, cutting can excise most of the roots attached to the stem. Before and after root cutting, the position of electrodes remained unchanged. $EC_{soil}$ was only measured before root excision (Fig. 1 a, b and c). For the Pot + Mixed topsoil and Field experiments, $EC_{root}$ and $EC_{soil}$ were measured both before and after root cutting (Fig. 1 a, b, c and d). Except for the Tube + Mixed topsoil experiment, all the $EC_{root}$ and $EC_{soil}$ measurements followed similar steps as the other three experiments (Fig. 1 a, b, c and d). When measuring $EC_{root}$, the negative electrode was inserted into the soil to 12 cm through a stainless steel rod (13 cm long and 3 mm in diameter), which was positioned 10 cm away from the stem base. The positive electrode (round rod stainless steel and 1 mm in diameter) was inserted into the center of the residual plant stem at 1 cm above the soil surface. Two stainless steel electrodes with a diameter of 3 mm and a distance of 20 cm were inserted into the soil to a depth of 12 cm to measure $EC_{soil}$.

For the Tube + Mixed topsoil experiment, the same positive electrodes were used as in the other three experiments. The negative electrode was inserted into the soil at the side of the pipe wall and 10 cm from

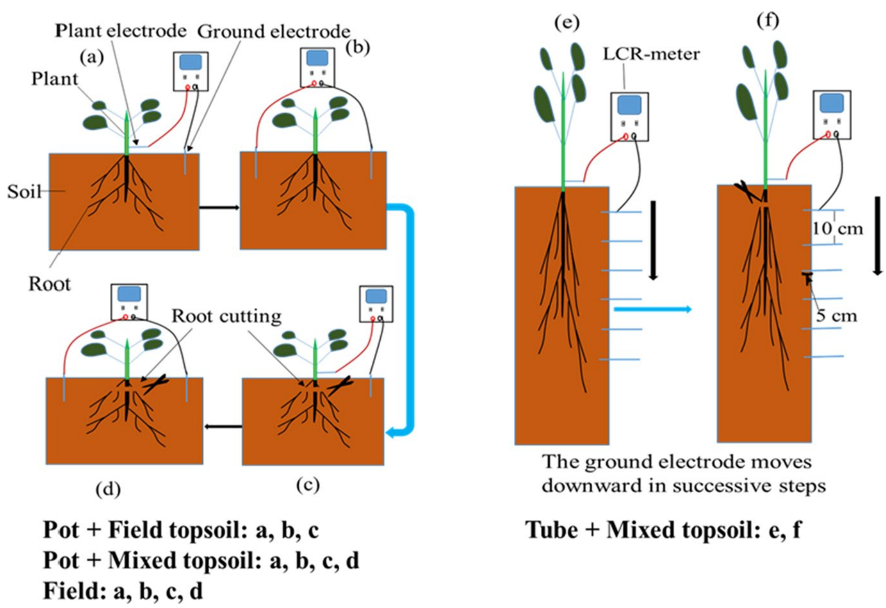



**Fig. 1** Capacitance measurement procedure before and after root cutting for the four experiments

the top end of the PVC pipe (Fig. 1e and f). After the measurement was completed, the plant electrode was fixed, and the ground electrode was sequentially moved 10 cm downward for the next measurement. Each PVC pipe was measured 6 times down to 60 cm. We pretested the effects of varying the distance of the soil electrode (at the same soil layer) on the capacitance measurements. The results did not indicate much difference among measurements, consistent with Ellis et al. (2013b). We compared the changes in $EC_{root}$ at depths of 0–60 cm after root cutting. This information may help inform the current flows in the root system.

### Root and soil moisture measurements

After the EC measurements were completed, the root systems were immediately removed from the pots, and the soil medium was gently washed for the Pot + Field topsoil and Pot + Mixed topsoil experiments. The PVC tubes were extracted and split with a small machine saw. Soil was separated into 10 cm depth intervals. In the Field experiment, a soil core (10 cm diameter) was used to collect soil samples to a depth of 60 cm at 10 cm increments.

All the roots were separated from the soil by rinsing through a stainless steel sieve (0.5 mm mesh). We carefully washed soil from the roots and retained most of the root material in the pot and Tube + Mixed topsoil experiments. Root samples were stored in a freezer (-20 °C) until they could be washed and separated. The debris was manually removed from washed root samples. The cleaned roots were scanned on a flatbed scanner (Epson Expression 12000XL, USA), and root length, surface area and volume were analyzed with WinRhizo software (ver. 2019a, Reagent Instruments Inc., Quebec, Canada). In the Pot + Field topsoil experiment, only the tap root and the attached lateral roots were scanned. Some roots broken during cleaning were not scanned. In the Pot + Mixed topsoil, Tube + Mixed topsoil and Field experiments, all roots were scanned. All roots were subsequently oven-dried to a constant mass (75 °C for 72 h), and the dry weight was recorded.

At the time of root harvest, a subsample of soil was collected, and the soil water content was measured by the oven-drying method (drying for 24 h at 105 °C).

### Statistics

R (version 3.6.0) was used for statistical analyses and plotting. Before variance analysis, all data were checked for a normal distribution (Kolmogorov–Smirnov test) and variance homogeneity (Levene's test). Regression analysis was conducted using the lm() function. Pearson correlation coefficients, means and standard errors were also calculated. Akaike information criterion (AIC) values, which can weigh the complexity of the estimated model and the goodness of the model fitting data, were used to evaluate the quality of the model. The differences in $EC_{root}$ measurements among cultivars were not compared as they are not the primary focus of this study and thus are presented in the supplementary data.

## Results

### $EC_{root}$ changes after root cutting

To describe the effects of root cutting on $EC_{root}$, we used $\Delta EC_{root}$, which is the percent difference (%) in $EC_{root}$ after root cutting. The results show that $EC_{root}$ changed dramatically after root cutting (Fig. 2). $EC_{root}$ decreased (averaged by -18%) significantly in most cases under dry conditions. The highest decrement (-66%) was observed in the Tube + Mixed topsoil experiment and lowest in the Field experiment (-9%). The averaged change rates under dry conditions were 33% at 100 Hz, 20% at 1 kHz and 25% at 10 kHz. After cutting, the largest $\Delta EC_{root}$ observations were obtained with 100 Hz frequency.

In contrast to the dry conditions, $EC_{root}$ increased (averaged by 10%) after root excision under wet conditions in the Pot + Field topsoil, Pot + Mixed topsoil and Tube + Mixed topsoil experiments (Fig. 2), although it declined after root cutting in the Field experiment. The change rates measured at higher frequencies decreased (averaged by -16% at 100 Hz, -11% at 1 kHz, and -4% at 10 kHz). There were no significant differences in $\Delta EC_{root}$ among cultivars (Supplementary Table S1). Thus, we will not further discuss cultivar effects. Overall, $EC_{root}$ declined after root cutting under dry conditions and tended to increase under wet conditions. Additionally, we found that the $\Delta EC_{root}$ measured at different depths in the

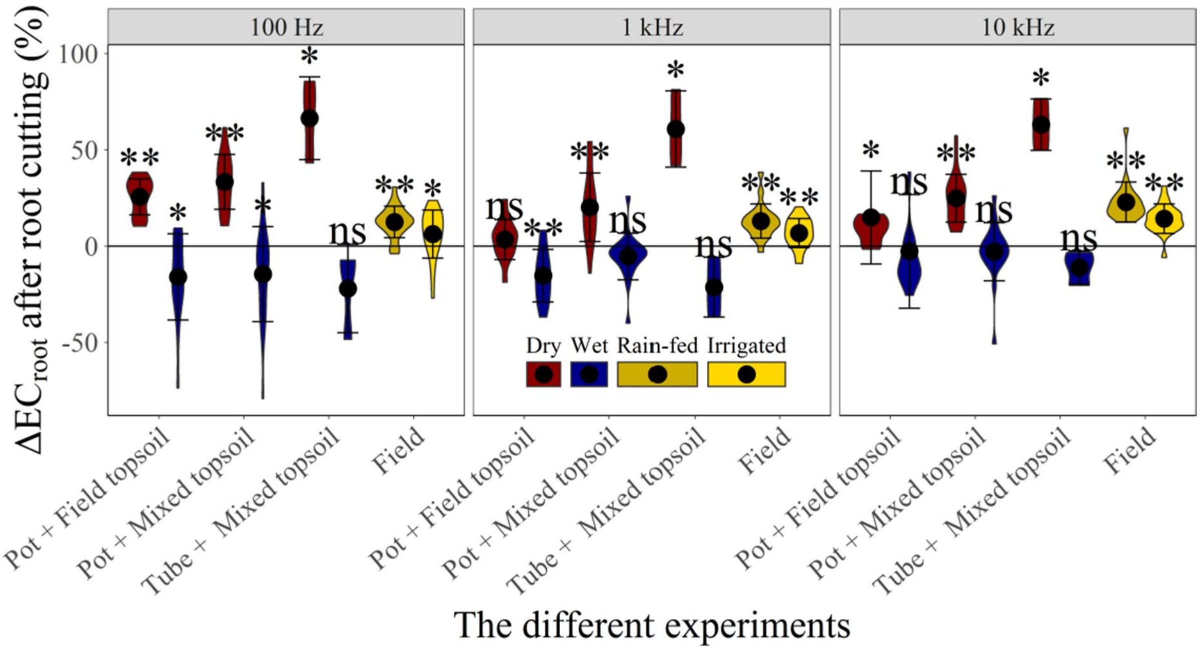



**Fig. 2** Changes in $EC_{root}$ before and after root cutting in the Pot + Field topsoil, Pot + Mixed topsoil, Tube + Mixed topsoil and Field experiments (ns indicates that there is no significant difference between the mean value and zero at $P = 0.05$; * and ** mean that the differences are significant at $P = 0.05$ and $P = 0.01$, respectively)

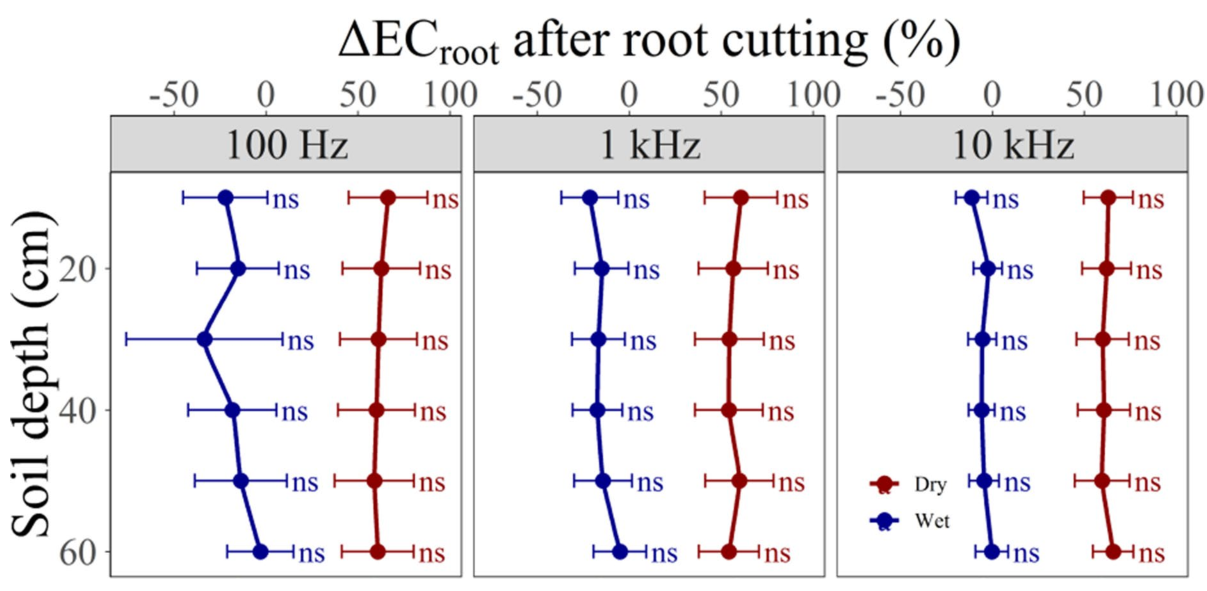



**Fig. 3** Changes in $EC_{root}$ before and after root cutting measured at different soil layers in the Tube + Mixed topsoil experiment (ns means there is no significant difference at $P = 0.05$ under different depths)

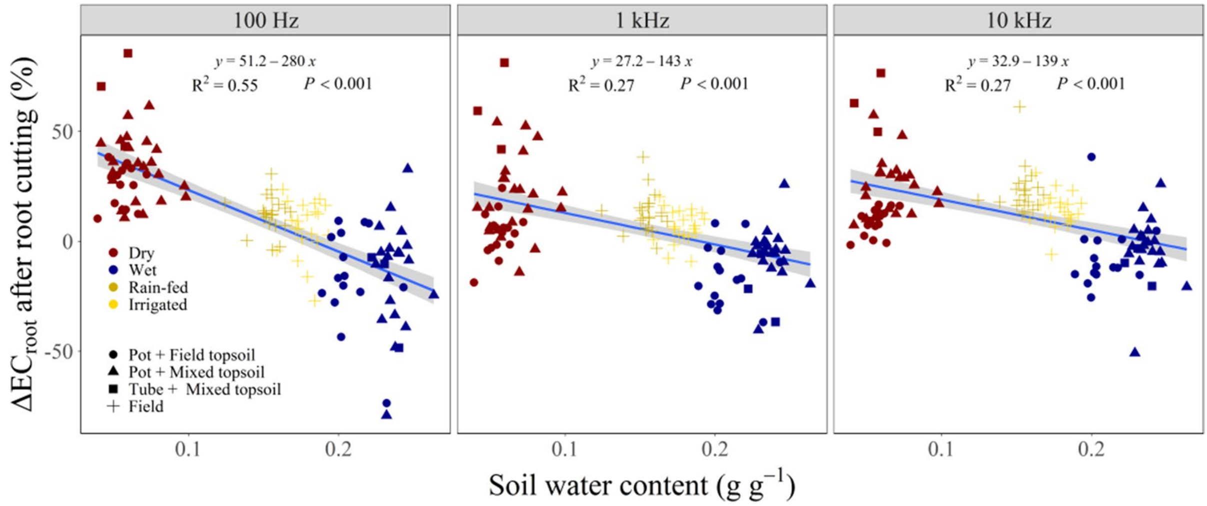



**Fig. 4** Relationships between soil moisture and the changes in $EC_{root}$ after root cutting

Tube + Mixed topsoil experiment did not show much difference under either treatment (Fig. 3).

Regression analysis of $\Delta EC_{root}$ with soil moisture was applied to better understand the effects of root cutting (Fig. 4). There were significant negative linear correlations between $\Delta EC_{root}$ and soil water content under the three measurement frequencies, indicating that soil moisture has a greater influence

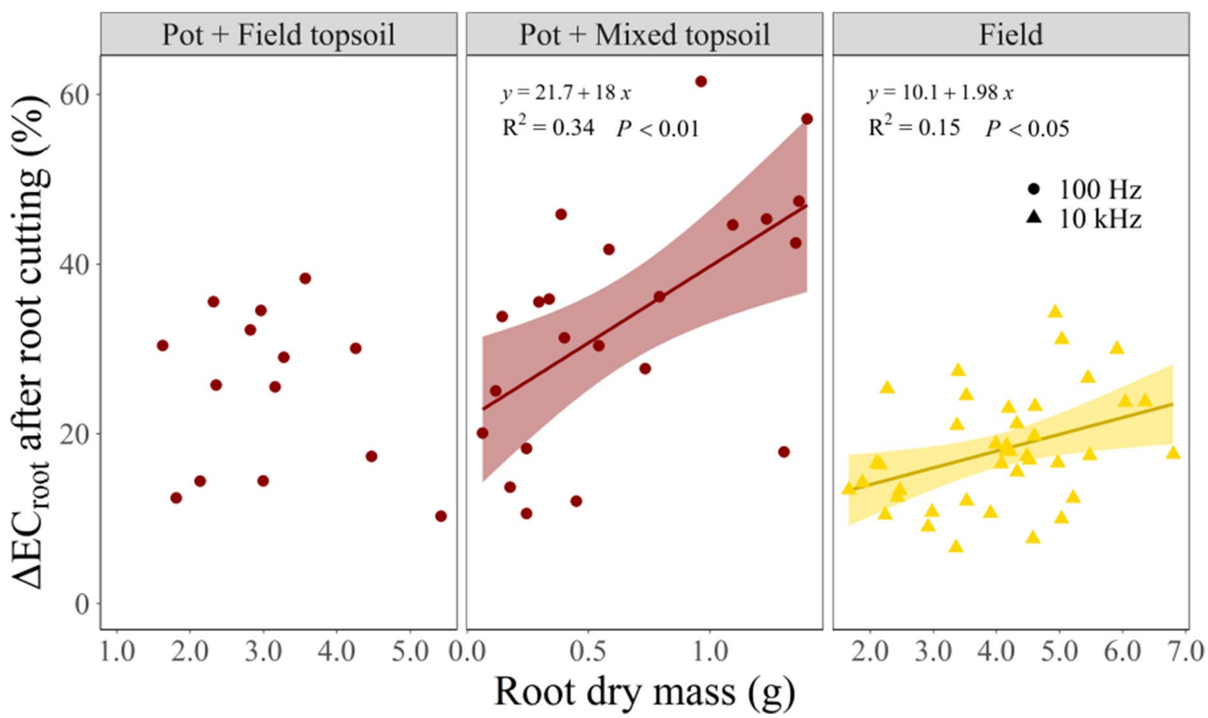



**Fig. 5** Relationship between root dry mass and $EC_{root}$ changes after root cutting under dry conditions in the Pot + Field topsoil, Pot + Mixed topsoil and Field experiments (only equations for significant relationships are presented)

on $\Delta EC_{root}$ under drier conditions. This may indicate that $EC_{root}$ has greater predictive potential for root characteristics under dry conditions, especially at 100 Hz (Fig. 4).

There were significant relationships between the root biomass and $\Delta EC_{root}$ in Pot + Mixed topsoil under dry conditions as well as in the Field experiment (Fig. 5). Taken together, Fig. 4 and Fig. 5, show that $\Delta EC_{root}$ appears to be affected by both soil moisture and root mass.

### $EC_{soil}$ changes after root cutting

Even though the soil was minimally disturbed during root cutting and changes in $EC_{soil}$ ($\Delta EC_{soil}$, percent change in $EC_{soil}$ after root cutting) were unexpected, we measured $\Delta EC_{soil}$ in the Pot + Mixed topsoil and Field experiments. $\Delta EC_{soil}$ was minimal in the Field experiment; however, it tended to increase under wet conditions (12% at 100 Hz, 7% at 1 kHz and 2% at 10 kHz) in the Pot + Mixed topsoil experiment (Fig. 6).

### The correlations between $EC_{root}$ and root traits

Since root traits such as dry mass, root length and root surface area are strongly correlated with each other, we only present the relationship between root dry weight and $EC_{root}$ as an example (Fig. 7). There were varied relationships between $EC_{root}$ and root dry weight under different water conditions (Fig. 7). Under dry conditions, there were parabolic relationships between the root dry weight and $EC_{root}$ with the best at 100 Hz ($R^2 = 0.49$, $P < 0.05$) in the Pot + Field topsoil experiment. The relationships under dry conditions were not significant ($R^2 = 0.11$, $P > 0.05$) in the Pot + Mixed topsoil experiment (Fig. 7). In the Field, the difference in soil moisture between irrigated and rain-fed soils was minimal (Fig. 4), so

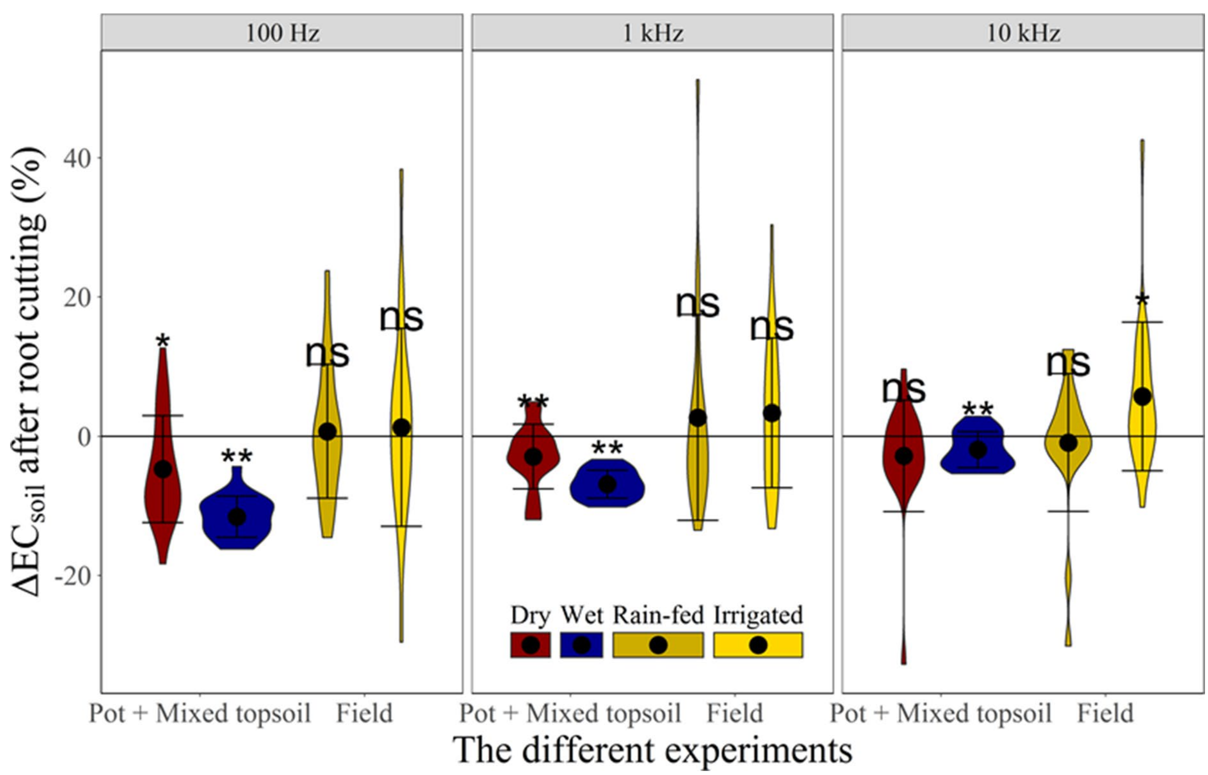



**Fig. 6** Changes in $EC_{soil}$ before and after root cutting in the Pot + Mixed topsoil and Field experiments (ns indicates that there is no significant difference between the mean value and zero at $P = 0.05$; * and ** mean that the differences are significant at $P = 0.05$ and $P = 0.01$, respectively)

we did not distinguish the relationships between $EC_{root}$ and root dry weight under different water treatments. When the data were pooled, significant linear relationships between $EC_{root}$ and root dry weight at 1 kHz and 10 kHz ($R^2 > 0.07$, $P < 0.05$) were found.

Under wet conditions, there were significant relationships between $EC_{root}$ and root dry weight in the Pot + Mixed topsoil experiment but not in the Pot + Field topsoil experiment. Considering the $\Delta EC_{root}$ results in the Pot + Mixed topsoil experiment (Fig. 2), roots only had a significant impact on $EC_{root}$ under dry conditions. Thus, these significant relationships may reflect an indirect measurement of root dry weight under wet conditions.

### The effects of RTD and soil water content on predicting $EC_{root}$

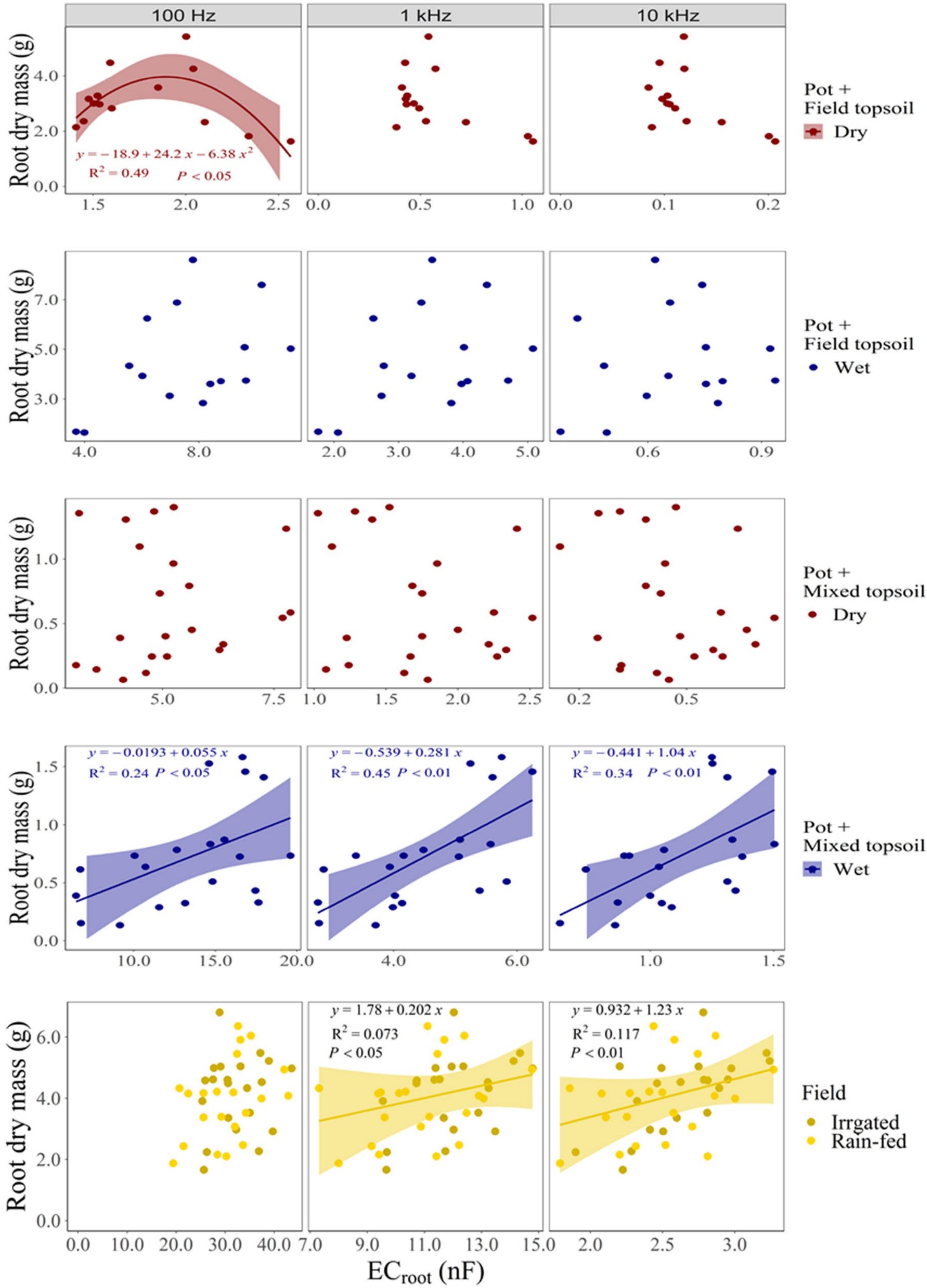
Using available data from the Pot + Mixed topsoil experiment, we incorporated RTD and soil water content into the regression model to predict $EC_{root}$ and found that all forward models were significant (Table 2) under dry conditions. Even though no significant linear correlations between root dry weight and $EC_{root}$ were found under the dry treatment in the Pot + Mixed topsoil experiment (Fig. 7), when we included RTD and soil water content in the forward model, the predicting models became significant (Table 2). At the same time, the AIC value of the model decreased, indicating that taking RTD and soil water content into account would optimize the correlation between root dry weight and $EC_{root}$ as a forward model. We also found that at 100 Hz, the coefficient of determination (0.41) of the predicting model was the highest when only RTD was added to the model. Similar results were found for root length and root surface area (Table 2). However, when soil water content was added to the model, 10 kHz was the best for the predicting model. In contrast, the new forward models that included RTD and soil water content did not improve the prediction accuracy of $EC_{root}$ under wet conditions (Table 3). This indicates that $EC_{root}$ is probably not a direct measurement of roots under wet conditions.

## Discussion

### EC tends to directly measure cotton roots under dry conditions

Our experiments showed that soil moisture conditions have a significant influence on $EC_{root}$ changes before and after root excision (Fig. 2). After root cutting, $EC_{root}$ decreased but $EC_{soil}$ did not change significantly under dry conditions, suggesting that the root system affected $EC_{root}$, namely, electrical current could enter the root system under these conditions. In addition, our results from the Tube + Mixed topsoil tube experiment showed that root cutting had little effect on $\Delta EC_{root}$ quantified at different soil depths under dry conditions (Fig. 3). Generally, placing the ground electrode deeper would force the current to flow deeper. The minimal changes in $\Delta EC_{root}$ at different soil depths may indicate that the current can flow deep into the root system under dry conditions; otherwise, it should have varied at deeper depths due to the presence of more polarized roots and a larger amount of accumulated roots (root mass from 30–60 cm accounted for 36.6% of the total root mass, Supplementary Fig. 2). This result further indicates that the root electrical longitudinal conductivity is higher than that of the soil under drought conditions and implies that $EC_{root}$ tends to directly measure roots under these conditions, as evidenced by the significant relationships of $EC_{root}$ and root traits as well as the forward predicting model (Fig. 7 and Table 2). In addition, the significant linear relationship between $\Delta EC_{root}$ and root dry mass further supports this point under drought conditions in the Pot + Field topsoil and Field experiments. At the same time, we also found that the correlations between $\Delta EC_{root}$ and root biomass were better than those between $EC_{root}$ and root biomass under drought conditions in some cases (Fig. 5 & Fig. 7). This result means that $\Delta EC_{root}$ has the potential to measure root biomass. However, further research is needed to estimate the influencing factors, such as the location of the root cuttings and the water content of the soil for the $\Delta EC_{root}$ measurements.

However, both $\Delta EC_{root}$ and $\Delta EC_{soil}$ tended to increase at 100 Hz under wet conditions (Fig. 2 and Fig. 6). There are two possible explanations: 1) a small cutting gap may slightly increase the soil



100 Hz
1 kHz
10 kHz
Root dry mass (g)
y = −18.9 + 24.2 x − 6.38 x²
R² = 0.49 P < 0.05
Pot + Field topsoil
Dry
Pot + Field topsoil
Wet
Pot + Mixed topsoil
Dry
y = −0.0193 + 0.055 x
R² = 0.24 P < 0.05
y = −0.539 + 0.281 x
R² = 0.45 P < 0.01
y = −0.441 + 1.04 x
R² = 0.34 P < 0.01
Pot + Mixed topsoil
Wet
y = 1.78 + 0.202 x
R² = 0.073
P < 0.05
y = 0.932 + 1.23 x
R² = 0.117
P < 0.01
Field
Irrgated
Rain-fed
EC_root (nF)

◂**Fig. 7** The relationships between $EC_{root}$ and root dry mass in the different experiments (only equations for significant relationships are presented)

volume, causing $EC_{soil}$ to increase (Mancuso 2012), and after root cutting, the area of the capacitor plate increases because of the increased soil volume. 2) Since we proved that current did not enter the root system under wet conditions (see the following discussion), root cutting may create a new interface (stem and soil) that also increases $EC_{root}$. However, we found that $\Delta EC_{root}$ was lower than $\Delta EC_{soil}$ at 100 Hz (Fig. 2 and Fig. 6) and that $EC_{root}$ measured at 1 kHz and 10 kHz did not change significantly under the wet treatment. This demonstrates soil disturbance is not the primary reason for the $EC_{root}$ changes. The marginal $\Delta EC_{root}$ after root cutting under wet conditions may indicate that electrical current leaks out from the stem-soil interface before entering roots. We also found that the correlations between stem diameter (an indicator for the stem-soil interface) and $EC_{root}$ were significant under wet conditions in the Pot + Field topsoil and Pot + Mixed topsoil experiments and were more significant than those under dry conditions (Supplementary Fig. 1). This may lead to significant correlations between $EC_{root}$ and root dry mass under wet conditions in the Pot + Mixed topsoil experiment (Fig. 7). This result is consistent with the results from Dietrich et al. (2012) that even though current leaks from the root system, it can indirectly measure roots under wet conditions. As a result, considering root tissue density in the forward model could not improve the correlation between roots and $EC_{root}$ under wet conditions because EC is not a direct measurement of roots under these conditions. However, Cseresnyés et al. (2020b) reported that although the stem base has a relatively high influence on EC, the roots of maize in the soil are the main determinant of capacitance detected at field capacity in arenosol soil. This result implies that EC has the potential to directly measure roots of other plant species and merits further study.

The different responses of $EC_{root}$ changes under dry and wet conditions indicate different current paths in the stem- root-soil system. After the current is applied to the stem, it will follow two paths, as the root and soil capacitors are in parallel. As mentioned in the introduction, most of the current follows the path of least impedance (Peruzzo et al. 2020) when different capacitors are incorporated in a parallel capacitor circuit. Therefore, most current enters the root path, as roots have a lower resistance than soil under dry conditions and flow to the soil path under wet conditions. EC of this parallel circuit (the sum of the EC for the two capacitors) is determined mostly by the preferable paths (Wu et al. 2017). This means that most current enters the root system under dry rather than wet conditions. Therefore, $EC_{root}$ after root cutting decreased significantly under dry conditions and exhibited minimal change under wet conditions. Drought stress can induce suberization and lignification, which makes the longitudinal conductivity significantly higher than the radial conductivity (Malavasi et al. 2016; Peruzzo et al. 2020; Suseela et al. 2020; Zhou et al. 2020). This could also explain the observation that $\Delta EC_{root}$ did not change significantly under wet conditions with the increasing depth in Tube + Mixed topsoil experiment (Fig. 3). However, no significant changes in $\Delta EC_{root}$ at different depths were neither found under dry conditions, showing that the current can flow to the deep roots under dry conditions because we proved that the current enters roots under these conditions in the earlier Pot + Field topsoil, Pot + Mixed topsoil and Field experiments.

However, the proposed circuit theory differs from those of Dalton (1995) and Dietrich et al. (2012). The Dalton (1995) model contends that all the roots are parallel capacitors and is able to predict the decreased $EC_{root}$ under dry conditions, but this model cannot explain the changes under wet conditions. The circuit model developed by Dietrich et al. (2012) can interpret the slight changes in $EC_{root}$ under wet conditions, but it cannot explain the decreasing trend under dry conditions. Furthermore, the Dietrich et al. (2012) model assumes that currents enter the root system. According to Peruzzo et al. (2020), current will not flow into the deep root in cotton under wet conditions. Therefore, we proposed that the current paths depend on the root and soil electrical characteristics.

We found that EC tends to estimate roots directly under dry conditions, but it may quantify roots indirectly under wet conditions. However, the preferable conditions under which stable correlations between EC and root size can be observed still need to be determined. For future studies of this method,

**Table 2** Forward models using root traits to predict $EC_{root}$ in the Pot + Mixed topsoil experiment under dry conditions

| Frequencies | Formula | Intercept | a | b | c | AIC | $R^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| 100 Hz | EC = 5.02 + 0.21×RDM | *** | ns | | | 81 | 0.005 | 0.756 |
| | EC = 3.89 + 9.16×RDM-43.7×RDM×RTD | *** | ** | ** | | 72 | 0.41 | 0.0066 |
| | EC = 4.22 + 90.7×RDM×SWC-25.5×RDM×RTD | *** | | *** | *** | 68 | 0.50 | 0.0014 |
| 1 kHz | EC = 1.88–0.23×RDM | *** | ns | | | 32 | 0.054 | 0.296 |
| | EC = 1.59 + 2.07×RDM-11.232×RDM×RTD | *** | * | * | | 28 | 0.29 | 0.038 |
| | EC = 1.57 + 28.0×RDM×SWC-9.17×RDM×RTD | *** | | *** | *** | 19 | 0.52 | 0.00092 |
| 10 kHz | EC = 0.51–0.074×RDM | *** | ns | | | -23 | 0.067 | 0.244 |
| | EC = 0.42 + 0.62×RDM-3.39×RDM×RTD | *** | * | * | | -28 | 0.33 | 0.022 |
| | EC = 0.42 + 8.68×RDM×SWC-2.85×RDM×RTD | *** | | *** | *** | -41 | 0.61 | 0.00012 |
| 100 Hz | EC = 4.43 + 0.017×RL | *** | ns | | | 79 | 0.11 | 0.139 |
| | EC = 3.86 + 0.106×RL-0.49×RL×RTD | *** | *** | ** | | 70 | 0.46 | 0.002 |
| | EC = 4.07 + 1.12×RL×SWC-0.30×RL×RTD | *** | | ** | *** | 65 | 0.57 | 0.00035 |
| 1 kHz | EC = 1.70 + 0.0007×RL | *** | ns | | | 33 | 0.0016 | 0.86 |
| | EC = 1.50 + 0.0318×RL-0.172×RL×RTD | *** | ** | ** | | 25 | 0.38 | 0.01 |
| | EC = 1.53 + 0.38×RL×SWC-0.13×RL×RTD | *** | | *** | *** | 16 | 0.59 | 0.00021 |
| 10 kHz | EC = 0.46 + 0.00022×RL | *** | ns | | | -22 | 0.0002 | 0.854 |
| | EC = 0.40 + 0.0095×RL-0.051×RL×RTD | *** | ** | ** | | -31 | 0.42 | 0.006 |
| | EC = 0.41 + 0.11×RL×SWC-0.038×RL×RTD | *** | | *** | *** | -42 | 0.64 | 0.000006 |
| 100 Hz | EC = 4.38 + 0.0017×RSA | *** | ns | | | 79 | 0.11 | 0.14 |
| | EC = 3.65 + 0.0106×RSA-0.048×RSA×RTD | *** | *** | ** | | 69 | 0.48 | 0.0019 |
| | EC = 3.88 + 0.11×RSA×SWC-0.03×RSA×RTD | *** | | *** | *** | 63 | 0.60 | 0.00017 |
| 1 kHz | EC = 1.72 + 9.07×$10^{-6}$×RSA | *** | ns | | | 33 | 7E-06 | 0.98 |
| | EC = 1.49 + 0.0029×RSA-0.0155×RSA×RTD | *** | ** | ** | | 26 | 0.35 | 0.0167 |
| | EC = 1.50 + 0.036×RSA×SWC-0.01×RSA×RTD | *** | | *** | *** | 17 | 0.57 | 0.00031 |
| 10 kHz | EC = 0.47 + 2.57×$10^{-7}$×RSA | *** | ns | | | -22 | 3E-06 | 0.998 |
| | EC = 0.40 + 0.00087×RSA-0.0047×RSA×RTD | *** | ** | ** | | -30 | 0.35 | 0.0167 |
| | EC = 0.40 + 0.011×RSA×SWC-0.003×RSA×RTD | *** | | *** | *** | -41 | 0.63 | 0.00007 |

RDM, RL, RSA, and SWC represent root dry mass, root length, root surface area and soil water content, respectively; RTD represents root tissue density; a is the coefficient of RL, RDM and RSA; b is the coefficient of the interactions between RL, RDM, RSA and RTD; c is the coefficient of interactions between RL, RDM, RSA and SWC *, ** and *** represent significance levels of 0.05, 0.01, and 0.001, respectively

a threshold or optimal range for soil moisture above which the EC method will not directly measure root size may need to be determined in a given soil type. Second, plants with different root types, such as maize and cotton, should be tested to confirm the current pathways in the root-soil system. In contrast to our findings in cotton, a recent study verified that roots of maize in the soil are the main determinant of capacitance detected at field capacity in arenosol soil, but the stem base has a relatively high influence (Cseresnyés et al. 2020b). With advanced technology, imaging current flows in the root-soil system is possible which can provide direct evidence other than the indirect measurements by root cutting (Peruzzo et al. 2020). Knowledge of root anatomical structure may help us further understand current pathways.

Future studies should also consider the soil depth. There is very limited work comparing the correlation between EC and root size at different depths (Nakhforoosh et al. 2014). We assume that by placing the return electrode deeper, the current goes deeper and polarizes additional root tissues. This implies that current will flow in the entire root system by placing the return electrode deep in the soil. When we compared the two water treatments in the Tube + Mixed topsoil experiment, we found

**Table 3** Forward models using root traits to predict $EC_{root}$ in the Pot + Mixed topsoil experiment under wet conditions

| Frequencies | Formula | Intercept | a | b | c | AIC | $R^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| 100 Hz | EC = 10.28 + 4.44×RDM | *** | * | | | 112 | 0.24 | 0.027 |
| | EC = 10.64–0.72×RDM-32.67×RDM×RTD | *** | ns | ns | | 114 | 0.25 | 0.083 |
| | EC = 11.11–31.64×RDM×SWC + 75.88×RDM×RTD | *** | | ns | ns | 113 | 0.26 | 0.073 |
| 1 kHz | EC = 3.334 + 1.61×RDM | *** | ** | | | 53 | 0.45 | 0.0012 |
| | EC = 3.283 + 2.368×RDM-4.802×RDM×RTD | *** | ns | ns | | 55 | 0.45 | 0.0058 |
| | EC = 3.13 + 21.48×RDM×SWC-22.56×RDM×RTD | *** | | ns | ns | 53 | 0.50 | 0.0029 |
| 10 kHz | EC = 0.88–0.32×RDM | *** | ** | | | -2 | 0.34 | 0.0073 |
| | EC = 0.851 + 0.77×RDM-2.83×RDM×RTD | *** | ns | ns | | -0.4 | 0.37 | 0.024 |
| | EC = 0.81 + 6.43×RDM×SWC-7.78×RDM×RTD | *** | | ns | ns | -2 | 0.42 | 0.0099 |
| 100 Hz | EC = 10.69 + 0.054×RL | *** | ns | | | 114 | 0.18 | 0.06 |
| | EC = 11.14–0.031×RL + 0.55×RL×RTD | *** | ns | ns | | 115 | 0.20 | 0.16 |
| | EC = 11.51–0.42×RL×SWC + 0.99×RL×RTD | *** | | ns | ns | 115 | 0.21 | 0.14 |
| 1 kHz | EC = 3.32 + 0.023×RL | *** | ** | | | 53 | 0.45 | 0.0012 |
| | EC = 3.30 + 0.028×RL-0.029×RL×RTD | *** | ns | ns | | 55 | 0.20 | 0.155 |
| | EC = 3.16 + 0.23×RL×SWC-0.21×RL×RTD | *** | | ns | ns | 53 | 0.49 | 0.003 |
| 10 kHz | EC = 0.88 + 0.0046×RL | *** | ** | | | -2 | 0.34 | 0.0074 |
| | EC = 0.85 + 0.01×RL-0.0345×RL×RTD | *** | ns | ns | | -0.3 | 0.35 | 0.024 |
| | EC = 0.81 + 0.072×RL×SWC-0.080×RL×RTD | *** | | ns | ns | -2 | 0.40 | 0.013 |
| 100 Hz | EC = 10.29 + 0.0058×RSA | *** | * | | | 113 | 0.21 | 0.045 |
| | EC = 10.78–0.037×RSA + 0.062×RSA×RTD | *** | ns | ns | | 114 | 0.23 | 0.11 |
| | EC = 11.18–0.044×RSA×SWC + 0.14×RSA×RTD | *** | | ns | ns | 114 | 0.24 | 0.09 |
| 1 kHz | EC = 3.206 + 0.0024×RSA | *** | *** | | | 52 | 0.47 | 0.0008 |
| | EC = 3.22 + 0.002×RSA + 0.0022×RSA×RTD | *** | ns | ns | | 54 | 0.47 | 0.004 |
| | EC = 3.09 + 0.019×RSA×SWC-0.013×RSA×RTD | *** | | ns | ns | 53 | 0.49 | 0.003 |
| 10 kHz | EC = 0851 + 0.00048×RSA | *** | ** | | | -3 | 0.36 | 0.005 |
| | EC = 0.83 + 0.0081×RSA-0.0021*RSA*RTD | *** | ns | ns | | -0.9 | 0.37 | 0.02 |
| | EC = 0.80 + 0.0061×RSA×SWC-0.00062×RSA×RTD | *** | | ns | ns | -2 | 0.42 | 0.011 |

RDM, RL, RSA, and SWC represent root dry mass, root length, root surface area and soil water content respectively; RTD represents root tissue density; a is the coefficient of RL, RDM and RSA; b is the coefficient of the interactions between RL, RDM, RSA and RTD; c is the coefficient of interactions between RL, RDM, RSA and SWC *, ** and *** represent significance levels at 0.05, 0.01, and 0.001, respectively

that $\Delta EC_{root}$ showed minimal changes with different depths under both conditions. The entry at the root system should be polarized under dry conditions, which is why the return electrode position did not affect $\Delta EC_{root}$, but if current follows the soil-path way, as it does under wet conditions, $\Delta EC_{root}$ can also show these minimal changes at different depths. One explanation may be that soil moisture is constant at different depths because we flooded these pots before the measurements for the wet treatments (Supplementary Fig. 2). Second, as noted in the methods, varying the distance between the negative electrode and the positive plant electrode would have little effect on the EC measurement. It is important to note that a higher terminal voltage of the equipment should be considered when measuring the deep soil layers although the terminal voltage did not affect the observation that root cutting significantly reduced the EC under dry conditions.

## Frequency effects

At present, most studies use 1 kHz to measure $EC_{root}$ (Cao et al. 2010; Cseresnyés et al. 2020a; Streda et al. 2020). Other studies assert that high frequency is more suitable for measuring root systems under drought conditions (Wu et al. 2017). With increasing frequency, the dielectric constant of plant tissue decreases, and the electrical conductivity increases. Therefore, at low frequencies, the current flows through the root system through the plasmid, while at high frequencies, the current can pass through the root system cells (Mancuso 2012). The contribution of the apoplast and symplast current pathways to the capacitance changes continuously with changing current frequency (Cseresnyés et al. 2013b) because the polarization mechanism of biomaterials goes from counterion or ionic polarization to interfacial polarization to dipole or orientational polarization as the frequency increases (Ehosioke et al 2020; Mancuso 2012). Our results from the Pot + Field topsoil and Pot + Mixed topsoil experiments indicate that 100 Hz is the best measurement frequency for measuring $EC_{root}$ under drought conditions in cotton (Fig. 7 and Table 2). Even though the current flows through the plasmid, the root-soil circuit can also be treated as a capacitor permitting root measurement (Dalton 1995; Ellis et al. 2013a). In the Field experiment (moderate dry conditions), however, higher frequencies were used, and better correlations between $EC_{root}$ and root traits were obtained. This may be because at higher frequencies, current flows more easily to deeper roots, as found under field conditions. However, at high frequencies, both the longitudinal conductivity and radial conductivity increase (Ehosioke et al. 2020; Mancuso 2012), which can also cause leakage problems. That is why the correlation between $EC_{root}$ and root dry weight was significant but the coefficient of determination was low in the Field experiment. The measurement frequency is an important consideration when using $EC_{root}$ to estimate root traits, although the underlying physical mechanism needs further study.

## A forward model to predict EC needs to be established

Many factors affect the accuracy of the EC method for measuring roots such as soil moisture, root type and the location of electrodes, as well as measuring frequency and temperature (Chloupek 1972; Cseresnyés et al. 2018a, b; Dalton 1995; Rajkai et al. 2005; Streda et al. 2020; Wu et al. 2017). However, in nearly 50 years of application of the EC method for detecting roots, the characterization of the root system was dependent on the inversion model (Streda et al. 2020). Simply fitting a linear model with EC and root system size (e.g., root dry biomass) to estimate root size would be attractive but would also limit its applications because plant types, developmental ages, soil environments and even measuring equipment can have significant influences on the correlations (Cseresnyés et al. 2020a; Ellis et al. 2013a; Streda et al. 2020). Second, because of complicating factors affecting EC, correlations with root size are not consistent (linear, parabolic or not significant), making the inverse model difficult to apply widely. In contrast, a forward physical model can help illustrate the importance of these factors for EC measurements and help us understand their mechanisms. Similar to the present study, Ellis et al. (2013a, b) showed that RTD has a significant effect on EC.

In this study, we used the simple forward model ($EC_{root}$, root traits and soil moisture) to evaluate whether it may be improved by including RTD and soil water content in the Pot + Mixed topsoil experiment. Our results did verify this hypothesis. Since RTD is inversely proportional to the free water content, which determines the relative dielectric constant of the root system (Guo et al. 2013; Paz et al. 2011), $EC_{root}$ can also reflect the activity of the root system under drought conditions (Cseresnyés et al. 2020a; Wu et al. 2017). Under drought conditions, RTD was positively correlated with root biomass, while RTD was negatively correlated with $EC_{root}$ (Table 2). This may be the reason for the parabolic relationship between capacitance and root biomass in the Pot + Field topsoil experiment under dry conditions.

The heterogeneity of the root system and soil conditions likely play significant roles in predicting EC. For example, soil water content is an important factor in improving the accuracy of the EC method in predicting root system size under drought conditions (Cseresnyés et al. 2018b, 2020a). Our results indicate that considering the interaction between soil moisture and root size parameters (root dry mass, root length and root surface area) can further improve the accuracy of predicting $EC_{root}$ (Table 2). Therefore, from what has been discussed above, understanding the influences of multiple factors on EC and establishing a quantitative model can improve the measurement accuracy. In the Pot + Mixed topsoil experiment, the change in

$EC_{root}$ was greater than that in the Pot + Field topsoil experiment, which might be because Pot + Mixed topsoil had a higher sandy content, leading to lower soil conductivity. Therefore, the influence of soil type on the current path needs further exploration.

## Conclusions

The flow of current through the root system is key to determining the feasibility of using EC to estimate plant root characteristics. We used the root cutting methodology to indirectly "image" the current paths in the woody cotton root system. $EC_{root}$ after root excision significantly decreased under dry conditions and marginally changed under wet conditions indicating that electrical current could flow through the cotton root system under dry conditions rather than wet soil conditions. $EC_{root}$ measured at 100 Hz has the greatest potential to estimate root traits under dry conditions in pot experiments, but higher frequencies are needed to measure roots under moderately dry conditions in the field. We also demonstrated that, under dry conditions, including RTD and soil water content in the forward model can significantly improve EC prediction accuracy.

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