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Measurement of micro-scale soil deformation around roots using four-dimensional synchrotron tomography and image correlation

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This study applied time lapse (four-dimensional) synchrotron X-ray computed tomography to observe micro-scale interactions between plant roots and soil. Functionally contrasting maize root tips were repeatedly imaged during ingress into soil columns of varying water content and compaction. This yielded sequences of three-dimensional densitometric data, representing time-resolved geometric soil and root configurations at the micronmetre scale. These data were used as inputs for two full-field kinematic quantification methods, which enabled the analysis of three-dimensional soil deformation around elongating roots. Discrete object tracking was used to track rigid mineral grains, while continuum digital volume correlation was used to track grey-level patterns within local sub-volumes. These techniques both allowed full-field soil displacements to be quantified at an intra-rhizosphere spatial sampling scale of less than 300 μm . Significant differences in deformation mechanisms were identified around different phenotypes under different soil conditions. A uniquely strong contrast was observed between intact and de-capped roots grown in dry, compacted soil. This provides evidence that functional traits of the root cap significantly reduce the amount of soil disturbance per unit of root elongation, with this effect being particularly significant in drier soil.

1. Background

More efficient breeding and cultivation of crops requires improved understanding of how plants interact with their environments, with the interplay of roots and soils being particularly important [1,2]. All agricultural activity is linked directly to the functional efficiency of the plant root system [3], and in soils the co-dependent factors of mechanical impedance and water stress are strongly implicated in limiting root growth [3,4]. However, most of our understanding of how these factors influence root–soil interactions has been derived from studies in which the roots and soil were not directly observed. The explicit mechanisms controlling soil deformation around roots are poorly understood for numerous reasons, including soil heterogeneity [5], the opacity of soils [6] and the difficulty of obtaining dynamic data [7]. As a result, little is known about the direct relationships between root traits, soil physical parameters, and the explicit mechanisms via which roots ingress soils. Modelling approaches to plant and soil interactions usually overlook rhizosphere-scale heterogeneity by treating the soil as a granular continuum [3].

There is thus a need for methods to quantify soil displacement at the single root scale, leading to mechanistic understanding of how phenotypic effects and soil parameters such as strength and water content influence root ingress [1]. Recent advances in imaging and image correlation have shown significant promise in this regard [8,9]. Vollsnes *et al.* used two-dimensional (2D) image correlation

(DIC) to show that contrasting conditions of soil compaction and root mucilage production led to quantifiable differences in local sand compaction around the root tips of maize seedlings [9]. Keyes *et al.* demonstrated a combination of four-dimensional (4D) X-ray computed tomography (XCT) and digital volume correlation (DVC), allowing the three-dimensional (3D) displacement fields around a growing maize seedling to be quantified in a field-derived soil [8]. However, neither was a strictly rhizosphere-scale approach, since both methods averaged displacements over sub-regions whose length scale was in the range of 2–3 mm. Furthermore, pertinent questions exist as to the applicability of what are fundamentally continuum approaches at the grain scale, where continuum assumptions break down [10].

Recent geotechnical research indicates the utility of applying discrete object tracking approaches to such questions. These approaches to deformation analysis of granular media quantify the kinematics of distinct grains, thereby negating the need for continuum assumptions. In sands, discrete approaches have quantitatively corroborated prior observations that important mechanical phenomena such as shear strain localization are in part controlled by grain-scale phenomena [10,11].

Recent synchrotron studies at the rhizosphere scale have allowed visualization of the soil structure surrounding roots in unprecedented detail [12–14]. At the micrometre scale, the field-derived soils used in these studies can be described using a conceptual description as shown in figure 1. According to this description, rigid grains of homogeneous, hard mineral composition (the ‘primary’ phase) are distributed within a compliant, porous matrix comprising the clay, silt and organic soil fractions (the ‘textural’ phase). To understand how soil–root mechanical interactions are influenced by the features evident at this scale, it is necessary to conduct kinematic analyses using dynamic sequences of high-resolution synchrotron X-ray computed tomography (SRXCT) data. These methods may improve modelling of root–soil interactions, and allow targeted screening of phenotypes for mechanisms of growth in different soil conditions. Since root cap removal is known to increase penetration resistance in soil by up to 70% [15], the breeding of plants better adapted to soils with deleterious impedances to root growth is also likely to benefit from understanding how root cap traits such as mucilage production control root ingress performance.

In this study, discrete and continuum approaches to 3D kinematic quantification were applied to study soil deformation around the root tips of maize (*Zea mays*) seedlings. *In vivo*, time-resolved (4D) SRXCT data were collected showing maize root tips growing *in situ*, using three soil treatments chosen to provide contrasts in soil compaction and/or water content. Two different maize cultivars were chosen to contrast in root tip morphology and mucilage production. The data permitted qualitative description of root–soil interactions, quantification of grain-scale mechanics, and comparison of continuum and discrete approaches for a range of conditions.

2. Material and methods

2.1. Growth chambers

A growth assay used in a previous kinematic study by Keyes *et al.* [8] was miniaturized (figure 2), using 1 ml syringe barrels as soil columns, which are known to be well suited to the constraints of SRXCT imaging [12–14]. To mechanically stabilize

Figure 1. Schematic of the root–soil system, as visualized by SRXCT imaging. (a) The system can be described according to the following definitions, with representative regions enclosed in hatched white lines: (i) the root; (ii) the primary mineral grains, which are rigid crystalline grains of quartz and similar phases (i.e. feldspars); (iii) the textural phase, comprising particles of clay, silt and organic matter whose discrete structure is largely irresolvable at the imaging resolution; (iv) fluid-filled pore spaces; (v) gas-filled pore spaces. (b) The phases can be extracted as discrete representations through appropriate segmentation. (c) The DVC approach (left) considers displacement of grey-level texture within cubic sub-volumes (represented by the red hatched line), and the discrete approach (right) considers the displacement of discrete primary mineral grains. Red crosses indicate centroid locations in each case. (d) The kinematics of each grain and sub-volume in response to a root elongation vector $\mathbf{r}$ is described by vectors $\mathbf{d}_g$ and $\mathbf{d}_s$ respectively (solid white arrows), having lateral and vertical components $(\mathbf{d}_{g,x}, \mathbf{d}_{g,y})$ and $(\mathbf{d}_{s,x}, \mathbf{d}_{s,y})$, represented by the hatched white arrows. (Online version in colour.)

roots for imaging, a plaster-of-Paris fixing protocol was adopted following Bengough & McKenzie [16], which fixes the radicle in space, proximal to the seed, so that pressure generated in the meristematic zone is fully exerted against the soil. A 3D printed root guide facilitated this step, and introduced the root tip into the soil chamber. The basal 5.85 mm of each syringe was plugged with a rubber stopper to prevent water egress. Between the top of this stopper and the base of the root guide, a 17.3 mm working length of chamber was able to be filled with growth media, giving a working volume of 0.3 ml.

2.2. Growth media

The soil material was a sand-textured Eutric Cambisol [12,14,17] collected from the surface horizon of an experimental plot at Abergwyngregyn, North Wales (53014'N, 4001'W), sieved to less than 5 mm, air dried for 2 days at $23 \pm 1^\circ\text{C}$ then sieved between upper and lower bounds of $1180 \mu\text{m}$ and $600 \mu\text{m}$. This soil comprised 52.7% sand, 32.8% silt and 14.5% clay. Three soil treatments were prepared: a loose texture with high water content, and two mechanically consolidated textures (with a bulk density of approximately 4/3 that of the unconsolidated treatment) prepared with contrasting (high and low) water contents.

To produce samples for the *wet loose* treatment, dry soil was introduced to each chamber, being lightly consolidated via tapping [18] to a volume of 0.3 ml. The soil was hydrated to a volumetric water content of approximately 25% via addition 0.075 ml of Milli-Q water.

Figure 2. Schematic of experimental set-up. (a) Seedlings were affixed to the root guide using plaster of Paris. (b) Samples were affixed such that a 2–3 mm length of root protruded from the base of the root guide (indicated by arrow). (c) An assembled assay immediately prior to imaging. The seed is surrounded by moist sand in region (i), and the root is free to elongate into the working volume of soil in region (ii). Scale bars are 15 mm in all instances. (Online version in colour.)

To produce samples for the *wet compacted* treatment, dry soil was introduced as before, to a volume of 0.2 ml, following which 0.0375 ml of Milli-Q water was added via pipette. Another 0.2 ml of soil was introduced, followed by another 0.0375 ml of water. The resulting 0.4 ml working volume was then compacted to a volume of 0.3 ml using a plastic piston, producing a volumetric water content of approximately 25%.

To produce samples for the *dry compacted* treatment, an approximately 3 mm depth of dry soil was first layered between

two moistened filter papers (Whatman No. 2) of 185 mm diameter, which were sealed between sheets of polythene film, and allowed to equilibrate for 5 h at $21 \pm 1^\circ\text{C}$. The soil layer was gently homogenized by re-scattering at 0.5 h intervals, producing a medium with a gravimetrically determined bulk volumetric water content of 11.2%. This soil was introduced to the working volume, consolidating lightly via tapping to a volume of 0.4 ml, following which it was compacted to a volume of 0.3 ml via a plastic piston, producing a volumetric water content of approximately 15%.

Following preparation, all samples were plugged with rubber bungs, and left at the imaging temperature of $21 \pm 1^\circ\text{C}$ for 6 h to allow mechanical settling and redistribution of soil water, being estimated via non-dimensionalization of Richard's equation [19].

2.3. Growth conditions

Two maize cultivars were selected to provide a functional contrast in mucilage production and root cap morphology: a wild-type (*Zea mays* cv. Earlybird), and a root-cap defective mutant (*Zea mays* cv. AGT1) [20]. AGT1 has a defective root cap junction, allowing the root cap to be removed. These functionally distinct root types are henceforth referred to as *decapped* and *intact*.

Seeds were sterilized in batches of 10, using 50 ml of sodium hypochlorite solution diluted to 5% b.v. in Milli-Q water to which three drops of surfactant (Triton 100X) had been added. Samples were agitated on rollers in 50 ml laboratory tubes for 15 min, following which they were rinsed 5 times and soaked for 24 h in Milli-Q water. Seeds were then transferred to 120 mm culture dishes between moist filter papers (Whatman No. 2), foil-wrapped and germinated for 3–5 days at $21 \pm 1^\circ\text{C}$. In order to promote gravitropic elongation and root straightness, seedlings were transferred to sheets of filter paper sandwiched between sheets of foam rubber. These were oriented vertically with root tips pointing vertically downwards. Seedlings with optimal radicle length (approx. 12 mm) were periodically taken from this assembly and transferred to a transparent growth-observation box. Elongation was monitored for approximately 2 h immediately prior to imaging, with decapped specimens having root caps excised under a dissecting microscope [9] immediately before transfer to the observation box.

To conduct SRXCT imaging of each sample, a seedling was introduced to a root guide, such that approximately 3 mm of root tip was emerging from the basal aperture (figure 2). A small bead (approx. 0.3 ml) of plaster of Paris, mixed to 1 : 1 by mass with water, was applied via pipette to fix the seedling to the upper face of the guide, and the seed surrounded with moist fine sand housed in a plastic collar. The root guide was then gently attached to the prepared soil chamber, such that the root tip penetrated 2–3 mm into the working soil volume (figure 2).

2.4. Imaging

Tomographic data were acquired at the TOMCAT beamline of the Swiss Light Source (Villigen, Switzerland), under a 19 keV monochromatic beam condition. To produce each tomogram, 750 2D projections were continuously acquired at a 75 ms exposure, with 30 dark- and 50 flat-field images acquired for radiograph correction. Each full acquisition took approximately 60 s in total. The attenuated beam was scintillated by a LuAG:Ce (cerium activated lutetium aluminium garnet) film of 20 μm thickness, and resultant images digitized by a PCO Edge 5.5 camera, following $4\times$ optical magnification. A sample-to-detector distance of 50 mm provided an intermediate degree of propagation phase contrast. A tomographic acquisition was automatically triggered every 6 min, with a sequence of 8 tomograms collected for each sample over a total time of 48 min.

An implementation of the *Paganin* single-distance phase retrieval algorithm [21] was applied to all projections, which were then reconstructed to 16-bit volumes using a high-power computing

cluster and an implementation [22] of the *Gridrec* algorithm [23]. This produced a time-series of 8 SRXCT volumes per sample, with a temporal spacing of 6 min and a voxel edge length of 1.6 μm . Three biological replicates of each soil/cultivar combination were imaged in this manner. Collecting sequences of 8 scans for each of 2 root types, three soil conditions and three replicates produced a total of 144 SRXCT volumes for analysis (representing approximately 3.5 TB of 16 bit volume files).

2.5. Image analysis

2.5.1. Pre-filtering

To quantify the mechanics of root–soil interaction, it was necessary to extract representations of soil and root morphology from the images. Due to the computationally intensive nature of the kinematic analyses and the large volume of data, all volumes were first subsampled in FIJI [24] by a factor of 2 along each axis, and downsampled to an 8-bit range, producing volumes of $1280 \times 1280 \times 960$ voxels, at a voxel edge length of 3.2 μm .

2.5.2. Root morphology

2.5.2.1. Three-dimensional root extraction

Due to an overlap in the grey-level distributions of the root and soil voxels, the morphology of the single root in each reconstructed volume was achieved using a manual approach [8]. Using the *Segmentation Editor* in FIJI, root cross sections were defined at a vertical spacing of 25 voxels, with this sampling frequency increased slightly in the immediate vicinity of the root tip. Automated 3D interpolation between cross sections generated binary label fields representing the root morphology. Root tip coordinates were exported during this step, using the *point* and *measure* tools in FIJI.

2.5.2.2. Root distance transform

From each root morphology, a Euclidean distance transform [25] was computed, allowing all kinematic data to be related to distance from the root surface. For every voxel in the soil domain, the algorithm returns the Euclidean distance (l) to the closest point on the root surface.

2.5.2.3. Root visualization

For qualitative interpretation of the SRXCT data, a vertical sectioning plane was defined in each image volume, coincident with both the root tip coordinates and the coordinates of the root centroid in the uppermost slice of the volume.

2.5.3. Soil morphology

The high-throughput approach necessitated a computationally inexpensive segmentation procedure. The Paganin filtering applied to the data provided sufficient contrast to allow the soil phases to be segmented via global thresholding between histogram peaks representing pore-space, mixed phase and primary mineral grains. Prior to segmentation, a 3D median filter with a 3×3 kernel was used to smooth image noise and enhance peak separation in the histogram. The details of this thresholding approach are detailed in the electronic supplementary material (S1). Example slices following three-phase segmentation are shown in figure 3.

Touching grains were separated using a 3D watershed algorithm in FIJI [26]. The soil segmentations were exported as three-phase 8-bit RAW volumes with pores, textural phase and primary grains represented by discrete grey-levels (figure 1).

2.5.4. Spatio-temporal analysis of soil volume fractions

The spatial distributions of the volume fractions of primary, mixed and pore phases around the roots were quantified using an annulus approach [27]. The Euclidean distance transform of each root

Figure 3. Example segmentations are shown for wet loose soil (a), wet compacted soil (b) and dry compacted soil (c). The left column shows the raw SRXCT data, and the right column shows the segmented representation, with discrete grey-levels representing primary mineral phase (white), the textural phase (grey), and the pore phase (black). The red boxes indicate positions of sub-regions shown in electronic supplementary material, figure S1. (Online version in colour.)

was used to subsample the three-phase soil segmentations, gridding the soil domain into 160 μm steps with respect to both distance from the root and depth down the soil column (approx. $3\times$ larger than the diameter of the smallest grains). The volume fraction of each phase at depth d from the top of the volume and distance from root l is denoted $\psi_{d,l}$. The change in volume of each phase at a given location is then given by

$$\Delta\psi_{d,l} = \frac{\psi_{d,l,t_2} - \psi_{d,l,t_1}}{\psi_{d,l,t_1}}, \quad (2.1)$$

where ψ_{d,l,t_1} and ψ_{d,l,t_2} are the volume fractions at location (d, l) at $t = 0$ min and $t = 42$ min respectively.

2.6. Soil deformation kinematics

This study applied two distinct methods of kinematic analysis, discrete grain tracking and digital volume correlation, identified schematically in figures 1 and 4.

2.6.1. Discrete grain tracking

To quantify the kinematics of rigid mineral grain motion, a discrete tracking approach was implemented in Matlab 2016b (Natick, MA, USA), following Ando *et al.* [10]. This method tracks 3D objects using the assumption that they undergo rigid body motion only, with no appreciable strain within the population that is being tracked (i.e. the grains are treated as simple fiducials).

Figure 4. The process for extracting kinematics from raw SRXCT data is shown in schematic form. The original data (a), with the root outlined in white, are spatially down-sampled to a computationally tractable size. (b) For grain tracking, grains above a certain volume threshold are extracted (i), and for DVC, the domain is gridded into sub-volumes (ii). In the DVC case, the array of centroid locations is illustrated with the grid of red dots, and a representative sub-volume (to scale) is shown in black. (c) The prepared data are passed through the respective tracking algorithms. (d) The results for each grain or sub-volume are combined with the Euclidean distance transform (i) and the distance transform of vertical column depth (ii), to quantify the spatial location of each sub-volume or grain centroid. (e) Example 3D vector fields are shown for the DVC (i) and grain tracking approaches (ii). (f) Plots show displacement intensity ($\bar{d}$) of sub-volumes (i) and grains (ii), as a function of distance (l) from the root (the colourbar indicates depth down the soil column). A decay with increasing distance is seen. (g) Plots show the displacement intensity ($\bar{d}$) for sub-volumes (i) and grains (ii) as a function of the angle (θ_{rd}) between the displacement vector of each grain/sub-volume ($\mathbf{d}$) and the root growth vector ($\mathbf{r}$) (the colourbar indicates distance (l) from the root surface). A maximum at approximately 0.4 radians is seen. (Online version in colour.)

To track grains between the time steps of each sample, each discrete grain must be uniquely identified in its initial (*reference*) and post-deformation (*deformed*) states. Grain displacement is then defined as the vector linking the centroid coordinates between these states. Following Ando *et al.* [10], grains were matched on the basis of a morphometric description. Discrete grain morphometry was quantified for the separated grain data using the *BoneJ* plugin in FIJI [24,28]. The parameters used were volume; surface area; first, second and third moments of inertia; major radius and minor radius, which were computed on a grain-wise basis for all grains in all 144 SRXCT volumes. Due to memory limitation in the kinematic processing step, very small grains with a volume of less than $3.28 \times 10^{-4} \text{ mm}^3$ were filtered out. Across all scans, this yielded a mean grain count of 1330 (s.d. = 67), with a mean grain volume of $5.7 \times 10^{-3} \text{ mm}^3$ (s.d. = $6.5 \times 10^{-4} \text{ mm}^3$), and mean equivalent-sphere diameter of 0.22 mm (s.d. = 0.05 mm). A CSV file of

morphometric data was exported for each step, with temporally sequential data pairs forming the basic input to the grain tracking algorithm.

2.6.1.1. Grain matching

The tracking algorithm was implemented in Matlab 2016b (Natick, MA, USA). For each time step, each of the grains in the *reference* set was paired to its most probable match in the *deformed* set via maximization of morphological similarity.

First, for each of the 7 morphometric measures ($p^{(1)}, \dots, p^{(7)}$), the morphometric difference was computed discretely for every permutation of reference and deformed grain pairs, producing a set of difference matrices ($\mathbf{P}^{(1)}, \dots, \mathbf{P}^{(7)}$) each of size ($n_{\text{ref}} \times n_{\text{def}}$). For a given matrix $\mathbf{P}^{(q)}$, each element is given by

$$\mathbf{P}_{i,j}^{(q)} = |p_{\text{def},i}^{(q)} - p_{\text{ref},j}^{(q)}|, \quad (2.2)$$

where $p_{\text{def},i}^{(q)}$ and $p_{\text{ref},j}^{(q)}$ are the values of morphometric parameter $p^{(q)}$ for the i th deformed grain and the j th reference grain respectively.

Each difference matrix was then converted to a normalized similarity matrix ($S_1, \dots, S_7$), by dividing through by the maximum difference:

$$S_{ij}^{(q)} = 1 - \frac{P_{ij}^{(q)}}{\|P^{(q)}\|_{\infty}}, \quad (2.3)$$

where $\|P^{(q)}\|_{\infty}$ is the infinity norm of $P^{(q)}$ (i.e. the magnitude of the maximum element value). Each element ($S_{ij}^{(q)}$) thus takes a value in the range 0–1, with 1 representing a perfect match between a deformed grain i and reference grain j for parameter ($p^{(q)}$).

Next a correlation table was computed from the full set of similarity matrices, given by

$$C = \sum_{q=1}^7 S^{(q)}, \quad (2.4)$$

where C_{ij} is the figure of merit for the morphometric similarity of reference grain i and deformed grain j . The indices of the element of greatest magnitude (i.e. $\|C\|_{\infty}$) denote the pair of grains for which the overall morphometric match is maximized.

Next, to filter out erroneously large displacements, entries in C were masked if they would result in displacements greater than the root tip displacement ($|r|$). An ($n_{\text{def}} \times n_{\text{ref}}$) matrix of grain displacements (V) was defined, where each element V_{ij} is the centroid-to-centroid distance between deformed grain i and reference grain j . To mask the correlation table, the condition was set over all i and j that if $V_{ij} > |r|$ then $C_{ij} \rightarrow 0$.

Grains were then matched. In most cases, $n_{\text{ref}} \neq n_{\text{def}}$ as a consequence of image noise and segmentation errors [10]. Thus, to avoid double-matching of grains, the matching procedure always matches the larger set of grains against the smaller set. For example, in the case of the reference $\rightarrow$ deformed direction ($n_{\text{ref}} > n_{\text{def}}$), for each grain i_{def} , the row $[C_{i,1}, \dots, C_{i,n_{\text{ref}}}]$ was searched for the maximum value. The reference grain j_{ref} corresponding to this element was taken to be the best morphometric match to grain i_{def} .

The measurements exported for kinematic analysis were the centroid coordinates of each grain in the reference state, the displacement vector (d) between reference and deformed centroid coordinates, the grain volume, and the angle between root tip and grain displacement vectors (θ_{rd}), given by

$$\theta_{rd} = \tan^{-1}(\|r \times d\|_2, r \cdot d), \quad (2.5)$$

where r is the root tip elongation vector for the given step. The displacement vector field showing all grain displacements was also exported in VTK format for 3D visualization (figure 4).

2.6.2. Digital volume correlation

Alongside the discrete grain tracking approach, DVC was also applied to all SRXCT data. DVC is a continuum, volume-averaged approach which tracks 3D grey-level distributions in local sub-volumes. Following Keyes *et al.* [8], the unfiltered data of all 126 sequential scan pairs were processed with a fast-Fourier DVC algorithm in DaVis 2.3.2 (LaVision, Goettingen, Germany), using cubic sub-volumes in a regular array, with an overlap of 75%. A multi-pass procedure was used to improve accuracy.

2.6.2.1. Digital volume correlation calibration

A noise study was first conducted to determine the optimal sub-volume size, using the procedure outlined in [8]. A suitable sub-volume edge-length was determined to be 288 μm . In the interests of brevity, further details of this procedure are found in the electronic supplementary material (S2).

To verify that the sub-volume size was suitable for tracking the largest possible displacements between reference and deformed states, a calibration volume pair was defined by translating a reference volume by $[0, 0, 80]$ voxels to produce a deformed volume. This produced a uniaxial offset to the reference volume of 256 μm , equal to the maximal stepwise root elongation magnitude observed in the study. The deviation of each sub-volume displacement vector from the displacement and orientation of the input translation vector was then computed and plotted. The grain tracking approach was applied to the same data for comparison, and the results shown in the electronic supplementary material (S2).

2.6.2.2. Computing strain data

To process the exported DVC data and compute the strain tensor for each sub-volume, the Matlab code used in [8] was applied, being modified to compute the average shear strain. The strain tensor at each sub-volume location was decomposed into the dilation tensor (i.e. the compressive components) and the deviator tensor, which characterizes the deformation without the influence of the dilation. The mean shear strain of each sub-volume was computed by taking the Euclidean norm of the deviator tensor. Due to the variability in per-step root elongation across time, and within sets of biological replicates, the mean shear strain (γ) of each sub-volume was normalized to the root tip elongation for the given step, giving a mean shear intensity ($\bar{\gamma}$) for each sub-volume, according to

$$\bar{\gamma} = \frac{\gamma}{|r|}, \quad (2.6)$$

where γ is the mean shear strain, and r is the root tip displacement vector for a given step.

DVC results from sub-volumes for which the correlation coefficient was in the range $c < 0.8$ were masked out to reduce error [8,29]. The metrics exported for kinematic analysis were the centroid coordinates of each sub-volume in the reference state, the displacement vector between reference and deformed sub-volume centroids, the angle between root tip and sub-volume displacement vectors, and the mean shear strain for each sub-volume. The 3D displacement vector field was also exported in VTK format for visualization.

2.7. Result visualization

3D displacement vector fields from grain tracking and DVC approaches were visualized in Paraview 5.1.2 (figure 4) [8,30].

Due to elongation rate heterogeneity, all displacement measurements (for grains and sub-volumes) were first normalized to the root elongation for the corresponding step, giving a displacement intensity ($\bar{d}$), computed according to

$$\bar{d} = \frac{|d|}{|r|} \times 100\%, \quad (2.7)$$

where d is the displacement vector of a given grain or sub-volume and r is the root tip growth vector at the given step. Thus, a grain or sub-volume which was displaced by the same magnitude as the root tip growth returned a displacement intensity of $\bar{d} = 100\%$.

Four key metrics were defined to describe and compare the kinematics of grains and sub-volumes: the displacement intensity ($\bar{d}$), the displacement intensities of the vertical and perpendicular displacement components ($\bar{d}_x$ and $\bar{d}_y$), and the angle between the root growth direction and the grain or sub-volume displacement (θ_{rd}). The Euclidean distance transform of the root defined the distance of each grain or sub-volume centroid from the root surface (l), permitting individual grain and sub-volume kinematics to be plotted with respect to distance from the root (figure 4).

All results were plotted with respect to (l), averaging over 0.3 mm steps [27], to a maximum of 3 mm, which represented the furthest extent of the imaged domain. For each of the six

Figure 5. Digital sections along the root axis taken at the start and end of SRXCT time-series. (a) An intact root extending into dry consolidated soil. The white arrow shows root elongation over the entire growth period. Two grains are highlighted in red, whose displacement is essentially perpendicular to the root growth orientation. (b) An intact root extending into wet consolidated soil. A series of grains initially situated lateral and basal to the original position of the root tip are translated to form a consolidated network via large displacements essentially aligned to the root growth orientation. (c) An intact root extending into dry consolidated soil. A large macropore (highlighted in red) situated beneath the initial root tip position allows the root to elongate with minimal soil disturbance. (Online version in colour.)

experimental conditions (decapped dry compact, decapped wet compact, decapped wet loose, intact dry compact, intact wet compact, intact wet loose) the mean of all time-steps over all three replicates was taken in each case.

3. Results

3.1. Qualitative observations

The 4D SRXCT data reveal the dynamic geometrical interaction of rhizosphere soil and single roots at a magnification an order of magnitude higher than previous 4D imaging studies [8]. Examples of these time-lapse data are seen in electronic supplementary material, video S1. Discrete primary mineral grains were clearly distinguishable from the textural phase, and complex grain-scale interactions between the root and the soil were observable (figure 5). In the interests of brevity, further discussion of these observations, including the impingement of primary grains on the root epidermis, are given in electronic supplementary material (S3).

Figure 6. The averaged elongation rate of the three biological replicates of each experimental condition ($n = 3$). Error bars show one standard deviation in the mean. Elongation rates were higher for intact versus decapped roots. In decapped samples, elongation was higher in wet consolidated versus dry consolidated soil, and higher in loose wet soil than consolidated wet soil. In intact samples, the differences between soil treatments were not so pronounced. (DC, dry compact; WC, wet compact; WL, wet loose.) (Online version in colour.)

3.2. Kinematic quantification

Welch's t -tests, accounting for unequal variances and sample sizes, were used to compare results. Comparisons were considered significant when $p < 0.05$. Analyses were conducted using Matlab 2016b (Natick, MA, USA). Only significant results are discussed, with other observations detailed in the electronic supplementary material.

3.2.1. Root elongation rates

Figure 6 shows the average root elongation per time-step over the full SRXCT sequence for each of the 18 samples considered. A generally decreasing trend was observed over time in all conditions. Elongation rates of different specimens were highly variable, even within the same condition, and only one comparison was significant; with the decapped dry compact condition showing lower elongation per step than the intact wet compact condition over all time-steps except 12 min. Individual elongation data are detailed in the electronic supplementary material (S4).

3.2.2. Comparison of digital volume correlation and grain tracking results

Statistical tests conducted between results for $\bar{d}$, $\bar{d}_x$, $\bar{d}_y$ and θ_{rd} between the two sets of kinematic data, for each conditions at all distances, showed only one statistically significant difference in 240 comparisons.

3.2.3. Soil densification

The results of the spatially resolved soil densification analysis revealed no significant differences. The results are reported and briefly discussed in the electronic supplementary material (S5).

Figure 7. Averaged results from the grain tracking quantification ($n = 21$): (a) vertical displacement intensity ($\bar{d}_y$), (b) lateral displacement intensity ($\bar{d}_x$), (c) overall displacement intensity ($\bar{d}$), and (d) angle between root and grain displacement orientation (θ_{rd}), all with respect to distance from the root surface (l). Values are averages of the three biological replicates over all time-steps, and the error bars show one standard deviation either side of the mean. (Online version in colour.)

3.2.4. Discrete grain kinematics

Figure 7 shows the averaged grain kinematic behaviour for each experimental condition, plotted with respect to distance from the root (l). For each condition, the kinematic parameters at each distance were compared with the values immediately proximate to the root surface. Only differences significant at $p < 0.05$ are reported.

The overall displacement intensity ($\bar{d}$) decreased over distance in the decapped dry compact and decapped wet compact cases. In the decapped dry compact case, this was observed for distances from the root in the range $l > 0.3$ mm, and in the decapped wet compact case for $l > 0.9$ mm. The vertical component of displacement intensity ($\bar{d}_y$) also exhibited a decreasing trend with distance from the root in the decapped dry compact and decapped wet compact cases. In the decapped dry compact case, this was observed for $l > 0.6$ mm, and in the decapped wet compact case for $l > 0.9$ mm. The intact wet compact case showed a decrease for $l > 1.8$ mm. The lateral component of grain displacement intensity ($\bar{d}_x$) showed no decrease with distance in any condition. The angle of grain displacement relative to the root growth

direction (θ_{rd}) increased with distance in the intact wet compact and decapped wet compact cases, for which θ_{rd} was elevated relative to the root-adjacent value at $l > 0.6$ mm.

Comparisons between conditions also revealed significant differences. The overall displacement intensity ($\bar{d}$) was higher for the decapped dry compact case compared to the intact dry compact case over the majority of the range of l ($0 \text{ mm} > l > 2.1 \text{ mm}$). The vertical component of displacement intensity ($\bar{d}_y$) was higher for the decapped dry compact case compared to the intact dry compact case for $l > 0.3$ mm. The lateral component of grain displacement intensity ($\bar{d}_x$) was higher for the decapped dry compact case compared to intact dry compact case for $0 \text{ mm} > l > 0.3 \text{ mm}$, and was elevated in the decapped wet compact case relative to the intact wet compact case for $0.9 \text{ mm} > l > 1.8 \text{ mm}$. The angle between root and grain displacement (θ_{rd}) showed no significant differences between conditions.

3.2.5. Sub-volume kinematics

Figure 8 shows the averaged sub-volume kinematic behaviour for each experimental condition, plotted with respect to

Figure 8. Averaged results from the DVC quantification ($n = 21$): (a) vertical displacement intensity ($\bar{d}_y$), (b) lateral displacement intensity ($\bar{d}_x$), (c) overall displacement intensity ($\bar{d}$), and (d) angle between root and sub-volume displacement orientation (θ_{rd}), all with respect to distance from the root surface (l). Values are averages of the three biological replicates over all time-steps, and the error bars show one standard deviation either side of the mean. (Online version in colour.)

distance from the root (l). For each condition, the kinematic parameters at each distance were compared with the values immediately proximate to the root surface. Only differences significant at $p < 0.05$ are reported.

The overall displacement intensity ($\bar{d}$) decreased over distance in the decapped dry compact, decapped wet compact, intact wet compact, and intact wet loose cases. In the decapped dry compact case, this was observed for $l > 1.2$ mm, in the decapped wet compact case for $l > 0.6$ mm, in the intact wet compact case for $l > 1.2$ mm, and in the intact wet loose case for $l > 0.9$ mm. The vertical component of displacement intensity ($\bar{d}_y$) exhibited a decreasing trend in the decapped dry compact, decapped wet compact, intact wet compact, and intact wet loose cases. In the decapped dry compact and decapped wet compact cases, this was observed for $l > 0.6$ mm. In the intact wet compact case, a decrease was observed for $l > 0.9$ mm. In the intact wet loose case, decreased decrease was observed for $l > 0.6$ mm. The lateral component of grain displacement intensity ($\bar{d}_x$) only showed a decrease with distance in the intact wet loose

case, observed for $l > 1.2$ mm. The angle of sub-volume displacement relative to the root growth direction (θ_{rd}) showed an increasing trend in the intact wet compact case, for which θ_{rd} was elevated at $l > 2.4$ mm.

Comparisons between conditions also revealed differences. As in the grain results, the overall displacement intensity ($\bar{d}$) was higher for the decapped dry compact case compared to the intact dry compact case over the majority of the range of l ($0 \text{ mm} > l > 1.8 \text{ mm}$). As in the grain results, the vertical component of displacement intensity ($\bar{d}_y$) was higher for the decapped dry compact case compared to the intact dry compact case, observed for $l > 0$ mm. As in the grain results, the lateral component of grain displacement intensity ($\bar{d}_x$) was higher for the decapped dry compact case compared to the intact dry compact case, observed for $0 \text{ mm} > l > 0.9 \text{ mm}$. The angle between root and grain displacement (θ_{rd}) was elevated in the intact dry compact case compared to the decapped dry compact case for $l > 1.2$ mm.

The shear results showed large variance, and are detailed in the electronic supplementary material (S6).

4. Discussion

4.1. General

This study investigated the mechanical dynamics of soil and root interactions at the rhizosphere scale. SRXCT is the only experimental technique that can provide such data at these fine spatio-temporal scales (approx. $1\ \mu\text{m}$, and $10\ \text{scans h}^{-1}$). Triple-replicated samples were imaged through time, with the sample panel designed to include contrasts in root tip traits (tip shape and presence of mucilage), soil wetting and soil compaction. The data reveal soil and root structures in detail similar to those observed in other SRXCT studies [12–14], but also show both soil deformation and root tip elongation over time thus allowing the link between root and soil kinematics to be observed and quantified. Reconfiguration of primary mineral grains, textural phase, pore gas and pore fluid were evident under the influence of root ingress (figure 5).

4.2. Soil kinematic quantification

The particular strength of the 4D SRXCT method is that it provides suitable data to parametrize kinematic analyses. In this study, 3D primary mineral grain geometry was extracted, separated, and classified using a discrete grain analysis. This approach has previously been applied to SRXCT data of geotechnical sands [10], but not to soils and/or root-related deformation. A DVC approach, previously applied to root–soil interactions [8], was applied in parallel, parametrized by unsegmented grey-level data, and using a spatial scale (i.e. sub-volume edge-length) an order of magnitude finer than the previous study. The results indicated virtually no statistically significant differences between the discrete tracking and DVC results (less than 0.5% of all comparisons were significantly different), indicating that despite the methods tracking different aspects of the soil geometry (i.e. the grains versus the textural phase) the fundamental characteristics of soil deformation were preserved across both methods at this scale. Both methods revealed significant differences both within and between different conditions of root type and soil condition, at substantially sub-millimetre scales of relevance. Across all samples, the matching efficiency was 89% (s.d. = 4.75%), which is slightly lower than measured in Ando *et al.* (92–94%), and can be accounted for by the increased complexity of the segmentation problem due to the multiple phases present.

4.2.1. Localization of soil deformation mechanisms

In wet and dry compacted soil conditions, decapped roots induced a vertical displacement intensity ($\bar{d}_y$) in the soil immediately proximal to the root ($l < 0.3\ \text{mm}$) that was elevated compared to the values at distances from the root of $l \approx 0.75\ \text{mm}$ and greater (figures 7 and 8). The physical interpretation of this result is that due to frictional effects, soil immediately next to the root tends to be translocated in the dominant direction of root growth, with this effect localized to the less than 1 mm closest to the root surface. The sub-millimetre scale is thus relevant for understanding at least some fundamental mechanics of root–soil interaction. The DVC results also returned significant surface-localized vertical displacements at the root surface in the intact wet consolidated and intact wet loose conditions, but these were not significant in the grain tracking results at the 95% confidence interval. Some differences of this nature are to be expected between the methods, given that the discrete grain method neglects

the textural phase, whereas the DVC method predominantly tracks this phase.

In the direction lateral to the dominant root growth direction, the displacement intensity ($\bar{d}_x$) quantified by the grain tracking approach did not vary significantly over distance from the root in any condition. The DVC results only indicated a reduction for the intact wet loose condition for distances of $l > 1.2\ \text{mm}$. Thus, the movement of soil in the dominant direction of growth (i.e. associated with friction at the boundary of the elongating root) appears to be a more highly localized mechanism than the displacement of soil in the lateral direction (i.e. that associated with radial compaction of soil during root ingress).

4.2.2. Comparison of mechanisms across conditions

Comparison of all conditions over the range of distance (l) from the root surface revealed the dominant difference to be between intact and decapped roots in the dry compacted soil condition (figures 7 and 8). In the dry compact soil, the vertical displacement intensity ($\bar{d}_y$) was significantly elevated for the decapped condition relative to the intact condition across virtually the entire range of l in both grain tracking and DVC results. Lateral displacement intensity ($\bar{d}_x$) was also significantly elevated for the decapped condition relative to the intact condition in the grain results, but only immediately proximal to the root ($l < \sim 1\ \text{mm}$). These observations indicate that in drier soil, decapped roots induced greater soil deformation per unit of root growth than intact roots, with soil displacements in the dominant direction of root growth being particularly significant. This correlates with 2D DIC results from sand-grown maize roots [9]. The putative explanation of prior studies appears to be borne out by these results; namely that the lack of mucilage, border cells and pointed cap geometry necessitate a decapped root to induce more soil deformation per unit of root growth than an intact root [9,15]. Furthermore, the current study indicates a particularly strong influence of soil water content on the significance of the differences between intact and decapped specimens. Because soil strength generally increases as soil dries [31], this corroborates the hypothesis that the presence of root cap is particularly important to penetrate high strength soils. The shear strain results, discussed in electronic supplementary material (S6), lend more tentative support to this conclusion.

Substantial heterogeneity was observed across the replicates in the study, evidenced by the relatively small number of significant comparisons between conditions. Though the individual replicates for each condition were prepared in an identical manner, the specific configuration of grains and pores at the rhizosphere scale was observed to differ from sample to sample. A particularly striking instance is seen in figure 5c, which shows a dry, compacted sample, in which a large macropore was present immediately beneath the root tip in the initial condition. This permitted elongation with little impedance by the soil, and may explain why this sample exhibited a high elongation rate compared to virtually all other samples across all conditions (electronic supplementary material, figure S4). As previously shown [32,33], heterogeneity at the macro-pore scale is particularly important for understanding root elongation. This complexity and variability of dynamics at the rhizosphere scale is also illustrated in figure 9, which shows orthogonal projections of grain displacements for a series of different samples.

4D SRXCT is the only technique for quantifying rhizosphere dynamics of root–soil mechanical interaction. As

Figure 9. 3D particle displacements shown for the initial growth step (6 min) of 6 samples, showing variation in shape of the displacement field. (a) A decapped sample in wet consolidated soil, with a uniformly distributed displacement field local to the root. (b) A decapped sample in wet loose soil, inducing extensive displacement across the majority of the domain. (c) An intact sample in wet consolidated soil, inducing localized and highly oriented displacement of material into a large macropore (seen on the left of the view in column 2). (d) An intact sample in wet loose soil, inducing localized and highly oriented displacement in the vicinity of a large grain (seen on the left of the view in column 1). (e) An intact sample in wet loose soil, inducing localized and highly oriented displacement with a strongly perpendicular component. (f) A decapped sample in wet loose soil inducing a localized and highly oriented displacement beneath the root tip, in the vicinity of a large macropore (seen towards the middle right of the view in column 1). (Online version in colour.)

with any technique, limitations exist. The scale of samples is small (i.e. less than 10 mm) to achieve sufficient spatial resolution, and to allow sufficient transmission of X-rays [34].

Though the rigid walls of the root chambers possibly exert a constraint on soil displacement and root elongation, it was nonetheless possible to quantify significant deformation mechanisms with characteristic length scales of less than a tenth that of the chamber diameter. It was not possible to determine if the observed decrease in elongation rate over time was influenced by factors beyond soil strength. X-ray doses were minimized as far as was practical, and are unlikely to produce significant effects over the very short timescales considered [35]. The soil kinematics were normalized to root elongation to allow the influence of variable extension rates to be neglected in the analyses.

The small samples required to conduct grain scale analyses entail greater heterogeneity in soil structure than at coarser scales. These limitations are difficult to overcome without larger sample sizes or more idealized growth media. Soil deformation has recently been quantified at much coarser spatio-temporal scales using benchtop XCT and DVC [8]. However, the poorer resolution and image noise precluded a grain tracking approach, and rhizosphere-scale dynamics could not be quantified. These factors indicate the merit of a complementary approach; using SRXCT data to probe the micro-scale fundamentals of root–soil interactions, and coarser-resolution XCT [19,36,37] to study deformation at whole root and root system scales. One fruitful avenue for future studies would be the design and fabrication of a triaxial rhizometer [38] suitable for *in situ* XCT imaging, allowing kinematic studies of root–soil interaction under controlled confining pressures. SRXCT studies in sampled cores rather than prepared soils may also be feasible, though collection of intact samples and repeatability of conditions is a substantial challenge at the approximately 5 mm scale required.

5. Conclusion

This paper has shown the possibilities extended by a fusion of 4D SRXCT and kinematic quantification approaches, with both discrete and continuum implementations considered. This has allowed dynamic, rhizosphere-scale soil–root interactions to be explicitly observed *in vivo* and in three dimensions. The methods were suitable to quantify significant differences between soil kinematic parameters, considered with respect to distance from the root. In a dry and compacted soil, roots without an intact root cap induced a significantly greater degree of soil deformation in the root growth direction than intact roots per unit of root elongation. For decapped roots in both wet and dry compacted soils, deformation was significantly localized proximal to the root surface, with these differences being significant over a sub-millimetre distance away from the root. The results produced by the continuum and discrete approaches did not differ significantly in this study. The scientific findings corroborate the indications of the only extant study to compare phenotypes using digital correlation, showing that an intact root cap improves ingress to soils, inducing less soil deformation per unit of root growth, probably due to the effects of lubricating mucilage and border cells [9]. The results of the present work show this effect to be particularly important in drier soil. The direct visualization and quantification of root-scale interactions will be valuable to the development and validation of explicit models of root–soil mechanical interaction, which can be up-scaled to the root-system scale.

Data accessibility. The data used for the analyses in this study are accessible under a creative commons licence (CC BY-NC 4.0) under DOI 10.5258/SOTON/D0176, and the analysis codes are accessible under a creative commons licence (CC BY-NC 4.0) under DOI 10.5258/SOTON/D0175.

Authors' contributions. S.D.K. co-designed the study, fabricated the growth environments, conducted the imaging, wrote the analysis scripts, analysed the data and co-wrote the manuscript. T.R. co-designed the study, analysed and interpreted the data, and prepared the manuscript. N.K. assisted with sample preparation and imaging, contributed to the development of the image analysis protocol, analysed and interpreted the data, and assisted with preparation of the manuscript. D.M.M.F. assisted with imaging, contributed to the development of the kinematic analysis protocol, analysed and interpreted the data, and assisted with preparation of the manuscript. C.P.S. assisted with imaging, analysed and interpreted the data, and assisted with preparation of the manuscript. L.C. assisted with imaging, analysed and interpreted the data, and assisted with preparation of the manuscript. S.D. assisted with statistical testing, analysed and interpreted the data. I.S. assisted with preparation of

the manuscript and provided technical assistance with the image analysis and kinematic protocols. A.v.V. analysed and interpreted the data.

Competing interests. We declare we have no competing interests.

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