Parameter sensitivity analysis of a root system architecture model based on virtual field sampling

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Abstract

Aims

Traits of the plant root system architecture (RSA) play a key role in crop performance. Therefore, architectural root traits are becoming increasingly important in plant phenotyping. In this study, we use a mathematical model to investigate the sensitivity of characteristic root system measures, obtained from different classical field root sampling schemes, to RSA parameters.

Methods

Root systems of wheat and maize were simulated and sampled virtually to mimic real field experiments using the root system architecture (RSA) model CRootBox. By means of a sensitivity analysis, we found RSA parameters that significantly influenced the virtual field sampling results. To identify correlations between sensitivities, we carried out a principal component analysis.

Results

We found that the parameters of zero order roots are the most sensitive, and parameters of higher order roots are less sensitive. Moreover, different characteristic root system measures showed different sensitivity to RSA parameters. RSA parameters that could be derived independently from different types of field observations were identified.

Conclusions

Selection of characteristic root system measures and parameters is essential to reduce the problem of parameter equifinality in inverse modeling with multi-parameter models and is an important step in the characterization of root traits from field observations.

Introduction

Agriculture faces the challenge of sustaining an increasing global food demand under increasingly limited resources such as water and nutrients. Since roots are the organs that plants use to acquire these resources, understanding plant root architectures and their interaction with the soil is particularly important.

Depending on the environmental conditions, different types of root systems are beneficial for the uptake of resources from soil. Regarding water uptake, shallow root systems are proposed to be beneficial in environments where water uptake is driven by rainfall supply while deep root systems are beneficial in environments where plants rely on water stored in deeper soil layers (Bodner et al. 2015; Wasson et al. 2014). Knowing the role of root traits or root architectural parameters for water and nutrient uptake is important for selecting optimal varieties in water and nutrient limited environments (Gregory 2006; Lynch 1995). Yet, the lack of phenotypic data of different genotypes is the main challenge in plant breeding programs (Kuijken et al. 2015). Furthermore, until recent years, most

breeding programs have focused on the shoot of plants, whereas the root system has been given less attention (Bardgett et al. 2014).

Root architectural models reconstruct representations of the root system of a plant based on its pre-defined root architectural parameters (Dunbabin et al. 2013). The root system architecture (RSA) represents the arrangement of primary, secondary and higher order root segments and how they are connected to each other. RSA varies from plant to plant and species to species depending on the genotype and soil environmental conditions (Comas et al. 2013). The parameters of these models can be considered to represent root traits or characteristics of the RSA of a certain plant in a certain soil environment. Lab based methods are widely used for determining root architectural parameters (Hamada et al. 2012; Kalogiros et al. 2016; Leitner et al. 2014) but also examples exist that used root systems that were carefully excavated from the soil (Kutschera 1960; Weaver et al. 1924). Using manual or automatized image processing, digitized root systems are obtained (Lobet et al. 2015), from which root system parameters can be derived directly (Bodner et al. 2017; Bucksch et al. 2014; Chen et al. 2017; Clark et al. 2011; Landl et al. 2018).

Next to 'static' structural parameters, RSA models also describe the dynamic root development and use parameters that represent root elongation rates. To estimate these parameters, dynamic root data are required (Pagès and Picon-Cochard 2014; Pagès et al. 2013). However, conditions in labs or greenhouses that have an important impact on plant and root growth may differ considerably from real field conditions such as precipitation, radiation, wind patterns, soil structure and soil depth (Gregory 2009; Leitner et al. 2010). Moreover, plant and root growth in small pots is not representative of root systems of large and/or mature plants. Traits of root systems at mature stage may differ considerably from traits observed during the early stages of plant development and are therefore difficult to predict from the early stage traits (de Dorlodot et al. 2007). The variables observed with different methods in the lab or greenhouse and the RSA parameters that are derived from these observations are therefore not directly transferable to plants grown under field conditions.

Studying root systems in the field is a difficult task due to a lack of direct access to the root system. It is impossible to get information about an entire root system without disturbing its original morphology because it is completely hidden beneath the soil surface. Several root sampling methods were developed to gain information about root system dynamics in the field. These methods are generally time consuming and labor intensive. Classical field methods are root intersection counting in vertical profile walls of a trench, soil coring, and non-destructive minirhizotron methods (Leitner et al. 2010). Soil coring is a widely used field sampling method to obtain the distribution of root length density with depth (Schroth and Kolbe 1994). The trench profile method is suitable for studying root distributions in 2-D. The main disadvantage of the trench profile method over core sampling is that the root biomass cannot be measured. The trench profile method has some difficulties when counting roots in deep layers and it may be difficult to distinguish between dead and living roots (Azevedo et al. 2011). Soil coring or trench profile methods do not provide direct information about the branching of a root system, but provide only the observed RLD or intersection counts. Although root counts and root length densities are correlated with each other, their relation depends on the orientation of the roots

with respect to the intersection surface. The orientation of the roots may change with depth and depends on the plant/crop type. Using RSA models, depth and plant specific calibrations could be derived that relate the two types of observation (Grabarnik et al. 1998; Vansteenkiste et al. 2014). Minirhizotron methods are useful to study the root growth at different times and depths at the same position without destroying the root system (Smit et al. 2000). Although minirhizotrons provide root arrival curves at specific depths in the soil profile, rhizotubes could act as obstacles to root growth and the observations can be altered slightly due to influence of rhizotubes installations (Garré et al. 2012).

However, those sampling techniques do not provide direct information about the RSA model parameters or of specific root traits of different phenotypes (Wasson et al. 2012). Root architectural models can be helpful to interpret data obtained from cost intensive field experiments. Techniques like parameter fitting or inverse modeling could be applied to derive RSA model parameters from field observations (Garré et al. 2012). In order to reconstruct realistic RSA, RSA models typically rely on a large number of parameters. The inverse estimation of a large number of parameters from indirect information may be hampered by equifinality, i.e., when several sets of parameter combinations lead to the same or very similar predictions of the observed variables. Equifinality may occur when model predictions of the data that are collected are not sensitive to changes of a certain parameter, or when changes in model predictions due to a change in one parameter can be compensated by changes of other parameters.

The main objective of this work is to define the sensitivity of different types of observations, which can be derived from field observations of root systems, to RSA parameters of the CRootBox model. This analysis identifies sensitive parameters for different observation types and gives insight in which observation types provide information about which parameters. This is important to design field sampling methods. Maize and wheat root systems will be considered as example crops. Root length densities (RLD) derived from soil cores, root impact counts (RIC) derived from vertical profile walls, and temporal distributions of root counts in horizontal rhizotubes that represent root arrival curves (RAC) will be considered as field observations.

In the material and methods part of the paper, we present the RSA model, how ‘virtual’ observations were derived from simulations with the RSA model, how the sensitivity analyses were carried out and how we interpret the sensitivities using principal component analysis (PCA). In the results section, we first present the simulated or ‘virtual’ observations and analyze the impact of the sample size on the uncertainty about the observations. Secondly, we present and discuss the sensitivity of different observations to the RSA parameters including PCA results.

Materials and Methods

Simulation of root systems: Despite the fact that virtual experiments are not limited to a certain crop type, we selected winter wheat and maize as crop types for virtual simulations and sampling due to their

importance as agricultural crops and the availability of root architectural parameters for these crops. We explicitly simulated the 3D root architectures of wheat and maize root systems over a vegetation period in a virtual field plot using the generic RSA model CRootBox (Schnepf et al. 2018b). CRootBox is a C++ version of the RootBox model (Leitner et al. 2010) which requires less computational time and can therefore be used to simulate multiple root systems over longer periods.

Fig. 1 Nomenclature of root architectural parameters used in CRootBox model to simulate root systems of wheat and maize crops. Zero order roots include all crown roots, seminal roots, brace roots and primary roots (adapted from ISRR nomenclature).

Fig. 1 illustrates the different RSA parameters used as input parameters of CRootBox, including basal (lb) and apical (la) zone lengths, branching distances (ln), and branching angles (theta). Numbers indicate root orders. Our simulated root systems consist of zero order roots and only two orders of branches. The branching angle represents the angle between a branch and its parent branch. For zero order roots, the branching angle theta0 represents the angle of the proximal root segment to the vertical axis. The radial angle is assumed to be random and uniformly distributed between 0 and 2π. Next to geometrical parameters, root elongation rates (r) and tropisms (tr) (in this study, we only considered gravitropism which defines to which extent the direction of the growth of a root tip changes due to gravity) have to be defined. Root diameters were not considered for simulations. For zero order and first order roots, the growth rate is a function of the root length and becomes zero when the root reaches a maximal length (maxl) (see (Schnepf et al. 2018b) for the implementation of gravitropism and growth rates as a function of root length). In order to reproduce the random nature of the root system, RSA parameters are stochastic parameters with a mean and a standard deviation (see (Leitner et al. 2010;

Schnepf et al. 2018b) for the implementation of the random RSA development). A complete list of CRootBox root architectural parameters is given in Table 1.

Table 1 List of root architectural parameters of wheat and maize used in root system simulations and sensitivity analyses (Schnepf 2017). Except the number of zero-order roots NB, each parameter is a stochastic parameter with a mean and a standard deviation (values inside the brackets indicate the standard deviations of the parameters).

No	Code	Root order	Name	Unit	Parameter values	
					wheat	Maize
1	lb0(std)	0	length of basal zone	cm	0.8 (1.2)	0.1 (0.1)
2	la0(std)	0	length of apical zone	cm	4.2 (6.4)	18 (1.8)
3	ln0(std)	0	branch spacing	cm	1.2 (0.5)	0.6 (0.06)
4	maxl0(std)	0	maximum length	cm	130 (30)	110 (30)
5	r0(std)	0	initial growth rate	cm/day	1.2 (0.6)	2.6 (0.6)
6	tr0(std)	0	tropism		1.2 (0.2)	1.0 (0.6)
7	theta0(std)	0	branching angle	rad	1.4 (0.2)	1.0 (0.5)
8	lb2(std)	1	length of basal zone	cm	0.8 (1)	0.2 (0.04)
9	la2(std)	1	length of apical zone	cm	1.8 (2.4)	0.4 (0.04)
10	ln2(std)	1	branch spacing	cm	1.0 (1.5)	0.4 (0.03)
11	maxl2(std)	1	maximum length	cm	2.0 (1.0)	2.6 (1.6)
12	r2(std)	1	initial growth rate	cm/day	0.4 (0.12)	2.0 (0.2)
13	tr2(std)	1	tropism		1.0 (0.4)	1.0 (0.3)
14	theta2(std)	1	branching angle	rad	1.2 (0.4)	1.2 (0.25)
15	la3(std)	2	length of apical zone	cm	2.2 (0.4)	0.5 (0.2)
16	r3(std)	2	initial growth rate	cm/day	1.0 (0.2)	2.0 (0.2)
17	tr3(std)	2	tropism		0.1 (0.6)	1.5 (0.35)
18	theta3(std)	2	branching angle	rad	1.12 (0.4)	1.2 (0.2)
19	NB	0	number of zero order roots	nos	20	30

We defined primary roots, seminal roots and crown roots as zero order roots in the simulations. First order roots emerge from the zero order roots and second order roots emerge from the first order roots. We assumed that zero order roots emerge all together, next to the seed at the beginning of the growing season. Lateral roots emerge from their parent branch only after the apical zone has reached a defined length.

A winter wheat (*Triticum aestivum*) root system typically consists of 10-30 zero order roots and a secondary fibrous root system with three orders of branching (Wasson et al. 2012). The root density of wheat is relatively homogeneous in the horizontal direction due to the high planting density (Couvreur et al. 2014). For some varieties of wheat and depending on the soil and weather conditions, roots can reach up to 170 cm. The root elongation rate is typically in the order of 1-1.5 cm/day (Colombi et al. 2017). The highest number of wheat roots are observed within the first 40 cm depth (Hoad et al. 2004). Maize (*Zea mays*) is a row crop and thus

field-measured root length density of maize varies in the horizontal direction perpendicular to the rows. The root system of maize consists of primary, lateral and shoot-borne roots and the elongation rates vary from 1-4 cm/day. Maize roots can reach up to 150 cm depth with lateral spreading of 120 cm, depending on soil texture (Feldman 1994; Hochholdinger and Tuberosa 2009). Root architecture parameters were generally derived from experimental data during the early stages of plants grown in laboratories under controlled environments but are not necessarily representative for older plants or for field conditions. Therefore, we followed a trial and error approach to derive parameters that are representative for plants grown under field conditions. We systematically changed some parameters (elongation rate (r_0), maximum length ($maxl_0$) and numbers (NB) of zero order roots such that resulting field-scale values of root length densities were realistic compared to known literature values. Table 1 shows the values of root architectural parameters of wheat and maize, used as input parameters in the root growth model. The parameter bounds for the sensitivity analysis were selected as 50% and 150% of the parameter value as the lower and the upper bounds, respectively.

Two different plant configurations were considered for the two crops. Plant density was determined according to common practices applied in Europe (Kolb et al. 2017). The inter row distance for wheat was 12 cm with 3 cm plant spacing within a row. 224 wheat plant root systems were simulated in a plot of 96 by 84 cm consisting of 8 rows with 28 plants in a row. Sowing density of maize is lower than that of wheat. The distance between the maize rows was 75 cm and the plant spacing in a row was 13 cm. 108 maize plants were simulated in a plot of 450 by 234 cm consisting of 6 rows with 18 maize plants in each row. The depth of the soil domains was 160 cm. Seeding depths were chosen as 3 cm and 5 cm for wheat and maize, respectively. The total life spans of wheat and maize plants were set to 240 and 180 days respectively. Table 2 shows the plant configurations and plot sizes of each crop type. Simulation results were saved as VTK files and Paraview (Ayachit 2015) was used to visualize the results.

Table 2 Virtual field simulation setup: plant seeding positions, plot size and plant density configuration of wheat and maize.

plant configuration	Wheat	Maize
Plot size (width*length*depth)	96 cm*84 cm*160 cm	450 cm*234 cm*160 cm
Number of rows	8	6
Number of plants in a row	28	18
Plant distance in a row	3 cm	13 cm
Distance between two rows	12 cm	75 cm
Total number of plants	224	108

Virtual field sampling: Classical field sampling schemes; soil coring, trench profiles and minirhizotron observations were implemented in the virtual field plots to obtain the information about root distributions (Fig. 2). We used signed distance functions to represent the geometries of rhizotubes, soil cores and trenches, and made use of the CRootBox feature that allows to explicitly represent obstacles for root growth

(rhizotubes). Table 3 shows the sampling methods, sampling size and sampling locations of virtual wheat and maize plots.

Fig. 2 Winter wheat plant positions, plant configuration, plot size and sampling schemes of virtual cores, trench profile and minirhizotron methods (maize sampling methods are similar to wheat except the sampling positions and the sampling size). 3D view of the wheat sampling schemes (A), aerial view of coring and trench profiles (B), vertical profile of a grid of trench root counting method (C).

Table 3 Description of sampling methods: number and sizes of cores, trenches and rhizotubes, used in virtual sampling schemes.

Sampling method		
	wheat	Maize
Coring ¹	15 (radius=2.1cm, depth=160 cm)	16 (radius=2.1cm, depth=160 cm)
Trenches ²	9 (width=24 cm, depth=160cm)	9 (width=150 cm, depth=160cm)

	grid size= 3*5 cm	grid size= 5*5 cm
Minirhizotron ³	6 tubes (radius = 3.2 cm)	6 tubes (radius = 3.2 cm)
	depths 10,20,40,60,80,120 cm	depths 10,20,40,60,80,120 cm
	20 images per tube	20 images per tube
	image size=1.65*2.35 cm	image size=1.65*2.35 cm

¹soil core sampling based on (Wasson et al. 2014).

²trench root counting method based on (Vansteenkiste et al. 2014).

³rhizotube setup according to Selhausen minirhizotron facility (Cai et al. 2016)

Virtual soil coring: Cylindrical cores of 4.2 cm diameter and 160 cm length were sampled and subsequently sliced horizontally in 5 cm intervals to determine the RLD of each sampling volume (69.72 cm³). We adjusted the sampling size similar to real field sampling schemes. For wheat we chose 3 locations in-between rows for 5 different rows, and for maize, we took 16 virtual cores with core positions in-between two plants within a row (Fig. 2). To avoid boundary effects, zones of 20 cm from the borders of the plot for wheat and 75 cm for maize were not considered for sampling. Core sampling was performed with monthly time intervals for 8 months for wheat and 6 months for maize. In order to account for the stochasticity of the model, this sampling procedure was repeated in 100 simulated field plots for each crop type, resulting in a total number of 1500 soil cores for wheat and 1600 for maize.

Estimation of sample size for root studies: In order to study the effect of sample size on the mean root length distribution with depth and to select a representative sample size for the root studies, we calculated the mean and standard deviation of RLD profiles that are based on a sample size of 1500 cores. With this sampling scheme for virtual coring, we are already at the upper limit of what is possible in real field experiments in terms of number of cores sampled per plot. As a measure of how good the sample mean root length density is compared to the “true” mean root length density, we use the relative standard error (RSE) for different sample sizes n with the sample size. We chose 10% of relative standard error (RSE) of the mean as the criterion to determine the number of required cores. The RSE was calculated in two ways. The first method, RSE1, used the theoretical relation between the standard error of the estimate of the mean, the number samples n , and the standard deviation of the samples, std . The second method, RSE2, calculated the standard deviations of sets of means, std_mean , that were obtained using different sample numbers.

$$RSE1 = \left(\frac{\left(\frac{std}{mean} \right) 100}{\sqrt{n}} \right) \quad \& \quad RSE2 = \left(\frac{std\_mean}{mean} \right) 100 \quad (1)$$

A smaller RSE requires a larger number of samples. Under field conditions, it may be necessary to find a compromise between the required precision and the number of samples that are economically feasible in practice.

Virtual trenching: Nine trenches 24 cm wide and 160 cm deep for wheat, and 150 cm wide and 160 cm deep for maize (TR1-9) were virtually constructed perpendicular to the rows (Fig. 3) and in each trench two profiles of root intersections below a single plant row were obtained. Similar to core sampling, trenches were positioned in the center of the plots to avoid boundary effects. For the wheat profiles, root intersections were counted in 3 by 5 cm grid cells and in the maize profiles in 5 by 5 cm grid cells (see Fig. 3 for the spatial arrangement of the grid cells with respect to the plant rows). The root system simulations and corresponding virtual samplings were repeated for 100 simulated field plots so that for each grid cell in a profile, a dataset of 1800 root intersection counts (all orders of roots intersections) was obtained from which a mean and standard deviation was calculated. Finally, maps of the mean intersection counts in the grid cells that illustrate the spatial root distribution in the profiles were made at the end of the growing period.

Fig. 3 Setup of the vertical trench profiles and the grid cells in which root intersections were counted for wheat (left) and maize (right).

Virtual minirhizotron

To account for the influence of rhizotubes, which act as obstacles to root growth, on the root distributions, root growth simulations were repeated for a setup with horizontally installed rhizotubes. To simulate the growth of roots around the rhizotubes or other obstacles, CRootBox uses signed distance functions to divert the growth direction of a root tip when it meets an obstacle (Schnepf et al. 2018b). The arrangement of the plants in minirhizotron simulations was identical to the one for the soil coring or trench profile methods.

The configuration of the rhizotube locations and the observations in the rhizotubes were similar to those at the minirhizotron facility in Selhausen (Germany) (see (Cai et al. 2016) for detailed information). At the Selhausen test site, rhizotubes of 6.4 cm outer diameter were installed horizontally at depths of 10, 20, 40, 60, 80 and 120 cm (Cai et al. 2016) and perpendicular to the plant rows. In order to avoid an impact of overlying rhizotubes on root observations in deeper rhizotubes, rhizotubes at different depths were 10 cm shifted horizontally with respect to each other (see Fig. 2). Root observations in a rhizotube were made by a camera that was positioned

at 10 different locations in the tube where 2 images were taken from both sides of the tube by turning the camera 80° clockwise and 80° counterclockwise with respect to the vertical. The size of the minirhizotron image was set according to the camera configuration of the Bartz system (VSI /Bartz Technology Corporation), which is 1.65 cm wide and 2.35 cm long along the arc circle of the rhizotube (Fig. 4) for the considered rhizotubes.

Fig. 4 Schematic cross section of a rhizotube installed in Selhausen test site and tube locations of the images used in the simulation study.

For the wheat and maize plots, images were taken from respectively a 50 cm and a 100 cm long stretch. Sampling positions were chosen within four inner rows of a plot to minimize the boundary effects. Images were taken weekly over a period of 240 and 180 days for wheat and maize respectively. Unlike coring or trench profile methods, roots are not directly sampled in the minirhizotron method. The camera can capture only the roots which are close to the rhizotubes but it is a challenging task to determine the viewing depth of the camera which varies with soil type and texture. According to our experience, we assumed that roots within 1 mm from the tube were seen in the images and the length of the root segments in the image was divided by the area of the image to derive the RLD (note that the RLD obtained from rhizotube images has a different unit (cm cm^{-2}) than the RLDs derived from soil coring (cm cm^{-3}) and can therefore not be compared directly). For each observation time and tube depth, a RLD was calculated from 2000 images (20 images in a tube for 100 field plots) and the time series of RLDs at a given depth represents the root arrival curve (RAC) at that depth.

Sensitivity analysis

As in (Schnepf et al. 2018a), we explicitly distinguish between the “model input parameters,” from which we compute the 3D root architecture using CRootBox, and “characteristic root system measures”, which we compute from the resulting 3D root architecture. The former corresponds to individual root traits which could be of interest in e.g. phenotyping. The latter corresponds to the aggregated information as it can be obtained from root field measurements. How these change with the parameters of the RSA model was evaluated in a sensitivity

analysis. From the simulated observations, a number of characteristics were derived. How these characteristics change with the parameters of the RSA model was evaluated in a sensitivity analysis. The characteristics that we considered for the sensitivity analysis are listed in Table 4. Since coring and trenching methods are destructive in real field sampling, we selected the results of last time step (240 and 180 days for wheat and maize respectively) for the sensitivity analysis. However, weekly time steps (total 32 measurements for wheat and 24 measurements for maize) were taken for the minirhizotron method.

Virtual soil coring provided us with the RLD profiles. From those profiles, we derived the following additional characteristic root system measures: D99, RMc, and Tc in the sensitivity analyses. The RLDs were calculated by averaging the root length densities in the 1500 (1600 for Maize) simulated soil cores for each of the 32 5-cm core segments. D99 represents the depth above which 99% of the total root length was observed. RMc is the maximum of the average RLD profile. Tc is the total root length per surface area and was obtained by integrating the RLD over depth (see Table 4 for the descriptions and units of the root system measures).

Table 4 Description of characteristic root system measures used in sensitivity analysis along with respective sampling methods.

Sampling method	Function	units	Description
Coring	RLD	cm cm ⁻³	vertical profiles of mean root length densities in 5cm thick slices of soil cores up to 160 cm depth
	D99	cm	the depth above which 99% of the total root length is observed
	RMc	cm cm ⁻³	maximum root length density in the RLD profiles.
	Tc	cm cm ⁻²	total root length per surface area
Trench	RX	counts cm ⁻²	root count density in 5 cm wide vertical transects of the trench profiles
	Rz	counts cm ⁻²	root count density in 5 cm thick horizontal transects of the trench profiles
	Tt	counts cm ⁻²	average root count density in the trench profiles
Minirhizotron	At	d ⁻¹	reciprocal of the median root arrival times at 10, 20, 40, 60, 80, and 120 cm depth
	IQR	d	interquartile range of root arrival times at 10, 20, 40, 60, 80, and 120 cm depth
	RMm	cm cm ⁻²	maximum root length density in a root arrival curve.

From the trench profile data, we derived three characteristic root system measures: Tt which is the total number of intersections in the profile, RX which represents the lateral root distribution in the direction perpendicular to the rows and Rz which represents root distribution with depth. RX was calculated for each vertical column of grid cells by summing up the root intersections in that column. Making use of the

symmetric behavior of the root growth with respect to the vertical axis below the seed, we averaged the values of the columns at the same distance left and right of the plant row.

From rhizotube observations of the root arrival curves (RAC), we derived the reciprocal of the median arrival time of the roots at a certain depth, At . The median arrival time was defined as the time at which the root length density in the RAC reached 50% of the maximal root density, RMm , of the RAC at that depth. The reciprocal was taken to avoid numerical complications for tubes that were below the maximal rooting depth for which At was equal to zero. The difference between the times at which the densities in the RAC reached 75% and 25% of the RMm represents the interquartile range of the root arrival times, IQR , which is inverse proportional to the slope of the RAC. For tubes below the maximum root depth, IQR was zero.

The Morris one-at-a-time sensitivity analysis method was performed to quantify the sensitivity of different characteristic root system measures to each parameter of the root architecture model (Morris 1991). Morris one-at-a-time sensitivity analysis method has been applied previously to perform sensitivity analysis of root architectural models (Garré et al. 2012; Pagès et al. 2012). The method calculates elementary effects, EE_i of a certain parameter p_i on a characteristic root system measure $Y(\mathbf{p})$, as:

$$EE_i = \frac{Y(\mathbf{p} + range_i \mathbf{e}_i \Delta_i) - Y(\mathbf{p})}{\Delta_i} \quad (2)$$

where $\mathbf{p}$ represents the model parameter vector with dimension k , $range_i$ is the range of parameter i , $\mathbf{e}_i$ is a unit vector with dimension k that points in the i^{th} direction and Δ_i is a perturbation factor of the i^{th} parameter. To account for non-linearities and interactions between different parameters, the elementary effects of a certain parameter was evaluated for r different parameter sets $\mathbf{p}$. The mean of the elementary effects, m_i and of the absolute values of elementary effects, m_i^* represent the overall influence of the parameter when it varies within its range on the considered characteristic root system measure:

$$m_i = \frac{\sum_{j=1}^r EE_i^j}{r} = \frac{\sum_{j=1}^r Y(\mathbf{p}^{(j)} + range_i \mathbf{e}_i \Delta_i) - Y(\mathbf{p}^{(j)})}{r \Delta_i} \quad (3)$$

$$m_i^* = \frac{\sum_{j=1}^r |EE_i^j|}{r} \quad (4)$$

To evaluate the non-linearity of the relation between Y and the parameter p_i and its interaction with other parameters, the standard deviation of the elementary effects, s_i , is calculated:

$$s_i = \sqrt{\frac{\sum_{j=1}^r (EE_i^j - m_i)^2}{r}} \quad (5)$$

In order to obtain accurate and precise estimates of the sensitivity parameters m_i , m_i^* and s_i , it is important to use a representative sample of the parameter space. However, for models with many parameters that require a long computation time, the number of parameter sets that can be used to calculate the parameter sensitivity is limited. We used the Method of Modified Optimized Trajectories that was proposed by (Ruano et al. 2012) and that is based on the methods of (Morris 1991) and (Campolongo et al. 2007). First, 500 parameter trajectories, which are sets of $k+1$ parameter vectors $\mathbf{p}$ (k is the number of parameters in the parameter vector, in our case 37), were generated according to the method proposed by (Morris 1991). Each parameter could take values on a fixed and uniform grid that was chosen to have $m = 4$ levels with the lowest and highest value that are, respectively, 50% lower and 50% higher than the mean parameter value given in Table 1. Each parameter trajectory was generated starting from a randomly chosen initial parameter vector on the m -level grid. In the parameter trajectory, only one parameter is changed between two consecutive parameter vectors and every parameter is changed once in the trajectory. The parameter change factor Δ_i in the trajectory was set according to (Morris 1991) to $m/(2*(m-1))$ or $2/3$. To calculate the elementary effects, only 10 trajectories ($r = 10$) were selected from 500 generated parameter trajectories so that CRootBox was run for 380 parameter sets. The 10 sets were selected based on the distances between the parameter vectors of the different trajectories. The largest distances were chosen so that the selected parameter vectors spanned the parameter space.

Finally, sensitivity indices or normalized mean absolute elementary effects and normalized standard deviations were calculated as a percentage of the overall effect of all parameters:

$$\mu_i^* = 100 \frac{m_i^*}{\sum_{i=1}^k m_i^*} \quad (6)$$

$$\sigma_i = 100 \frac{s_i}{\sum_{i=1}^k m_i^*} \quad (7)$$

If the parameter sensitivity is equally distributed among all 37 parameters, each parameter should contribute 2.7% ($100/37$) to the overall effect. Therefore, we selected 5%, which is approximately double the equal parameter sensitivity, as the margin to determine the sensitivity levels. We defined parameters with sensitivity indices (μ^*) greater than 10, between 5 and 10, and below 5 as the most sensitive, moderately sensitive and non-sensitive parameters, respectively. Parameters with a sensitivity index $\mu^* > \sigma$ and $\mu^* > 2\sigma/\sqrt{r}$ were defined as parameters with respectively high and severe non-linear effects (Morris 1991).

The parameter sensitivities, μ_i^* , were presented as the percentage of the total variation of the observed output variable (root system characteristic measure) due to variation in all the input parameters (RSA) that can be attributed to the variation in parameter i . To evaluate the parameter sensitivity against the uncertainty of an observed variable due to sampling uncertainty, the standard deviation of the observed variable was normalized by the overall effect of all parameters:

$$R^* = 100 \frac{stdev}{\sum_{i=1}^k m_i^*} \quad (8)$$

where $stdev$ is the standard deviation of the observed variable or characteristic root system measure (averages of RLD, of Rz, or of RMm). When $R^* < \mu_i^*$, the effect of parameter i on the observed variable can be distinguished from the uncertainty of the observation.

Principal component analysis of sensitivities

The sensitivity analyses reveal parameters that have a strong influence on certain aggregated root system characteristics that are derived from field sampling. But, the effects of different parameters on these characteristic root system measures may be strongly correlated. This implies that a change that is induced by one parameter may be compensated by another parameter. Parameters with strongly correlated effects cannot be derived independently by inverse modelling. In order to assess which parameters could be derived from the observed characteristic root system measures, we carried out a principal component analysis of the sensitivities.

The relation between model parameters $\mathbf{p}$ and model output $\mathbf{Y}$ can be represented using a linear approximation of the model as:

$$\mathbf{Y}(\mathbf{p}) = \Delta \mathbf{p}^T \mathbf{J} + \mathbf{Y}(\mathbf{p}_0) \quad (9)$$

Where $\mathbf{p}_0$ is a reference parameter set and $\mathbf{J}$ is the Jacobian of the model that corresponds with the sensitivity of the model output $\mathbf{Y}$ to the parameters $\mathbf{p}$. This linear approximation is for instance used to estimate parameters using non-linear regression. In analogy to linear regression, the Jacobian corresponds with the independent variable matrix $\mathbf{X}$. When the independent variables (columns of $\mathbf{X}$) are strongly correlated with each other, the estimates of the parameters become very uncertain (multicollinearity). This implies that not all parameters of the model can be inferred from the regression and that the model is overdetermined. In order to avoid multicollinearity, new independent variables that are linear combinations of the original variables and that are not correlated with each other can be derived using a principal component analysis (PCA). The principle components (PC) explain a certain part of the variability of the independent variable dataset and because of their mutual independence, the total variability in the dataset is the sum of the variabilities explained by each of the PC's. The PC's are ranked in function of the variability that they explain with the first PC explaining the most.

By selecting only the first PC's as independent variables for the regression, the dimension of the problem and hence the number of parameters that are estimated can be reduced. Turning the problem around, the principal component analysis (PCA) provides information about how many and which parameters of the model can be estimated from the observed dependent variables Y and its sensitivities to the parameters $\mathbf{p}$. The coefficients that relate the different independent variables (sensitivities to the parameters $\mathbf{p}$) to the PC's, i.e. the loadings, can be plotted for two PC's in biplots. The vectors shown in a biplot for different independent variables (sensitivities to the parameters $\mathbf{p}$) indicate how much of the variability of the two PC's is linked to the variability of the independent variables (length of the vector) and how strongly independent variables are correlated among each other.

As an estimate of the Jacobian, we used the average value of the sensitivities, m_i . When different observation types with different dimensions (e.g. root length in soil cores vs root counts in a profile or in a rhizotube) are combined, the sensitivities have different dimensions as well. In order to evaluate the impact of combining different observation types on the amount of parameters that can be estimated independently, the sensitivities were normalized by the average length of the sensitivity vectors for a certain observation type. In other words, we calculated the length of the vectors in each row of $\mathbf{X}$ for a certain observation type Y and took the average of these lengths. Then the values in the $\mathbf{X}$ matrix for that observation type were divided by this average length. We considered as observation types: root lengths in soil cores; average arrival times, IQRs of arrival time distributions, and maximum root counts derived from root counts in rhizotubes; and the amount of roots counted in vertical transects at different distances from the rows. In order to account for the different number of observations for the different observation types, weights were assigned to the sensitivities corresponding with different observations. For the cores, 45 observations were available, for the rhizotube observations 18 (3 types and 6 depths) and for the counts in vertical transects 2 for wheat and 8 for maize.

A flowchart with the simulations of the root system architectures, sampling methods, derivation of characteristic root system measures, the sensitivity and PCA analyses is given in Fig 5.

Fig. 5 Flow chart showing the main steps used in the methodological approach.

Results

Root systems simulations

Fig. 6 presents a 3D visualization of the root systems of maize plants at 180 days after sowing that were simulated using CRootBox in a field plot. Similar simulations were done for wheat. Sampling methods and the locations of sampling are also shown in the figure. Computation time for the virtual field simulations significantly varied with the RSA parameters and number of root systems that were simulated in the virtual field plots. Simulation of root growth and sampling in one field plot took 177 seconds for wheat and 878 seconds for maize on a desktop computer with Intel(R) core (TM) i3-4150 cpu @ 3.50GHz processor and 8.00 Gb RAM.

Fig. 6 Simulated maize root systems in a virtual field plot until 180 days after sowing (color scale represents the appearance time of the root segments) Vertical transparent cylinders represent the soil cores, horizontal cylinders the rhizotubes, and vertical planes the trenches. Winter wheat simulations followed a similar simulation procedure with different plant density and sampling locations.

Root sampling

Coring: Fig. 7 shows the core sampling results at one-month intervals for maize and wheat, respectively. Each line of the graph represents the mean root length density and the gray shaded bands represent the standard deviation of the estimate of the mean root length density that is based on 15 cores in one realization of a field plot. The standard deviation of root length densities in the individual soil cores is represented by the red shaded band. The results of the two plant types, maize and wheat, show different patterns. The maximum simulated rooting depth of wheat is 10-20 cm deeper than that of maize whereas the highest root length density for maize is reached about 10 cm deeper than for wheat.

Fig. 7 Root length density profiles obtained from 15 soil cores in a field plot with wheat (left), and maize (right) at 30-day time intervals. The lines represent the average of the profiles that are simulated in 100 realizations of the plots. The grey shaded areas represent the standard deviation of the obtained profiles from 15 soil cores in the different realizations. Red shaded area represents the standard deviation of the root length densities in the 1500 soil cores at the end of the growing season.

Fig. 8 Range of root length density profiles obtained from 15 soil cores in a field plot with wheat (left), and maize (right) at 240 and 180 days respectively, based on the full set of parameter variations used in sensitivity analysis.

The individual root length density profiles that are shown in Fig. 8 represent the distributions that were simulated for the reference RSA parameter set in Table 1. In order to demonstrate the variability of the root distributions in the sensitivity analysis, we plotted in Fig. 8 root length density profiles for the 380 considered parameter sets. This plot illustrates the flexibility of the RSA model to simulate different root length density distributions by changing its parameters between 50% and 150% of the standard parameters set. Depending on the parameters, different shapes of root density distributions: bell shaped, sigmoid, nearly uniform and exponential; different

maximal root depths ranging from 30 cm to over 160 cm depth; and different maximal root densities: 20 cm/cm³ compared to approximately 2 cm/cm³ for the standard parameter set are obtained.

Estimation of representative sample size

Fig. 9 shows the relative standard error of the mean root length density, RSE, versus the number of cores that was used to calculate the mean. To reach a RSE smaller than 10%, 10 cores are sufficient for wheat except for the deeper soil horizons where root densities are smaller. This is in agreement with results of wheat field core samples (Hang Lai Thi Thu et al. 2011). For maize, around 50 cores would be required to reach the 10% RSE threshold in the upper soil layers, whereas an even higher number of cores would be required for the layers below 60 cm. RSE may increase due to selection of sampling position such as positions just below the seed or in between two rows in maize sampling because higher number of roots are observed close to the seed position and root density decrease with increasing distance from the plant base. It is important to note that the sample volume also contributes to the precision of the root length density measurements which increases with the volume of selected sampling size (larger diameter cores increase the precision of the selected number of cores, and vice versa).

Fig. 9 Relative standard error of the estimated mean root length density of wheat (left) and maize (right) at different depths obtained from soil cores as a function of the sample size: lines represent the theoretical relation between the standard error of the mean and the sample size and symbols are standard errors of sets of means.

Trenching: Root counting in trenches of the two considered crops shows relatively diverse results for wheat and maize (Fig. 10). The winter wheat root distribution is laterally homogeneous below 10 cm depth, whereas maize shows higher lateral heterogeneity of root counts; root counts decrease with the increasing distance from the seed position. The highest root count density was observed close to the seed position.

Fig. 10 Spatial distribution of wheat and maize grid-based root counts in virtual trench profiles. Dots represent the standard deviation of the mean root counts (winter wheat at 240 days after sowing (left) and maize at 180 days after sowing (right)).

Minirhizotrons: Simulated root arrival curves to the rhizotubes at different depths represent the variation of root distribution and density with time (Fig. 11). Depth profiles of root length densities derived from rhizotubes had a different shape than those derived from soil cores. For instance, for wheat at 240 days there is almost no variation of root densities between 10 and 60 cm depth observed in the rhizotubes whereas the profiles derived from soil cores show a considerable variation with depth. In maize, the rhizotubes indicate the highest root density at 10 cm depth, whereas the profiles derived from soil cores show the highest root length density at 20 cm depth. The reason for these differences can be due to the different locations below the plant rows that are sampled by the rhizotubes and the soil cores and the impact of the root orientation on the root densities that are observed in the rhizotubes.

Fig. 11 Mean root arrival curves (RAC) in horizontal rhizotubes at different depths (T1-10cm, T2-20cm, T3-40cm, T4-60cm, T5-80cm, T6-120cm) for winter wheat (left) and maize (right). Lines are means of root length densities obtained from 2000 images per observation time (20 locations in 100 tubes in different realizations of the field plots) and shaded areas represent standard deviations of means estimated from 20 images in one realization of the field plot (shaded areas)

Sensitivity Analysis

Fig. 12 Summary of the sensitivities of the characteristic root system measures, which are derived from virtual field observations (see Table 4), to parameters of the root system architecture model (Table 1) (W: winter wheat, M: maize)). Classes of parameter sensitivities and non-linearity effects; parameters are categorized into six zones based on the elementary effects and standard deviations. The continuous line represents 1:1 line and the dashed line represents $\mu^* = 2\sigma/\sqrt{r}$ with $r=10$ is the number of parameter trajectories.

Fig. 12 shows which characteristic root system measures are influenced by the RSA parameters. Each parameter is characterized by its normalized mean of absolute elementary effects and normalized standard deviation and illustrated by the color-code and font type shown in Fig. 12. Parameter sensitivities of root system measures that vary with depth and that are obtained from all three sampling methods; coring (RLD), trenching (Rz) and minirhizotron methods (RMm, At) also vary with depth and the change of the parameter sensitivity with depth differs between different parameters and between the two crops (Fig. 13). For instance, for wheat and maize, the sensitivities of RLD, Rz and RMm to NB (number of zero order roots) decrease with depth whereas their

sensitivities to maxl0 (maximal length of zero order roots) increase with depth. The latter is consistent with the maximum rooting depth ($D99$) that shows the highest sensitivity to maxl0 (see Fig. A. 2). RLD and R_z in maize are sensitive at greater depths to tr0 (gravitropism of zero order roots) whereas RLD and R_z in wheat close to the soil surface are sensitive to θ_0 (insertion angle of zero order roots) (see Fig. 13). The sampling uncertainty in one realization of the field plot (i.e. 15(16) cores, 9 trenches, 1m long rhizotubes) relative to the total effect of all parameters, R^* , was for RLD, R_z and RMm always lower than 2% so that the parameter sensitivities, μ_i^* shown in Figure 13 could be distinguished from sampling uncertainty.

Fig. 13 Normalized means of absolute elementary effects (μ^*) of the most sensitive RSA parameters on root length densities at different depths in soil cores (RLD, first row), on root impact densities at different depths in trench profiles (TZ, second row), and on the maximum amount of roots observed in rhizotubes at different depths (RMm, third row) for winter wheat (left) and maize (right) 240 days and 180 days after sowing, respectively.

Fig. 14 Normalized means of absolute elementary effects (μ^*) and standard deviations of elementary effects (σ) of the most sensitive RSA parameters on median root arrival times (At, top) and on the IQR of root arrival times (bottom) observed in rhizotubes at different depths (red, green, blue, black, cyan and magenta colors represent the depths 10, 20, 40, 60, 80, 120 cm respectively) for winter wheat (left) and maize (right).

The sensitivities of root system measures that quantify the total amount of roots such as the total root length, Tc, the total number of root impacts in a trench, Tt, or the maximal root length density, RMc are shown in Figure A 1. These measures show in general largest sensitivities to NB, ln0 (branching distance on zero order roots),

maxl1 (maximal length of first order roots), and maxl0 (Tt and Tc). From the trench observations, a characteristic that represents the lateral variations in root impacts, RX, and that is related to the lateral extent of the root system was derived. For maize, the sensitivity of Rx to NB, tr0 and maxl0 varies with the lateral distance from the plant row whereas for wheat RX and the sensitivity of RX to the RSA parameters do not vary with lateral distance (see Fig. A. 3). Finally, root characteristics that represent the dynamics of the root system development such as the reciprocal of the median root arrival time, At, and the interquartile range of arrival times, IQR, at different depths were derived. The sensitivities of At and IQR to RSA parameters are shown in Fig. 14. In contrast to the other root system characteristic measures that were derived from measurements at the end of the simulated root growth period, these dynamic root characteristics are sensitive to the growth rate of the zero order roots, r0. The difference between uncertainty of the observed variable to the total sensitivities of the parameters R*, which is lower than the detectable parameter sensitivities, except Rz measure of maize trench root counting method (results are not shown).

Principal Component Analysis

The variability of the sensitivities of the core data to the different RSA parameters can be explained for the largest part by the first two PC's whereas the third PC explains less than 5% of the variability (Table 5). When rhizotube data are used or when all data are combined, the variability of sensitivities is spread more over the different PCs. This indicates that the impact of certain parameters on certain observation can less be compensated by changing other parameters. The parameter with the highest PC loading for the first PC is the number of zero-order roots (NB). For the other PCs, the parameters with the highest loadings differ between the crop and between the type of data that are considered. However, for the rhizotube data, the growth rate of the zero-order roots, r0, has the highest loading for the second PC for both wheat and maize. Fig. 15 and 16 show biplots of parameter loadings for the 1st and 2nd PCs and the 2nd and 3rd PCs using data from respectively soil cores (see Fig. 15) and rhizotubes (Fig. 16) for wheat and maize. The alignment of the loading vectors of NB and ln0 with the first PC axis in the biplots indicates that the first PC is correlated with the NB and ln0 sensitivities whereas the opposite orientation of the vectors shows that ln0 has the opposite effect compared to the effects of NB. A larger distance between branches leads to a lower root length density whereas a larger number of zero order roots leads to a higher root density. The close alignment of loading vectors to the second PC (e.g. maxl0 and tr0 for the core data from respectively wheat and maize and r0 for the rhizotube data) identifies parameters that have an effect on the observations that is independent of the parameters that are related to the first PC and that cannot be compensated by changing these parameters.

Table 5 Percentage of variance explained by the first 5 principle components of the sensitivities when only data from coring (cores), rhizotube measurements (rhizotubes) are used and when rhizotube, core and impact data are combined (All) The parameter names with the highest PC loading are given in parenthesis.

PC	Cores	Rhizotubes	All
Wheat			
1	73.3 (NB)	51.9 (NB)	55.6 (NB)
2	18.9 (maxl0)	27.5 (r0)	19.0 (r0)

3	4.6 (theta0)	6.5 (la1)	9.4 (maxl0)
4	1.0	3.7	4.9 (theta0)
5	0.6	2.7	2.6
Rest	1.6	7.7	8.5

Maize

1	70.5 (NB)	35.7 (NB)	57.2 (NB)
2	17.9 (tr0)	28.4 (r0)	15.3 (tr0)
3	3.9 (maxl0)	11.7 (la0)	8.1 (r0)
4	2.2	8.0	4.3 (la0)
5	1.5	4.2	4.2
Rest	4	12	10.9

Fig. 15 Biplots of parameter loadings for the 1st and 2nd PCs (first row) and the 2nd and 3th PCs (second row) using data from soil cores for the wheat crop (left column), and for the maize crop (right column).

surface area or corresponding soil volume that is sampled by the images is relatively small so that a large number of images is required to obtain a precise estimate of the RAC. This can be achieved by taking more images using longer tubes and/or using more tubes at one depth. The standard error of the mean root density that was calculated from 120 images taken from three tubes at the same depth in a winter wheat plot was estimated to be 8% by (Cai et al. 2016). This would correspond with a standard error of 20% when the mean is estimated from 20 images, which is slightly larger than what we simulated for winter wheat. In our model simulations, we did not consider soil heterogeneity which may be an additional factor influencing the spatial variability of root densities that is observed in the field. The larger variability of RAC estimated from 20 images for maize can be attributed to the larger lateral variation of root densities of maize. Rhizotubes have often been installed with a certain insertion angle instead of horizontally. The large number of images that is required to obtain a precise estimate of the RAC at a certain depth would imply that a large number inserted rhizotubes to obtain a precise estimate of the RAC.

Sensitivity analyses of the characteristic root system measures that were obtained from the three different root sampling methods demonstrate that the parameter sensitivity varies not only with the sampling method, but also with each characteristic root system measure, which reflect properties such as rooting depth, root mass, and lateral spreading of the root system in soil. Also, of note are the differences in sensitivities for the two crops. This can be related on the one hand to different cropping density. On the other hand, the non-linearity of the model also leads to different sensitivities for root systems with a different architecture (or a different set of RSA parameters). The variance of the sensitivities is distributed more over the PCs of the rhizotube data (Table 5) which indicates that more parameters can be derived from rhizotube data than from soil core data or trench profile observations. Rhizotube data also provided information about the dynamics of the root growth. This information could also be obtained when soil cores or trench profiles were taken at several times but parameter sensitivities were in our study only calculated for soil coring and trench profile data that were obtained at a single time. When all datasets were combined, additional information about root length densities from deeper soil layers (maize) or from closer to the soil surface (wheat) and information about the lateral root distributions (maize) in the soil core and root impact data, which was not present in the rhizotube data, gave extra loading to the RSA parameters $tr0$ (maize) and $\theta0$ (wheat).

Regarding the rankings of sensitivities (Fig. 12), the number and maximal length of the zero order roots, NB and $maxl0$, have a strong influence (red and green numbers) on a large number of characteristic root system measures. Next come the growth rate $r0$ and the branching distance, $ln0$, of the zero order roots which have a moderate influence on a large number of characteristic root system measures; and the branching angle, $\theta0$, and gravitropism, $tr0$, of the zero order roots and the maximal length of the first order roots, $maxl1$, with a moderate influence on a few characteristic root system measures. Of note is that the parameters that describe the first (except $maxl1$) and second order roots have a less strong direct influence but their influence depends strongly on other parameters as indicated by the large normalized standard deviations, σ (Eq. 7), of their effects. The higher order root parameters have a severely non-linear impact on some of the characteristic root system measures (magenta numbers).

Looking at the distribution of the influences of the parameters on the RLD with depth (Fig. 13), it seems that the second PC reflects the sensitivity of the root densities deeper in the soil profile. For wheat, PC2 is strongly

linked to the maximal length of zero order roots, maxl0 , whereas for maize, especially the gravitropism of the zero order roots, tr0 , is important. For both maize and wheat, maxl0 and tr0 have positive PC2 loadings which indicates that these parameters influence root densities at larger depths in the same way, i.e. higher root densities at greater depths can be obtained by longer primary roots or by roots that grow downwards more vertically due to a higher gravitropism. But the importance of both parameters for root densities at greater depths differs for the two crops. Maxl0 influences strongly the maximal rooting depth (D99) for both maize and wheat (Fig. A. 2).

The third PC is for wheat linked to the angle of the zero order roots, theta0 , which influences the root densities close to the soil surface (see Fig. 13). It should be noted that for both maize and wheat, theta0 has negative PC2 loadings which indicates that an increase in theta0 (more horizontal roots) leads to a lower root density at larger depths. For maize, the third PC is more strongly related to maxl0 than to tr0 and reflects the root distributions at intermediate depths, which are more sensitive to maxl0 (see Fig. 13).

The total root length T_c , which can be considered to be the product of the root depth and the root density, is sensitive to all the parameters that are related to PC1, PC2 and PC3. For wheat, these are NB, $\ln0$, maxl0 , theta0 and maxl1 , whereas for maize these correspond to NB, $\ln0$, maxl0 , tr0 and maxl1 (Fig. A. 1). The $\sigma\text{-}\mu^*$ plot for T_c also shows a severe non-linear sensitivity of the total root length of wheat to the parameters that characterize higher order roots (maxl1 , la1 , lb1). Since in the PCA the mean sensitivities m_i are considered, the non-linear effects are not expressed by the PCs.

Comparing the $\sigma\text{-}\mu^*$ plot for T_c (total root length) with that for T_t (total number of intersections in a trench profile), (Fig. A. 1) the two plots look very similar. This indicates that T_c and T_t contain similar information about RSA parameters. Similar conclusions could be drawn when comparing sensitivity depth profiles for RLDs derived from soil cores (Fig. 13), for RLDs derived from minirhizotube observations (RMm in Fig. 13), and for RIDs derived from trenches (RZ in Fig. 13). Although there are some differences in depth profiles of RLDs/RIDs that are derived from the different methods, these three types of aggregated information about root systems show a similar sensitivity to RSA parameters. This indicates that they contain similar information about RSA parameters. This suggests that adding root coring data to minirhizotube data for the same depth range will not lead to a lot of additional information that can be used to estimate RSA parameters.

Looking at the sensitivities of the lateral distribution of root impacts densities (RID) in trench profiles (Fig. A. 3), it is obvious that for wheat, with a uniform lateral distribution of root counts, the sensitivity of the RID averaged with depth, RX , to the RSA parameters does not vary a lot with lateral distance and is very similar to the sensitivity of the total number of root impacts T_t . However, for maize the sensitivity of RX varies considerably with lateral distance and RX is more sensitive to tr0 than to NB, which is opposite to the sensitivity of T_t to tr0 and NB. Tr0 defines how strongly zero order roots are growing downward or can grow laterally, which explains the sensitivity of RX to tr0 . Combining soil core with trench profile data provides extra information for estimating RSA parameters. The PCA of the combined dataset showed that the loadings of the parameters tr0 and maxl0 to PC2 and PC3 increased which means that tr0 and maxl0 could be better discriminated when trench profile data are combined with soil core data (results not shown).

The biplots of the parameter loadings of the PCs that were derived from the sensitivities to the rhizotube data (RMc, At, and IQR at different depths) show that the first PC is related to the same parameters as the first PC of the soil cores (Fig. 16). This PC is therefore also related to the average root densities in the soil profile. The second PC is related to the growth rate, r_0 , and corresponds with the median arrival time of the roots at different depths (Fig. 15). The third PC is related to la_1 (the length of the apical zone of the first order roots) for wheat and to la_0 for maize. Since the median root arrival time near the surface is sensitive to la_1 (wheat) and la_0 (maize) (Fig. 15), the third PC is related to the root arrival time near the soil surface. The influence of the parameters on the slope of the arrival curve is strongly or severely non-linear, which indicates that the effect of parameters on the slope of the arrival curve depends strongly on the values of the other parameters (Fig. 15).

Summary and Conclusions

Root systems of a large number of plants in a field setting and different field sampling methods such as soil coring, root impacts on vertical soil trenches, and images taken from horizontal rhizotubes were simulated using the RSA model CRootbox. As an example, two crops with a different planting density and root architecture, maize and wheat were considered. Making use of the improved computational efficiency of CRootbox, it was possible to repeat the simulations for a large number of realizations of the field plots and a large set of RSA parameters. Based on these simulation data, the uncertainty or variability of root sampling data and the sensitivity of characteristic root system measures, which can be inferred from field data, to RSA parameters could be assessed. The most sensitive parameters were those of the zero order roots whereas the parameter of higher-order roots showed non-linear sensitivities, which means that their influence depended on the values of other parameters. Similar characteristic root system measures that were obtained with different methods, e.g. depth profiles of root density obtained from soil coring, from root impacts in vertical trenches, or from images taken in horizontal rhizotubes at different depths, showed similar sensitivity to the RSA parameters. However, the parameter sensitivity depended on the crop and the observation types, which implies that specific results cannot be generalized. Nevertheless, our results are consistent with an earlier sensitivity analysis of rhizotube observations to RSA parameters of spring barley (Garré et al. 2012). Although another RSA model was used, RootTyp (Pagès et al. 2004), the number of zero order roots (NB) and the root growth rate of the zero order roots (r_0) were, as in our study, found to be the most sensitive parameters for, respectively, the root density observed in the rhizotubes and the median arrival time of the roots. As also found in our study, the sensitivity of the slope of the root arrival time to RSA parameters was very non-linear.

Using principal component analyses of the parameter sensitivities, groups of parameters that had similar or counteracting effects on characteristic root system measures could be identified and linked to specific characteristic root system measures. The analyses demonstrated that the total number of zero order roots NB, the branching distance on the zero order roots (ln_0), and the maximal length of the zero order roots ($maxl_0$) influenced characteristic root system measures that are related to the overall root density (maximal root density, total amount root impacts) in a similar way. An increase in for instance NB could be compensated by a decrease in $maxl_0$ or an increase in ln_0 . This provided insight into which RSA parameters could be identified independently from specific characteristic root system measures by inverse modeling and which characteristic root system measures provide extra information to infer additional RSA parameters. Using information about root development over time, which can be obtained non-invasively from rhizotube images, is important to

estimate root growth rates (r_0). Root density depth profiles obtained from horizontally installed rhizotubes can be extended with data from soil coring or from root impact counts in vertical trenches that provide a higher spatial resolution or give additional information about the lateral distribution of root densities. The PCA and sensitivity analyses showed the additional value of combining these datasets for the estimation of parameters like maximum length ($maxl_0$), insertion angle (θ_0) and gravitropism of zero order roots (tr_0) from field measurements.

Outlook

The development of the root system also depends strongly on environmental factors like soil temperature (Nagel et al. 2009), soil strength (Bingham and Bengough 2003), shape and size of soil grains (Lipiec et al. 2016), and the presence of biopores (Perkons et al. 2014). Some studies showed that root architecture and characteristic root system measures of a certain plant genotype reflect its adaptation to environmental conditions (Hamada et al. 2012) such as the effect of tillage system on soil strength, the soil type and soil moisture condition (Cai et al. 2018; Muñoz-Romero et al. 2010; Fan et al. 2017). Therefore, it is required to include soil information into RSA models. CRootBox does have the capability to simulate root growth affected by static soil condition as well as to dynamically changing soil conditions via coupling to soil models (see e.g. example 5 in Schnepf et al. (2018b)). At the cost of increasing the number of parameters, this shall be considered in future sensitivity analyses. In this study, our sensitivity analysis results provide a broad insight about the sensitivities of classical field sampling data to different root architecture parameters. We highly recommend investigating the application of root architecture models to retrieve the specific root traits of different genotypes and their response to environmental conditions with the aid of an inverse modeling algorithm and to test the suitability of real sampling schemes to retrieve the root system architecture of a plant. This approach could further valorize field phenotyping data by linking them to individual root architecture parameters or root traits. These architectural traits could be plugged into functional structural models that predict the functional traits of root systems that could be of interest to breeders (Meunier et al. 2017; Passot et al. 2018).

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Supplementary material

The supplementary material consists of all the scripts and data required to reproduce the analysis and is available online at: <http://www.tr32db.uni-koeln.de/data.php?dataID=1862>

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Appendix

Fig. A. 1 Normalized means of absolute elementary effects (μ^*) and standard deviations of elementary effects (σ) of the most sensitive RSA parameters on the maximum root length density in vertical root length density profiles derived from soil cores (RMc) first row), on the total root length per surface area derived from soil core measurements (Tc second row), and on the average root count density vertical profile walls (Tt third row). Blue line: 1:1 line, red line: $\mu^* = 2 \sigma/\sqrt{r}$ line (winter wheat (A) and maize (B) after 240 days and 180 days of simulation time respectively).

Fig. A. 2 Normalized means of absolute elementary effects (μ^*) and standard deviations of elementary effects (σ) of the RSA parameters on the depth above which 99% of the total root length is observed (D99) for winter wheat (left) and maize (right) 240 days and 180 days after sowing, respectively. Blue line: 1:1 line, red line: $\mu^* = 2 \sigma/\sqrt{r}$.

Fig. A. 3 Normalized means of absolute elementary effects (μ^*) of the most sensitive RSA parameters on root impact densities in vertical transects of trench profiles (RX) at different distances from the stem for winter wheat (left) and maize (right) 240 days and 180 days after sowing, respectively.

Fig. A. 4 Biplots of parameter loadings for different PCs when all data are combined for the wheat crop.

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917 Fig. A. 5 Biplots of parameter loadings for different PCs when all data are combined for the maize crop.