Soil texture and structure heterogeneity predominantly governs bulk density gradients around roots

Maxime Phalempin1 | Eva Lippold1 | Doris Vetterlein1,2 | Steffen Schlüter1

1 Dep. of Soil System Science, Helmholtz Centre for Environmental Research—UFZ, Theodor-Lieser-Strasse, 4, Halle/Saale 06120, Germany
2 Soil Science, Martin-Luther-University Halle-Wittenberg, Von-Seckendorff-Platz 3, Halle/Saale 06120, Germany

Correspondence
Maxime Phalempin, Dep. of Soil System Science, Helmholtz Centre for Environmental Research—UFZ, Theodor-Lieser-Strasse, 4, 06120 Halle/Saale, Germany.
Email: maxime.phalempin@ufz.de

Abstract
Rhizosphere soil is known to differ from the bulk soil due to numerous physicochemical processes induced by root growth. The spatial extent and magnitude of the influence of roots on the surrounding soil is still debated controversially. To date, most studies focused on a limited number of soil types and plant species and were carried out under homogeneous soil structure conditions (i.e., finely sieved and repacked soil). With the help of X-ray computed tomography (CT), we present the results of an image processing workflow, which enabled to analyze soil structure around roots of maize (Zea mays L.) plants under different degrees of soil structure heterogeneity. We analyzed >400 samples extracted during laboratory and field experiments covering various combinations of texture, bulk density, packing heterogeneity, maize genotype, and soil moisture. We show that soil texture and structure heterogeneity predominantly governs the magnitude of bulk density alteration around roots. In homogeneous soil structure, roots had to create their own pores by pushing away soil particles, which confirms previous findings. Under more heterogeneous conditions, we found that roots predominantly grew in existing pores without inducing compaction. The influence of root hairs, root length density, and plant growth stages had no or little impact on the results. The effect of root diameter was more pronounced in sand than in loam. Fine roots caused sand grains to align along their axis, whereas big roots broke the fragile arrangement of grains. Our findings have implications for water and solute transport dynamics at the root–soil interface, which may affect plant productivity.

1 | INTRODUCTION

Characterizing soil properties at the root–soil interface is key to a better understanding of environmental processes and to the promotion of sustainable agriculture. Indeed, this interface mediates water and nutrient uptake as well as root exudates distribution and diffusion. The zone of the soil in the vicinity of roots which is influenced by them is termed the “rhizosphere,” whereas the zone which is not affected by the roots is referred to as the “bulk soil.” The rhizosphere soil is known to differ from the bulk soil due to the numerous physical, chemical, and biological processes induced by root growth (Hinsinger et al., 2009; Rugova et al., 2017; Vetterlein et al., 2020; Whalley et al., 2005). Such processes include

Abbreviations: 3D, three-dimensional; BD, bulk density; CT, computed tomography; EDT, Euclidian distance transform; GV, gray value; RLD, root length density; ROI, region of Interest; rth3, root hair defective mutant; WT, wild type
particle rearrangement, which may lead to soil compression or loosening (i.e., to an increase or decrease of local bulk density [BD]), both of which are known to alter water regimes and gas exchanges in the soil (Menon et al., 2015; Pla et al., 2017). The earliest evidences of the influence of soil particle rearrangement by roots suggested a compaction effect, as assessed visually with microscopy (Barley, 1954; Bruand et al., 1996). More recently, the advent of X-ray computed tomography (CT) has offered the possibility to investigate the rhizosphere with unprecedented accuracy and nondestructively. In the last decade, this motivated researchers to determine rhizosphere soil properties for different soil types and plant species. To date, however, the literature is still controversial regarding the magnitude and spatial extent of the influence of roots on the surrounding soil. Some studies reported root-induced soil compaction (Aravena et al., 2011; Vollnes et al., 2010), whereas some studies observed a zone of looser soil around the roots (Burr-Hersey et al., 2020; Helliwell et al., 2017). Some other studies reported both (i.e., a zone of higher porosity at the direct root–soil interface followed by a zone of lower porosity farther away [Helliwell et al., 2019; Koebernick et al., 2017, 2019; Lucas et al., 2019]). Moreover, most of these studies have been carried out in laboratory conditions with repacked and finely sieved soil. There is currently a lack of knowledge as to how the results acquired under laboratory conditions can be transferred to field conditions where the soil heterogeneity is greater.

On one hand, root-induced soil compaction can be attributed to the fact that the volume that roots occupy in soil has to be accommodated by an equal loss of the pore space volume. Dexter (1987) was the first to mathematically describe root-induced compaction when considering the radial expansion of roots in soil. On the other hand, an increase of macroporosity in the rhizosphere was attributed to fluctuations in water content due to root water uptake and/or to wetting and drying cycles, which were shown to be responsible for crack formations (Ma et al., 2015), especially in clayey soil (Yoshida & Hallett, 2008). Increase of porosity in the vicinity of roots was also attributed to the phenomenon of root shrinkage occurring during soil desiccation (Carminati et al., 2009). Another mechanism, which can potentially explain the macroporosity increase at the root–soil interface is the packing of soil particles at the root surface (Koebernick et al., 2019). The influence of packing of convex particles around a larger rigid surface is known to generate a zone of higher porosity at the interface between the particles and the rigid surface. This phenomenon is known as the “surface wall effect” and it has been studied both experimentally and mathematically for randomly packed beds of spheres in cylindrical containers (Guo et al., 2017; Mueller, 2010; Reimann et al., 2017; Suzuki et al., 2008).

The initiation of root hairs is one root trait among many, which enables plants to adapt to environmental conditions. Root hairs provide a significant advantage for plant growth, particularly under conditions which restrict root elongation (e.g., in compacted soils; Haling et al., 2013). The role of root hairs in root tip anchorage was suggested by Stolzy and Barley (1968) and later experimentally demonstrated by Bengough et al. (2016) for very young primary roots of maize (Zea mays L.) plants still lacking laterals. The advantage in anchorage provided by root hairs may suggest that by penetrating soils having a higher mechanical resistance, roots of a hair-bearing plant genotype may compact the soil more because of their greater ability to push away soil particles or to ingress denser soil aggregates instead of being deflected by them. Root hairs are also known to influence the rhizosphere soil by promoting soil aggregation (Moreno-Espíndola et al., 2007). Root hairs enable higher transpiration rates under drying conditions (Carminati et al., 2017), which would, in turn, increase the likelihood of crack formation and thereby increase macroporosity. The effect that root hairs may exert on soil particles is still debated controversially. Using X-ray CT scanning, Koebernick et al. (2017) found that a barley (Hordeum vulgare L.) genotype with root hairs had a significantly greater soil pore volume fraction at the root–soil interface, as compared with the root hair defective mutant. However, no differences in pore structure were found for the same genotypes investigated in a subsequent experiment in which the sieving procedure was modified (Koebernick et al., 2019).

Based on the literature, it seems that the extent to which the soil around roots differs from the bulk soil might be the result of interacting factors such as soil type, plants species (Helliwell et al., 2019), and the presence of root hairs (Koebernick et al., 2017). In an attempt to reconcile contrary views on the subject, Lucas et al. (2019) introduced a new conceptual model, which suggests that the initial soil structure (i.e., the availability of pores big enough for roots to grow into) is the governing factor to predict root-induced soil compaction.

In this work, we present an extensive study of BD distribution extending from the root surface to the bulk soil using X-ray CT. Specifically, we investigated the following hypotheses:

**Core ideas**

- Roots rearrange soil particles during ingression into the soil.
- Bulk density distribution around roots is predominantly governed by soil texture and structure.
- Our findings have implications for water and solute transport at the root–soil interface.
Changes in soil structure around roots depend on the particle size (i.e., the texture) and the spatial arrangement of soil particles (i.e., the structure). Following up on the work of Lucas et al. (2019), we hypothesize that root-induced compaction, if observed, will be greater in a structurally homogenous environment (i.e., finely sieved and repacked soil) as compared with a heterogeneous environment (coarsely sieved and structured soil).

Modification of soil structure induced by root growth depends on root diameter. In line with the mathematical background provided by Dexter (1987), we hypothesize that root-induced compaction, if observed, will be greater for big roots than for fine roots due to the increased volume of big roots.

The presence of root hairs has an influence on the modification of soil structure induced by root growth. Bearing the experimental results of Bengough et al. (2016) in mind, we hypothesize that root-induced compaction, if observed, will be greater for roots with root hairs as compared to roots without root hairs due to the role of root hairs in providing anchorage.

In order to address these hypotheses, we compiled data from several laboratory and field experiments. Specifically, we characterized BD distribution extending from the root surface into the bulk soil for two Zea mays genotypes (i.e., one root hair-bearing genotype and one root hair defective mutant) grown in two different soil textures (i.e., loam and sand), in experiments in which the soil sieving, packing procedure, and BD differed. Thanks to the sampling at different plant growth stages and to the different plant growth conditions in the field and in the laboratory, additional insights are provided regarding the influence that the age of the rhizosphere, its desiccation history, and the root length density (RLD) has on rhizosphere soil structural properties. With the help of X-ray CT, we present here the results of an image processing workflow, which enabled to analyze BD in the vicinity of roots for >400 samples.

2 | MATERIAL AND METHODS

2.1 | Genotypes

In order to determine the influence of root hairs on rhizosphere soil structural properties, the Zea mays genotype root hair defective mutant (rth3) and the corresponding B73 wild type (WT) were selected for the growth experiments. The mutant rth3 shows normal root hair initiation but disturbed elongation. It shows no aberrant shoot phenotype, but grain yield is reduced by 19–42% as compared with the WT (Hochholdinger et al., 2008).

2.2 | Soil material

The substrate loam was obtained from the upper 50 cm of a haplic Phaeozem soil profile. The substrate sand constitutes a mix of 83% quartz sand (WF 33, Quarzwerke Weferlingen) and 17% of the loam. Details on chemical and physical properties are provided elsewhere (Vetterlein et al., 2021). The loam and sand substrates were the same for all experiments carried out in this study. However, soil structure differences across experiments arose due to differences in sieving and packing procedures.

2.3 | Experimental setup and sample preparation

2.3.1 | Field experiment

The field experiment was established in Bad Lauchstädt, Germany (51°22′00″ N, 11°49′60″ E). In order to ensure homogeneity in terms of soil structure at the start of the experiment, 24 individual rectangular pits (11 × 3.1 m) were excavated to a depth of 1 m. The pits were filled with a 25-cm-thick gravel layer and a 75-cm-thick layer of the loam or sand packed to a BD of 1.36 and 1.5 g cm⁻³, respectively. The loam was sieved using a heavy duty double deck vibrating screen (Keestrack Combo; 3.2-m² area per deck) having a mesh aperture of 20 mm. After sieving, the loam was gradually placed in layers of 15 cm by a wheel loader (Volvo EW140C), evened out with the wheel loader bucket, and compacted with a vibrating plate (weight 70 kg with a pressure of 80 kPa at reduced speed). For the sand plots, a similar procedure was used, but no vibrating plate was used. Treatments were established in 12 replicates for each soil type. Of the 12 plots of one soil type, six were planted with the WT genotype, the other six with the rth3 genotype. More information regarding the field plot setup and the plant growth conditions in the field experiment is provided by Vetterlein et al. (2021). In the field experiment, two approaches were chosen to obtain the samples. Figure 1 provides an overview of the experimental design of the two sampling approaches in the field. Table 1 summarizes the difference between the two approaches in terms of sieving and packing procedure.

The first approach entailed the sampling of undisturbed cores at different plant growth stages during the growing season. The cores were extracted with aluminum cylinders of 5 cm in height and diameter. The sampling campaigns were carried out at different growth stages (i.e., BBCH14, BBCH19, BBCH59, and BBCH83), in accordance with the German coding of phenological growth stages of maize (Bleisholder et al., 2001). At BBCH14, the sampled depth intervals were 2.5–7.5 and 12.5–17.5 cm considering the shallow
FIGURE 1 Overview of the experiments conducted in the field and in the laboratory where the number of samples extracted is specified for each treatment. The drawing is not to scale. Dimensions are expressed in millimeters. The letters “L” and “S” refers to loam and sand, respectively, whereas the acronyms “WT” and “rth3” refer to the wild type and root hair defective mutant, respectively. Note that the number of samples refers to the total number of samples extracted and not to the number of samples analyzed for the characterization of rhizosphere soil properties (i.e., samples with no roots were excluded).

rooting depth of the plants at this early growth stage. For the later growth stages, cores were taken at the depth intervals 2.5–7.5, 12.5–17.5, 22.5–27.5, and 32.5–37.5 cm in the same pit in between the maize rows. Once the cores were extracted, they were stored at 4 °C in sealed plastic bags in order to avoid desiccation prior to CT scanning. In total, 384 undisturbed cores were extracted from the field. The first approach investigated the effect of soil structure heterogeneity, plant growth stages, and soil moisture conditions on rhizosphere soil structure properties and aims at capturing field conditions as well as possible.

The second approach entailed placing ingrowth cores underneath the growing plants and leaving them during the entire growing season in order to allow the roots to populate the cores. The cores were packed in plastic cylinders of 5 cm in height and diameter. The wall, top, and bottom of the cylinders were perforated with a drill bit of 2 mm in diameter so that roots could enter the cores from all sides. The loam in the ingrowth cores was filled in the laboratory with soil sieved down to 2 mm (i.e., it is more homogenous than in the undisturbed cores). The sand originated from the sieved material used to fill the plots in the first field experiment (i.e., sieved down to <20 mm) and was not further sieved down as all sand grains that make up the typical granular structure would have passed the 2-mm sieve anyway. The BD to which the ingrowth cores were packed was the same as in the field plots. The soil was gradually filled by layers of 1 cm and gently consolidated at each filling step. Once packed, the cores were buried in field plots and two cores per plot were placed next to each other at 15-cm depth right underneath a plant shortly after germination. The location was accessed laterally through an excavated tunnel so that the topsoil in the field plot was not disturbed. In total, 48 ingrowth cores were buried in the field plots. At harvest, they were extracted and stored at 4 °C in sealed plastic bags in order to avoid desiccation prior to X-ray CT scanning. The motivation behind the establishment of the second approach was to create a different growing environment for roots by combining a finer sieving (for loam) and a more uniform packing of the soil as compared with the undisturbed cores and to still capture field conditions.
| Experiments                     | Soil texture | Plant genotype<sup>a</sup> | BD  | Sieve aperture | Filling method                                                                 |
|--------------------------------|--------------|----------------------------|-----|----------------|--------------------------------------------------------------------------------|
|                                |              |                            | g cm<sup>−3</sup> | mm             |                                                                                |
| Laboratory experiment 1        | Loam         | WT                         | 1.26| 1              | Pouring and settling                                                            |
|                                | Sand         | rth3                       | 1.47|                |                                                                                |
| Laboratory experiment 2        | Loam         | WT                         | 1.26| 1              | Filling by layers and gently consolidating after each layer                      |
|                                | Sand         | WT                         | 1.36|                |                                                                                |
| Undisturbed cores in the field | Loam         | WT                         | 1.36| 20             | Filling by layers and compacting with a vibrating plate                          |
|                                | Sand         | rth3                       | 1.5 |                |                                                                                |
| Ingrowth cores in the field    | Sand         | rth3                       | 1.5 |                |                                                                                |
|                                | Loam         | rth3                       | 1.36| 2              |                                                                                |

<sup>a</sup>WT, wild type; rth3, root hair defective mutant maize genotype.

### 2.3.2 Laboratory experiments

The laboratory experiments were conducted with soil columns in a climate chamber. The climate chamber was set to 22 °C during the day and 18 °C at night with a 12-h light period, 350 μM m<sup>−2</sup> s<sup>−1</sup> of photosynthetically active radiation, and a constant relative humidity at 65%. Harvest was conducted on Day 22 after planting. In the laboratory, this growth duration corresponded to the BBCH14 plant growth stage (i.e., four leaves unfolded). Water content was tested in trial experiments in order to ensure appropriate soil moisture conditions for plant growth and to avoid water logging in the bottom part of the sand columns. The retained average volumetric water content values were 22 and 18% for loam and sand, respectively. At the end of the growing period, subsamples were taken using a custom made subsampling device (UGT). In the laboratory, two experiments were conducted. For both experiments, six subsamples per column were extracted (two per depth at −5, −10, and −15 cm). Differences between the two experiments arose from differences in treatments, sample size, and soil packing procedure (Table 1). Figure 1 provides an overview of the experimental design of the two experiments conducted in the laboratory. The motivation behind the establishment of the laboratory experiments was to compare rhizosphere soil structure under well-watered conditions with field conditions for which fluctuating water content as well as drought stress occurs.

For the first experiment, acrylic glass tubes (25-cm height, 7-cm i.d.) were filled with soil and packed carefully in order to avoid particle sorting and hence the presence of layers. The soil was sieved down to 1 mm and poured into the columns up to a height of 23 cm. The loam columns were packed to a BD of 1.26 g cm<sup>−3</sup>, whereas the sand columns were packed to a BD of 1.47 g cm<sup>−3</sup>. Filling the columns to the target BD was achieved by repeatedly and delicately tapping the column on a flat surface to provoke particle settlement and slight compaction. The subsamples extracted at the end of the growing period consisted of aluminum cylinders of 1.6 cm in height and diameter. Six columns of each combination of soil type (i.e., loam and sand) and plant genotype (i.e., WT and rth3) were sampled. This sampling procedure yielded a total of 144 subsamples, which were stored at 4 °C in sealed plastic bags prior to X-ray CT scanning. The first laboratory experiment aimed at evaluating the effect of texture and root hairs on rhizosphere soil structure properties.

For the second experiment, plants were grown in the laboratory in the same fashion as for the first experiment in terms of growth duration and conditions. However, subsample size, column size, and soil packing methodology differed. The packing procedure was changed from pouring the soil into the columns at once to gradually placing layers of soil and by gently consolidating the soil at each filling step. In the second experiment, only the WT genotype was used, but the columns were packed to different BD in order to investigate the effect
TABLE 2

| Variable          | Unit | Subsamples (Exp. 1) | Subsamples (Exp. 2) | Undisturbed cores | Ingrowth cores |
|-------------------|------|---------------------|---------------------|-------------------|----------------|
| Core diameter     | mm   | 16                  | 30                  | 50                | 50             |
| Image resolution  | μm   | 10                  | 19                  | 25                | 25             |
| Beam energy       | kV   | 115                 | 130                 | 140               | 140            |
| Beam current      | μA   | 85                  | 150                 | 200               | 200            |
| No. of projections|      | 2,748               | 2,500               | 2,400             | 2,400          |
| Exposure          | ms   | 1,000               | 700                 | 500               | 500            |
| Duration of scan  | min  | 46                  | 29                  | 20                | 20             |

Core diameter, image resolution, beam energy and current, number of projections, exposure time for each projection and time required per sample for all samples analyzed in the experiments conducted.

of different degrees of soil compaction. The columns (20-cm height, 10-cm i.d.) were filled up to 18 cm in 10 layers step. For the low BD treatment, four columns per soil type were packed to 1.26 and 1.5 g cm

-3 for the loam and the sand, respectively. For the high BD treatment, four columns per soil type were packed to 1.36 and 1.6 g cm

-3 for the loam and the sand, respectively. At the end of the growing period, six subsamples of 3 cm in diameter and height were extracted per column. This sampling procedure yielded a total of 96 subsamples to analyze, which were stored at 4 °C in sealed plastic containers prior to X-ray CT scanning. The second laboratory experiment aimed at evaluating the effect of texture and BD on rhizosphere soil structure properties.

2.4 X-ray computed tomography scanning

X-ray CT scanning was performed with an industrial μCT scanner (X-TEK XTH 225, Nikon Metrology) having an Elmer-Perkin 1620 detector panel (1,750 × 2,000 px). The obtained images were reconstructed into a three dimensional (3D) tomogram having an 8-bit grayscale via a filtered back projection algorithm with the CT Pro 3D software (Nikon Metrology). The conversion to 8-bit allowed saving considerable space without losing considerable information. During the 8-bit conversion, the grayscale range was normalized with a percentile stretching method. This method sets the darkest and brightest 0.2% voxels to 0 and 255, respectively, and performs a linear stretching in between. Considering the different size of the samples analyzed in this study, the image resolution, beam energy, beam current, number of projections, and exposure time for each projection were adjusted for all experiments (Table 2).

2.5 Root segmentation and diameter differentiation

Root segmentation was performed with a modified version of the root segmentation algorithm “Rootine v.2” (Phalempin et al., 2021). Rootine v.2 is a free macro available for the free image analysis software ImageJ (Schindelin et al., 2012). In addition to gray value (GV) information, Rootine v.2 is based on the shape detection of cylindrical roots. Some of the key steps of Rootine v.2 and modifications of the original version for this study (when applicable) are briefly described below.

After X-ray CT scanning, all images were visually analyzed and samples devoid of roots were not considered for analysis. For the remaining samples, circular regions of interest (ROIs) were defined. The circular ROIs were created in order to exclude the wall from the analyzed region. Subsequently, the size of the circular ROI was reduced by 10% in order to exclude regions where potential soil structure disturbance due to sampling was present.

After defining the ROI, the images were subjected to preprocessing steps. First, the images were filtered with a 3D nonlocal means filter (Tristán-Vega et al., 2012) available in the ITK library (McCormick et al., 2014), except for the samples having a resolution of 10 μm for which the two-dimensional (2D) nonlocal means filter available in ImageJ was used. After filtering, a step of edge enhancement was performed with the “Unsharp Mask” filter available in ImageJ. Then, a background removal step was applied via an “absolute difference transform” whose rationale and technical aspects are described by Phalempin et al. (2021). The obtained images served as input for the root segmentation per se.

Rootine v.2 is tailored for the segmentation of roots in whole-column scans where the image resolution is close to the smallest root diameter to segment. For whole-column scans, the distribution of root diameters is continuous within a range that is plant species dependent. For the samples used in this study, the distribution of root diameters is not continuous but rather discrete, considering that roots of different types and orders can be present within a sample and vary substantially amongst all samples in a dataset. To cope with this discreteness, Rootine v.2 was modified in order to perform a “root diameter targeted approach” instead of using a “root diameter incremented approach,” as implemented in the original version of Rootine v.2. With the new approach, every image was visually analyzed and the diameter of the roots in
the image was measured using the “Measure” tool available in ImageJ. The sigma values of the tubeness filter implemented in ImageJ were then calculated based on the measured root diameters according to the formalizing steps described elsewhere (Phalempin et al., 2021). The results of the tubeness filter were segmented using the “3D Hysteresis Thresholding” (Ollion et al., 2013) available in ImageJ.

After root segmentation, the obtained images were subjected to post-processing steps. First, a 3D median filter available in ImageJ was applied in order to smoothen the root surface. In the original version of Rootine v.2, small isolated objects are discarded by using a connectivity criterion of the root branches from top to bottom, which is tailor-made for whole-column scans. For the samples as used in this study, roots can enter the ROI from all sides. In order to cope with this, Rootine v.2 was modified and the connectivity criterion was replaced by a size exclusion criterion in order to get rid of every object whose size fell under a user-defined threshold. The size exclusion step was performed with the “Size Opening (2D/3D)” plugin available in the “MorphoLibJ” plugin suite (Legland et al., 2016). The size exclusion threshold was adapted for every dataset since the number of voxels comprising a false positive depends on the image resolution. With the obtained images, the root length was calculated after a step of skeletonization with the “Skeletonize (2D/3D)” plugin available in the BoneJ plugin (Doube et al., 2010).

Additional image processing steps were applied on the segmented root images in order to analyze rhizosphere soil structure properties for different root diameter classes. To differentiate root diameter classes, the root diameter was first computed with the “Local Thickness” plugin available in ImageJ. This plugin relies on the “Maximum Inscribing Sphere” method and assigns to every root voxel a value corresponding to the diameter of the largest sphere, which locally fits into the root. The results were then segmented with threshold values corresponding to the defined diameter classes. Two diameter classes were defined, one class with roots having a diameter smaller than 200 μm and one class with roots having a diameter superior to 350 μm.

2.6 Pore segmentation, and pore and grain size distribution analysis

Pore segmentation was performed with a simple thresholding method. This resulted in images where two distinct phases were segmented (i.e., one phase for the pore space and one phase for the soil matrix). The threshold detection methods and the preprocessing steps were adapted in order to obtain the best results possible for each dataset. The accuracy of the pore segmentation was evaluated visually by comparing the original grayscale data and the outline of the segmented pores. For the samples having a resolution inferior or equal to 19 μm, the original grayscale images were subjected to edge enhancement with the “Unsharp Mask” filter available in ImageJ. This step enhances the local contrast between the matrix and the pores (Schlüter et al., 2014) and improved the pore segmentation results. The “Otsu” method (Otsu, 1979) in ImageJ was then used to determine an adequate threshold for each image. For the samples having a resolution of 25 μm, the images were filtered with a 3D nonlocal means filter and the filtered image served as input for the pore segmentation. The threshold values were determined with the fuzzy c-means clustering method (Jawahar et al., 1997) using Quantim v.4 (www.quantim.ufz.de). After segmenting the pores, the analysis of pore and grain size distribution was conducted on the binary pore and grain images with the “Local Thickness” plugin available in ImageJ.

2.7 Characterization of rhizosphere soil properties

The characterization of the rhizosphere soil structure properties was performed by assuming that changes in GVs are a suitable proxy for changes in BD. This rationale is based on the intrinsic properties of the X-ray attenuation (i.e., a feature of low density [e.g., an air-filled pore] exhibits a low X-ray attenuation and is hence assigned a low GV after reconstruction of the tomogram). In contrast, an object of high density (e.g., a soil aggregate) exhibits a high X-ray attenuation and is therefore assigned a high GV after reconstruction. In addition, we consider GV as a proxy for BD as more reliable than porosity because GVs contain information on subresolution features and analyzing GVs does not require the choice of thresholding values for pore segmentation.

The characterization of the rhizosphere soil structure properties was performed in a similar fashion as in Lucas et al. (2019). This characterization relies on the computation of the so-called “Euclidian distance transform” (EDT). The EDT was computed on the binary root system images with the dedicated “exact Euclidian distance transform (3D)” method available in ImageJ (Supplemental Figure S1). The result of this operation is an image in which every soil voxel is assigned a value corresponding to its distance to the closest root in a 3D volume. The EDT result is then combined with the original grayscale image into a composite image. This combination yields an image in which every voxel contains the grayscale information in one channel and the distance to the closest root information in another channel. On the composite image, a loop in the X and Y dimensions is then initiated for all Z slices, and the information of both channels is retrieved simultaneously. This results in an output file in which the average GV for each distance class from the root i (GVi) is stored. Additionally, the number of voxels (Ni) used for the calculation of
GV is available for each distance class. The distance class interval is a multiple of the image resolution and is therefore different across datasets.

The EDT was computed separately for the two root diameter classes considered. In order to exclude the influence that roots from one root diameter class have on the other root diameter class, the EDT images were masked with the dilated root images of the other root diameter class (e.g., to analyze the class <200 µm, the roots having a diameter >350 µm were dilated and served as a mask for the EDT image of the root diameter class < 200 µm). The root images were dilated such that the EDT mask extended at least up to 500 µm from the root surfaces. This masking step is important when analyzing the rhizosphere properties of a lateral root belonging to the class <200 µm, which emerge from a root belonging to the class >350 µm. In such instance and if no masking was done, the influence of the lateral root on the soil in its vicinity would be confounded by the influence of the root belonging to the class >350 µm at the point of emergence of the lateral.

2.8 | Data and statistical analysis

In order to analyze the output data described above, the following data processing steps were applied. First, the first statistical moment of the GV distribution was calculated using the number of voxels as the number of observations for each root distance class i (Equation 1):

\[
GV_{im} = \frac{\sum^n_i GV_i N_i}{\sum^n_i N_i}
\]  

(1)

where \(GV_{im}\) is the mean GV of an image, \(GV_i\) and \(N_i\) are the average GV and the number of voxels counted for each distance class i from the root, respectively, i is the distance class from the root, and n is the number of root distance classes (i.e., \(n = 256\) with an 8-bit resolution). With \(GV_{im}\), a normalization step was carried out (Equation 2):

\[
de_{vi} = \left( \frac{GV_i}{GV_{im}} - 1 \right) \times 100
\]

(2)

where \(de_{vi}\) is expressed in percentage and is the deviation from \(GV_{im}\) for each root distance class i. This normalization step is necessary because the image contrast can differ due to the percentile stretching method used during reconstruction and conversion to 8-bit. Applying a normalization step eases the comparison between two images having different contrast (i.e., having different \(GV_{im}\)). Negatives values of \(de_v\) indicate zones of lower BD, whereas positives values indicate zones of higher BD, as compared with the mean BD of the sample. The bulk soil is defined as the zone of the soil where the deviation from the mean GV is equal to 0. Note that, since a decrease or increase of \(GV_i\) necessarily means a decrease or increase of \(de_v\), the words “GV” and “deviation from the mean GV” will be used interchangeably throughout the rest of the manuscript. The deviation from the mean GV for each treatment was obtained by calculating the weighted average of \(de_v\) of each sample within a treatment using the number of voxels \(N_i\) as weighting factors. An example of the output data and of the data analysis steps is shown in the supplemental information (Supplemental Figure S2). Data analysis was carried out in R Studio 3.5 using the multcomp, car, plyr and ggplot2 libraries.

3 | RESULTS

The different packing and sieving procedures induced visual differences in initial soil structure (Figure 2). For loam, the most noticeable differences can be seen when comparing the samples extracted in the field for which the soil was prepared with large-scale machinery as compared with the samples for which the soil was prepared in small volumes in the laboratory. For the undisturbed cores extracted in the loam plots, aggregates of sizes up to 20 mm were observed and the spatial arrangement of those aggregates generated an increased heterogeneity of the pore space (i.e., creating bigger and more irregular pores). Noticeable differences are also visible when comparing loam and sand across all experiments. For loam, the structure is predominantly caused by the presence of aggregates, a fine-textured phase containing sub-resolution pores (i.e., pores that cannot be resolved because they are smaller than the image resolution) and the visible pore space. For the undisturbed and ingrowth cores in sand, the only heterogeneity consists of occasional loam aggregates in an otherwise homogeneous sand matrix composed of rigid quartz grains without internal pores. For the subsamples from column experiments with sand, the embedded loam aggregates were much smaller due to the fine sieving down to <1 mm.

The sampling at different growth stages, under different growth conditions and at several depths led to very different RLD values in the analyzed samples (Supplemental Figure S3). Due to the constrained volume available for root growth in the laboratory experiments, RLD was, on average, much greater for the samples extracted in the laboratory as compared with the samples extracted in the field. In the laboratory, the loam treatments exhibited more growth than the sand treatment, whereas this was the opposite in the field. For loam in general and for the WT genotype growing in loam in particular, drought symptoms were observed before BBCH 59 was reached. For the sand treatments in the field, drought stress was not as severe as plants were smaller at this stage (data not shown). In those laboratory and field experiments in
FIGURE 2  Two-dimensional cross sections of X-ray computed tomography images highlighting the differences in soil structure observed for the experiments carried out in the field and in the laboratory and for the two soil textures investigated. Differences in sample size and scanning resolution are denoted by the scale bar. The circles around the images denotes the mesh aperture of the sieve (blue = 1 mm, green = 2 mm, orange = 20 mm). Bulk density (BD) of the sample is annotated at the top of each image. Note the increase of structure heterogeneity from top to bottom for the loam samples. For sand, the only heterogeneity consists of occasional loam aggregates in an otherwise homogeneous sand matrix composed of rigid quartz grains without internal pores.
FIGURE 3  Deviation from the mean gray value (GV) as a function of the distance from the root surface. Root diameter class inferior to 200 μm is depicted with a solid line whereas the root diameter class superior to 350 μm is indicated by a dashed line. The semitransparent ribbon denotes the standard error. The number of samples and bulk density for each treatment is given with n and BD, respectively. The mentions “WT” and “rth3” refers to the wild type and root hair defective mutant maize genotypes, respectively.

which the influence of root hairs was investigated, there was no genotype effect as root growth was not statistically different between WT and rth3 in both soil textures. In the laboratory experiment in which the effect of soil compaction was investigated, the initial BD had no significant effect on root growth for both sand and loam (Supplemental Figure S3c).

For all experiments, genotypes, root diameter classes, and soil textures, the deviation from the mean GV close to the root surface (i.e., <100 μm) was very negative (Figure 3). It indicates the presence of a zone of lower BD close to the roots as compared with the bulk soil. The extent of this zone varied slightly across experiments but rarely exceeded 0.5 mm except for the loam samples with the greatest soil structure heterogeneity (i.e., the undisturbed cores extracted from the field). The biggest differences in the deviation from the mean GV mainly arose between soil textures and experiments. The most obvious difference between the sand and the loam treatment is noticeable for fine roots. For the root diameter class <200 μm in sand, the presence of an overshoot past the mean GV was observed at a distance of approximately 0.2 mm from the root surface. Past the overshoot, an abrupt decrease of GV below the mean GV was observed and another minimum was attained. Past this minimum, the deviation from the mean GV gradually increased again towards zero. Interestingly, this damped oscillation was typical for the root diameter class < 200 μm and was observed in most of the sand samples. For the root diameter class > 350 μm in sand, the presence of the overshoot was absent and only a decline of GVs towards the roots was observed. For the loam treatments, the only difference between the root diameter classes is that the GV distribution from the bulk soil towards the root surface was steeper for the root diameter class > 350 μm for the undisturbed cores. Analyzing BBCH stages separately revealed that this difference in loam is mainly contributed by the diameter class > 350 μm of BBCH19 (Supplemental Figure S4).

For the loam treatments, the biggest difference arose across experiments rather than between genotypes or root diameters. For the samples prepared with finely sieved soil in small volumes and which had a more homogeneous soil structure, an overshoot past the mean GV was observed directly after the zone of lower BD close to the root surface. This overshoot suggests a zone of soil compaction at a distance from the root surface ranging from 0.25 to 1 mm. The peak deviation from the mean GV is in the range of 1 to approximately 2.5%.
the experiment where the effect of BD was investigated, the deviation from the mean GV for the low and high BD treatment is very similar in terms of peak deviation and extent from the root surface. For the undisturbed cores, the GV overshoot is absent and only a zone of lower BD is observed up to a distance 1 mm. Comparing treatments within a given experiment revealed almost no differences between the WT and rth3 genotypes for both soil textures.

4 | DISCUSSION

4.1 | Influence of soil texture and structure on rhizosphere soil structure properties

The main and first hypothesis of our work was that changes in soil structure in the vicinity of roots depend on the soil texture and antecedent structure. Salient differences were observed between sand and loam, which is why the results obtained for both soil textures are treated distinctively in the discussion part.

For loam, two distinct patterns emerged when analyzing the results of the samples having an antecedent homogeneous soil structure as compared with the samples for which the soil structure heterogeneity was greater. In a homogeneous soil structure, no or few preexisting pores were present because of the fine sieving and careful packing in the laboratory (Figure 2). Under these circumstances, the zone of lower BD close to the roots was short. The presence of this zone is well in line with previous studies using high resolution X-ray CT in similar homogeneous conditions (i.e., finely sieved and repacked soil) (Burr-Hersey et al., 2020; Helliwell et al., 2017, 2019; Koebernick et al., 2019; Lucas et al., 2019). The zone of lower BD close to the roots extended up to 0.5 mm from the root surface, which is also in the range of values reported in the literature. In the study of Helliwell et al. (2017), the decrease in BD close to the root surface in a clayey soil was attributed to the formation of micro-cracks due to plant water uptake. In our study, the laboratory experiments were conducted under well-watered conditions. A visual assessment of the images from these datasets revealed virtually no cracks formed radially around the roots. We therefore exclude soil swelling and shrinking as potential factors causing a decrease in BD close to the root surface. This decrease in BD at the root surface is more likely the result of the surface wall effect, as already suggested by Koebernick et al. (2019) and Lucas et al. (2019). Past the zone of lower BD, a zone of compaction was observed. This zone of compaction can be attributed to the fact that roots predominantly had to push away soil particles when no existing pores were present. This is supported by the visual analysis of the soil in the vicinity of a primary root for which a typical compacted zone was observed (Figure 4). The extent and the peak deviation from the mean GV in the zone of compaction is well in accordance with the values that Helliwell et al. (2019) and Lucas et al. (2019) reported at approximately similar initial bulk densities and soil textures. In the experiment where the effect of initial BD was investigated, there was no difference in the extent and magnitude of the root-induced compaction between the high and the low BD treatment.

In an attempt to understand which factors influenced root-induced soil compaction in loam under homogenous soil structure conditions, correlations were established between the peak deviation from the mean GV (dev_{peak}, see Supplemental Figure S2c) and the RLD and the mean root diameter within a sample, the mean porosity, and mean pore size of the bulk soil (Supplemental Figure S5). The coefficients of correlation calculated were low for all four properties (i.e., $R^2 \leq .18$), which suggested that none of these properties was robust enough to explain root-induced soil compaction in finely sieved and repacked loam.

In a more heterogeneous soil structure, the zone of lower BD close to the roots extended twice as far and there was no zone of compaction farther away. The difference with the BD distribution with finely sieved and repacked soil can be explained in part by the presence of existing pores for roots to grow into in a more heterogeneous soil structure. This is supported by a local analysis of BD distribution in dense or loose areas that were identified according their pore size distribution analyzed for three undisturbed cores (Figure 5). Based on Figure 5, it seems that the propensity of roots to induce soil compaction depend on the initial local soil structure that roots encounter (i.e., a decline of GV towards the...
root surface was mainly observed when the structure in which roots grew was initially loose and where pores big enough for roots to grow into were present). With that in mind, the absence of a zone of compaction for the undisturbed cores extracted in the loam field plots could be attributed to the high local pore space heterogeneity induced by the coarse sieving and packing procedure. Under these conditions, roots predominantly penetrated existing pores without inducing compaction. Note that our observations consolidate the findings of Lucas et al. (2019) who suggested that the initial soil structure is an important factor for predicting root-induced compaction.

Another potential factor explaining the decrease in BD close to the root surface is root shrinkage as a consequence...
of soil desiccation. In the field, drought stress increased over the whole growing season and dry conditions could have led to root shrinkage, especially for the WT genotype growing in loam. In this treatment, plants showed the largest shoot biomass early on and were hence affected by severe drought earlier than other treatments. Permanent wilting point was reached at BBCH 59 (13.6% volumetric water content, corresponding to a matric potential of approximately −30,000 cm). A more detailed look at the BD distribution obtained for the undisturbed cores extracted at different growth stages revealed differences, in particular for the roots with >350-μm diameter (Supplemental Figure S4). Very likely, roots were partly desiccated and shrunken at BBCH59 and, to a lesser extent, at BBCH83. Shrunken and/or partially degraded roots would induce an offset in the distance from the root surface whose effect could be confounded with the surface wall effect. Also, this offset distance would mean that the rather constant position of the GV peak at 0.2–0.3 mm from the root surface is smeared across a larger distance range, when samples with and without drought stress are lumped together, thus potentially removing the GV overshoot altogether. This phenomenon cannot be ruled out for the ingrowth cores in the field.

For sand, the results across experiments and genotypes are very similar. A possible explanation for this similarity is that sand was originally very consistent in terms of granulometry and that homogeneous structure conditions were guaranteed despite the different sieving and packing procedures. A careful inspection of Figure 2 supports this statement. The results obtained for the low and high BD treatments are very similar as well. This suggests that there is no impact of initial BD on the results, at the BD investigated in this study. The main differences in sand are noticeable for the different diameter classes, and these differences will be treated in the dedicated section below.

4.2 Influence of root diameter on rhizosphere soil structure properties

The second hypothesis of our work was that root-induced compaction, if observed, will be greater for big roots than for fine roots due to the greater volume of big roots. Here again, differences were observed between sand and loam, and the results obtained for both soil textures are treated separately down below. Before discussing the effect of root diameter for each soil texture, it is worth noting that there were great differences in root diameter distribution for plants growing in loam and in sand. Indeed, plants growing in sand had systematically a larger share of big roots and bigger roots, on average, as compared with plants growing in loam (data in preparation). This observation was true, both in the field and in the laboratory experiments.

In loam, there was no or only small effects of root diameter on root-induced soil compaction under homogeneous conditions (Figure 3 and Supplemental Figure S5b). That is in contradiction with the results obtained by Lucas et al. (2019) and the model of soil compression around roots suggested by Dexter (1987). A possible explanation for this is that the difference in root diameter for the two classes investigated (i.e., <200 and >350 μm) might not have been large enough to cause a detectable difference in compaction. Broadening the range of root diameter classes could have helped to capture those differences but would have resulted in having too few roots in the upper diameter class to construct reliable statistical analysis.

For sand, root diameter had a much greater effect on rhizosphere soil structure properties. This effect manifested itself by the presence of a damped oscillation for the root diameter class <200 μm, which was absent for the root diameter class >350 μm. To understand these differences, a local analysis targeting roots of different diameter was carried out (Figure 6a). By visual evaluation only, it is noticeable that the big root created bigger pores in its surroundings, whose presence evoked a longer tailing of the pore diameter frequency distribution. In contrast, the small root caused the sand grains to align along the root surface. In order to understand the nature of the alignment of sand grains, an analysis of a sample scanned at the highest resolution (i.e., 10 μm) was carried out. By analyzing a zone around a small root for this sample (Figure 6b), it appears that the GV distribution has the shape of an oscillatory function. This observation is well in line with the model established by Suzuki et al. (2008), who first mathematically described this damped oscillatory function of void distribution as a function of the distance from the wall in particle packed bed reactors. On this oscillatory function, several zones can be delineated. The closest zone to the root (i.e., Zone a–b) shows a substantial drop of GVs that we now confidently attribute to the surface wall effect (Suzuki et al., 2008). At point b, the GV reaches a maximum, which we can now attribute to the center of grains aligned at the root surface. From point b to c, the GV decreases again because of the interstitial porosity between sand grains. This oscillatory pattern is then repeated from point d to e, d to e, and so forth but is damped as the distance from the root surface increases and the grain packing becomes more randomized. By conducting an analysis of the sand grain diameter, we found that the periodicity of this oscillatory function is approximately equal to the median sand grain diameter ̄d (i.e., ̄d ≈ distance a to b). Mueller (2010) highlighted that this oscillatory behavior is observed when particles are regularly shaped and have similar sizes. In our study, almost all sand grains had a size comprised between 100 and 250 μm, which is a fairly narrow size range (Figure 6b). The absence of the oscillatory pattern for the root diameter class >350 μm could be explained by the fact that big roots do not only force particles to align by
FIGURE 6  (a) Local analysis of the deviation from the mean gray value (GV) as a function of the distance from the root surface and the pore size distribution around a small root (red line) or a big root (blue line), in comparison with the whole sample (black line) for one undisturbed core extracted in the sand field plots. The horizontal red and blue dashed lines represent the deviation from the mean GV of the zone around the big and the small root, respectively, as compared with the mean GV of the whole sample. (b) Local analysis of the deviation from the mean GV as a function of the distance from the root surface for one subsample sand sample scanned at a resolution of 10 μm. The gray value distribution has the shape of a damped oscillatory function, whose periodicity is approximately equal to the median grain diameter

rotating and tilting but push them farther away than their own diameter.

4.3 Influence of root hairs on rhizosphere soil structure properties

The third hypothesis of our work was that roots with root hairs would induce a greater soil compaction than roots without root hairs because of the anchorage provided by the hairs. Contrary to our hypothesis, the presence of root hairs did not influence BD distribution in the vicinity of the roots. Our results are supported by the findings of Koebernick et al. (2019), who also found no differences in rhizosphere soil structure properties between a root hair bearing and a root hair defective barley genotype. Bengough et al. (2016) suggested that the role of root hairs in providing anchorage was mitigated when BD was ≥1.3 g cm⁻³. However, BDs lower than 1.3 g cm⁻³ were investigated in the laboratory. Therefore, root hair influence was expected to be observed in those two experiments. The absence of the effect of root hairs could be explained by two factors (i.e., the root hair length and the age of the plant). A microscopic examination of root segments was carried out, and we found that the mean root hair length was 0.24 mm with no significant difference between loam and sand. Frequently, higher values in the range of 0.7–0.9 mm are reported for maize. Shorter root hairs decrease the ability to anchor the growing root tip in soil if an insufficient length of each root hair is secured within the rhizosphere soil pores (Bengough et al., 2016). In our study, the ability of the root hairs to provide anchorage might have been lessened by their relatively short size. Bengough et al. (2016) studied the role of root hairs using the same maize genotypes as in the present study. However, the authors studied juvenile plants for which laterals were absent. In our study with older plants, we speculate that lateral and seminal roots took over the role of the root
hairs in providing anchorage, which might also have lessened their importance (Bailey et al., 2002).

4.4 Relevance for rhizosphere research

Our findings have implications for water and solute transport dynamics at the root–soil interface. Under heterogeneous conditions such as the one investigated in the field, the root–soil contact and the volume of solid in the vicinity of roots was less as compared with under homogeneous soil structure as the one investigated in the laboratory. Under water-limited conditions, heterogeneous soil structure might result in patchy water uptake (i.e., root water uptake is favored in dense local zones where the root–soil contact is greater than in loose zones). To investigate such structure related heterogeneities in root function, more dedicated experiments comparing different structures are required.

To the best of our knowledge, only few studies have considered the differences in soil structure properties in the vicinity of roots when trying to model water flow in the rhizosphere. Aravena et al. (2014) showed that root-induced soil compaction led to an increase in water flow towards the root in a very loose soil (i.e., 1 g cm$^{-3}$). Landl et al. (2021) showed that the lower BD around the roots, as observed in our study, had the effect of reducing water flow to the roots. By modeling the coupled effect of rhizosphere BD and mucilage concentration gradients in the rhizosphere at the single root segment scale, they showed that transpiration levels were kept lower during longer times, and that this could be regarded as beneficial since it would prevent fast dehydration. Modeling approaches of water flow should orient towards integrating explicitly the rhizosphere soil structure and to evaluate its effect on soil water dynamics at the plant scale (Landl et al., 2021). Rhizosphere soil structure properties, and how they differ from bulk soil properties, should also be considered for the interpretation of chemical imaging data, the study of diffusive processes such as mucilage exudation, and the assessment of the suitability of the rhizosphere as a habitat for microorganisms.

5 CONCLUSION

With the help of X-ray CT, we presented the results of an image processing workflow, which enabled us to analyze the BD changes in the vicinity of roots for >400 samples extracted during laboratory and field experiments. Our study consolidated previous knowledge regarding the conditions under which a zone of denser or looser soil is observed in the vicinity of roots. In homogeneous soil structure such as in finely sieved and repacked soil, roots had to create their own pores by pushing away particles. Under these conditions, the presence of a zone of lower BD close to the root surface was attributed to the surface wall effect, and this zone was followed by a zone of compaction farther away. In more heterogeneous conditions such as in field experiments, the zone of lower BD close to the root surface was attributed to the fact that roots predominantly grew in existing pores, thereby reducing the root–soil contact and thus the ability of roots to compact their surroundings. The effect of root diameter was found more pronounced in sand than in loam. In sand, fine roots caused sand grains to align along their axis, whereas bigger roots broke the fragile arrangement of sand grains. In contrast with rigid sand particles, loamy aggregates could also be deformed plastically, leading to different patterns of BD changes. We thereby showed that soil texture and structure heterogeneity predominantly governs BD distribution around roots and that other factors such as the presence of root hairs or RLD had no or little impact on the results. Our findings have implications for water and solute transport dynamics at the root–soil interface, as well for the interpretation of chemical imaging data, the study of diffusive processes from the root to the soil, and the assessment of the suitability of the rhizosphere as a habitat for microorganisms.

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AUTHOR CONTRIBUTIONS

Maxime Phalempin: Data curation; Formal analysis; Investigation; Methodology; Software; Writing-original draft; Writing-review & editing. Eva Lippold: Methodology; Writing-review & editing. Doris Vetterlein: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing-review & editing. Steffen Schlüter: Conceptualization; Funding acquisition; Methodology; Software; Supervision; Validation; Writing-review & editing.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

ORCID
Maxime Phalempin https://orcid.org/0000-0003-1198-807X
Steffen Schlüter https://orcid.org/0000-0002-3140-9058

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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