Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale

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Abstract

The numerous feedback loops between roots, microorganisms, soil chemical and physical properties, and environmental variables result in spatial parameter patterns which are highly dynamic in time. In order to improve our understanding of the related rhizosphere processes and their relevance at the soil–plant system scale, experimental platforms are required. Those platforms should enable (1) to relate small scale observations (nm to dm) to system behaviour, (2) the integration of physical, chemical and biological sampling approaches within the same experiment, and (3) sampling at different time points during the life cycle of the system in question. Here we describe what requirements have to be met and to what extent this has been achieved in practice by the experimental platforms which were set up within the framework of DFG priority programme 2089 "Rhizosphere Spatiotemporal Organisation—a key to rhizosphere functions". It is discussed to what extent theoretical considerations could be accommodated, in particular for the comparison across scales, *i.e.*, from laboratory to field scale. The latter scale is of utmost importance to overcome the trade-off between fraction of life cycle covered and the avoidance of unrealistic root length densities.

Key words: destructive sampling / rhizosphere / root / undisturbed sampling / X-ray CT / *Zea mays*

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Supporting Information available online

1 Introduction

The processes at the soil–root interface are key to unraveling plant resource acquisition, soil carbon storage and soil structure formation as well as the communication of plants with the soil microbiome which is thought to be the basis for plant health (*Hinsinger* et al., 2009; *Bardgett* and *van der Putten*, 2014; *Keiluweit* et al., 2015; *Roose* et al., 2016; *York* et al., 2016).

The numerous feedback loops between roots, microorganisms, soil chemical and physical properties, and environmental variables result in spatial parameter patterns which are highly dynamic in time. It was recently hypothesized that such patterns are the result of self-organization and that they are highly relevant for understanding the properties of soil–plant systems such as stability under disturbances and change (*Vetterlein* et al., 2020). A detailed discussion of current knowledge gaps in rhizosphere research and how applying the concept of self-organization as a system approach can contribute to advance rhizosphere research is found in *Vetterlein* et al. (2020). Here we focus on the experimental prerequisites required to follow such a system approach. The experimental platforms should enable to relate small scale observations (nm to dm) to system behavior, the integration of physical, chemical and biological sampling approaches within the same experiment and, ideally, sampling at different time points and at selected sites during the life cycle of the system in question (*Oburger* and *Schmidt*, 2016; *Roose* et al., 2016).

In the following, we describe what requirements have to be met by joined experimental platforms, and provide the experi-

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mental details of a laboratory and a field platform that were established within the framework of DFG priority programme 2089 "Rhizosphere Spatiotemporal Organisation—a key to rhizosphere functions". In retrospect, we discuss the outcomes of these approaches and sum up in how far theoretical considerations could be accommodated.

Specifically, the following points will be addressed and respective requirements derived:

- Gradients of interest and associated sampling strategies
- Relevance for system behavior
- Integration of physical, biological and chemical sampling strategies within the same experiment
- Suitable drivers for challenging spatiotemporal rhizosphere patterns
- Boundary conditions *suitable for rhizosphere studies—avoid luxury supply*
- Homogeneous initial conditions—*work load versus statistical requirements*

1.1 Gradients of interest and associated sampling strategies

Gradients can be observed along roots, *i.e.*, with changing root ontogeny and topology or with increasing distance from the root surface (*Hinsinger* et al., 2009). These gradients constitute the rhizosphere, which can be defined in the most general terms as the zone of soil affected by roots. However, it is not straightforward to sample the rhizosphere. There are operationally defined ways of sampling the rhizosphere, which are applied if the methods require a certain amount of homogenized soil material for analyses. Here, the rhizosphere soil is the amount of soil adhering to the roots after extracting them from bulk soil. It is thereafter removed from the root surface by brushing or washing. For these sampling methods the rhizosphere soil sample is a mixed sample across the whole root system. It does not refer to a certain distance from the root surface, but rather to the binding strength of soil to the root surface. The properties of such "rhizosphere samples" are conventionally compared to the properties of the bulk soil samples, *i.e.*, the soil remaining after the roots with adhering soil were removed.

Alternatively, *in situ* approaches can be applied, for which the spatial context between root and soil is not altered by sampling and the distance between sampling location and root surface is known. This is the case for all imaging approaches, whether they are 2D, requiring a transparent access window, or 3D, requiring a specific geometry. For the latter, we can distinguish between completely non-invasive, like intact roots within soil columns being scanned during growth, and undisturbed soil columns with intact root and soil structure extracted from the field. All *in situ* approaches provide information on spatial gradients without predefining the extent of the rhizosphere. 2D approaches come with the risk of confounding diffusion in soil with diffusion in the contact medium between the soil and the measurement device. Moreover, roots may be altered by the growth along a solid plate. 2D approaches can however easily address root topology (root branching pattern), whereas 3D approaches mostly only provide root architecture.

A third category of sampling approaches aims at obtaining spatial information from destructive samples by carefully dissecting volume samples to identify a root and physically sampling at increasing distance from the root channel (*Barej* et al., 2014). Similarly, spatial resolution for destructive sampling can be obtained by extracting such samples along roots after removing the transparent access window.

Finally, there is a range of sampling and monitoring devices (*e.g.*, micro suction cups, tensiometers, TDR moisture sensors) which can be installed in the soil at a known position from the root surface or from a compartment within which root growth is constrained (*Vetterlein* and *Jahn*, 2004). The results obtained can be then interpreted in relation to the distance from the root surface or to the compartment.

Requirement 1: Allow access for a range of non-invasive and destructive sampling approaches.

1.2 Relevance for system behaviour

Frequently, physiological properties are investigated under controlled conditions in the laboratory to exclude uncertainties introduced by fluctuating environmental parameters (*e.g.*, temperature, light, water availability, pests). As a result, most of our current knowledge on rhizosphere processes is derived from young plants two to six weeks of age. Growth limitation within small pots is recognized as a potential artifact (*Passioura*, 2006). Most laboratory experiments show high root length densities and hence are not comparable to the field situation in terms of potential pay-off of resource acquisition strategies.

Requirement 2: Run laboratory (short term) and field (growing season) experiment with similar treatments.

1.3 Integration of physical, biological and chemical sampling strategies within the same experiment

For many research questions, it is indispensable to work with the same sample, or at least to sample the same system in order to advance our understanding. This is true whether one wants to relate the composition of the rhizosphere microbiome to the release of root exudates, the extent of P depletion zone to root age, or the root water uptake rate to aquaporin expression, to name just a few examples. If sampling techniques are mutually exclusive, as is the case for destructive sampling of rhizosphere microbiome and sampling of root exudates requiring an intact root system, at least parallel setups should be used.

Partly, subsampling strategies can be used to overcome such difficulties; this is particularly promising for combining non-invasive methods (X-ray computed tomography—X-ray CT, Magnetic resonance imaging—MRI) with destructive sampling approaches (plant gene expression, microbiome analy-

sis) or *in situ* sample collection (soil solution sampling). Subsampling can also be used to combine analyses at different scales like detailed chemical mapping at the nanometer to the micrometer scale with the whole plant nutrient uptake.

Requirement 3: Standardized experimental protocols. Non-invasive and in-situ measurements as reference points for destructive sampling.

1.4 Suitable drivers for challenging spatiotemporal rhizosphere patterns

In order to motivate scientists of different disciplines to find a common specific experimental platform, drivers expected to fundamentally alter physical, chemical and biological parameter spatial patterns in the rhizosphere have to be identified.

The most prominent soil property affecting water and nutrient fluxes and hence parameter spatial patterns is texture (*Jarvis*, 2007). Indeed, textural fractions shape the pore network as well as the number of sorption sites relevant for chemical gradients to develop (*Tinker* and *Nye*, 2000).

For root traits, the spatial extent of patterns, such as depletion zones for a certain chemical element or the zone impacted by root exudation, is expected to vary with the presence of root hairs. Fortunately, there are root hair mutants available for some plant species that facilitate questioning their importance for the establishment of rhizosphere gradients.

Requirement 4: Drivers of general interest, one related to soil property, one related to plant property.

1.5 Boundary conditions suitable for rhizosphere studies – avoid luxury supply

Rhizosphere processes are in most cases associated with an investment of carbohydrates (root growth, specific root traits, release of substances, symbioses, expression of genes) in order to improve resource acquisition. Such investment will only be advantageous if resource availability is limiting growth. Hence, the system boundary conditions have to be selected accordingly, *i.e.*, avoiding over-supply and luxury consumption of nutrients, in particular P.

Requirement 5: Marginal nutrient supply.

1.6 Homogenous initial conditions – work load *versus* statistical requirements

Ideally, chemical, physical and biological parameters should initially be homogeneous at all scales, as unwanted heterogeneity would modify rooting patterns at the profile scale but would also be detrimental to the interpretation of the observed gradients at the nano or micrometer scale. Practically, such homogeneous conditions are unrealistic. One way of coping with heterogeneity is to increase the amount of replicates (independent samples, biological replicates), and/or number of sampling points within one replicate (technical replicates, pooled subsamples). The limit to these workarounds is not just the work load but likewise the large soil area and volume consumption.

Requirement 6: Homogenous initial conditions.

In the following, we describe the experimental platforms in the laboratory and the field of the DFG priority programme 2089 "Rhizosphere Spatiotemporal Organisation—a key to rhizosphere functions". For the design of these platforms, we tried to fulfill the requirements 1–6. In this review, we provide the design, initial chemical and physical properties of the substrates as well as climatic conditions. We further discuss as to how far requirements 1–6 could be fulfilled for laboratory *versus* field scale and provide a first comparison of plant growth at laboratory and field scales for the common growth stage (BBCH 14).

2 Material and methods

2.1 Experimental design

The laboratory based experiments will be denoted as soil column experiments (SCE) throughout this review, the field based experiment as soil plot experiment (SPE). For both scales, the basic design is a two factorial, randomized block design with six replicates (Fig. 1). The term replication here refers to six individual soil columns (SCE) or six individual field plots (SPE). Factor one is substrate with two levels [loam (L), sand (S)]. Factor two is the *Zea mays* genotype with two levels comprising wild type (WT) and a root hair defective mutant (*rth3*).

2.2 Genotypes

For the experiments, the *Zea mays* root hair defective mutant *rth3* and the corresponding wild-type siblings were selected (*Wen* and *Schnable*, 1994).

The monogenic mutant *rth3* is transposon induced and shows normal root hair initiation but disturbed elongation. The mutant does not display an aberrant shoot phenotype, but yield is reduced by 20 to 40% compared to the wildtype (*Hochholdinger* et al., 2008). The mutated gene encodes a GPI-anchored COBRA-like cell wall protein RTH3 that is involved in the organization of the synthesized cellulose (*Hochholdinger* et al., 2018). The *rth3* mutants used in these experiments are genetically highly homozygous because they have been backcrossed to the inbred line B73 for > 8 generations. Seeds were multiplied at the experimental station Endenich of the Faculty of Agriculture of the University of Bonn.

2.3 Substrate

The substrate loam (L) was obtained by excavating 700 t of a haplic Phaeozem soil (from 0 to 50 cm depth) in Schladebach, Germany (51°18′31.41′′ N; 12°6′16.31′′ E). The haplic Phaeozem has been under agricultural use until excavation. Before the excavation, the area was planted with oilseed rape. The excavated material was sieved at a gravimetric water content of 14% utilizing a heavy duty double deck

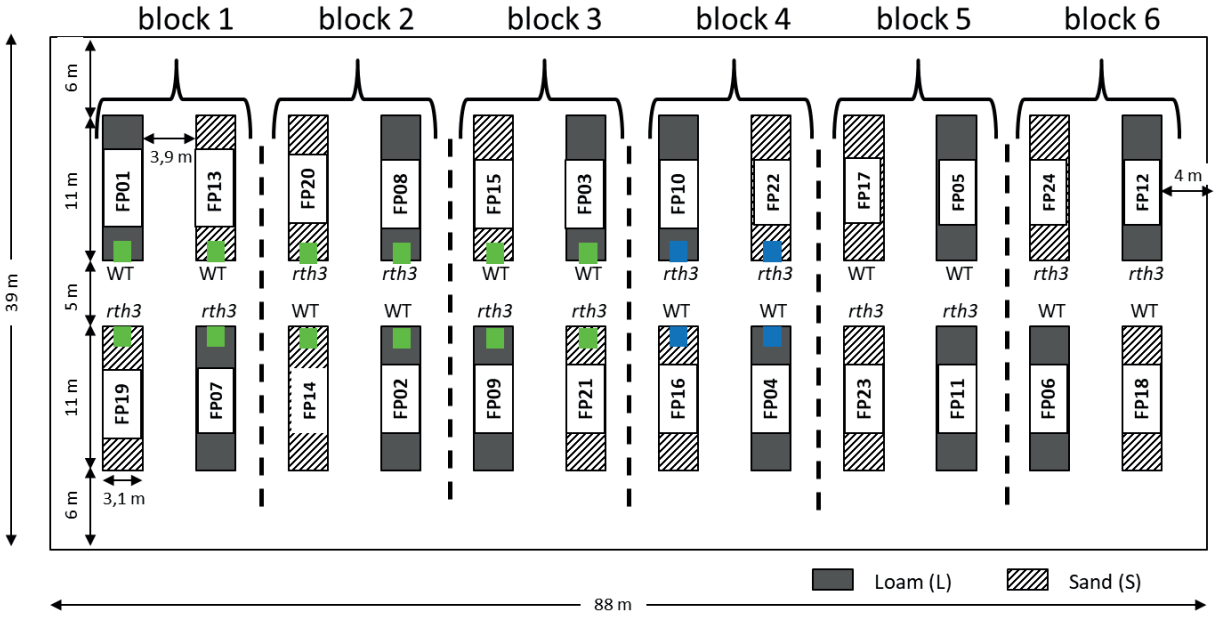

Figure 1: Soil plot experiment established as randomized block design with 6 replicates per treatment. Factor one: substrate with two levels [Loam (L), Sand (S)]; factor two: plant genotype with two levels [*Zea mays* B73 wild type (WT), respective hairless mutant (*rth3*)]. Position of root windows and monitoring devices for water relations are indicated in green and blue, respectively.

vibrating screen (Keestrack Combo; 3.2 m^2 area per deck). An upper level was mounted with a 40 mm mesh; a lower level had a 20 mm mesh. The throughput was in the range of 15 tons substrate per hour. Out of the three fractions obtained, the coarse one (> 40 mm) was discarded (about 5% of the total), and the medium one was added back to the ongoing sieving process. The whole amount was sieved within one week and stored by wheel loaders under a carport sheltered from rain. Setting up the field plots started right after the sieving operation in October 2018.

The substrate sand (S) was obtained by repeated mixing and sieving of (L) with quartz sand (550 t, WF 33, Quarzwerke Weferlingen, Germany). This procedure was chosen as no suitable sandy substrate (no carbonate, dominated by medium sand fraction, clay in the range of 5%) was available within a distance < 80 km (transport costs) from the research station. Initial mixing was conducted with wheel loaders based on the volume of their buckets (about 9:1). For homogenization of the pre-mixed material a drum screening plant (20 mm mesh) was used. Within one week, about 600 t of substrate (S) were produced and stored protected from rain, in the same fashion as for the loam substrate.

Stocks of initial material loam and quartz sand were kept for the SCE. These were stored in plastic containers (610 L, Kiga Kunststofftechnik GmbH) in a shelter protected from rain and sun.

2.4 Field plot and soil column design

Field plot experiment (SPE) was established in the research station Bad Lauchstädt, Germany (51°22′0′′ N, 11°49′60′′E). Individual field plots (11 × 3.1 m) were excavated to a depth of 1 m (Fig. 2a). Vertical side walls were covered with a root barrier (420 Xavan® 420 g/m^2, RootBarrier BV, Netherlands). The bottom of the plots was filled with a 25 cm gravel layer (0/32) and a drainage textile was then placed on top of the gravel (Fig. 2b). Substrates were then filled up to the original soil surface with a thickness of 75 cm (Fig. 2a). For weed growth prevention, the plots were covered thereafter with a nonwoven fabric made of 100% continuous polypropylene filaments (Plantex®Gold 125 g/m^2, DuPont, Luxembourg). The prevention canvas is permeable to air and water. Plots were established in October and November 2018 and remained covered until April 2019.

Individual soil columns for the SCE consist of an acrylic glass tube (25 cm height, 7 cm inner diameter). A nylon mesh (30 μm mesh size) is placed at the bottom of the column in order to retain the soil. The columns were then filled up to 23 cm height with the investigated substrates. With such a set-up, the volume available for plant growth is 885 cm^3.

2.5 Sieving and packing

For field plots treatment (L), loam was gradually placed in layers of 15 cm by a wheel loader, evened out with wheel loader bucket and compacted with a vibrating plate (weight 70 kg, support pressure 8 N/cm^2, reduced speed). This procedure proved to be suitable in a pilot experiment, as X-ray CT scans of extracted undisturbed soil cylinders showed no layering (Fig. 3). For treatment (S), sand was packed similarly; however, no vibrating plate was used.

For SCE, the loam with a moisture content of 10% is sieved step by step to < 4 mm, < 2 mm, and finally < 1 mm. The substrate sand corresponds to a mixture of 16.7% loam and 83.3% quartz sand, and it is established for each individual experiment (Tab. 2).

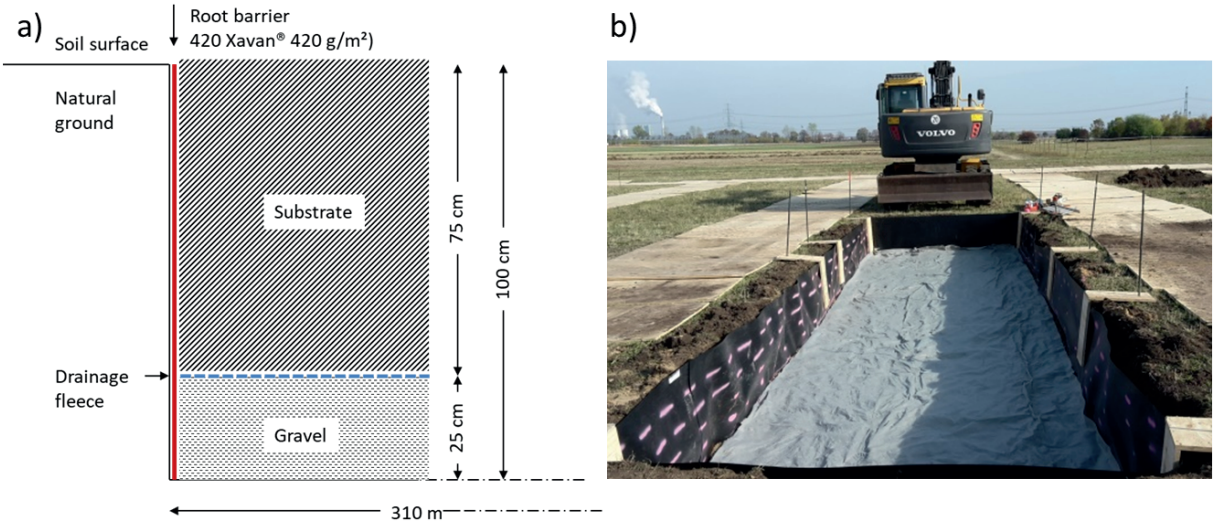

Figure 2: (a) Field plot cross section with root barrier and drainage layer, (b) field plot during establishment, drainage fleece is still visible.

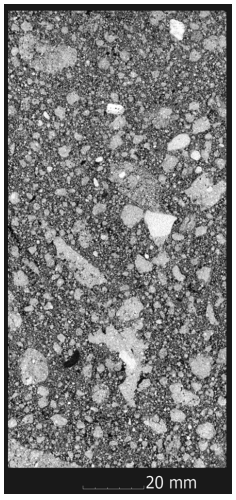

Figure 3: X-ray CT scan of an undisturbed soil column (20 cm height, 10 cm diameter) extracted from the field in a pilot study assessing different packing options. The substrate loam was packed with a vibrating plate in layers of 15 cm. Boundary between two subsequent layers would be expected halfway up the image.

Columns are packed carefully avoiding particle sorting by introducing a coarse sieve (4 mm) during filling which is continuously moved laterally. Compaction is achieved by "stamping" the entire column on a flat surface. For details regarding the packing of the columns, see https://www.ufz.de/spp-rhizosphere/index.php?en=43229.

2.6 Fertilization

In order for investment into resource acquisition to pay-off, the aim was to achieve a nutrient level which is in the range between slightly nutrient deficient to adequate nutrition for the WT genotype. Substrate as a driver was chosen primarily to introduce differences in water and nutrient transport; hence, differences in nutrient availability should ideally be compensated by fertilization.

Pre-trials were run with different doses of NPK application to achieve similar shoot growth rates for the WT on both substrates in column experiments, without visible symptoms of nutrient deficiency. These trials were associated with tissue analyses and the determination of plant available P and K in the substrates.

As a result N, P, K, and Mg were added at a dose twice as high in (S) compared to (L), and Ca as well as micronutrients were only applied to (S) (Tab. 1). This was the case for SCE and likewise for SPE.

The type of fertilizer differs between SCE and SPE as for column experiment laboratory grade chemicals of high purity are used, while for the field conventional fertilizers were applied (Tab. 1). For SCE, fertilizers were mixed with the soil prior to filling the columns, but for SPE, fertilizers were surface applied, 50% prior to seeding (24/04/2019), and the remaining 50% after first sampling (14/06/2019).

2.7 Agronomic measures – growth conditions

For field plots maize was sown (24/04/2019) to a depth of 5 cm by hand using a V-shaped planting device for single grain seeding. Distance within row was 20 cm, between rows 45 cm. This resulted in six rows with 54 plants each and a planting density of 9.5 plants m^{-2}, corresponding to a soil volume per plant of 78.935 dm^3 down to a depth of 75 cm.

During the first year, no pesticides were applied and weeding was done by hand. No heavy machinery was allowed to pass over the plots to avoid modification of soil structure.

The climatic conditions at the research station Bad Lauchstädt (average for the years 2008–2018) are depicted in Fig. 4 (Meteorological data of Bad Lauchstädt, Helmholtz Centre for Environmental Research–UFZ, Department of Soil System Science).

Table 1: Substrate specific fertilization in soil column experiments (SCE) and soil plot experiments (SPE). Data for SPE are for the first year after establishment of plots. 50% of the fertilizer was applied prior to seeding (24/04/2019), 50% after first sampling (14/06/2019).

(a) SCE

Substrate	Nutrient	Application rate (mg nutrient kg^{-1})	Type
Loam	N	50	NH_4NO_3
	P	40	$CaHPO_4$
	K	50	K_2SO_4
	Mg	25	$MgCl_2 \times 6\ H_2O$
Sand	N	100	NH_4NO_3
	P	80	$CaHPO_4$
	K	100	K_2SO_4
	Mg	50	$MgCl_2 \times 6\ H_2O$
	Ca	100	$CaSO_4 \times 2\ H_2O$
	Mn	3.25	$MnSO_4 \times H_2O$
	Zn	0.79	$Zn(NO3)_2 \times 4\ H_2O$
	Cu	0.5	$CuSO_4 \times 5\ H_2O$
	B	0.17	H_3BO_3
	Fe	3.25	Fe-EDTA

(b) SPE

Substrate	Nutrient	Application rate (kg nutrient ha^{-1})	Type
Loam	N	50	Calcium ammonium nitrate
	P	12	Triple superphosphate
	K	50	60s corn potash
	Mg	18	Epsom salt
	Ca	27	(applied with other fertilisers)
Sand	N	100	Calcium ammonium nitrate
	P	24	Triple superphosphate
	K	100	60s corn potash
	Mg	33	Epsom salt
	Ca	52	(applied with other fertilisers)
	Micronutrients	100	Excello 331 Spezial

Weather data are available from the DWD weather station which is located at the research station Bad Lauchstädt (ID 02878), https://opendata.dwd.de/climate_environment/CDC/observations_germany/climate/).

For column experiments, the maize seeds are surface sterilized (10% H_2O_2) and sown to a depth of 1 cm. Surface is covered with inert quartz gravel to reduce evaporation. Columns are carefully watered from top and bottom to an average volumetric water content of 22% for loam and 18% for sand. Fluctuation of water content is low as watering intervals are shortened as plant transpiration increases. Plants are grown at 22°C during the day and 18°C at night with 12 h light-period, 350 μM m^{-2} s^{-1} photosynthetically active radiation in a climate chamber. Relative humidity is kept constant at 65%. Growth duration in SCE is 21 d, *i.e.*, harvest is conducted on day 22 after seeding.

2.8 Definition of growth stages

As a common definition of growth stages for the field, BBCH-scale (*Bleiholder* et al., 2001) is used for defining sampling points in time. BBCH 14 (four leaves unfolded) was selected

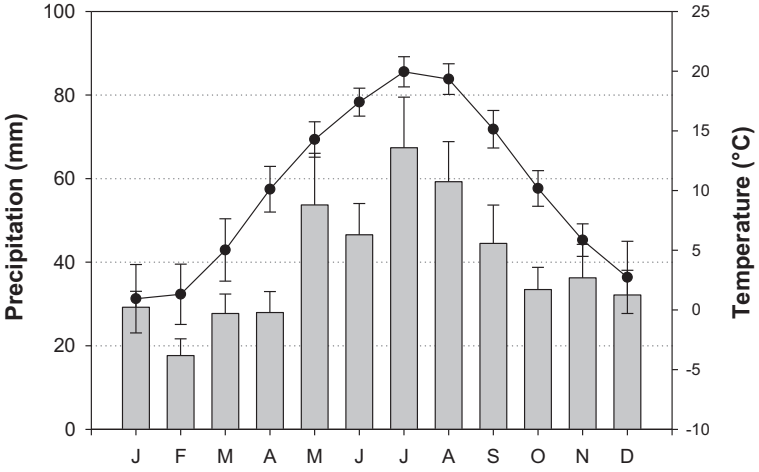

Figure 4: Mean temperature (symbols) and precipitation (bars) for the field station Bad Lauchstädt for 2008–2018. Source: Meteorological data of Bad Lauchstädt, Helmholtz Centre for Environmental Research–UFZ, Department of Soil System Science.

as the first sampling point as this corresponds to the developmental stage achieved after 21 d in SCE. BBCH 19 (nine or more leaves unfolded) was selected as second time point representing exponential growth. BBCH59 (end of tassel emergence) was selected as time point during the transition from vegetative to generative growth and BBCH 83 (early dough) as a growth stage representing ripening phase.

2.9 Installations for sampling and monitoring

2.9.1 Soil and plant water status monitoring (Fig. 5)

Sensors for the measurement of soil temperature, water-content (TEROS 10; Meter Group AG) and matric potential (T5 and TEROS 21; Meter Group AG) were installed at a depth of 10, 20, 40, and 60 cm at three different positions in relation to the planted maize rows. T5 is used to acquire accurate measurements of soil matric potential in the wet range, whereas TEROS 21 provides reliable measurements in the dry range. In addition, four plants per field were equipped with leaf psychrometers (PSY1; ICT International, Armidale, NSW, Australia) and sap flow sensors (Dynamax Inc., Houston, TX) to monitor respectively plant water status (as ψ_{leaf}) and transpiration. Measured data are logged every 10 min and transmitted to University of Bayreuth, which are responsible for the monitoring network. Due to high costs of installation only one out of the six blocks of SPE (Fig. 1) is equipped with these sensors.

2.9.2 Root windows (Fig. 6)

Inspired by the installation of root observation windows in forest sites (*Marschner* et al., 1991) Oburger et al. (pers. comm., manuscript with construction details in preparation) developed new root windows providing higher flexibility for obtaining partial access to roots growing along the transparent plane. These windows with a width of 60 cm and a depth of 60 cm were installed in three out of six field blocks (Fig. 1).

2.9.3 Isotope labelling (Fig. 7)

Stable isotope probing is used increasingly in different disciplines to trace uptake or release of substances and their subsequent distribution in functional networks (*Heinrich* et al., 2015). To enable such investigations in the soil plot experiments, an area of 70 × 120 cm was reserved within each plot for the three growth stages BBCH 14, 19, and 59. To trace nitrogen uptake, ^{15}N was applied to the soil surface as KNO_3 dissolved in water 60 h prior to sampling. The amount of water was calculated to allow infiltration to at least 0–10 cm. Application rates of 0.1, 0.2, 0.4 g m^{-2} were chosen at BBCH 14, 19, 59, respectively (99% ^{15}N KNO_3, Euriso-Top GmbH, Germany).

To trace the fate of assimilated carbon in the rhizosphere, ^{13}C pulse labeling was conducted on the same subplots 24 h prior to sampling. Gas tight chambers (70 × 120 cm, covering nine plants) with increasing height were set up and ^{13}C-CO_2 (99% ^{13}C Na_2CO_3, Euriso-Top GmbH, Germany) was released from Na_2CO_3 by adding sulfuric acid to the initial solution following a protocol adapted from *Heinrich* et al. (2015). Two short term pulses were applied over a 4-h period from 9 am to 1 pm. 8, 16, 32 g of $Na_2{}^{13}CO_3$ tracer were applied within the 4-h period to the nine plants in the chambers for BBCH 14, 19, and 59, respectively. Soil surface was sealed during ^{13}C labeling with a plastic foil to avoid direct gas exchange (Fig. 7).

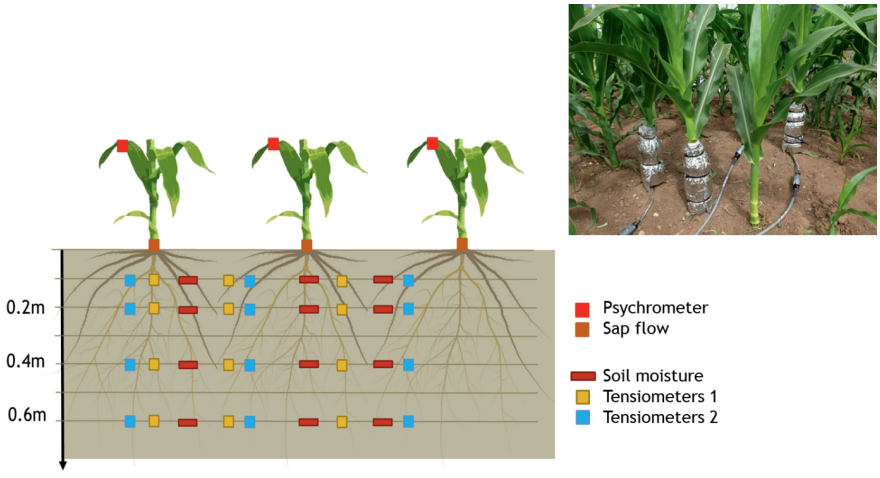

Figure 5: Sensor installation for the measurement of soil temperature, water content (TEROS 10), and matric potential (T5 and TEROS 21) at different depths and different positions in relation to the planted maize rows. In addition, plant water status is monitored with psychrometers (PSY1) and sap flow sensors (Dynamax TX).

Figure 6: Root windows allowing visual monitoring, as well as repeated and non-destructive access to field-grown maize roots during the entire vegetation period. Idea and design: Eva Oburger, BOKU—University of Natural Resources and Life Sciences; Technical construction: Gottfried Wieshammer (gottfried.wieshammer@aon.at), Technisches Büro für Bodenkultur, Austria.

2.10 Characterization of initial soil conditions

Initial chemical characteristics were determined for SPE after establishment of plots in November 2018. For each plot, a bulk sample from 5 to 10 cm depth was obtained by mixing material collected at nine points per plot. Samples were air dried, homogenized and sieved to < 2 mm prior to chemical analyses.

2.10.1 Chemical analyses (Tab. 2, Tab. S1)

Soil pH was measured using a 0.01 M $CaCl_2$ solution (soil:solution ratio of 1:2.5). Total contents of elements were determined by X-ray fluorescence analysis (Spectro XEPOS HE, SPECTRO Analytical Instruments and S4 PIONEER, Bruker-AXS). Cation exchange capacity was determined with ammonium acetate method at pH 7 according to Lavkulich (*Gregorich* and *Carter*, 2007). Carbonate was below detection limit, hence, organic carbon (C_{org}) contents and total nitrogen (N_t) were determined using a CNS analyzer (Vario Max CN Element Analyzer, Hanau, Germany) without further pretreatment. Plant available P and K were determined by CAL-method (*Schüller*, 1969). Iron-oxides were characterized by oxalate (Fe_o) and dithionate (Fe_d) extraction (*Mehra* and *Jackson*, 1960; *Schwertmann*, 1964).

Mineral nitrogen (N_{min}) was analyzed for the field plots in April 2019 (NO_3-N and NH_4-N according to VDLUFA-Methods).

2.10.2 Physical analyses

2.10.2.1 Soil texture

Texture analysis was carried out according to International Standards (*ISO 11277*, 1998; *ISO 11277*, 2002). In brief, samples were air-dried and sieved down to 2 mm. Soil organic matter was oxidized with hydrogen peroxide solution (30 Vol%). A solution of tetra-sodiumdiphosphate decahydrate was added as a dispersing agent and samples were put in an overhead shaker for 18 h. The suspension was sieved with mesh sizes of 630 μm, 200 μm, or 63 μm. The residues on the sieves were oven-dried at 105°C to determine the fractions of coarse, medium, and fine sand, respectively. The remaining suspension contained particles below 63 μm in diameter and was shaken for 1 min prior to sedimentation. Aliquots of 10 mL were taken at defined time steps, now containing only particles smaller than 20, 6.3, or 2 μm. These aliquots were transferred into glass beakers and dried at 105°C. All fractions were weighed out and their proportion to the dry mineral matter of the sample was calculated, with a correction for the weight of the dispersing agent being applied for the sedimented fractions.

2.10.2.2 Water retention curves—bulk density

In order to characterize the water retention curve (WRC) of the substrates, we used a simplified evaporation method coupled with the HYPROP apparatus (UMS GmbH Munich). For the SCE, the soil was sieved to < 1 mm and then packed to a bulk density of 1.26 g and 1.50 g, respectively, for the loam and sand. Five replicates were packed for each substrate. For SPE, one sample was extracted per plot at a depth of –15 cm prior to seeding (03/04/2019). All 250 cm^3 samples were then saturated overnight by raising a water table gradually before being placed in the HYPROP device. After the measurements, the samples were oven-dried overnight at 105°C to determine bulk density. The obtained WRC are shown in Fig. 11.

The saturated hydraulic conductivity (Ks) of the investigated soils was characterized by using a falling head method with the KSAT device (UMS GmbH Munich) for the SCE substrates. To do so, another set of five samples per soil type was prepared using the same packing procedure as for

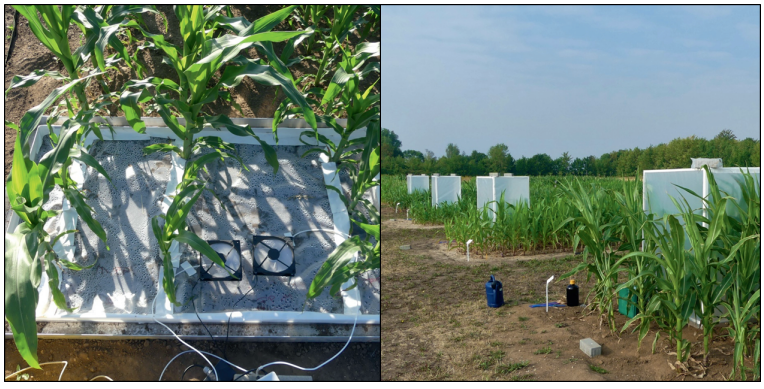

Figure 7: ^{13}C Isotope labeling in the soil plot experiment at BBCH 19. On the right, chambers in the field during pulse labeling; on the left, sealing of soil surface with a plastic foil to avoid direct diffusion into the soil.

Table 2: Selected characteristics of the substrates 'loam', 'sand' after establishment of soil plot experiment in the field. Treatments were established in 12 replicates for each substrate. Initial values for loam, quartz sand and the mixture 'sand' (16.7% loam, 83.3% quartz sand) are provided for comparison. The 'loam' was obtained by sieving and homogenizing 700 t of a haplic Phaeozem excavated from 0–50 cm depth at Schladebach (51°18′31.41″ N, 12°6′16.31″ E). The quartz sand is WF33 from Quarzwerke GmbH. Standard errors of mean are given in brackets for the field plots (n = 12). *calculated based on results of loam and quartz sand and the mixing ratio 16.7:83.3.

	Depth	Bulk density	pH ($CaCl_2$)	Carbonate	Sand	Silt	Clay	CEC	C_{org}	N_t	P plant available	K plant available	Fe_o
	(cm)	(g cm^{-3})		(g kg^{-1})	(%)	(%)	(%)	(mmol$_c$ kg^{-1})	(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	(g kg^{-1})
SPE 'loam'	5–10	1.39 (*0.01*)	6.37	< 1 (*0. 0*)	32.5 (*0.36*)	47.9 (*0.17*)	19.5 (*0.26*)	98.6 (*4.7*)	0.85 (*0.01*)	0.083 (*0.001*)	32.69 (*0.40*)	28.51 (*0.72*)	1.32 (*0.01*)
SPE 'sand'	5–10	1.50 (*0.01*)	6.29	< 1 (*0. 0*)	91.8 (*0.51*)	5.6 (*0.35*)	2.6 (*0.17*)	33.1 (*2.6*)	0.15 (*0.01*)	0.017 (*0.001*)	8.29 (*0.37*)	7.84 (*0.61*)	0.25 (*0.01*)
SCE 'loam'		1.26	6.21	< 1	33.2	47.7	19.1	76.1	0.84 (*0.01*)	0.084	33.41	26.67	1.32
SCE 'sand'*		1.47	6.25	< 1	88.6	8.1	3.3	13.0	0.14	0.014	5.67 (*0.01*)	5.44	0.22
Quartz sand WF33			5.80	< 1	99.8	0.1	0.1	0.33	0.00	0.000	0.11	1.19	0.00

the WRC determination. For the SPE substrates, we used tension disk infiltrometers and performed two measurements per plot next to each other at a depth of –15 cm on the 15/04/2019. During the infiltration, a tension of –0.5 cm and –2 cm was imposed on the disk and the Ks was then derived by solving the equation proposed by *Reynolds* and *Elrick* (1991).

In order to fit the experimentally acquired data of both the SCE and SPE, we used the software HYPROP-fit (UMS GmbH Munich). The experimental data was fitted to the bimodal Mualem–van Genuchten model (*Durner*, 1994) and by allowing all parameters of the model to be fitted, except the Ks. The resulting fitted curves and parameter sets are respectively shown on Fig. 11 and in Tab. 3.

Equation of *Durner* (1994) for multimodal pore size distribution:

$$\frac{\theta(h)-\theta_r}{\theta_s-\theta_r}=\sum_{i=1}^{k} w_i\left[\frac{1}{1+(\alpha_i|h|)^{n_i}}\right]^{m_i} \quad (1)$$

where h is the capillary pressure, θ_r and θ_s are respectively the residual and saturated VWC, k is an integer number equal to the number of subcurves considered (k = 2), w_i are the weighting factors between the subcurves, α_i is a parameter related to the inverse of the air entry capillary pressure for the subcurve i, n_i and m_i are empirical parameters for the subcurve i (note that we consider $m_i = 1–1/n_i$).

2.11 Sampling strategies and documentation

Combining monitoring approaches and invasive sampling with different strategies over time requires rigorous documentation of activities. This is done by tagging any activity in the field and recording the position by a GPS device (Leica Viva GNSS-GS08 plus) with a precision of ≤ 2 cm in x, y and z direction. As a coordinate system EPSG 25832 (ETRS89/UTM zone 32N) was used and positions are all kept in specific GIS files. These are used to avoid unintended overlap of destructive/invasive sampling activities on the one hand and to relate data sampled from close proximity on the other hand.

To accommodate a multitude of activities despite the limited size of plots, individual investigators are encouraged to share samples or access to samples whenever possible. At the same time this improves the possibility for data integration and joint interpretation.

For soil column experiments, the basic approach is the same, *i.e.*, as far as possible different groups are encouraged to share samples from the same SCE. Subsampling schemes are developed to spatially register data from different groups to each other and to integrate results obtained with different approaches (see sect. 3.3).

Table 3: Fitted parameters for the bimodal Mualem–van Genuchten model for substrates loam (L) and sand (S) for soil plot experiment (SPE) and soil column experiment (SCE). Equation (1) for establishing water retention curve according to *Durner* (1994).

Parameters	Units	Loam		Sand	
		SPE	SCE	SPE	SCE
θ_s	cm^3/cm^3	0.411	0.482	0.346	0.414
θ_r	cm^3/cm^3	0	0.004	0	0
n_1	–	3.103	3.609	9.996	7.706
α_1	1/ cm	0.0332	0.0256	0.0338	0.0315
n_2	–	1.108	1.334	1.060	1.505
α_2	1/ cm	0.1670	0.0137	0.5	0.0221
w_2	–	0.833	0.732	0.327	0.328
K_s	cm/d	441	245	1174	1864
τ	–	–1.089	1.309	–0.125	1.939

3 Results and discussion

3.1 Selected drivers and their implementation

Seeds of the two plant genotypes (WT, *rth3*) had been produced in sufficient quantity in the summer season 2018 at the experimental station of the University of Bonn to supply all SCE and the SPE with seeds from the same lot. Hence, differences in seed quality can be excluded as explaining variable.

For the substrates, providing equally homogenous quality was much more difficult. Comparing two substrates under the same environmental conditions in the field offers unique possibilities, but meant that substrates had to be homogenized as in soil column experiments and soil profiles had to be established artificially. This resulted in tremendous amounts of substrate required (700 t of loam, 550 t of quartz sand) and hence tools (wheel loader, heavy duty vibrating screen, vibrating plate) not commonly used in rhizosphere research. Substrate selection could not only be based on scientific criteria (textures sand and loam, free of carbonate, low content of organic matter, small differences in pH) but had to account for local availability in large quantity at a given time point, transport cost and handling. The selected substrates (Tab. 2) show, as intended, large differences in texture with small variability between field plots, and likewise, between field plots and soil column experiments. Along with texture, there are strong differences in cation exchange capacity and concentrations of plant available P and K. Values in (L) are by a factor of 3–4 higher than those in (S). The levels of plant available P and K are low (B) and very low (A) according to the rating of the agricultural classification system (*LLFG*, 2008). These differences were accounted for by the selection of fertilization dose, being twice as high in (S) compared to (L) for the macronutrients. Resulting tissue concentrations for column experiments are in the range of 2.1–5.4 mg g^{-1} for P and 26–45 mg g^{-1} for K for (L) and (S), respectively. This classifies them as low to adequate (*Bergmann*, 1986) and, hence, the goal to avoid luxury supply for these nutrients was achieved. N_{min} was likewise higher in (L) (1.1 mg kg^{-1}) compared to (S) (0.4 mg kg^{-1}) and in general very low. Soil pH is in the neutral range for both substrates showing low variability (Fig. 8).

C_{org} and N_t concentrations for (L) are in a range typical for a loess derived substrate under the given climatic conditions (*LLFG*, 2008) and differ by a factor of 5–6 between substrates. C_{org} (being zero in quartz sand) values were used to calculate the actual mixing ratio achieved for the soil plot experiment (Fig. 8). The median derived for the twelve field plots with substrate (S) is used as the mixing ratio for soil column experiments.

Bulk density values differ between substrates in both SPE and SCE. While similar bulk densities at laboratory and field scale could be achieved for (S), this was not possible for (L). The field bulk density of 1.39 g cm^{-3} for loam cannot be established under laboratory conditions without using special devices.

A major difference between SPE and SCE is found for soil structure (Fig. 9) as mixing and sieving of the total batch of substrate required for the field could not be done in a similar fashion as it is done for SCE. Utilizing the substrates as they were packed in the field plots for SCE without further sieving would have been an option; however, this would have introduced a large heterogeneity in SCE which might hinder the detection of chemical and physical gradients at nanometer to the millimeter scale.

A column experiment in which the two structures (Fig. 9 b, d) were compared for (S) showed no impact of the structural differences on shoot or root growth, at least not at the whole column scale (Fig. 10).

Water retention curves differ, as expected, fundamentally between (L) and (S) (Fig. 11). The differences between laboratory and field scale are a result of differences in structure and bulk density as discussed above. The substrate specific volu-

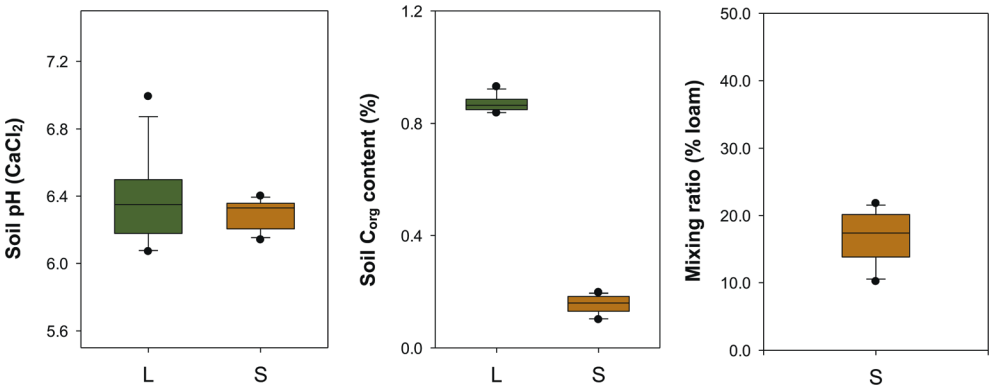

Figure 8: Soil pH and soil C_{org} content for 5–10 cm in the soil plot experiment (SPE) after establishment (November 2018). The mixing ratio for substrate sand (S), which was created by mixing substrate loam (L) with quartz sand (Q), was calculated based on C_{org} values. The median of 16.7% L is used for establishment of respective treatments in soil column experiments (SCE).

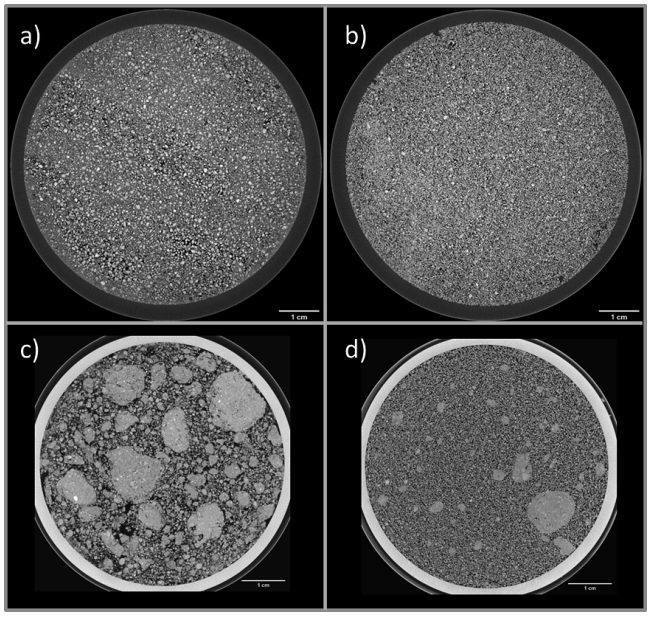

Figure 9: Soil structure in soil column experiment (SCE) and the soil plot experiment (SPE) after establishment (November 2018). Structure was visualized by X-ray CT scanning of whole columns (SCE; 7cm diameter) and extracted undisturbed soil cores (SPE, 5 cm diameter), respectively. (a) SCE–loam, (b) SCE–sand, (c) SPE–loam, (d) SPE–sand.

metric water content of 22 and 18% for (L) and (S) in SCE takes into account the steeper water potential gradient in (S) across the column height induced by lower capillarity, and the associated risk of water logging at the bottom of the sand-columns, as well as the higher share of water content below the permanent wilting point for (L). With the chosen watering levels, plant available water content averaged across the column is similar between (L) and (S) for SCE.

For SPE being exposed to natural rainfall, soil hydraulic conditions are different. The lower ability of (S) to store water is modified by the fact that a drainage layer was introduced at the lower boundary. The gravel layer acts as a capillary barrier and therefore reduces groundwater recharge and retains more water in the sand layer above.

The decision for limiting the soil profile to 75 cm and establishing a drainage layer at that depth was governed by practical considerations. The total amount of substrate to be handled had to be limited and a depth larger than 75 cm would have required construction of concrete side walls. This would have incurred substantially higher costs and time requirement.

3.2 Extrapolation from laboratory to field scale – relevance of rhizosphere processes for system behaviour

Addressing both the laboratory and field scale for rhizosphere research was motivated by two main issues: (1) the strong discrepancy in soil volume available for root growth and the potential consequences for the obtained results (*Hess* and *De Kroon*, 2007; *Poorter* et al., 2012; *Ray* and *Sinclair*, 1998), and (2) the need to judge the relevance of small scale rhizosphere processes for system behavior and the problem of extrapolating from experiments with very young plants to the whole plant life cycle.

3.2.1 Soil volume

A fundamental problem for any type of pot experiment is that available soil volume is by order of magnitude smaller in pot than in field trials. For the present example soil volume for one plant in SCE is 0.885 dm^3 while it is 78.935 dm^3 for SPE. This discrepancy remains even if we assume that for the early growth stage (BBCH 14) roots are not found below 20 cm (= 21.049 dm^3). The resulting effect for studies of root growth or rhizosphere processes is reflected in the root length density (RLD) data shown in Fig. 12. While in the field values are in the range of 0.1 cm cm^{-3}, they are 100 times higher in the SCE even at this very early growth stage (BBCH 14). Taking the classical simple concept of half mean distance (*Gardner*, 1960), assuming that all roots within a given cube are aligned in parallel [Eq. (2)], this corresponds to half mean distance

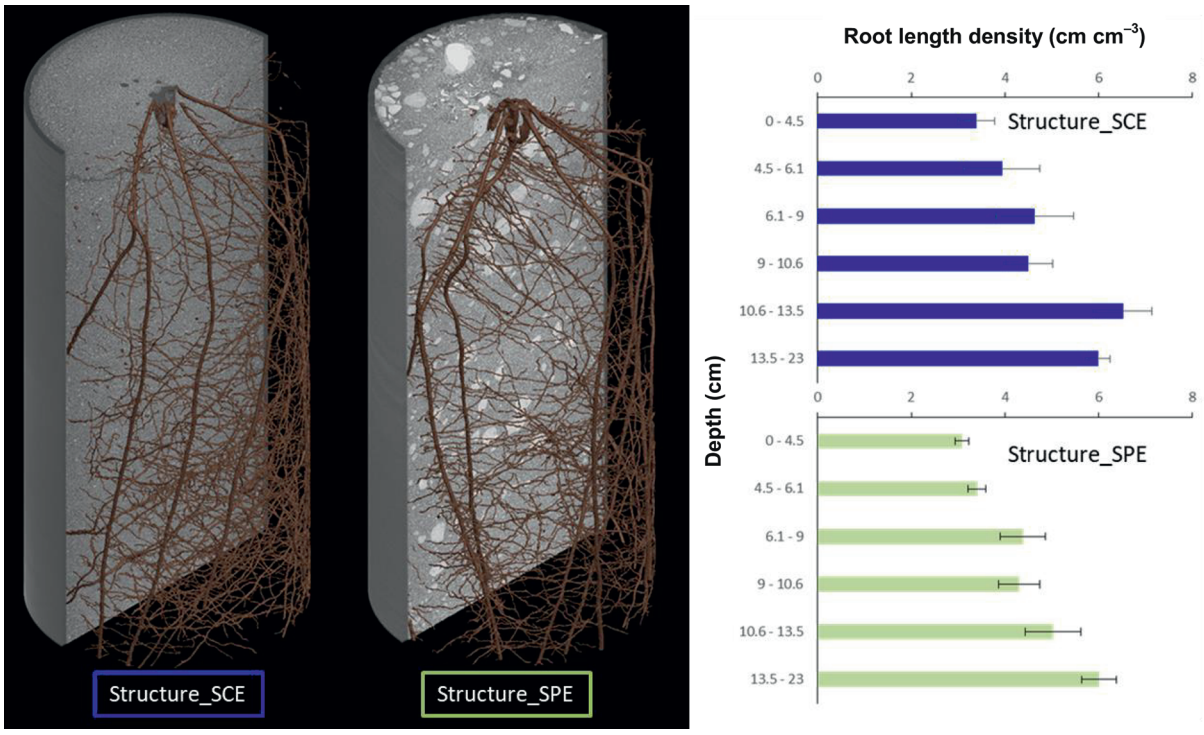

Figure 10: Root growth of *Zea mays* (B73 wild-type) in a column experiment (diameter 7cm) in substrate sand (S) for soil structure as it was established in soil plot experiments (Structure_SPE) *versus* soil column experiments (Structure_SCE). Root system architecture segmented from X-ray CT scans (left) and root length distribution with depth (right) derived from destructive root sampling. One-factorial ANOVA, $n = 3$ for individual depths, bars refer to standard error, comparison of means by Tukey-test, no significant differences were observed in any of the depths.

(HMD) of 1.78 mm for the SCE compared to 10 mm for the SPE. In other words, for a RLD of 10 cm cm^{-3} potential radial gradients around roots would start to overlap if they extent further than 1.78 mm.

$$HMD = \frac{1}{\sqrt{\pi\, RLD}}. \quad (2)$$

Alternatives are shortening the growth duration or enlarging pots. Both options have their drawbacks. Shortening the growth period means restricting the investigations to the very early growth stage in which interactions with the microbiome just start and part of the nutrients are still derived from the seed (*Julia* et al., 2018). Enlarging pots results in loss of resolution for all non-invasive imaging techniques (X-ray CT, MRI, neutron tomography). However, high resolution is required in order to detect fine roots. For the genotypes selected here depending on the substrate and age, up to 40% of the roots are smaller than 100 μm in diameter. Nominal resolution obtained with the geometry of SCE (7 cm diameter) is at best 45 μm.

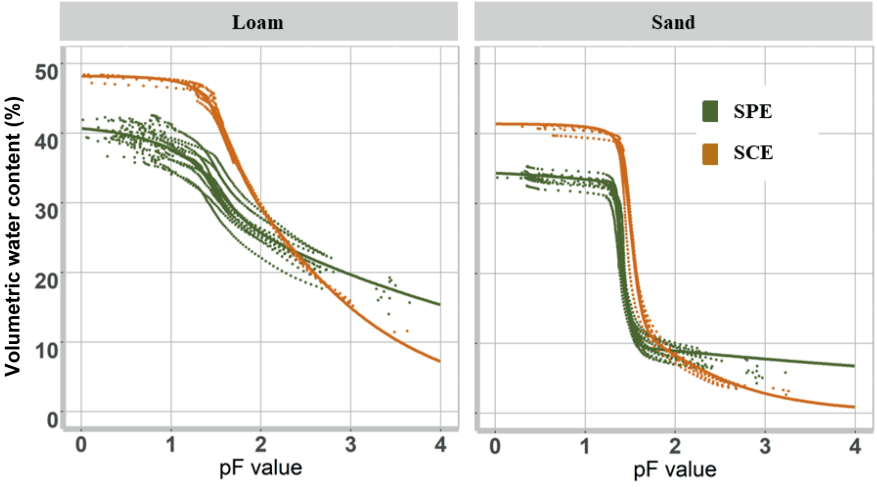

Figure 11: Measured (symbols) and fitted (solid lines) water retention curves for substrates loam (L) and sand (S) for soil plot experiment (SPE) and soil column experiment (SCE). Fitted parameters for the bimodal Mualem–van Genuchten model are presented in Tab. 3.

3.2.2 Life cycle

Accepting limitation of pot experiments to early developmental stages leads to the necessity to investigate later stages in the field. As it is well known that root function and hence associated rhizosphere processes are changing with root ontogeny and with plant age (*Vetterlein* and *Doussan*, 2016), extrapolation from the early growth stage to the whole life cycle is not justified. In addition, some of the prominent plant

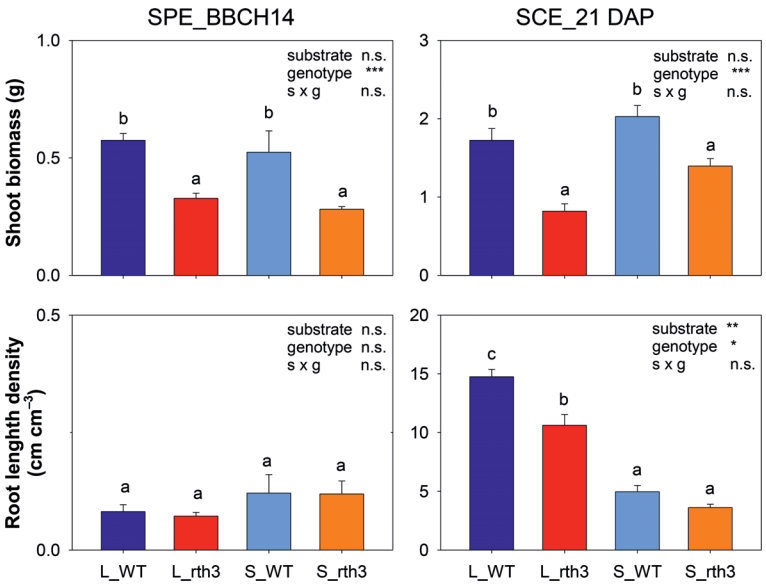

Figure 12: Impact of substrate (L, S) and genotype (WT, *rth3*) on shoot biomass and root length density in soil plot experiment (SPE) and soil column experiment (SCE) for comparable growth stage BBCH 14, which is obtained in SCE 21 d after planting (DAP). Two-factorial ANOVA, $n = 6$, bars refer to standard error, comparison of means by Tukey-test, significant differences are indicated by different letters.

microbiome interactions, like mycorrhizal symbioses, need two to several weeks for establishment, depending on, *e.g.*, soil temperature, nutrient levels, and plant host (*Jakobsen* and *Nielsen*, 1983; *Abbott* and *Robson*, 1991).

Under field conditions, plants are typically confronted with soil characteristics changing with depth and this is shaping root distribution and hence the rhizosphere (*Lynch*, 2013). This is not the case in SPE at the initial state except for soil water distribution driven by gravity and capillarity. However, starting with these homogenous conditions will allow studying changes induced by plant growth in general and more specifically by root activity. It is expected that depth gradients in soil structure, nutrient availability, carbon input and microbial activity and composition will develop over time. SPE is expected to run for six consecutive years.

A further difference between laboratory and field scale is the fact that SCE investigates the individual plants while in the field (SPE) the plant stock is investigated. It cannot be ruled out, that differences arise from interactions of roots from the same individual (SCE) *versus* roots from neighboring plants (SPE), even though neighboring plants share the same genotype (*Hess* and *De Kroon*, 2007; *Poorter* et al., 2012).

3.3 Integration of sampling strategies across disciplines

The easiest compromise for joint interpretation of data across different disciplines and between different working groups is using the same drivers (here substrate and genotype) for independent experiments. A higher degree of integration is achieved by using whenever possible the same experimental platforms (here SPE, SCE). The optimal situation for integrative research is to sample the same experiment at the same point in time and, when applicable, at the same location. This final step poses a particular challenge. In order to obtain accurate information of rhizosphere activities, two further aspects of sampling are taken most seriously: short duration and accurate timing of harvests. Particular challenge for sampling in the field is that increasing sampling time lowers the quality of enzyme activity or metabolite or gene expression level analyses. For instance, inconsistencies in RNA quality causes widespread changes in RNA sequencing based gene expression levels (*Romero* et al., 2014). Sample collection timing is another important factor for the analysis of activities, which follow diurnal rhythms, such as root gene expression (*Covington* et al., 2008) and rhizosphere microbiome structure and function (*Hubbard* et al., 2018), albeit not significant for others, such as root exudation (*Badri* et al., 2010).

3.3.1 Non-invasive approaches for data integration and guiding of sampling

For soil column experiments, a workflow was developed to provide temporally resolved information on root architecture based on X-ray CT (*Schlüter* et al., 2018). This workflow can be used to describe the spatial context of *in situ* sampling over time, such as soil solution sampling (*Gao* et al., 2019; *Lohse* et al., 2020), but they can also be used as a guide and provide the spatial context for destructive sampling and subsampling (Fig. 13). To obtain information from different depths, 1.6 cm thick layers are cut with a sharp steel blade without disturbing soil structure. Such layers have been used for the harvest of rhizospheres for microbiome analyses and sampling of roots for RNA sequencing (*Ganther* et al., 2020). The layers are further divided into undisturbed subsamples (diameter 1.6 cm) which, after sample fixation, high resolution 3D X-ray scanning, and resin embedding, are used for a range of 2D chemical mapping techniques working at the nm and μm resolution. This will enable visualization, quantification and merging of chemical and physical gradients around individual roots of known age. These data can be embedded into information on the gene expression and microbiome composition data derived from the same layer from the material surrounding the subsamples and the root age and root spatial distribution data from CT analysis. The data set can be complemented by information describing the whole system like overall root and shoot growth, plant nutrient and water uptake or root exudation. The list is not exhaustive and it is not always possible to perform all measurements simultaneously in the same system. In particular, measurement of root exudation requires intact root systems (*Oburger* and *Jones*, 2018). For biological parameters, potential artifacts like irradiation damage caused by X-ray CT scanning has to be quantified or ruled out prior the establishment of the workflow (*Schmidt* et al., 2015; *Blaser* et al., 2018; *Ganther* et al., 2020).

For the field, X-ray CT cannot fulfill the same purpose in terms of integration of data. It can still be used for investigation of

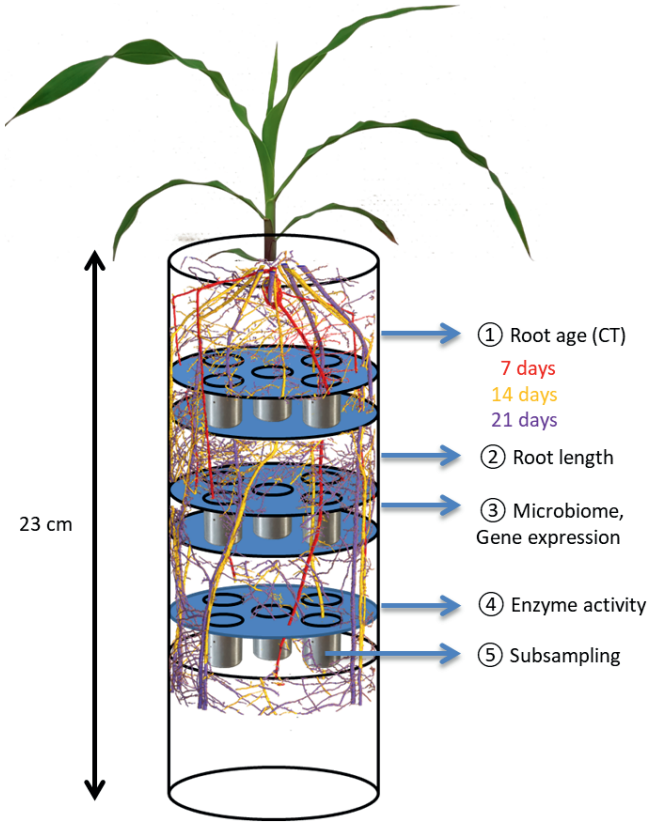

Figure 13: Illustration of a workflow for integrated sampling in soil column experiments (SCE) guided by X-ray CT scanning.

root architecture and for the work-flow associated with subsampling, but root age cannot be derived since *in vivo* application is not possible. Transparent interfaces in a soil profile, root windows, are used to describe root growth and architecture and to merge different techniques as it has been shown for rhizoboxes (*Neumann* et al., 2009; *Spohn* et al., 2015; *Kreuzeder* et al., 2018).

Another issue arises in the field experiment, *i.e.*, a given variability within a plot that needs to be captured by an appropriate sample size or number of technical replicates. An increased number of technical replicates is in stark conflict with the objective of minimal field area consumption and the idea of keeping working time and costs reasonable. Here, the compromise is oriented towards keeping both space consumption and workload as low as possible by sharing the sample material among scientists. Further benefit of this approach is that using the same sample facilitates the joint interpretation of the data. Similarly to the column experiment, detailed multidisciplinary foci on rhizosphere processes are complemented by information describing the whole plant–soil system, like overall root and shoot growth, plant nutrient and water uptake, soil carbon and nitrogen and microbial biomass levels.

First results on root and shoot growth from SCE and SPE (Fig. 12) show that, among them, the relative differences between treatments are similar regarding shoot growth at the early growth stage (BBCH 14). In both experimental platforms, the maize WT plants grow better than the *rth3* plants. This observation is in line with the expectation that under marginal P supply, implemented in both systems, root hairs contribute to P uptake. That the substrate showed no impact on shoot growth, is in part explained with our choice of fertilization level for the two substrates aiming at similar shoot size for the WT. Surprisingly, root growth at this early stage shows contrasting trends for laboratory and field scale. While at the laboratory scale root growth is higher in loam compared to sand, we observed an opposite trend in the field. For root length density, differences between genotypes are absent in the field and small in the laboratory compared to the impact of substrate.

4 Conclusion and outlook

With the establishment of the experimental platforms, a basis for interdisciplinary cooperation was laid. We consider this interdisciplinarity as indispensable for a fundamental gain of knowledge in rhizosphere research. Carrying out joint sampling and exchanging samples from the same system not only facilitates interpretation of the complex interactions and feedbacks between soil, root system and microorganisms, but also raises awareness of the needs of the different discipline-specific methods. The combination of laboratory and field scale is a great challenge but indispensable (see also *Darrah* et al., 2006; *York* et al., 2016). In principle, it is expected that although the absolute values differ, the relative differences between treatments point in the same direction in both experimental platforms. Whenever this is not the case, theories must be questioned, which can lead to the conception of new experiments and advancing the field to new areas. For the field scale, SPE provides the unique possibility to follow long-term changes of soil properties induced by a rhizosphere trait specific difference which is related to a mutation of a single gene and affects only root hair formation.

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