An improved formula for evaluating electrical capacitance using the dissipation factor

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Abstract

Background and aims The measurement of electrical capacitance in root–soil system (C_R) is a useful method for estimating the root system size (RSS) *in situ*; however, C_R–RSS regressions are often poor. It was hypothesized that this weak relationships could be partly due to the variable energy-loss rate, indicated by the dissipation factor (DF).

Methods The values of C_R and the associated DF were measured in six plant species grown in quasi-hydroponic pumice medium, arenosol and chernozem soil. The dielectric properties of the plant growth media were also recorded. A modified root–soil capacitance, C_{DF}, was calculated from each C_R/DF pair according to the formula $C_{DF} = C_R \cdot (DF/DF_{mean})^{\alpha}$ by estimating α with a standard nonlinear minimization of the sum of squared residuals for C_{DF}–RSS regressions.

Results The capacitive behavior of the medium improved (mean DF decreased) but fluctuated increasingly as the substrate became more complex. The mean DF values in plant–substrate systems were chiefly determined by the plant and were the most variable in chernozem soil. This strengthening substrate effect on C_R measurements appeared as a decreasing trend in the R^2 values obtained for the C_R–RSS regressions. The regression slope was influenced by plant species and medium, while the y-intercept differed only between substrate types. The proposed use of C_{DF} in place of C_R could significantly improve the R^2 of C_{DF}–RSS regressions, particularly in chernozem soil (R^2 increased by 0.07–0.31).

Conclusions The application of C_{DF} will provide more reliable and accurate RSS estimations and more efficient statistical comparisons. The findings are worth considering in future investigations using the root capacitance method.

Abbreviations: AIC – Akaike's Information Criterion; C – Electrical capacitance; C_p – Electrical capacitance of the planting substrate; C_R – Electrical capacitance of the root–soil system; C_{DF} – Electrical capacitance of the root–soil system corrected with dissipation factor; DF – Dissipation factor; NP – Number of model parameters; RDM – Root dry mass; RL – Root length; RSA – Root surface area; RSS – Root system size

Introduction

The reliable estimation of the extent and functionality of the root system is undoubtedly important not only for modeling and characterizing water and nutrient uptake, but also for determining many plant phenomena related to root development. It is thus essential for various plant physiological, agricultural and ecological studies. Due to the hidden nature of the root system, many conventional investigation methods (*e.g.* monoliths, soil cores, in-growth cores, pits or excavation) are time- and labor-intensive, expensive and inherently destructive, making them unsuitable for the continuous monitoring of the same plant. The results may also represent only part of the whole root system. Therefore, the application and improvement of non-intrusive techniques will have an increasing role in obtaining information about root size, morphology and functions *in situ* (Rewald and Ephrath 2013). Though several methods of this type have been developed for the quantification of root characteristics (*e.g.* minirhizotron, MRI, tracers or X-ray imaging), their adaptability is greatly limited in many cases (Milchunas 2012). They often give poor resolution of the root structure (chiefly root hairs), tending to produce uncertain data, if any, on the actual activity or absorptive surface area of the root system.

The measurement of electrical capacitance in root–soil systems (C_R) is one non-destructive method that is capable of providing an assessment of root system size (RSS) and functionality without damaging the plant. The process was developed by Chloupek (1972) using several crop species (maize, sunflower, oat, onion and rape) under greenhouse and field conditions. By fixing one electrode to the plant stem, embedding the other in the soil, and connecting them to a capacitance meter operating with a low-voltage alternating current (1V, 1 kHz AC), the measured C_R is directly correlated with root dry mass (RDM), root length (RL) and root surface area (RSA).

Capacitance is formed by the polarization and relaxation phenomena of living root membranes and cells, leading to changes in the amplitude and phase of the AC signal applied (Dvořák et al. 1981; Repo et al. 2000). Dalton (1995) was the first to present a conceptual model for the interpretation of the plant root–soil system, in which RSA was considered, at the macro-scale, to be the surface area of a group of parallel-connected cylindrical condensers having the same average diameter as the cellular system constituting the roots (Fig. 1). Dalton (1995) hypothesized that, within the root–soil–electrode network, the xylem and phloem sap in the roots form a low-resistance electrical conduit separated from the low-resistance external soil or nutrient solution by isolating root membranes. Thus, the polarized membrane plays the role of a dielectric in a capacitor, where the plant sap and soil solution provide the two conduit plates. The root–soil interface has a capacitance proportional to the charges accumulated on the membrane surfaces. In cylindrical condensers like plant roots, the plate distance (d) is determined by the radii of the xylem (r_1) and rhizodermis (r_2), analogous to the internal and external electrodes, respectively (Fig. 1). If r_{i1} approaches r_{i2} using the Taylor series expansion of logarithmic function, the expression in Fig. 1 can be reduced to a form describing the capacitance of the sum of parallel-plate condensers (Dalton 1995). The capacitance (C) of a parallel-plate condenser is commonly expressed by the formula

[Eq. 1] $C = \varepsilon_0 \cdot \varepsilon_r \cdot A \cdot d^{-1}$

where ε_0 is the permittivity of free space (8.854 F m^{-1}), ε_r is the relative permittivity of the dielectric, A is the plate area and d is the plate separation (thickness of the dielectric).

Though Dalton's model still remains the main concept for the physical description of root–soil circuitry, some of its assumptions have since been amended. Rajkai et al. (2005) and Dietrich et al. (2013) highlighted the fact that the substrate around the roots also provides capacitance, and thus recommended a two-dielectric model consisting of charge-storing conductive capacitor surfaces and two dielectric media with different permittivity. The resulting capacitance measured between the ground and plant electrodes combines as the component capacitors wired in series. Provided that the capacitance of the root tissue is much smaller than that of the rooting substrate, the capacitance of the plant–substrate system is determined by the root tissue. Dietrich et al. (2012, 2013) found that C_R was dominated by the tissue between the plant electrode and the solution (or soil) surface and was proportional to the cross-sectional area or circumference of the root at the solution (soil) surface. Thus, the authors modified the conceptual framework of Dalton's model: the revised model approximated the root tissue as a continuous dielectric, and considered the capacitances of tissues along an unbranched root to be connected in series and those of the whole root system in parallel. Ellis et al. (2013a) proposed a new empirical model relating RL to C_R and root tissue density (ρ) which, in turn, estimated the ε_r of the root cortex. They demonstrated also that the increasing proportion of the finest roots reduced the correlation. However, we need to complement our understanding of electrical aspects of fine roots. Methodological specifications regarding sample size, preparation, washing method or sieve mesh size vary widely between studies, resulting in large differences of recovered root biomass and root length (Oliveira et al. 2000; Muñoz-Romero et al. 2010).

The main limitation for the generalization of the capacitance method is the sensitivity of C_R to edaphic factors, such as soil water saturation, ionic status and soil texture (Dalton 1995; Ozier-Lafontaine and Bajazet 2005). Dalton (1995) and Ellis et al. (2013b) highlighted the need for careful and consistent placement of the stem electrode, demonstrating a marked decrease in C_R as the electrode was fixed at increasing distances above the root neck. The considerable effect of the shape and size of the ground electrode on C_R has recently been shown in a pot experiment (Kormanek et al. 2016). Nevertheless, under standardized conditions (soil moisture content corresponding to at least field capacity, homogenized medium with constant salinity and consistent electrode placement) the method can provide a good estimation of RSS. The reliability of the technique was demonstrated in various pot and field experiments focused on crop genotypes (Beem et al. 1998; Chloupek et al. 2006; Cseresnyés et al. 2013b, 2014, 2016) and young tree cultivars (Preston et al. 2004; Cao et al. 2010; Pitre et al. 2010; Kormanek et al. 2016). Chloupek et al. (2010) emphasized that C_R data are relative, making them comparable only for plants of the same species, grown in the same substrate at the same moisture level in the same time frame.

Previous studies clearly indicate the varying degrees of success with which the capacitance method was applied in root investigations (Aulen and Shipley 2012). In several cases, C_R proved to be an insignificant or poor predictor of RSS, particularly when the measurements were performed not in hydroponic or mineral substrates, but in more complex and heterogeneous natural soils (Postic and Doussan 2016). The reason for this is that, while ideal physical capacitors store energy electrostatically with an infinitesimal effective energy loss, root tissue – being an imperfect dielectric – acts as a leaky (poor) capacitor (Dalton 1995; Rajkai et al. 2005). Additionally, soil constituents, particularly colloids, also possess dielectric character (Hilhorst 1998; Arulanandan 2003), making the root–soil–electrode system more complicated electrically, and moreover, while the Dalton model assumes homogeneous ε_r for the root cortex, the empirical allometric relationship between RL and C_R revealed by Ellis et al. (2013a,b) was verified in the case of a root dielectric with variable ε_r.

Living tissues, including plant roots, can be considered as a parallel resistance–capacitance (RC-) circuit that is a dielectric with losses (Ozier-Lafontaine and Bajazet 2005; Grimnes and Martinsen 2015), which can be characterized by complex relative permittivity ε_r* (Fig. 2):

[Eq. 2] $\varepsilon_r^* = \varepsilon_r' - i \cdot \varepsilon_r''$

where ε_r' is the real part of permittivity (energy stored electrostatically), ε_r'' is the imaginary part of permittivity (energy dissipation or energy loss due to conduction, *i.e.* to the motion of the charges), and i is the imaginary unit, $i^2 = -1$. Thus, a complex capacitance C* can be expressed as:

[Eq. 3] $C^* = \varepsilon_0 \cdot (\varepsilon_r' - i \cdot \varepsilon_r'') \cdot A \cdot d^{-1}$

The value of the tendency of dielectric materials to absorb some of the energy during AC application is defined as the dissipation factor (DF) or loss tangent ($\tan(\delta)$), which is the ratio of dielectric losses to energy storage (Fig. 2):

[Eq. 4] $DF = \tan(\delta) = \varepsilon_r''/\varepsilon_r' = G/(\omega \cdot C)$, where G is the electrical conductance (= 1/R), ω is the angular frequency and C is the capacitance. The loss angle δ is the complementary angle of the phase angle (Φ) of capacitive impedance:

[Eq. 5] $\delta = 90° - \Phi$

A former study (Cseresnyés et al. 2013a) revealed that even-aged plant populations with fairly uniform RSS tended to show considerable variance in their impedance response (in Φ, thus in DF) during electrical measurements, and higher Φ (lower DF) values were generally associated with higher C_R and vice versa. It was hypothesized that the changeable values of DF and C_R could be attributed to the change in ε_r*, caused by variations in either ε_r' or ε_r'' or both. Moreover, to obtain a better prediction of RSS by the C_R method, Ellis et al. (2013a) also suggested considering the mass density of the root tissue, which is related to dielectric properties (Aulen and Shipley 2012) and thus presumably to DF.

It was hypothesized that, in some cases, the low efficiency of C_R measurements and the insignificant or weak C_R–RSS relationship are at least partly due to the variability of electrical impedance derived from the variability of ε_r*, which influenced the measured DF and C_R. Therefore, the measurement of DF when the C_R method is applied and the use of DF to modify C_R data will presumably contribute to enhancing the predictive capability of C_R for RSS.

The present study aimed to provide an improved empirical formula for the capacitance method, giving a practical basis for the more reliable estimation of RSS. The use of DF seemed to be suitable for this purpose, because this parameter can be displayed simultaneously with electrical capacitance using a precision LCR instrument, without the need for any additional work. The influence of the plant species and growth substrate on the mean value and standard deviation of DF were first investigated. Secondly, the effect of species and substrate on the parameters, *i.e.* the slope, y-intercept and coefficient of determination (R^2) of linear regressions between C_R and RSS variables (*i.e.* RDM, RL and RSA) was studied. Finally, the aim was to find a mathematical formula comprising both C_R and DF, with which the R^2 of C_R–RSS regressions could be improved.

Materials and methods

Plant cultivation

The experimental work was performed on six crop species, namely bean (*Phaseolus vulgaris* L. Cv. Goldrush), cucumber (*Cucumis sativus* L. cv. Perez-F1), maize (*Zea mays* L. cv. DC 488F1), soybean (*Glycine max* L. Merr. cv. Martina), tomato (*Lycopersicon esculentum* Mill. cv. Kecskeméti 549) and wheat (*Triticum aestivum* L. cv. TC33). Each crop was grown in three contrasting types of planting substrate: the soil-analog pumice medium, natural arenosol and chernozem. Pumice – a porous, chemically inert vitroclastic perlite – is a commercially available hydroponic medium, which allows good water retention and aeration during plant cultivation. The coarse-textured arenosol (IUSS 2015) and the chemically and structurally more complex chernozem were collected from the field, then spread on large trays and completely air-dried at room temperature. The dried soils were passed through a coarse sieve to remove large clods and plant material. The main physical and chemical properties of the substrates were determined according to Buzás (1988) (Table 1).

A total of 540 (for 30 replicates of 6 species in 3 growing media) 3.75 L plastic pots were lined with plastic mesh to stop the substrates leaking through the drain holes, and then filled with pumice or soil. The crop seeds were germinated by placing them on moistened paper towels in Petri dishes and keeping them in the dark at 25 °C for 2–4 days (depending on the species). Three germinated seeds were placed in each pot, then the seedlings were thinned to one per pot five days after planting (DAP). Plant cultivation was carried out in a large growth room at 28/18 °C day/night temperature and 16/8 h photoperiod, with a photon flux density of 800 µmol m^{-2} s^{-1} and relative humidity of 50–80%. The substrates were irrigated daily with tap water to field capacity: the pots were placed on a balance (±1 g) and watered to a weight calculated from the soil volume and the water content at field capacity. The volumetric water content was measured with a Trime-FM3 TDR meter (IMKO GmbH, Ettlingen, Germany) and then adjusted precisely to field capacity by adding more water as required (owing to the increment of plant biomass in the pots). Furthermore, the pumice was fertilized twice a week from DAP 5 with 100 mL of Hoagland's solution to prevent nutrient deficiency in the plants.

Electrical measurements

The electrical impedance response was measured with a GW-8101G precision LCR-bridge (GW Instek Co. Ltd., Taiwan) with 1 V terminal voltage at 1 kHz AC frequency. DF and C_R were displayed for a parallel RC-circuit. One terminal of the instrument was connected to the ground electrode, a stainless steel rod (6.3 mm in diameter and 18 cm long) inserted to a depth of 15 cm into the potting medium at a distance of 8 cm from the stem base. The other terminal was linked to the plant with a spring tension clamp fixed through a 5 mm wide aluminum strip that bent the stem to avoid any plant injury (Beem et al. 1998; Rajkai et al. 2005). Since the placement of the plant electrode is known to influence C_R (Dalton 1995), a distance of 10 mm was consistently maintained between the lower edge of the aluminum strip and the substrate surface. Electrocardiograph paste (Vascotasin®; Spark Promotions Co. Ltd., Budapest, Hungary) was smeared under the clamp to maintain electric contact (Rajkai et al. 2005). Two hours before the measurement the plants were brought into the laboratory (22 °C) and watered to field capacity (see above). In this manner, the soil moisture values measured by the TDR instrument at each measuring date did not differ significantly among the treatments. Prior to the C_R measurement, the parallel capacitance, C_p and DF of the planting media were also detected in the pots between two identical ground electrodes embedded in the soil at 8 cm distance and attached to the LCR-bridge.

For each plant species, electrical measurements were executed over a 30-day period: between DAP 6 and 35 in bean, cucumber, maize and soybean, and from DAP 11 to 40 in tomato and wheat (in the latter cases, fastening the electrode to the thin plant stem was not feasible earlier). One plant from among the 30 replicates of each species and substrate type was chosen daily for electrical measurement and subsequent harvest in order to obtain ranges of RSS for data evaluation.

Plant harvest and RSS evaluation

Immediately after the electrical measurement, the selected plants were destructively sampled. The shoots were cut at the substrate surface, after which the roots were separated from the substrate by hand washing with a water sprinkler carefully (to avoid the breaking of roots) over a 0.5-mm mesh sieve followed by the root-flotation method (Oliveira et al. 2000). Great care was also taken during flotation to minimize the loss of fine roots. The washed roots were stained with methyl violet solution for 48 h, then rinsed with water. To assess RL and RSA, the stained root systems were laid in a rectangular glass tray containing water and subjected to scanning and image analysis (Delta-T Devices Ltd., Cambridge, UK). Finally, the roots were oven-dried at 70 °C to constant weight and weighed (±0.001 g) to determine RDM.

Data analysis

Statistical evaluation was performed using "R package nloptr, ver. 1.0.4." software (Johnson 2014). The measured DF data were analyzed by testing the homogeneity of their variances using a modified robust Brown–Forsythe Levene-type test based on absolute deviations from the median (Quinn and Keough 2002, p. 195). The effect of plant species, substrate type or their interactions on mean DF was evaluated by two-way ANOVA. The distribution of DF proved to be non-normal (with heavier right tail than the normal), thus a robust two-way ANOVA for median was applied with confidence intervals calculated by bootstrapping (Wilcox 2012, p. 201). The analysis was also performed using standard two-way ANOVA.

The relationship between electrical capacitance and RSS variables (RDM, RL or RSA) was analyzed using the linear regression method by minimizing the sum of squared deviations. As a first step, the root–soil capacitance, C_R–directly measured by the LCR instrument–was used for these regression analyses to obtain separate regression equations for the RSS variable, species and substrate type (C_R–RSS regressions). Thereafter, a mathematical formula was created to convert the measured C_R into a corrected value, C_{DF} using the DF value. Since the measured C_R data associated with lower and higher DF tended to appear above and below the regression line, respectively, in the course of C_R–RSS regression, the following formula was chosen to improve the fit of the regression model: $C_{DF} = C_R \cdot (DF/DF_{mean})^{\alpha}$ where C_{DF} is the root–soil electrical capacitance corrected with the dissipation factor, C_R is the measured root–soil electrical capacitance, DF is the measured dissipation factor, DF_{mean} is the mean dissipation factor for a given plant in a given substrate (n = 30) and α is a nonlinear correction factor. For each C_{DF}–RSS regression, α was estimated with a standard nonlinear minimization of the sum of squared residuals (quasi-Newton method BFGS; Quinn and Keough 2002, p. 151). There were 3·3·6 = 54 regressions: 3 types of RSS variables (RDM, RL or RSA), 3 growing substrates and 6 plant species. The number of replications was n = 30 for each, giving a total of N = 30·54 = 1620 data points. If the number of parameters in a model is denoted as NP, then the degrees of freedom of the residual sum-of-squares ResDegF = N – NP (here the statistical term "degrees of freedom" is abbreviated as DegF to avoid the confusion with the symbol DF used for the dissipation factor).

The more detailed version of the correction formula is

[Eq. 6] $CDF_{p,r,s} = CR_{p,r,s} \cdot (DF_{p,s} / DFmean_{p,s})^{\alpha}$

where CDF stands for C_{DF}, DFmean for DF_{mean}, p = 1..6 for the plant species, s = 1..3 for the substrate, r = 1..3 for the type of RSS variables (*i.e.* RSS_1 = RDM, RSS_2 = RL and RSS_3 = RSA) and α will be specified later. For each (p,r,s) group, CR, DF and RSS variables are vectors composed of the 30 replications performed in each situation during this experimental campaign.

The following five models were taken into account (see Table 2 for constraints on model parameters):

Model 1 (M1): $CDF_{p,r,s} = a_{p,r,s} + b_{p,r,s} \cdot RSS_{p,r,s}$ and Eq 6. with $\alpha = \alpha_{p,r,s}$

where $a_{p,r,s}$ (the y-intercept), $b_{p,r,s}$ (the slope) and $\alpha_{p,r,s}$ are free parameters. The number of parameters in M1 was NP1 = 3·3·3·6 = 162, and the residual degrees of freedom for M1 was ResDegF1 = N – NP1 – 1 = 1457.

Model 2 (M2): $CDF_{p,r,s} = a_{p,r,s} + b_{p,r,s} \cdot RSS_{p,r,s}$ and Eq 6. with $\alpha = \alpha_{p,s}$
where $a_{p,r,s}$, $b_{p,r,s}$ and $\alpha_{p,s}$ are free parameters. The number of parameters in M2 was NP2 = 3·(1+3+3)·6 = 126, so the residual degrees of freedom for M2 was ResDegF2 = N – NP2 – 1 = 1493.

Model 3 (M3): $CDF_{p,r,s} = a_{p,s} + b_{p,r,s} \cdot RSS_{p,r,s}$ and Eq 6. with $\alpha = \alpha_{p,s}$
where $a_{p,s}$, $b_{p,r,s}$ and $\alpha_{p,s}$ are free parameters. The number of parameters in M3 was NP3 = 3·(1+1+3)·6 = 90, so the residual degrees of freedom for M3 was ResDegF3 = N – NP3 – 1 = 1529.

Model 4 (M4): $CDF_{p,r,s} = a_{s} + b_{p,r,s} \cdot RSS_{p,r,s}$ and Eq 6. with $\alpha = \alpha_{p,s}$
where a_{s}, $b_{p,r,s}$ and $\alpha_{p,s}$ are free parameters. The number of parameters in M4 was NP4 = 3·6+3+3·3·6 = 75, so the residual degrees of freedom for M4 was ResDegF4 = N – NP4 – 1 = 1544.

Model 5 (M5): $CDF_{p,r,s} = a + b_{p,r,s} \cdot RSS_{p,r,s}$ and Eq 6. with $\alpha = \alpha_{p,s}$
where a, $b_{p,r,s}$ and $\alpha_{p,s}$ are free parameters. The number of parameters in M5 was NP5 = 3·6+1+3·3·6 = 73, so the residual degrees of freedom for M5 was ResDegF5 = N – NP5 – 1 = 1546.

In order to choose the best model, the Akaike Information Criterion (AIC) was calculated for each model listed above as AIC = N·ln(SSQResid) + 2·NP – N·ln(N), where N is the total number of data points, NP is the number of parameters in the model and SSQResid is the residual sum-of-squares of the model (Quinn and Keough 2002, p. 139). The basic idea was to eliminate unnecessary parameters using an optimization function that balanced model fit and parsimony.

Results

Electrical properties of substrates

The ANOVA procedure revealed highly significant differences between the parallel electrical capacitance values of the planting substrates: the lowest (6.5 ± 0.8 nF; mean ± SD), medium (18.5 ± 0.7 nF) and highest (31.1 ± 1.4 nF) C_p values were measured in pumice, chernozem and arenosol, respectively (Fig. 3). All three media exhibited relatively high DF, indicating their poor charge storage capacity and predominant ohmic resistance. The mean DF also differed significantly among the substrates: the highest (29.7 ± 1.2), medium (24.1 ± 1.5) and lowest (14.9 ± 1.7) mean values were obtained for pumice, arenosol and chernozem, respectively. Though the Brown–Forsythe test showed that the group SDs did not differ significantly, it is worth mentioning that SD increased (from pumice to chernozem) as the mean DF decreased.

Dissipation factor (DF) in plant–substrate systems

The DF values detected in plant–substrate systems proved to be considerably smaller than those measured for the substrates, and showed great variability among plant species (Fig. 4). Irrespective of the substrate used, the lowest and highest mean DF values were obtained for wheat and soybean, respectively. The mean DF ranged from 2.51 to 3.79 in pumice, from 2.69 to 3.92 (0.12–0.18 higher for each species) in arenosol and from 2.30 to 3.81 in chernozem. The SDs of the above data groups were the lowest (0.46–0.66) in pumice and the highest (0.63–0.92) in chernozem for all the species.

Standard two-way ANOVA was first used for the statistical analysis of the data. This test revealed that the plant species had a highly significant effect and the substrate type a significant effect while their interaction was non-significant (Table 3). As the Brown–Forsythe test indicated heterogeneity of variance, influenced significantly by the plant ($F = 2.75$; $p = 0.018$), the substrate ($F = 3.47$; $p = 0.032$) and their interaction ($F = 1.77$; $p = 0.029$), data analysis was repeated using a robust two-way ANOVA for medians, using the "R package WRS 2, ver. 0.4." software (Mair et al. 2015). The latter procedure showed that the effect of the plant on DF was highly significant, while the effect of the substrate and their interaction were non-significant (Table 3).

Root–soil capacitance (C_R) and root system size (RSS)

The minimum value of C_R, detected in the youngest plants, was within the range of 0.363–0.459 nF, 1.616–1.908 nF and 1.323–1.783 nF in pumice, arenosol and chernozem, respectively (Table 4). The maximum C_R, generally measured in the oldest plants, showed great variability not only between the substrate types but also between species. In each medium, the maximum C_R was the highest in maize (pumice: 5.871 nF; arenosol: 14.85 nF; chernozem: 12.10 nF) and the lowest in bean (1.174 nF; 3.515 nF and 3.292 nF).

RSS was strongly dependent on the plant species. Soybean showed the highest RDM (1.837–2.012 g) in all the substrates. The largest RL was produced by soybean in pumice (142.2 m) and by maize in arenosol (147.7 m) and chernozem (201.6 m). The species with the highest RSA was soybean in pumice (1793 cm^2) and arenosol (1313 cm^2), but maize in chernozem (1475 cm^2). Depending on the substrate type and the RSS variable, the smallest root system was developed by bean or tomato by the end of the experiment.

C_R–RSS regressions

Linear regression revealed significant ($p < 0.01$) positive relationships between C_R and RSS for each substrate, species and RSS variable (R^2 = 0.451–0.942; F = 23.0–450.6; DegF = 29; Table 5). From among the numerous regressions obtained, the C_R–RDM relationships for the dicot bean and the monocot wheat grown in different substrate types are graphically represented in Fig. 5 and 6, respectively (left panels). The calculated y-intercept (in nF) clearly depended on the planting medium: 0.463–0.597 in pumice, 2.048–2.203 in arenosol and 1.582–1.788 in chernozem.

The slope of the regression line proved to be strongly dependent on the plant species, differing by almost an order of magnitude in some cases. Irrespective both of the substrate and the RSS variable used, the smallest slope was always obtained for soybean: 0.573–1.238 nF g^{-1} RDM, 0.008–0.017 nF m^{-1} RL and 0.0007–0.0016 nF cm^{-2} RSA. The steepest slope was shown by wheat for RDM (7.375–11.10 nF g^{-1}), by tomato or wheat for RL (0.053–0.102 nF m^{-1}) and by maize or wheat for RSA (0.0057–0.0094 nF cm^{-2}) in the different media. In terms of the substrate types, the greatest slope was obtained for all species and RSS variables in pumice, and the smallest mostly in arenosol, but in some cases in chernozem.

Interesting tendencies were seen in the R^2 values calculated for the regressions. With regard to the species, the best fit, with R^2 of 0.757–0.942, was obtained for maize in each case, followed by wheat or tomato, while the lowest R^2 value (from 0.451 to 0.796) was found for bean, the only exception being soybean RSA in pumice. When considering the substrate type, the highest R^2 values (from 0.751 to 0.942) were found in pumice and the lowest (from 0.451 to 0.830) in chernozem for each species and RSS variable (the only exception being the RL of wheat in arenosol). No relationship was observed between R^2 and the RSS variables.

C_{DF}–RSS regressions and model selection

Linear regressions between RSS variables and C_{DF} (calculated for each electrical measurement from the detected C_R and associated DF data using Eq. 6) were fitted according to M1 (Table 6). The application of M1 resulted in R^2 values of 0.866–0.972 and 0.818–0.954 for pumice and arenosol, respectively, and 0.696–0.936 for chernozem for the majority of species, with the exception of tomato (R^2 = 0.551–0.675). The correction factor α, estimated from a standard nonlinear minimization of the sum of squared residuals using C_{DF} and RSS data, generally varied from 0.39 to 1.09 (but was between 1.63 and 1.72 for tomato in chernozem) and showed no relationship with the potting media ($p = 0.079$) or species ($p = 0.082$). Since $\alpha = 0$ corresponds to the $C_{DF} = C_R$, correlation coefficients found in C_{DF}–RSS regressions are at least equal to those found in C_R–RSS regressions. In consequence, all 54 regressions of model M1 gave more reliable estimates for RSS, as indicated by higher R^2 values, than for the corresponding relationships based on C_R (Table 5). The coefficient increased by 0.011–0.195 in pumice and by 0.042–0.242 in arenosol. In chernozem the increase was 0.036–0.177 for tomato and 0.070–0.312 for the other species. The three-way ANOVA showed that the effect of substrate type on the y-intercept was extremely significant and that the effect of plant species was also significant, but the RSS variable had no influence on the y-intercept (Table 7). The same test for slope revealed that the effect of the RSS variable was extremely significant and the effect of species was significant, but the substrate type had no influence on the slope.

Linear regression involved two parameters (y-intercept and slope) and an additional y-correction parameter α was used (Eq. 6), so the aforementioned model was somewhat overparameterized with $54 \cdot 3 = 162$ parameters. In order to find the optimal subset of parameters, a sequence of five models was taken into consideration, starting with that explained above. The summarized statistics of the initial model, designated M1, are given in the first line of Table 8. Smaller AIC values indicate better models, so M4 proved to be the best model in the series. NP decreased from 162 to 75, while the R^2 values remained almost as good as in M1. The finite sample size corrected version of AIC (AICc) and Evidence Ratio (Burnham and Anderson 2004) were also applied to characterize the relationships between models M1 to M5. AICc gave almost the same values as AIC due to the relatively high sample size (N = 1620). Model M4 proved to be the only reasonable choice from the set of models M1 to M5, as the Akaike Weight of M4 was 0.999. Evidence Ratios and their logarithms confirmed this decision (Table 8). The authors do not claim to have tested all possible models, but present the results of an AIC controlled stepwise model selection procedure. ANOVA analyses on the estimated parameters are given in Table 7.

M4 included a common α factor for all three RSS variables for a given species in a given substrate, varying from 0.41 to 1.03, though a value of 1.66 was found for tomato grown in pumice, as in M1 (Table 9). The y-intercept only differed between the substrates, being 0.529, 2.129 and 1.600 nF for pumice, arenosol and chernozem, respectively. The R^2 values achieved with M4 were exactly the same or only slightly lower (by at most 0.013) than those obtained using M1. The C_{DF}–RDM regressions for bean and wheat are graphically shown in Fig. 5 and 6, respectively (right panels).

Discussion

Effect of plant and substrate on C_R–RSS regressions

The experimental results suggest that plant species and substrate type had a great influence on the regression between electrical capacitance and RSS. This finding is consistent with previous studies describing the necessity of specific calibration for each plant–substrate system (Dalton 1995; Chloupek et al. 2006; Ellis et al. 2013b). As in the present work, Aulen and Shipley (2012) reported highly variable slope estimates for RDM (2.0–43.3 nF g^{-1}) in ten herbaceous species grown in the same organic soil mixture. Chloupek (1972) obtained a slope of 0.59 nF g^{-1} RDM for maize in sand, which is an order of magnitude lower than the value of 5.4 nF g^{-1} obtained here. The discrepancy with our results can no doubt be attributed to differences in the soil moisture and soil composition and in the type and placement of the ground and plant electrodes. Dietrich et al. (2012, 2013) also found a significant linear relationship between the C_R and RDM in wheat plants of different root sizes, but their experiments revealed that C_R was determined by the cross-sectional area of roots at the substrate surface. Thus, the linear C_R–RDM relationship appeared to result from allometric relationships between RDM and the cross-sectional area of roots near the substrate surface. Though cross-sectional area was not measured in the present study, a close relationship was found in general between RSS variables of the same species (data not shown), which is indirectly indicated by the relatively similar R^2 values obtained in many cases for different C_R–RSS regressions for the same species and growth media. The considerable species-specific differences in the slope of regression are likely to be attributable to the great differences between species both in root cross-sectional area and in the morpho-anatomical properties of the root system and the stem base. Dietrich et al. (2012) demonstrated that the gradient of the relationship was much (4.3-fold) steeper for seminal than for nodal roots of the same barley cultivar. The small regression slopes for soybean were probably caused by the strong lignification of the stem base from the early vegetative stage of plant ontogeny, which may influence the capacitive response. The C_R–RSS regressions have a positive y-intercept (Table 5, Fig. 5 and 6); the "accompanying" capacitance is thought to be a function of substrate type and water status (Chloupek 1977; McBride et al. 2008; Chloupek et al. 2010).

All the relationships between capacitance and root properties were highly significant ($p < 0.001$), but the predicted variance was dependent on the species and substrate. The higher R^2 values obtained for maize and wheat were presumably due to the fact that monocots have a fibrous root system with no thick taproots, the contribution of which to the electrical circuit is uncertain (Ellis et al. 2013a). In relation, the smaller mean DF displayed by the two cereals indicated more efficient charge storage, probably caused by the different root structure and tissue properties compared to the dicots (Wachsman et al. 2015). The better regression fit for the monocots can also be interpreted according to the improved model reported by Dietrich et al. (2012), if a closer allometric relationship existed between the size of the fibrous root system and the root cross-sectional area at the soil surface (which is proportional to C_R).

Although high R^2 values were obtained for the regressions in pumice (quasi-hydroponic) medium, capacitance became a poorer predictor of root attributes as the soil complexity increased. The present results correspond with previous findings indicating weaker correlations in structurally and chemically complex soils or organic substrates (manure and compost) than in hydroponics or sand-based cultures (Chloupek 1972; Aulen and Shipley 2012), making it difficult to extrapolate the capacitance method from pot studies to the field. On the one hand, a possible explanation for these observations was the greater difficulty faced when removing fine roots from substrates that tend to adhere to the roots. A field study by Muñoz-Romero et al. (2010) demonstrated that wheat root separation from vertisol cores using a sieve with a 0.5 mm mesh screen led to a marked (and consistent) underestimation of root biomass compared to using a 0.2 mm mesh screen. On the contrary, Livesley et al. (1999) found that maize roots passing through the 0.5 mm sieve, but recovered by the 0.25 mm sieve contributed only slightly to root biomass. Consequently, in future studies, it is definitely important to clarify how the various root extraction (sieve mesh size, flotation) and investigation (scanning and image analysis) methods influence the size estimation of intact root systems growing in soil media in order to increase the reliability of the results.

Soil water content was considered to be another major constituent in the reliability and accuracy of C_R measurement, adding noise to the electrical relation if variable (Postic and Doussan 2016). Water status locally around the stem base and on the top layer of the substrate is of crucial importance for measuring C_R (Dietrich et al. 2013). In more complex rooting media (soils), the heterogeneity in water content resulted in variable contact between roots and soil solution, influencing the capacitive response.

Role of DF in data evaluation

The results convincingly demonstrated the considerable role of DF in the evaluation of C_R data. An apparent capacitance (C_{DF}) normalized with DF according to the scheme set out in Eq. 6 proved to be a more reliable predictor of RSS than directly measured C_R.

According to the ANOVA results, in plant–substrate systems DF is mostly determined by the species, but is probably also influenced by the substrate (Fig. 4): standard ANOVA showed a significant substrate effect ($p = 0.011$), whereas robust ANOVA indicated borderline significance ($p = 0.087$). Considering the substrates themselves, capacitive loss was found to be the smallest but the most variable for chernozem and the highest but the least variable for pumice (Fig. 3). It is suspected that the unstable capacitive character of chernozem soil may confound the root measurements and cause higher fluctuation in DF and thus in the C_R, leading to lower R^2 for the linear model. This can be mitigated by using the α factor and the C_{DF} parameter.

The application of the correction factor α aimed to reduce the magnitude of the residuals found in the linear regression between electrical variables and RSS variables. The value of α was roughly between 0.4 and 1.1 in most cases and showed no dependence on any of the variables tested. The transformation described by Eq. 6 proved to be optimal when α was <1.1, since this led to an overall reduction in C_R values and the number of outliers. This was true in each case, the only exception being tomato in pumice, where the optimum was attained at $\alpha = 1.6–1.7$. A closer look revealed that there was a negative correlation between C_R and DF in this case and extremely low C_R values when $DF > DF_{mean}$ (data not shown), so the values of the product on the right side of Eq. 6 remained low when α was > 1. Former studies showed that DF tended to depend on the plant phenological stage, owing to the characteristic biochemical and physical changes in the root tissue (Aubrecht et al. 2006; Cseresnyés et al. 2013a). In the present study, despite the short cultivation time, which only covered the vegetative growth stage, the increasing trend of C_R measured in tomato plants developing in pumice proved to be significantly associated with decreasing DF. This finding implies that soil conductivity has a contribution in the DF measurements. More detailed investigations will be required to explain the exceptional value of α in this case and to test the repeatability of this phenomenon.

Relation of C_p and DF with substrate properties

The fluctuation in C_R appears to be associated with the fluctuation in electrical impedance (shown by DF), probably due to the unsteady components of complex relative permittivity ε_r*. The observed variability in dielectric characteristics between and within the substrates is attributable to their different physicochemical properties (Hilhorst 1998; Arulanandan 2003). Pumice is mainly composed of amorphous silicon dioxide and aluminum oxide, which are relatively poor in charged colloidal particles, so the dielectric behavior is predominantly governed by the solution that fills the pores. The fluid phase contains a small quantity of charges with high mobility, resulting in low C_p and high capacitive loss with low variance (due to the homogeneous, ground medium). The high C_p exhibited by arenosol is related to the greater amount of polarizable charges carried by the colloidal surfaces of the constituent clay minerals and organic substances (Singh and Uehara 1999). In this case, the moderate value of DF is indicative of the decreased conductivity (σ) caused by the reduced mobility of charge carriers owing to counterion adsorption and hydration shell formation (Grimnes and Martinsen 2015). Among the planting media used, chernozem has the highest percentage of colloidal clay and organic matter incorporated into diverse organo-mineral complexes (Brady and Weil 2007). The smaller C_p compared to arenosol is likely due to the reduced polarizability of the bound particles, whereas the lower rate of dielectric loss shows the more retarded charge migration. The diverse pool of clay minerals and organic compounds assembles into aggregates of various shapes and sizes, generating inhomogeneous structure and thus water distribution, which appears as the increased variance in detected DF. The aforementioned differences in substrate properties are clearly seen in their parallel electrical conductance (G), calculated from the measured C_p and DF values according to Eq. 4: conductance proved to be the smallest in pumice (1.22 mS; due to the low amount of movable charges), somewhat higher in chernozem (1.72 mS; large amount of ions but retarded migration) and much higher in arenosol (4.70 mS; high quantity of mobile charges). Parallel G for the plant–substrate systems was one or two orders of magnitude lower than that of the substrates, ranging from 5.68 μS to 0.25 mS depending on plant size, species and substrate type. Dalton's model assumes that electric current flows between the ground and the plant electrodes through the root system (radially in root cortex and axially along xylem vessels). However, a possible consequence of high soil conductivity is that current could flow preferentially through the soil instead of passing through the root tissues, as suggested by Dietrich et al.'s model. Therefore, further investigations are needed about the current path between the electrodes (particularly inside roots) in order to resolve the contradiction between the two models and maybe to interpret some former results.

The present observations on C_p and C_R are in accordance with the two-dielectric (series-connected root and soil dielectric) capacitor model. An accurate estimation of the root capacitance requires that the capacitance of the plant-growth medium is substantially higher than that of the root system (Rajkai et al. 2005; Dietrich et al. 2012, 2013). This criterion was met in the present experiments, as much higher capacitances were measured for the substrates (Fig. 3) than for the plant–substrate systems (Table 4), with a difference of more than an order of magnitude in some cases (depending on plant size). This confirmed that the C_R values were dominated by the plant tissue.

Effect of root traits and electrode placement on capacitance response

As roots comprise component materials with various ε_r (Ellis et al. 2013b), natural differences in root system properties between plants of the same species are also obviously responsible for the variable capacitance losses. Several chemical and structural features of roots are thought to, or have been observed to influence electrical behavior, including the following:

(i) Individual plants may differ in their root dry matter content in relation to the cell-wall fiber content and tissue density (ρ), which influence the capacitance response of the root system by affecting the preferential pathways (apoplastic or symplastic) of the electrical current (Dvořák et al. 1981; Aulen and Shipley 2012; Ellis et al. 2013a).

(ii) Root systems are complicated hierarchical structures with various distributions of root segments with different length, diameter, internal architecture and cell-wall chemical composition (which is associated with permeability). Root segments of different ages contain very different amounts and proportions of suberin and lignin in the endo- and exodermal cell walls (Hose et al. 2001). Lignin and suberin have lower permittivity (ε_r ~ 2–2.4) than the other main component materials of the root, such as water (ε_r ~ 80) and cellulose (ε_r ~ 7.6; Ellis et al. 2013b), so the variability in their quantity is likely to cause considerable variation in the dielectric properties of the root tissue. Capacitance behavior is strongly determined by the geometric properties (morphology and branching order; Fig. 1) of the roots as well (Dalton 1995; Cao et al. 2010).

(iii) Aulen and Shipley (2012) described the intra-individual root density effect: most species have a propensity for concentrating fine roots in a small soil volume, such as in nutrient- or water-rich microsites. Dense root clustering has an adverse influence on root–soil electrical contact, thus confounding the capacitance response.

(iv) Electrical signal loss is likely to increase with the distance the electrical current travels along the root "circuit". Therefore, the resulting capacitive loss is influenced by the relative distribution of RL at different distances from the root neck (Urban et al. 2011; Ellis et al. 2013a). This characteristic is related to root depth distribution, which may be variable within species.

(v) The majority of vascular plants form root associations with arbuscular mycorrhizal (AM) or ectomycorrhizal fungi. Mycorrhizae often result in changes in root morphology (*e.g.* absorptive area, root length density or root architecture), water and nutrient uptake rate and hydraulic conductivity (Bárzana et al. 2012), leading to marked changes in root electrical properties, including capacitive behavior (Cseresnyés et al. 2013b) and the real and imaginary parts of impedance spectra (Repo et al. 2014). The intensity and frequency of AM colonization exhibit substantial differences not only between plants of the same species, but even between different regions of the same root system (Füzy et al. 2015), contributing to the varied capacitive response.

In addition to the differences in root properties outlined above, the placement of the stem electrode may also be responsible for fluctuations in capacitive loss. Although the stem electrode was consistently fixed at the same height of 10 mm above the substrate surface, the electrodes may not have been equidistant from the root neck of the plants. Dietrich et al. (2012) showed a linear relationship between C_R and the reciprocal of the distance between the plant electrode and the surface of the rooting medium, which was that expected for capacitors connected in series along the root axis. For this reason, variability in the stem length included in the stem–root–substrate circuit induces further uncertainty in C_R measurements (Ellis et al. 2013b; Postic and Doussan 2016). Although several root traits having an influence on electrical measurements have been discussed, a true assessment of their contribution to the intraspecific variability in capacitive behavior will require detailed investigations on a root scale.

Proposals for field applications

Though several studies (Beem et al. 1998; Preston et al. 2004; Chlopek et al. 2006, 2010) demonstrate the relevance of the C_R method in the field, the measurement is only reliable under homogeneous soil conditions and soil water status. Data can be compared only when soil water contents are statistically equal around all plant root systems studied. During field application, it is advisable to perform C_R measurements simultaneously with the detection of soil moisture content (using a TDR meter) in the root zone. If the investigation covers a relatively large area, the soil electrical properties should be systematically measured. Variability in soil temperature is suggested to affect the measured data. Field conditions are expected to require a higher number of replicates to cover the greater heterogeneity of the plant population, but the rapid and simple capacitance method allows a large number of plants to be measured in a short time. It could be advantageous *e.g.* for plant breeders screening numerous plant genotypes from segregating populations. Repeated C_R measurements during plant ontogeny may improve the RSS estimation. Nevertheless, our results imply that the technique is better suited for use in pot experiments carried out in growth chambers and greenhouses or outdoors under semi-controlled conditions.

Conclusions

It was shown in this work that although direct measurements of root–soil capacitance were always significantly related to RSS, the predictive power was poor in some cases. The experimental results suggest the possible importance of the variable electrical impedance response due to the variable complex relative permittivity (ε_r*) in the root–substrate–electrode electrical network. By measuring relative dielectric losses (DF, associated with permittivity components) and applying a newly developed formula (Eq. 6), a better predictor, C_{DF}, was obtained to improve the reliability of RSS estimates. The transformation had greater significance when a well-structured soil was chosen for plant cultivation instead of a quasi-hydroponic medium or a coarse sand-based substrate. The capacitive behavior fluctuates even in pots containing mineral substrate or bulk soil and is expected to vary even more under real field conditions, *i.e.* in soils with well-developed horizons and great structural and spatial heterogeneity. The main advantage of our approach that DF can be displayed simultaneously with the magnitude of C_R using a precision LCR instrument. No relationship was found between factor α and the plant species or growth medium, but α was easy to calculate for any plant–substrate system and the same α value could be used for all RSS variables. The transformation of C_R into C_{DF} and knowledge of the parameters of C_{DF}–RSS regression models provided an improved prediction of root extension. In comparative studies, such as monitoring the root growth of plants subjected to different treatments or conditions, C_{DF} is expected to have lower variance within groups than C_R, which is undoubtedly advantageous for statistical discrimination.

However, although various models and improvements have been developed and investigated, the lack of accurate knowledge on the complex electrical circuit of the system still remains the main drawback to root capacitance measurement (Dietrich et al. 2012; Ellis et al. 2013b). The root–substrate–electrode continuum is described as a serial circuit represented by a heterogeneous medium composed of a large array of elements possessing resistance and capacitance variously associated and interfered (Ozier-Lafontaine and Bajazet 2005; Urban et al. 2011). Although the present study may not contribute greatly to a better understanding of the basic physics of the capacitance method, the findings could be of significance for more reliable root measurements under greenhouse conditions. This is important due to the strong influence of numerous external factors which complicate both the efficient use of the technique and the transferability of measurement data between sites and species. The capacitance method is rapid, labor-saving and nondestructive, so notwithstanding the problems influencing its reliability, it will certainly be of interest for future applications and further development.

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Appendix

Some mathematical formulae have been treated in a simplified form in order to stress the relevant points of our method. Each of our models M1 to M5 were defined by two equations simultaneously: the first one is a linear regression and the second one is a nonlinear correction formula for the electrical capacitance. We must admit that it is a little bit unusual way of model declaration. For the sake of correctness we provide here an example of a full equation consisting all the parameters, although a rather cumbersome equation is resulted by this rigorous formulation. The full equation of model M1:

$$L(\alpha, a, b) = \sum_{t=1}^{30} \sum_{p=1}^{6} \sum_{r=1}^{3} \sum_{s=1}^{3} \left(CR_{prs}(t) \cdot \left(\frac{DF_{ps}(t)}{DFmean_{ps}} \right)^{\alpha_{prs}} - a_{prs} - b_{prs} \cdot RSS_r(t) \right)^2$$

where L(α,a,b) is the quadratic loss function to be minimised in all parameters.

Table 1. Main physical and chemical properties of the plant growth substrates used in the experiments.

	Pumice	Arenosol	Chernozem
Sand/silt/clay content [%]	–	80.9/11.9/7.2	20.1/56.5/23.4
pH_{H2O}/pH_{KCl}	6.53/5.85	7.52/7.05	7.86/7.27
Cation exchange capacity (CEC) [mmol 100 g^{-1}]	2.20	8.39	11.71
Lime content [%]	0	0.29	4.09
Humus content [%]	0	1.18	4.18
Bulk density [g cm^{-3}]	0.92	1.55	1.37
N/P/K content [mg kg^{-1}][(a)]	70/0/179	730/438/222	1830/167/345
Field capacity [cm^3 cm^{-3}][(b)]	0.179	0.190	0.359
Dry mass per pot [g]	3360	5650	5000

[(a)] Total organic and mineral N content; ammonium lactate-acetate extractable P and K
[(b)] Determined with a pressure membrane apparatus at h = 20 kPa

Table 2. Overview of the applied models M1 to M5 described by constraints on parameters.

Model	Parameter		
	a	b	α
M1	none	none	none
M2	none	none	does not depend on type of RSS[a] variables
M3	does not depend on type of RSS variables	none	does not depend on type of RSS variables
M4	does not depend on type of RSS variables and plant species	none	does not depend on type of RSS variables
M5	does not depend on type of RSS variables, plant species and growing substrate	none	does not depend on type of RSS variables

[a] Root system size

Table 3. Summarizing table of standard and robust two-way ANOVA. Effect of plant species, substrate type and their interaction on the dissipation factor (DF) measured in plant–substrate systems.

Effect	Standard two-way ANOVA					Robust two-way ANOVA[a]	
	DegF	Sum Sq.	Mean Sq.	F	p	F	p
Plant	5	132.3	26.46	57.82	0	255.1	0
Substrate	2	4.11	2.05	4.49	0.0116	4.46	0.087
Plant:substrate	10	1.66	0.166	0.363	0.9618	3.39	0.770
Residuals	522	238.9	238.9				

[a] Robust two-way ANOVA for median by percentile bootstrap method (function pbad2way of R package WRS2, see technical details in Wilcox 2012, p. 201 and p. 351.)

Table 4. Minimum and maximum values of measured root–soil electrical capacitance (C_R in nanofarads, nF), root dry mass (RDM in g), root length (RL in m) and root surface area (RSA in cm^2) in various potting substrates (Su) and plant species (Sp: B – bean; C – cucumber; M – maize; S – soybean; T – tomato; W – wheat).

Su	Sp	C_R (nF)		RDM (g)		RL (m)		RSA (cm^2)	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Pumice	B	0.426	1.174	0.012	0.339	0.483	30.33	5.810	214.5
	C	0.383	1.882	0.009	0.477	0.745	73.88	7.653	637.6
	M	0.459	5.871	0.015	1.166	0.414	136.4	7.408	907.7
	S	0.387	1.610	0.022	1.837	0.886	142.2	12.11	1793
	T	0.401	2.688	0.003	0.490	0.423	33.38	3.451	543.1
	W	0.363	5.569	0.016	0.619	0.933	71.50	12.72	689.6
Arenosol	B	1.661	3.515	0.009	0.352	0.321	25.72	3.742	250.0
	C	1.616	4.971	0.017	0.589	1.021	54.30	12.70	763.9
	M	1.908	14.85	0.027	1.974	0.775	147.7	9.658	1253
	S	1.732	4.258	0.020	2.012	0.867	113.6	9.938	1313
	T	1.880	3.872	0.002	0.290	0.166	15.66	1.552	217.1
	W	1.805	10.60	0.009	0.867	0.378	78.03	3.955	840.5
Chernozem	B	1.528	3.292	0.030	0.489	2.295	43.80	18.67	391.6
	C	1.323	4.040	0.013	0.652	1.101	72.06	13.14	873.4
	M	1.783	12.10	0.029	1.835	1.035	201.6	6.718	1475
	S	1.435	4.458	0.019	1.896	0.931	141.5	13.35	1394
	T	1.574	4.270	0.006	0.448	0.473	75.38	4.578	674.7
	W	1.336	10.63	0.027	0.749	0.733	160.7	5.258	1521

Table 5. Estimated parameters of regression equations describing the relationship between measured root–soil electrical capacitance (C_R in nanofarads, nF) and the root dry mass (RDM in g), root length (RL in m) and root surface area (RSA in cm^2) in various potting substrates (Su) and plant species (Sp). See Table 4 for plant species symbols.

Su	Sp	RDM			RL			RSA		
		y-int.	Slope	R^2	*y*-int.	Slope	R^2	*y*-int.	Slope	R^2
Pumice	B	0.489	1.859	0.751	0.493	0.020	0.769	0.469	0.0027	0.799
	C	0.547	2.624	0.794	0.500	0.019	0.871	0.476	0.0021	0.870
	M	0.574	4.700	0.942	0.551	0.042	0.920	0.464	0.0057	0.941
	S	0.501	0.573	0.785	0.503	0.008	0.786	0.514	0.0007	0.753
	T	0.522	4.601	0.824	0.463	0.067	0.792	0.548	0.0042	0.773
	W	0.568	7.375	0.879	0.597	0.066	0.866	0.467	0.0072	0.897
Arenosol	B	2.112	3.208	0.635	2.132	0.042	0.612	2.125	0.0046	0.629
	C	2.172	3.869	0.688	2.143	0.044	0.726	2.203	0.0032	0.683
	M	2.192	5.808	0.875	2.197	0.076	0.822	2.099	0.0094	0.889
	S	2.156	1.054	0.724	2.189	0.016	0.687	2.178	0.0015	0.712
	T	2.086	5.961	0.751	2.107	0.102	0.734	2.123	0.0070	0.738
	W	2.048	8.614	0.812	2.108	0.093	0.711	2.187	0.0093	0.733
Chernozem	B	1.651	2.228	0.451	1.660	0.027	0.469	1.693	0.0027	0.447
	C	1.625	3.035	0.627	1.643	0.031	0.685	1.783	0.0022	0.523
	M	1.704	4.534	0.830	1.729	0.045	0.801	1.685	0.0057	0.757
	S	1.718	1.238	0.592	1.757	0.017	0.536	1.736	0.0016	0.562
	T	1.582	5.021	0.498	1.662	0.037	0.541	1.647	0.0037	0.508
	W	1.759	11.096	0.809	1.716	0.053	0.722	1.691	0.0055	0.721

Table 6. Estimated parameters and R-squares of model M1. See Table 4 for symbols.

Su	Sp	RDM				RL				RSA			
		y-int.	Slope	α	R^2	*y*-int.	Slope	α	R^2	*y*-int.	Slope	α	R^2
Pumice	B	0.505	1.566	0.67	0.946	0.514	0.017	0.61	0.909	0.495	0.0023	0.56	0.914
	C	0.492	2.892	0.89	0.938	0.480	0.020	0.56	0.929	0.454	0.0022	0.56	0.926
	M	0.574	4.460	0.57	0.972	0.817	0.040	0.73	0.970	0.484	0.0054	0.39	0.952
	S	0.500	0.531	0.99	0.932	0.509	0.007	0.89	0.895	0.513	0.0007	0.99	0.890
	T	0.562	3.531	1.68	0.916	0.509	0.052	1.63	0.866	0.567	0.0032	1.72	0.866
	W	0.516	7.551	0.96	0.951	0.553	0.067	0.90	0.929	0.340	0.0072	0.76	0.941
Arenosol	B	2.106	2.932	0.46	0.877	2.125	0.038	0.46	0.838	2.119	0.0042	0.45	0.862
	C	2.056	4.102	0.65	0.903	2.061	0.045	0.59	0.903	2.138	0.0032	0.55	0.828
	M	2.327	5.175	0.76	0.954	2.241	0.068	0.81	0.899	2.202	0.0084	0.62	0.931
	S	2.149	0.972	0.66	0.901	2.192	0.014	0.63	0.818	2.184	0.0013	0.62	0.842
	T	2.053	5.936	0.48	0.902	2.079	0.101	0.47	0.871	2.097	0.0069	0.46	0.867
	W	2.013	8.036	0.68	0.934	1.938	0.089	0.84	0.883	2.092	0.0087	0.77	0.863
Chernozem	B	1.507	2.634	0.44	0.753	1.565	0.030	0.39	0.696	1.543	0.0034	0.44	0.703
	C	1.425	3.561	0.70	0.892	1.566	0.032	0.50	0.815	1.558	0.0028	0.66	0.835
	M	1.625	4.335	0.73	0.922	1.687	0.042	0.67	0.871	1.546	0.0055	0.81	0.867
	S	1.685	1.172	0.98	0.813	1.667	0.018	1.09	0.843	1.688	0.0015	1.01	0.800
	T	1.468	5.317	0.85	0.675	1.658	0.034	0.58	0.577	1.636	0.0035	0.62	0.551
	W	1.651	10.600	0.60	0.936	1.591	0.051	0.63	0.834	1.527	0.0053	0.67	0.859

Table 7. Summarizing table of three-way ANOVA. Effect of plant species, substrate type and root system size (RSS) variable on the 54 y-intercept and slope parameters estimated by model M1 and listed in Table 6.

Effect	DegF	y-intercept				Slope			
		Sum Sq.	Mean Sq.	F	p	Sum Sq.	Mean Sq.	F	p
Plant	5	0.14	0.030	5.72	0.0004	36.57	7.31	3.99	0.0045
Substrate	2	23.88	11.94	2366.4	0	1.79	0.89	0.488	0.6170
RSS variable	2	0.011	0.006	1.137	0.3332	207.7	103.9	56.78	6.49×10^{-13}
Residuals	44	0.222	0.005			80.47	1.83		

Table 8. Summarized statistics for models M1 to M5. Residual sum of squares (SSQ_{Res}), residual degrees of freedom (ResDegF), number of free model parameters (NP), values of Akaike Information Criterion (AIC), the finite sample size corrected AIC (AICc), the Akaike Weights, the Evidence Ratios and Log10 of Evidence Ratios.

Model	SSQ_{Res}	ResDegF	NP	AIC	AICc	Akaike weight	Evidence Ratio	Log10 EviRatio
M1	349.5	1457	162	-2160.4	-2124.1	1.835×10^{-36}	5.449×10^{35}	35.43
M2	354.1	1493	126	-2211.5	-2190.1	3.910×10^{-22}	2.558×10^{21}	21.41
M3	355.4	1529	90	-2277.5	-2266.8	1.742×10^{-5}	5.740×10^{4}	4.76
M4	357.9	1544	75	-2296.1	-2288.7	0.999	1.000	0.00
M5	654.8	1546	73	-1321.4	-1314.4	2.742×10^{-212}	3.674×10^{211}	211.5

Table 9. Estimated parameters and R-squares of the best model (model M4). See Table 4 for symbols.

Su	*y*-int.	Sp	RDM		RL		RSA		α
			Slope	R^2	Slope	R^2	Slope	R^2	
Pumice	0.529	B	1.456	0.946	0.016	0.907	0.0020	0.906	0.64
		C	2.728	0.925	0.018	0.928	0.0020	0.925	0.64
		M	4.256	0.972	0.044	0.968	0.0053	0.947	0.57
		S	0.508	0.932	0.007	0.893	0.0006	0.890	0.53
		T	3.661	0.916	0.051	0.865	0.0034	0.868	1.66
		W	7.506	0.950	0.068	0.929	0.0068	0.940	0.87
Arenosol	2.129	B	2.824	0.877	0.038	0.834	0.0041	0.862	0.46
		C	3.910	0.900	0.043	0.903	0.0032	0.827	0.59
		M	5.365	0.954	0.070	0.890	0.0084	0.927	0.73
		S	0.991	0.901	0.015	0.818	0.0014	0.841	0.63
		T	5.508	0.901	0.096	0.871	0.0066	0.867	0.47
		W	7.768	0.931	0.085	0.882	0.0087	0.863	0.76
Chernozem	1.600	B	2.348	0.748	0.029	0.688	0.0031	0.698	0.41
		C	3.135	0.882	0.031	0.813	0.0027	0.829	0.60
		M	4.353	0.922	0.043	0.869	0.0055	0.866	0.74
		S	1.244	0.812	0.018	0.841	0.0016	0.799	1.03
		T	4.841	0.662	0.036	0.571	0.0036	0.549	0.68
		W	10.681	0.936	0.051	0.844	0.0053	0.859	0.63

Fig. 1a. Schematic representation of root electrical capacitance measurement. AC – alternating current. **1b.** Equivalent electrical network of the root system, according to Dalton's (1995) conceptual model. Each element (i) consists of a parallel R_i–C_i (resistor–capacitor) circuit. **1c.** Representation of plant root as a cylindrical capacitor (Ellis *et al*. 2013a,b) with the equation for the electrical capacitance, in which, ε and A are the permittivity and surface area of root tissue, respectively, r_1 is the radius of the xylem and r_2 is that of the rhizodermis.

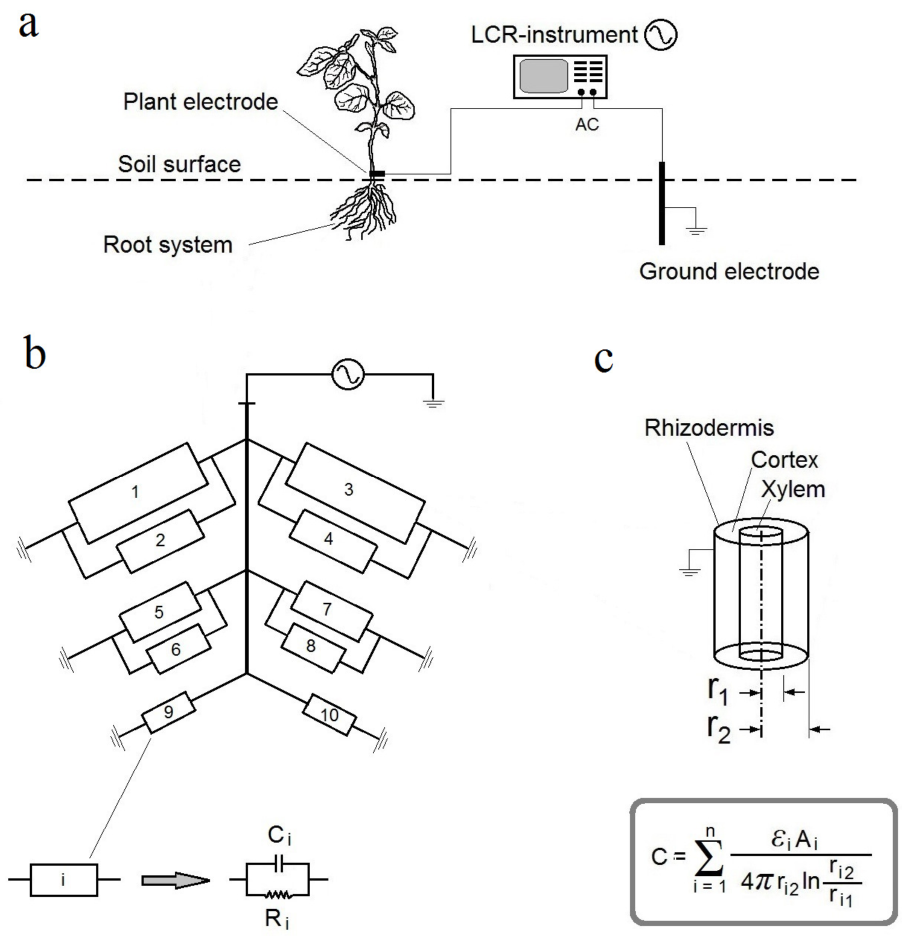

Fig. 2. Schematic representation of complex relative permittivity (ε_r*) by the components of real (ε_r') and imaginary (ε_r") parts and loss angle (δ), and the expression of complex electrical capacitance (C*) in a parallel equivalent circuit. In the equation, ε_0 is the permittivity of free space, A and d indicate the area and distance of the capacitor plates, respectively.

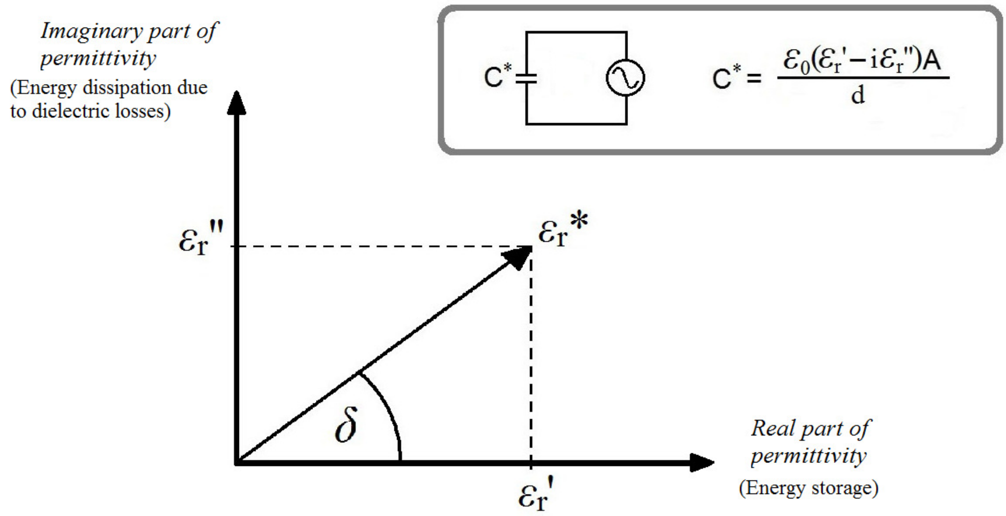

Fig. 3. Mean and standard deviation (n = 30) of electrical capacitance (C_p in nanofarads, nF) and dissipation factor (DF) measured for different substrates between two ground electrodes at 1 kHz current frequency. ANOVA showed highly significant differences ($p < 0.001$) among substrates for C_p and DF.

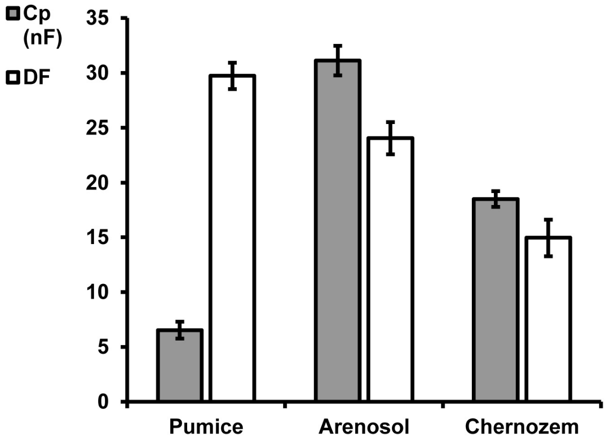

Fig. 4. Mean and standard deviation (n = 30) of dissipation factor (DF) measured in plant–substrate systems for different plant species and different substrate types at 1 kHz current frequency. Robust two-way ANOVA revealed a highly significant effect of the plant species on DF ($p < 0.001$) and a non-significant effect of the substrate type ($p = 0.087$) and the plant:substrate interaction ($p = 0.770$).

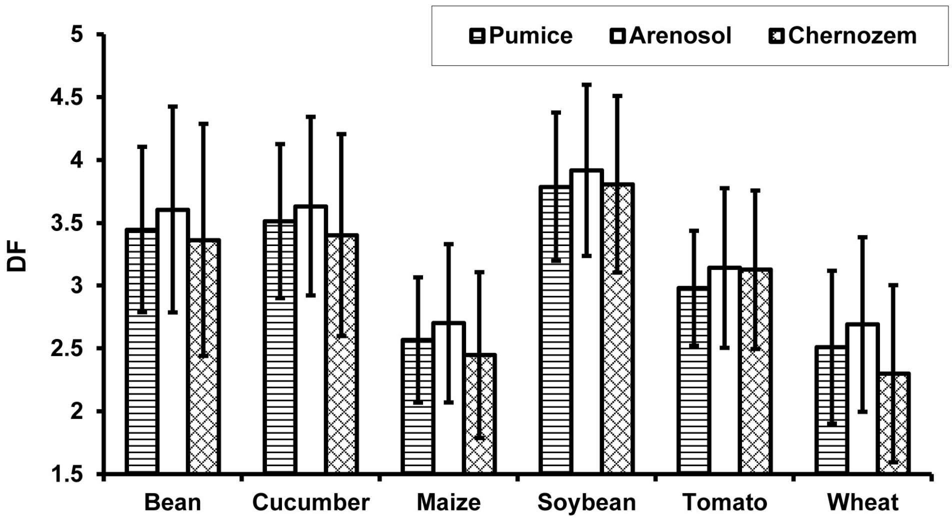

Fig. 5. Improvement of the root mass estimation by the proposed formula for bean of different ages grown in different substrates. C_R – Electrical capacitance of the root–soil system; C_{DF} – Electrical capacitance of the root–soil system corrected with dissipation factor

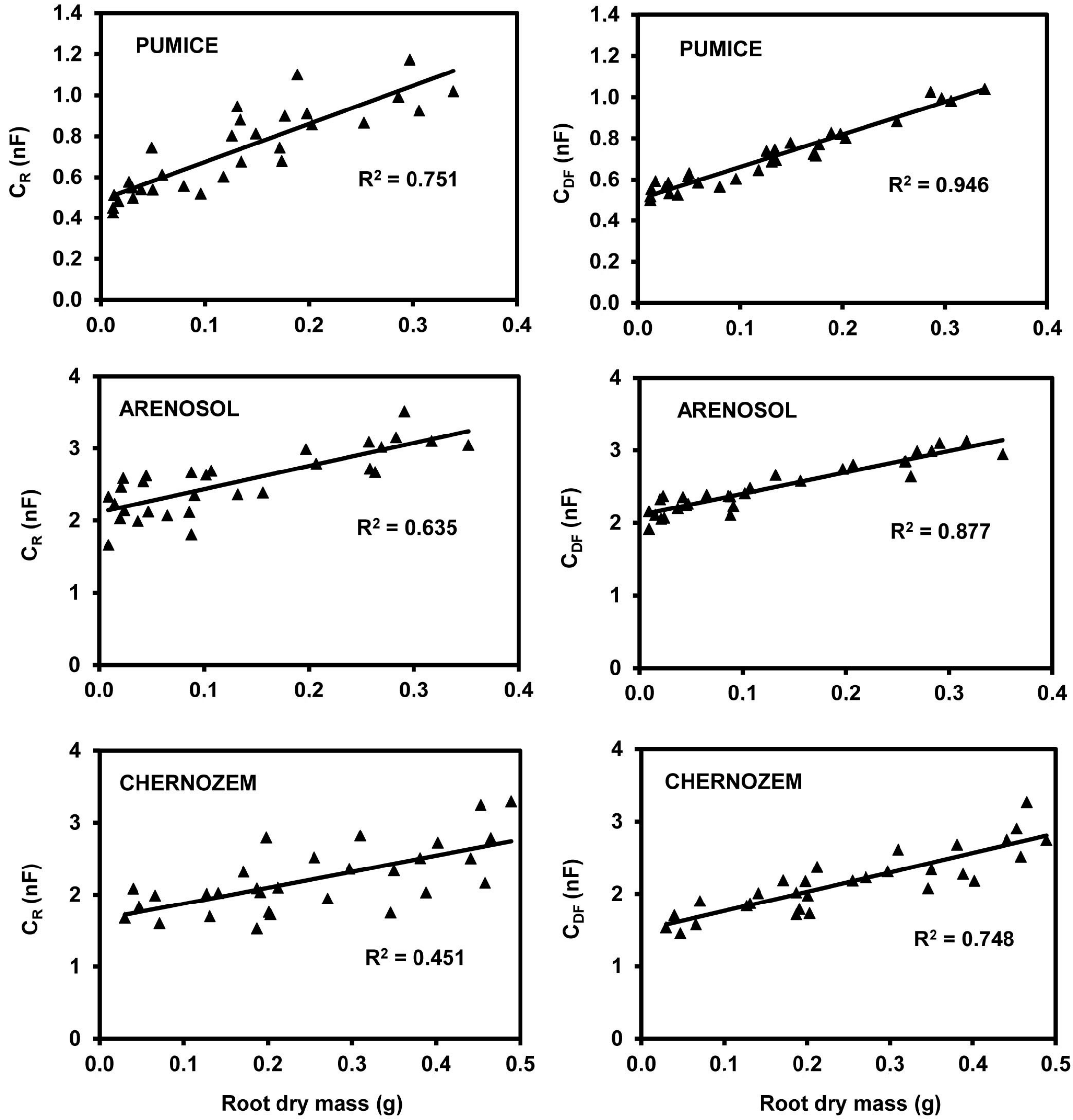

Fig 6. Improvement of the root mass estimation by the proposed formula for wheat of different ages grown in different substrates. See Fig. 5 for symbols.