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1 **The helical motions of roots are linked to avoidance of particle forces in soil**

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15

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23

24 **Abstract**

- 25 • Limitation to root growth results from forces required to overcome soil
26 resistance to deformation. The variations in individual particle forces affects
27 root development and often deflects the growth trajectory.
- 28 • We have developed Transparent Soil and Optical Projection Tomography
29 microscopy systems where measurements of growth trajectory and particle
30 forces can be acquired in a granular medium at a range of confining pressures.
31 We developed image processing pipelines to analyse patterns in root
32 trajectories and a stochastic-mechanical theory to establish how root
33 deflections relate to particle forces and thickening of the root.
- 34 • Root thickening compensates for the increase in mean particle forces but does
35 not prevent deflections from 5% of most extreme individual particle forces
36 causing root deflection. The magnitude of deflections increases with pressure
37 but assemble into helices of conserved wavelength in a response linked to
38 gravitropism.
- 39 • The study revealed mechanisms for the understanding of root growth in
40 mechanically impeding soil conditions and provides insights relevant to
41 breeding of drought-resistant crops.

42 **Keywords**

43 Biophysics, root, granular, mechanical stress, deflection, Transparent Soil

44

45 **Introduction**

46 To improve crop water and nutrient efficiency of crops, significant plant breeding
47 interest is now focused on modifying the architecture of the root system to improve
48 rooting depth (Lynch, 2011). Much of the attention is given to genetically controlling
49 rooting angles to enhance drought resistance (Uga *et al.*, 2013) or to enhance nutrient
50 acquisition (Liao *et al.*, 2001). While most crop research takes molecular genetics
51 approaches to study the development of root system architectures, limitations arise
52 because suitable traits for deep rooting are difficult to identify.

53 There is consequently a growing interest in understanding the biomechanical factors
54 that limit root growth through soil (Colombi *et al.*, 2017b). Physical limitations to
55 tissue expansion are linked to the physiology of the cell, in particular the cells ability
56 to sustain turgor pressure and to soften the properties of cell walls (Mirabet *et al.*,
57 2011). Turgor pressure results in the build-up of tension forces within cell walls and
58 growth occurs because the network of cellulose microfibrils permit extension and
59 rearrangement (Braidwood *et al.*, 2014) through a mechanism termed polymer creep.
60 Although the extensibility of primary cell walls is biochemically controlled (through
61 pH, production of enzymes and free radicals, Cosgrove, 2005), growth can be
62 mechanically arrested because external pressure exceeds turgor pressure, as initially
63 shown by early biophysicists (Green *et al.*, 1971) and later studies (Geitmann &
64 Ortega, 2009).

65 This view of the biophysics of growth is challenged in soils where plant roots grow
66 under considerable levels of external pressure from an inhomogeneous soil medium.
67 Turgor pressure in plant cells is rarely measured above 1 MPa, even when growth has
68 been arrested (Meshcheryakov *et al.*, 1992; Clark *et al.*, 1996). This level of pressure
69 corresponds well to the maximum axial pressure a root can exert on a rigid obstacle
70 (Misra *et al.*, 1986) but not to the soil penetrometer pressure at which growth is
71 arrested (5 MPa). Differences are attributed to lubrication by border cells, or flexibility
72 of the root tissue (Bengough & Mullins, 1990). Still, turgor pressure within root cells
73 is an order of magnitude less than known physiological limits of turgor, for example
74 in fungal appressoria where turgor pressure can exceed 10 MPa (Howard *et al.*, 1991).
75 Turgor pressure itself may not be the basis for limitation to deep rooting, and it is

76 reasonable to question why plants are not generating larger growth forces to penetrate
77 hard soils.

78 The inhomogeneity of soil makes it difficult to understand the forces experienced by
79 roots. Soils are comprised of particles held together by forces at the contact points
80 between adjacent particles (repulsion, friction, cohesion). Even when dry and
81 monodisperse, packings of particles are disordered (Majmudar & Behringer, 2005).
82 Large variations in particle forces arise because of the heterogeneous distribution of
83 contact points between particles, with the tail of the probability distribution of particle
84 forces following an exponential distribution (Radjai *et al.*, 1998). It is not clear how
85 such a stochastic distribution of forces will affect the growth of a root. The mechanics
86 of root penetration has been the subject of recent computational studies (Fakih *et al.*,
87 2019), but conceptual frameworks to understand the nature of root responses to
88 granular forces are still lacking.

89 We report here an analysis of microscale deflections of growth due to interactions with
90 the soil granular structure. We develop an experimental system that captures the
91 statistical distribution of particle forces exerted on the root and analyse growth
92 responses to these forces. We propose a theory that links root elongation to particle
93 force and show that root responses to mechanical interactions with particles are linked
94 to the statistical distribution of these forces.

95 **Materials and methods**

96 **Transparent Soils.** Transparent Soil is a soil surrogate made of Nafion™, a
97 transparent low refractive index polymer. It was prepared as described in (Downie *et al.*,
98 2012). Nafion pellets (4 mm×3 mm NR50 1100, Ion Power Inc., USA) were
99 freezer milled and sieved with 1250 μm and 250 μm mesh size and polydispersity
100 further characterised by image analysis (Methods S1-1). Particles were immersed in
101 stock solutions of Hoagland No 2 basal solution (H2395, Sigma, USA) to adjust the
102 pH and titrate the particles with mineral ions. These were shaken at 30°C for 30
103 minutes before replacing the nutrient solution (Downie *et al.*, 2012), and the operation
104 was repeated until the pH was 6.5. The particles were rinsed with dH₂O to remove
105 excess Hoagland media and autoclaved in dH₂O at 30% water content. The resulting
106 particles had sizes ranging from 0.20 to 2.21 mm (Figure 1A).

107 **Pressure chambers.** Chambers were made of Sterilin™ Quickstart Universal
108 Polystyrene 30mL tubes, and the piston applying the force at the surface of the soil
109 was made of Sterilin™ Polystyrene 7 mL tubes (Methods S1-2). The piston was
110 transparent to allow penetration of light and fitted with negligible friction with the
111 inner wall of the chamber. A 3 mm hole was drilled in the cap of the inner tube (piston)
112 to introduce the seed or the penetrometer needle for measuring penetration resistance
113 forces. Compression in the chamber was applied by moving the base of the stage
114 (Methods S1-2) and monitoring soil confining pressure with a 20 N load cell (Applied
115 Measurements Ltd). The intensities of the compression applied to the soil were 0 N (0
116 kPa, control), 10 N (25 kPa) and 20 N (50 kPa).

117 **Resistance to penetration.** Penetrometer resistance was measured within chambers
118 under confining pressure of 0 kPa, 25 kPa and 50 kPa (n=6). Penetrometer resistance
119 was measured using an Instron 5544 universal test frame (Instron, MA, USA) fitted
120 with a 50 N load cell accurate to ± 2 mN (Methods S1-3). The penetrometer needle
121 used was a cone shaped tip of 30° semiangle with a base of 1.72 mm in diameter and
122 a cross-sectional area of 2.32 mm² and a 20% rebated shaft to minimise shaft friction.
123 Shaft cones of 30° semiangle are commonly used as root analogues because they
124 compromise well between cone friction and formation of soil bodies (Bengough &
125 Mullins, 1990). Crosshead displacement for penetrometer testing was performed at a
126 rate of 2 mm min⁻¹ to maintain quasi-static conditions (Methods S1-2). It has been
127 shown experimentally that dynamic effects are observed for penetration rates that are
128 one or two orders of magnitude larger (Bengough & Mullins, 1990). Depths of 20 to
129 40 mm were tested because the mean force was approximately constant over this range
130 and shaft friction negligible. We define the particle force F_i as the i^{th} peak of force
131 recorded in this experiment. Mean force $\langle F \rangle$, third quartile $F_{75\%}$, and probability
132 distribution of particle forces were characterised.

133 **Root biomechanics.** The mechanical resistance of seedling roots (n=7) was tested
134 under compression. Seeds were germinated using germination paper until the roots
135 were approximately 2 cm long. Seedling primary roots were then anchored in plaster
136 of Paris and tested under axial compression (Methods S1-3) using an Instron 5544
137 universal test frame as described above.

138 **Plant growth.** Lentil (*Lens culinaris*) cv. Peridot seeds were sterilised in 10% solution
139 of sodium hypochlorite for 20 minutes. Seeds were pre-germinated on germination
140 paper at 25°C and photoperiod of 16 h. After root protrusion (36 h), the seeds were
141 transferred to a cylindrical chamber containing Transparent Soil held at 30%
142 volumetric water content. The germinated seeds grew for 3 days at 25°C in soil
143 maintained at 0 kPa, 25 kPa and 50 kPa of confining pressure (n=5). Roots were then
144 washed and digitally scanned with root diameter measured along the first 2 cm of the
145 root from the apical meristem.

146 **3D Microscopy.** After 3 days of growth, a solution of 20% trehalose was added for
147 refractive index matching with the soil particles. A vacuum pump (RelChron PPROB-
148 10398) was used to remove air bubbles present in the samples. Images were collected
149 15 hours after the addition of trehalose under laboratory conditions with an Optical
150 Projection Tomography microscope (Sharpe *et al.*, 2002). The microscope was made
151 from a Leica MZ16 FA stereomicroscope fitted with a 0.5X plan achromatic objective
152 for long working distance (135 mm) and a Leica DFC350FX camera (Figure 1B). Each
153 scan consisted of 720 projections taken every 0.5 degree. Scans were obtained at three
154 different depths and achieved 13 to 20 μm resolution and 3 cm field of view out of 5
155 cm of soil depth. The image data obtained from three different depths were combined
156 using Fiji sequence stitching (Schindelin *et al.*, 2012), and 3D reconstructions were
157 performed in Matlab (MathWorks Inc.) using the filtered backprojection algorithm.
158 Confocal laser scanning imaging was done on a Nikon A1R microscope. Roots were
159 stained with calcofluor (fluorescent brightener 28; Sigma F3543) and imaged using
160 either x4 or x10 magnification.

161 **Signal processing.** We developed a pipeline to reconstruct the root centreline with
162 precision (Figure 2A). First, a 3D vessel tracing algorithm was used to obtain a coarse
163 representation of the root centreline (Friman *et al.*, 2010). To improve the accuracy
164 and precision of the root centreline a multiplane tracing approach was developed
165 (Methods S2-1) by generalisation of bi-plane snakes (Canero *et al.*, 2000). Root
166 centrelines were subsequently centred along the z-axis. This was achieved using a
167 spline regression with 3 anchor points and fitted values were subtracted from the
168 original signal. Centred signals $f(t)$ were then analysed for helical patterns. The
169 analysis was based on a modification of the Fourier transform to include orthonormal
170 helix forming basis functions (Figure 2B),

$$\psi_0(t) = (0, 0, \sqrt{3}t) \text{ and } \psi_k(t) = (e^{i2\pi kt}, e^{i(2\pi kt - \pi/2)}, 0), \quad (1)$$

$$k \in [-N, N].$$

171 t represents the rooting depth and k the spatial frequency. The sign of k indicates
 172 clockwise or anti-clockwise helices. The coefficients of the transform C_k are then
 173 obtained by projection on the set of basis functions,

$$C_k = \int_0^T (f_x; f_y; f_z/T^2) \cdot \overline{\psi_k} dt. \quad (2)$$

174 The wavelength of the helix is $\lambda_k = |1/k|$. $\tilde{\lambda}_k$ is the length of root contained in a helix
 175 of one period is therefore

$$\tilde{\lambda}_k = \sqrt{1 + (2\pi k r_k)^2} \lambda_k, \quad (3)$$

176 where $r_k = \sqrt{C_k \overline{C_k}} + \sqrt{C_{-k} \overline{C_{-k}}}$ is the radius of the waveform of frequency k . We also
 177 introduce the asymmetry ratio R_a ,

$$R_a = \frac{C_{-j} \overline{C_{-j}}}{C_k \overline{C_k}} \quad (4)$$

178 where $k > 0$ and $j > 0$ are respectively the clockwise and anticlockwise dominant
 179 frequencies of opposite sense of rotation. The asymmetry ratio $0 \leq R_a \leq 1$ indicates
 180 whether the helix change its sense of rotation during growth. When clockwise and
 181 anticlockwise spectra have identical peaks, R_a is equal to 1, and in the case of a perfect
 182 helix of infinite length, it is equal to 0. When analysing real data, extremal values are
 183 not reached but relative values of R_a indicate the degree of consistency of the sense
 184 of rotation of a root helical trajectory. Changes in root directions were identified as
 185 local maxima in the root curvature. A custom software RootHix was developed to
 186 perform the analyses (www.archiroot.org.uk/tools.html). Full mathematical derivation
 187 of the analysis can be found in Methods S2-2. The data generated in this study is
 188 available for download on the following Zenodo repository
 189 <https://zenodo.org/record/889946#.WbgwrsiGO-4>.

190 **Theory for root-particle interactions.** Root deflection occurs when the energy of
 191 axial elongation U_e becomes larger than the energy U_b required for bending and lateral
 192 displacement of particles (Figure 2C),

$$U_b(E, I, \langle F \rangle, d, \delta) < U_e(F, \delta). \quad (5)$$

193 F (N) is the force required to displace the particle in front of the root, E (Pa) is the
 194 Young's modulus of the root tissue, I (m^4) is the second moment of area of the root,
 195 $\langle F \rangle$ is the mean particle force, δ is the mean displacement between two peak forces in
 196 a penetrometer test, and d is the distance between particles determined as the mean
 197 particle diameter (Methods S3).

198 Since U_e is a growing function of F , it is possible to calculate from equation 5 a
 199 critical force F_{crit} above which the deflection of the root will occur,

$$F_{crit} = A(EI)^{1/4} \langle F \rangle^{3/4} d^{1/2} \delta^{-1}, \quad (6)$$

200 with a bending constant $A \approx 0.237$. A similar calculation can be made if the tissue
 201 has viscoelastic properties. In this case, E is replaced with a time-dependent coefficient
 202 $E^*(t)$, termed creep function, which we obtain from the Kelvin Voigt viscoelastic
 203 model,

$$\frac{1}{E^*(t)} = \frac{1}{E} \left(1 - \exp - \frac{E}{\eta} t \right). \quad (7)$$

204 t here refers to the time required for the root growth to overcome a particle and is
 205 determined as d/v where v is the root tip velocity (m s^{-1}). The probability of a
 206 deflection occurring can then be expressed from the distribution of particle forces
 207 obtained from penetrometer test. Because deflections are rare, they must be caused by
 208 large particle forces which occurrence is described by the tail of the distributions of
 209 particle forces. These follow an exponential law,

$$q = \frac{1}{4} \exp[-b(F_{crit} - F_{75\%})]. \quad (8)$$

210 The theory then provides the probability distribution (pdf) σ and κ of the occurrence
 211 of deflections and curvature of the root respectively,

$$\sigma(x) = \frac{q}{\delta} \exp\left(-\frac{xq}{\delta}\right), \quad (9)$$

$$\kappa(x) = \frac{q^2 d^2}{\langle l \rangle^2 \delta^2 x^3} \exp\left(-\frac{qd}{\langle l \rangle \delta x}\right). \quad (10)$$

212 $\langle l \rangle = B(d^2 EI / \langle F \rangle)^{1/4}$ is the expected bending length with $B \approx 2.06$. The pdf of root
 213 curvature follows an inverse gamma distribution with shape parameter 2 and scale

214 parameter $qd/\langle l \rangle \delta$. Full mathematical derivation for models can be found in Methods
215 S3.

216 When the tissue is anisotropic, root reorientation occurs preferentially along a given
217 axis of rotation (blue cone, Figure 2D). The axis of rotation defines two equally
218 probable bending directions V_1 and V_2 . Because the anisotropy is helical, V_1 and V_2
219 are not constant but rotate along the roots (red arrow, Figure 2D). To test whether
220 deflections occur preferentially in certain directions, we assign the probability q_1 for
221 direction V_1 and $q_2 = 1 - q_1$ for V_2 . We used equations 5-8 to simulate the
222 occurrence of deflections in an elongating root based on experimental data and
223 assumed three scenarios for the direction of deflection. If the deflection has no
224 predefined direction (random deflection) then $q_1 = q_2 = 0.5$. If there is an intrinsic
225 sense of rotation then $q_1 = 1$ or $q_2 = 1$. If the direction of deflection occurs
226 preferentially towards gravity (gravitropic deflection), the probability is

$$q_1 = \frac{\tanh(-G\Delta\alpha) + 1}{2} \quad (11)$$

227 $\Delta\alpha = \alpha_1 - \alpha_2$ is the difference in verticality between V_1 and V_2 , i.e. α_1 (respectively
228 α_2) is the positive angle between V_1 (respectively V_2) and the downward vertical. G
229 defines the sensitivity of the response to gravity.

230 Results

231 **Resistance to penetration.** Penetrometer tests produced stochastic data (Figure 3A).
232 Analysis of the data showed soil confining pressure (0 kPa, 25 kPa, and 50 kPa,
233 generated by loads of 0 N, 10 N and 20 N respectively) increased the mean penetration
234 force. In soils that were not held under compression, the mechanical resistance to
235 penetration was the lowest with $\langle F \rangle = 0.15$ N (± 0.01), $F_{75\%} = 0.19$ N. In soils held
236 under a confining pressure of 25 kPa, the resistance to penetration increased to $\langle F \rangle =$
237 1.11 N (± 0.03), $F_{75\%} = 1.18$ N. The highest resistance to penetration, $\langle F \rangle =$
238 2.14 N (± 0.03), $F_{75\%} = 2.26$ N, was obtained in soils held at a confining pressure of
239 50 kPa (Figure 3B).

240 We analysed the sequence of forces recorded during penetration (Figure 3C&D). The
241 tail of the statistical distribution of transformed particle forces (Figure 3C) showed an
242 exponential decline, with characteristic force values ($1/b$) measured as 36.3 mN, 75.9

243 mN and 95.3 mN for respectively 0 kPa, 25 kPa and 50 kPa of soil confining pressure.
244 Permutation tests showed the exponential tail of the distribution was not affected by
245 the increase in confining pressure from 25 kPa to 50 kPa ($p=0.88$), but there was a
246 statistical difference between uncompressed and compressed soils ($p<0.001$).

247 We also studied the distances δ between peak forces (Figure 3D). The probability
248 density of δ followed an exponential decline with a characteristic distance of 0.13 mm.
249 No statistical differences were found between soils under compression ($p=0.94$) nor
250 between compressed and uncompressed soil ($p=0.12$). The distance between the peaks
251 of force was substantially smaller than the size of the particle diameter (1 mm on
252 average).

253 **Roots helical response to mechanical forces.** Plant roots grew healthily in all
254 experiments (Figure 4A). Soil confining pressure reduced root elongation rates and
255 increased root diameters. Roots growing in soil without confining pressure exhibited
256 the fastest elongation rate of $1.99 \text{ cm}\cdot\text{d}^{-1}$ (± 0.40) and had diameters of 0.64 mm (± 0.04)
257 at 2 cm from the root tip. Roots growing in soil under 25 kPa of confining pressure
258 exhibited an elongation rate of $1.60 \text{ cm}\cdot\text{d}^{-1}$ (± 0.42) and had diameters of 0.69 mm
259 (± 0.03). Roots growing in soil under 50 kPa of confining pressure exhibited an
260 elongation rate of $1.36 \text{ cm}\cdot\text{d}^{-1}$ (± 0.28) and had diameters of 0.75 mm (± 0.07).

261 Root centrelines exhibited helical morphologies (Figure 4A) which could be detected
262 by helical transformation (Figure 4B). The analysis identified a dominant wavelength
263 that is not affected by soil confining pressure. Wavelength values ($\tilde{\lambda}$) were 13.7 mm
264 (± 1.4), 12.1 mm (± 0.5) and 12.8 mm (± 0.6) for pressures of 0 kPa, 25 kPa and 50 kPa
265 respectively (Figure 4C). However, the radius of the helix r_k significantly increased
266 from 0.17 mm (± 0.03), to 0.24 mm (± 0.03) and 0.30 mm (± 0.04) for pressures of 0
267 kPa, 25 kPa and 50 kPa. These helical shapes had curvatures of respectively 0.034
268 mm^{-1} , 0.063 mm^{-1} and 0.071 mm^{-1} . Helices were clockwise, anti-clockwise and
269 occasionally changed direction of rotation along the same root axis.

270 Compression tests were used to characterise the mechanical properties of roots (Figure
271 4D). All roots tested deformed into a helical shape at an average force of 20.0 mN
272 (± 1.5). Helical shapes were more pronounced near the tip where the root was thinner.
273 Roots retained their helical shape after removal of the axial forces. The wavelength of
274 the helix ($\tilde{\lambda} = 12.2 \text{ mm} \pm 2.0$) closely matched those measured in Transparent Soil, but

275 the radius of the helix ($0.9 \text{ mm} \pm 0.1$) was about three times as large as the value
276 measured under 50 kPa of confining pressure. The mechanical test was interrupted
277 before roots were visibly fractured. Roots were subsequently moved into water and
278 recovered their shape within 20 minutes.

279 **Soil particles influence root deflections.** Roots curvatures at deflections exceeded
280 those of fitted helices by an order of magnitude (Figure 5A). The distance between the
281 sites of deflections was not influenced by the pressure acting on the soil. The
282 distribution of the distance between the sites of two successive deflections was
283 approximately uniform, with distances spanning between 500 μm and 6 mm with an
284 average of 2.5 mm (Figure 5B). Since the characteristic distance between peaks of
285 forces produced by soil particles is $\delta = 0.13 \text{ mm}$ and one deflection occurs every 2.5
286 mm of root growth in average, thus 5% of particle force events produced root
287 deflections.

288 The curvature of the root where deflection occurs was influenced by soil confining
289 pressure (Figure 5C). In the absence of confining pressure, curvatures were below 0.2
290 mm^{-1} . When 25 kPa of pressure was applied, curvatures measured were below 0.3 mm^{-1}
291 ¹, whereas when 50 kPa of pressure was applied, curvatures above 0.4 mm^{-1} were
292 measured. Confocal laser scanning microscopy observations showed sharp deflections
293 extending over a length of root of around 700 μm . The curvature of these deflections
294 increased with increases in confining pressure. There was little evidence of tissue
295 torsion. Angle in the files of epidermal cells relative to the local longitudinal root axis
296 was rarely observed and did not correlate with the sites of deflection (Figure 5C Inset).

297 **Linking root gravitropism and deflections to helix formation.** The model described
298 the overall root responses to confining pressure for the distribution of curvatures
299 (Figure 6A Top) but overestimated the deflection in the high frequency domain
300 (Figure 6A Middle). The Young's modulus required to predict the range of curvatures
301 varied between 1.5 and 5.0 MPa ($3.7 \text{ MPa} \pm 1.4$, $1.64 \text{ MPa} \pm 0.62$ and $2.05 \text{ MPa} \pm 0.78$
302 for respectively 0 kPa, 25 kPa and 50 kPa of confining pressure) and declined with
303 confining pressure (Figure 6A Bottom). Apparent variations in the Young's modulus
304 may be due to the viscoelasticity of the tissue, with Kelvin Voigt model best fitting
305 data with $E = 21.5 \text{ kPa}$ and $\eta = 1.7 \text{ GPa} \cdot \text{s}$.

306 There was strong evidence of the role of gravitropism in the maintenance of helical
307 shapes. Experimental results showed the asymmetry ratio
308 R_a is decreasing linearly with root deviation from verticality (black curves,
309 Figure 6B top right). Roots that grew more horizontally had therefore a greater
310 tendency to maintain a consistent helix and conserved sense of rotation. Simulations
311 demonstrated this is caused by a bias in the direction of growth when a deflection
312 occurs. When random deflections were imposed in the simulations (red curves, Figure
313 6B), roots lost their ability to maintain helical forms due to the sense of rotation of the
314 helix changing randomly when hitting an obstacle: R_a was larger than in all other cases
315 and also independent of the deviation from verticality. Roots which deflected
316 following a fixed direction V_1 or V_2 (green curves, Figure 6B) naturally produced helix
317 with unchanged sense of rotation (asymmetry ratio ≈ 0.3). Here too, the model could
318 not predict the experimental effect of deviation from verticality on the asymmetry
319 ratio. Roots which direction of deflection was influenced by root verticality
320 (gravitropic simulation, Figure 6B, blue curve) produced more realistic growth
321 patterns and induced the formation of helices with occasional switches in the sense of
322 rotation, but these switches were less frequent.

323 **Discussion**

324 **Linking root responses to soil structure.** Biophysical theories link growth response
325 to soil pressure, cell wall rheology, and water potential (Greacen & Oh, 1972; Dexter,
326 1987). If the soil pressure on the root and the tensile stresses in the cell walls exceed
327 turgor pressure, then growth must be arrested (Plant, 1982; Dexter, 1987). Our
328 approach departs from this view and links root physical limitations to the mechanical
329 stability and deflections of the root tip due to surrounding particle forces. Therefore,
330 critical root elongation forces define the ability of a root to remain mechanically stable,
331 and this ability is linked to the rigidity of the tissue and the distribution of particle
332 forces.

333 The emergence of theories for the prediction of critical elongation forces has been
334 largely limited by the ability to characterise the mechanical environment of a growing
335 root. Experimental systems using compression chambers of various sorts have been
336 extensively used in the 1960's and later (Barley, 1962; Materechera *et al.*, 1991;
337 Abdalla A *et al.*, 1969), but simultaneous measurements of particle forces were not

338 made. The first attempts of Whiteley and Dexter (1982) showed measuring the drag
339 force of a particle of known size and traction speed is possible but precise control of
340 particle displacement is not. More recently, research on the physics of granular media
341 has characterised interparticle mechanics using planar force sensors or computational
342 methods (Mueth *et al.*, 1998; Hurley *et al.*, 2016), but application of such techniques
343 within biological systems remains difficult. Use of photo-elastic materials has been
344 successful (Kolb *et al.*, 2012; Wendell *et al.*, 2012) but current materials do not allow
345 fabrication of realistic soil-like substrate, and for this reason, penetrometer resistance
346 tests remain the preferred approach to characterise the resistance to root elongation
347 (Clark *et al.*, 2003). Root responses to particle forces are equally difficult to monitor.
348 Particle displacements have been measured alongside root deflections in glass bead
349 substrates using tracking algorithms (Bengough *et al.*, 2009). More recently X-ray
350 computed tomography has achieved similar results in 3D (Keyes *et al.*, 2017).
351 Deriving measurements of forces from such data is not currently possible because it
352 would require detection of the deformation of individual particles (Brodu *et al.*, 2015).

353 In this study we have addressed some of these challenges and characterised how root
354 deflections occur in relation to the distribution of particle forces in the growth medium.
355 Our pressure chamber shares similarity with the system used by (Materechera *et al.*,
356 1991), but we additionally monitored 3D growth trajectories and measured
357 penetrometer resistance. More importantly, we have developed signal processing
358 technologies to retrieve the wavelength and radius of root helices and to study the
359 frequency and magnitude of root deflections, e.g. using multiplane tracing and helical
360 transform (Figure 2 A&B). Previously, few studies have utilised the variations
361 observed in penetration resistance. Geostatistical tools were used to analyse periodic
362 variation in penetration resistance in relation to changes in soil structure (Grant *et al.*,
363 1985; Hadas & Shmulewich, 1990), but none of these studies linked variations in
364 particle forces to growth trajectories and root responses to mechanical stress.

365 **Theory for growth in confined soil environments.** Using our experimental system,
366 it was possible to identify factors that heavily influence root responses to interactions
367 with soil particles.

368 Granular media appear to cause frequent deflections of the root trajectory. Growth
369 response to soil heterogeneity has been widely documented (Goss & Russell, 1980),

370 and can be commonly observed in the form of tortuous morphologies, for example in
371 compacted soil (Popova *et al.*, 2016). Our results showed the presence of root
372 deflections appears to be independent of soil mechanical resistance and may prevail
373 in granular media. Deflections occur by bending at the root tip as observed on other
374 root species (Bizet *et al.*, 2016), and it is predominantly the magnitude of deflection,
375 not the frequency, that is affected by soil confining pressure (Figure 5). The study was
376 limited to roots and particles within a narrow range of sizes. It is unclear how the
377 mechanisms described here translate across spatial scales. Root behaviours in finer or
378 cohesive soils are notable. Arguably, some degree of homogenisation of particle forces
379 would apply (Kolb *et al.*, 2017), but granular media are also known to exhibit
380 macroscopic behaviour such as arching or clustering (Delenne *et al.*, 2004; Aranson
381 & Tsimring, 2006). Therefore, root trajectories in these conditions may also exhibit
382 sequences of deflections that are similar to those observed in our study.

383 Root deflections are linked to a mechanical process controlled by the fluctuations of
384 particle forces acting at the root tip. We have characterised the nature of particle forces
385 and found patterns that confirm this hypothesis. The distance between peak particle
386 forces (δ) is conserved across a range of confining pressures, and the tail of the
387 statistical distribution of particle forces experienced by a root is exponential, as is
388 commonly found in granular media (Figure 3C, Radjai *et al.*, 1998). Because the
389 distance between the sites of deflections is larger than δ and larger than the size of
390 particles (Figure 5B), we conclude that in our experimental set-up, a root can often
391 displace soil particles axially, but that on rare occasions the growth trajectory is
392 deflected. Deflection also requires mechanical energy to bend the root and displace
393 particles laterally (Gordon *et al.*, 1992), and therefore it is both the distribution of axial
394 and lateral particle forces that determine if a deflection will take place.

395 Root deflections are mechanically viscoelastic and anisotropic, and may be influenced
396 by gravitropism. Results show roots did not fully recover their shape following
397 mechanical tests (Figure 4D). Also, there was time dependence of the apparent
398 Young's modulus determined experimentally from the model (Figure 6A). This
399 behaviour is typical of viscoelastic materials (Findley & Davis, 2013). Since the time
400 required to overcome a soil particle (between 9 and 14 minutes) exceeds the duration
401 of mechanical testing, visco-elastic deformation must affect the nature of the
402 deflection. This is also consistent with the requirements for tissues to elongate

403 (Braidwood *et al.*, 2014) and for fluids to move across cells (Nonami *et al.*, 1997). We
404 observed the formation of helices with wavelengths similar to those observed during
405 the waving phenomenon observed at the surface of agar (Rutherford *et al.*, 1998). The
406 curvature of helices is an order of magnitude smaller than the curvature at the sites of
407 a deflection and we conclude helical shapes are due to the combined anisotropy of the
408 tissue (Lloyd & Chan, 2002) and the frequency of deflections. Unlike what was
409 proposed by Silverberg *et al.* (2012), torsion pre-stress is not required for helices to
410 form.

411 We have formalised the conditions for the occurrence of a deflection. Our theory
412 predicts how roots respond to soil confining pressure in granular media, and it shows
413 that roots deflect at frequencies that are maintained across increasing levels of soil
414 confining pressure (Figure 5B). This is counterintuitive because the forces required to
415 displace soil particles are increasing too. However, root thickening and subsequent
416 stiffening of the tissue (Materechera *et al.*, 1991; Bengough *et al.*, 2006) prevent the
417 increase in frequency of deflections. The thickening is not sufficient to limit the
418 magnitude of deflections which, as predicted by the mechanics of embedded structures
419 (Mojdehi *et al.*, 2016), results in shorter bending length and increased curvature
420 (Popova *et al.*, 2016).

421 Our theory also showed limitations. The mean field approximation used to establish
422 the critical particle force led to oversimplified predictions of distances between
423 deflections (Figure 6A). The model itself did not address either how the soil structure
424 or the displacement of particles affects the stochasticity of forces. However, various
425 experimental and theoretical studies are now addressing these limitations. The
426 stochasticity of soil penetrometer resistance is now being linked to soil structure (Ruiz
427 *et al.*, 2017). Theoretical work on snow mechanics has also successfully demonstrated
428 how understanding the microstructure of a granular media can lead to improved
429 predictions of forces applied on a penetrating structure (Schneebeil *et al.*, 1999; Löwe
430 & van Herwijnen, 2012). In the future, our theory could therefore be improved by
431 better accounting for variations in particle forces, and this could allow prediction of
432 root growth and morphology to be made directly from the knowledge of soil structure.

433 **Root navigation through paths of least resistance in soil.** Roots are known to
434 mobilise various sensing mechanisms in response to obstacles. For example, response

435 to touching an obstacle has been found to mobilise also gravity sensing (Massa &
436 Gilroy, 2003) and the root cap is believed to play a key role in the reorientation of the
437 tip. Skewing and waving patterns observed during growth on gels are also thought to
438 result from gravitropism and touch stimuli (Migliaccio & Piconese, 2001; Oliva &
439 Dunand, 2007). Similar mechanisms also prevail in the field where roots are known
440 for example to grow in macropores (Moran *et al.*, 2000; White & Kirkegaard, 2010).
441 The phenomenon was recently found, for example, to compensate for the effects of
442 soil compaction (Colombi *et al.*, 2017a).

443 In this study we found evidence that root responses to soil pressure results from
444 interactions taking place at the level of the soil particle. Helical shapes were formed
445 more consistently when roots deviated from verticality (Figure 6B), and this was due
446 to gravitropic effects during deflections from particles. Simulations predicted smaller
447 asymmetry ratio than those measured experimentally (Figure 6B) , which indicates
448 that simulated root morphologies are more helical. This could be explained by
449 constraints imposed in the model on the direction of deflections. The model did not
450 include biological responses linked to the anisotropy of the tissue either. For example,
451 we did not include changes in the mechanical properties of the tissue in response to
452 gravitropic stimulation. Such responses were not studied experimentally here, but
453 work on *Arabidopsis thaliana* have shown that a number of biomechanical factors can
454 affect the waving mechanism observed in roots (Buer *et al.*, 2003). Mechanical
455 properties of root tissues are also known to vary in response to mechanical stresses
456 and consequently to confining pressure (Loades *et al.*, 2013).

457 The conservation of the root deflection frequency also hints at a potential mechanism
458 for growth through paths of least resistance in soil. Because deflections limit exposure
459 to large particle forces, they reduce the overall resistance opposed to elongation.
460 Deflections have also undesirable consequences on root foraging dynamics. For
461 example, models predict that stochastic deflections result in a transition from a
462 convective to a dispersive propagation through soil (Dupuy *et al.*, 2018), and a
463 subsequent reduction in rooting depth.

464 The development of deep roots requires mechanical stability of elongating tissues.
465 Extreme pressures, deformations, or deflections of the root apex are known to affect
466 the cellular organisation of the meristem (Jackson *et al.*, 2017). In such conditions, the

467 developmental functions of the meristem may be affected, and the ability to explore
468 new regions of soil or access resources may become limited. Some control of the
469 morphological and structural properties of tissues in response to soil mechanical
470 properties must therefore prevail. The way sensing of and response to the
471 micromechanical environment operate, however, requires additional study.

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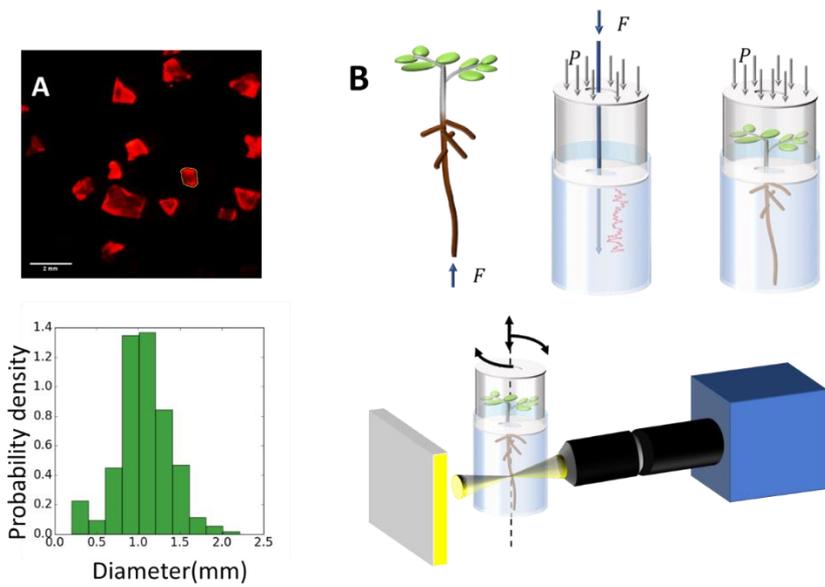
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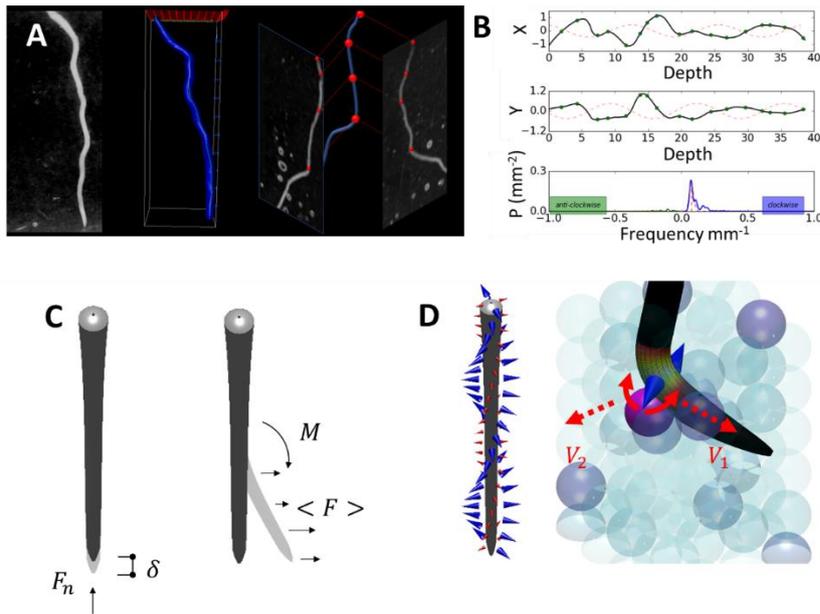
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660 **Figures**



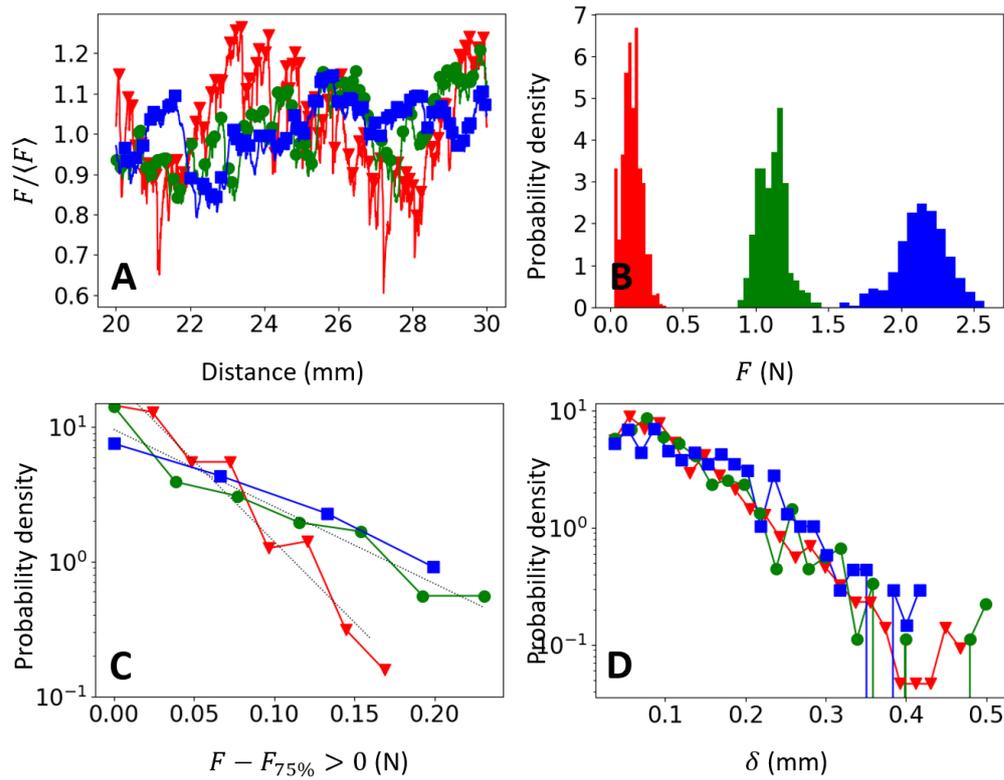
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662 Figure 1. Experimental system for the study of root trajectories in response to soil
663 particle forces. A) Transparent Soils are used as soil analogue. Stained particles can
664 be used for visualisation and here also to quantify particle size distribution. The
665 histogram of particle size distribution (below) shows particles have an average
666 diameter of 1.07 mm \pm 0.32. B) Biomechanical analysis of root soil interactions is
667 based on three experiments. First the buckling of root tips in compression was
668 characterised on living root tips using a universal test frame (left). Measurements and
669 control of particle forces were obtained from plants growing in a cylindrical chamber
670 with the Transparent Soil maintained under controlled mechanical pressure using a
671 piston fitted with a load cell (Methods S1). The transparent piston has a 3 mm opening
672 to (i) monitor changes in particle forces using a penitrometer needle (middle) and (ii)
673 to allow for emergence of the shoot (right). B) An optical projection tomography
674 system with two degrees of freedom (rotation and vertical translation) is used to image
675 the roots over large fields of view. The microscope assembles 720 projections of a root
676 taken every 0.5 degree and at three depths.



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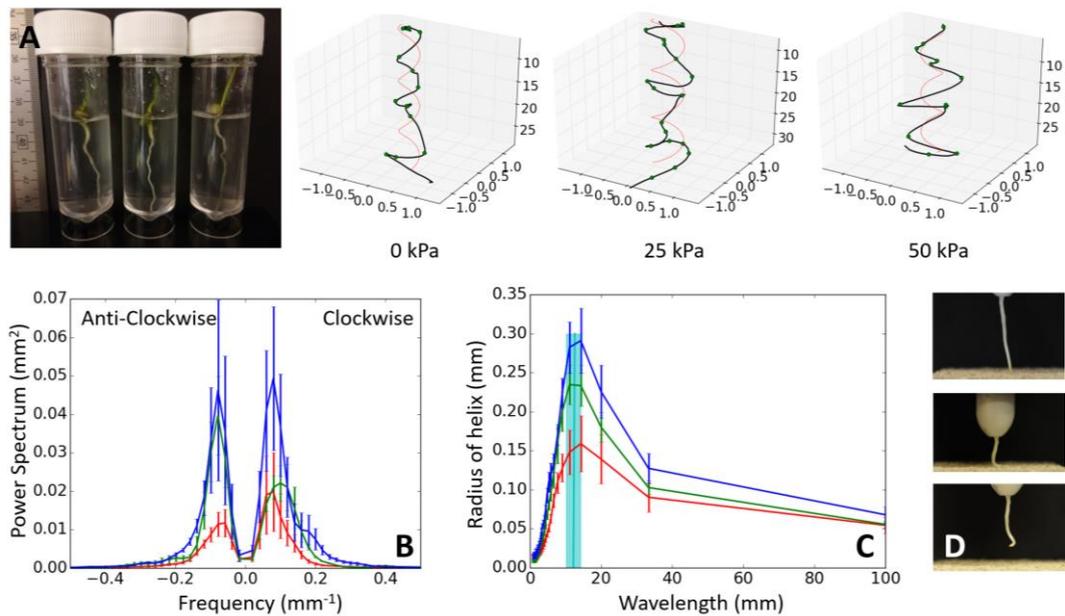
678 Figure 2. Pipeline for mechanistic understanding of root growth trajectories. A) The
 679 morphology of the root is characterised using a 3D image processing pipeline. (left)
 680 projection data are assembled by stitching followed by 3D reconstruction using the
 681 filtered back projection algorithm. A coarse representation of the centreline is first
 682 obtained using automated tracing (centre) and fine mapping of the root centreline is
 683 then obtained using multiplane tracing (right). B) Signal processing tools were
 684 developed to mine for local deflection of the root and detect helical waveforms. Here
 685 the root trajectory is projected in the XZ (top) and YZ (middle). The helical transform
 686 then provides the power spectrum of spatial frequency of the helical waveforms
 687 (bottom). The curve in red indicates the dominant wavelength waveform extracted by
 688 the study. A theory is also developed to understand root responses to soil particle
 689 forces. C) The theory considers two response modes. First, the root may overcome the
 690 resistance F of the particle resisting straight elongation (left). When F reaches a critical
 691 value, bending $\langle M \rangle$ and lateral displacement of particles $\langle F \rangle$ offer less resistance and
 692 a deflection occurs. D) Because a root has inherent helical anisotropy (principal axis
 693 of rotation as blue arrows, minor axis of rotations shown as red arrows), deflection
 694 occurs in the 3-dimensional space where two directions of deflections of least
 695 resistance V_1 and V_2 are equally probable. The sense of direction of the deflection is
 696 then determined by other biological factors.



697

698 Figure 3. Granular physics of Transparent Soil. A) Variation in the force F (N) resisted
 699 by a penetrometer cone of similar size to a root (1.72 mm diameter) for different levels
 700 of confining pressure and measured at depths between 20 mm and 30 mm. The forces
 701 have been normalized by the averaged force $\langle F \rangle$ obtained over a travelled distance of
 702 20 mm. The averaged force increases with confining pressure P . The markers indicate
 703 local maxima of the forces at different confining pressure (red triangle 0 kPa, green
 704 circle 25 kPa and blue square 50 kPa). To avoid sensitivity to sensor noise, only
 705 maxima that are absolute on a neighbourhood of 30 μm are identified. B) Probability
 706 density distribution of the forces (red triangle 0 kPa, green circle 25 kPa and blue
 707 square 50 kPa). C) Tail of the probability distribution of particle forces shows
 708 exponential decline, where $F_{75\%}$ is the third quartile. D) Probability density
 709 distribution of the distance between identified of forces F (markers in A) with red
 710 triangle 0 kPa, green circle 25 kPa and blue square 50 kPa.

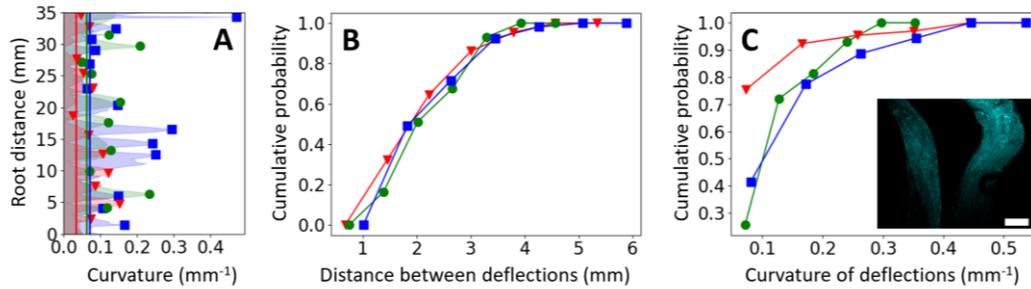
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713 Figure 4. A) Lentil seedlings grown at increasing levels of confining pressure (from
 714 left). Typical centrelines (black curves) of roots grown under increased confining
 715 pressure (right). The horizontal X-Y coordinates are multiplied by 4 to enhance
 716 visibility. Markers indicate the sites of local maxima in root curvature and red lines
 717 show the dominant helix obtained by helical transform. B) The power spectrum of the
 718 helical transform from roots grown at respectively 0 kPa (red), 25 kPa (green) and 50
 719 kPa (blue) shows helices can be both clockwise and anti-clockwise. C) Radius of the
 720 helical waveforms is influenced by the pressure from the soil respectively (same
 721 colour code as above) but the wavelength $\tilde{\lambda}$ is conserved at approximately 13 mm of
 722 root length. D) Root deformations in response to compression forces and buckling also
 723 exhibit helical patterns. The wavelength of the helix observed in these roots (12 mm)
 724 closely matched those grown in soil and is shown in cyan in figure (C) as a vertical
 725 line and a surrounding shadow indicating the confidence interval.

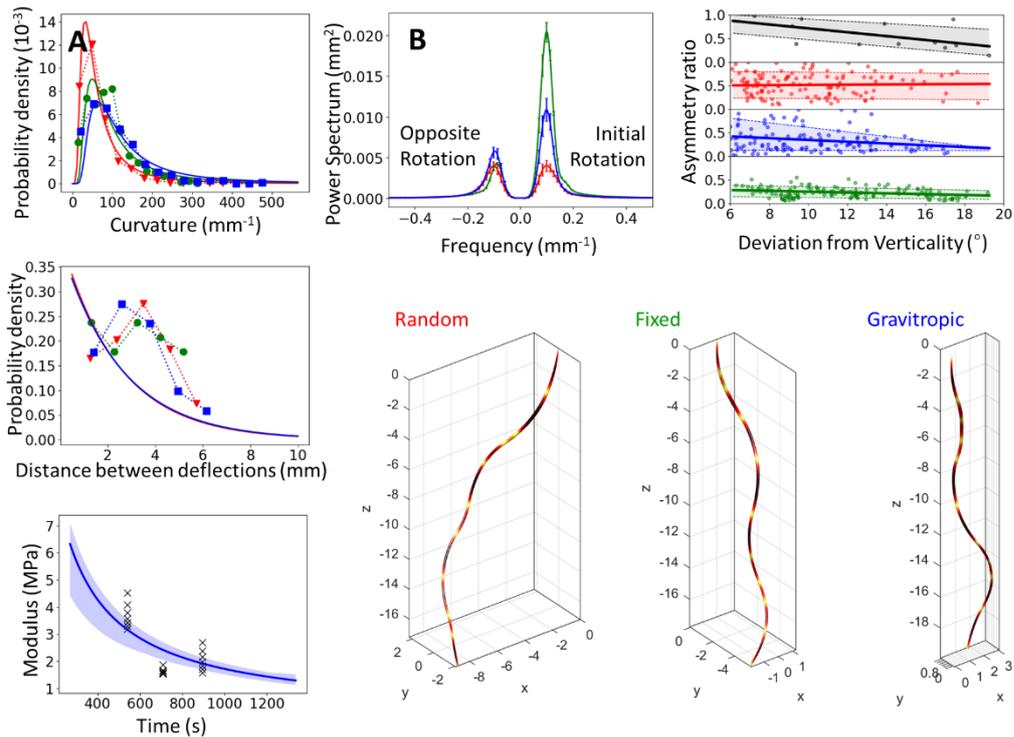
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728 Figure 5. Development in granular media induces microscale deflections of the growth
 729 trajectory. A) The profile of root curvature along the root (shaded area) shows that the
 730 overall growth trajectory of the root is dominated by a sequence of local changes in
 731 direction. The sites of deflections (markers) have high curvature with comparison to
 732 the expected curvature from the global helix (vertical lines). B) The frequency of
 733 deflections expressed as the distribution of the distance between the sites of two
 734 consecutive deflections. The frequency follows an approximate uniform distribution
 735 and is not influenced by soil confining pressure. C) The curvature at the site of the
 736 deflection is increased with the soil confining pressure. Observations made with a
 737 Confocal Laser Scanning Microscope shows localised bending of the root, here under
 738 25 kPa and 50 kPa (bottom left, scale bar 500 μm). Red, green, and blue markers
 739 indicate confining pressures of the Transparent Soil, respectively 0 kPa, 25 kPa and
 740 50 kPa.

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743 Figure 6. Predictions of root responses to particle forces. A) The theory predicts
 744 occurrence and magnitude of deflections, linking the distribution of root curvatures
 745 (top) and the frequency of occurrence of deflections (middle) to critical particle force,
 746 particle size and mean particle force. Suitable prediction can only be achieved with
 747 relaxation of the root stiffness with time, here modelled with the Kelvin Voigt
 748 viscoelastic model (bottom). Experimental data is plotted with dotted lines and
 749 theoretical predictions are plotted with plain lines. Red, green, and blue markers
 750 indicate confining pressures of respectively 0 kPa, 25 kPa and 50 kPa. B) Experiments
 751 and simulations showed gravitropic response is also required to obtain realistic 3-
 752 dimensional root trajectories, with both helical transform analysis (top) and
 753 visualisation (bottom) showing the effect of gravitropism in the formation of helices.
 754 Experimental data (black) shows the asymmetry ratio is influenced by deviation from
 755 verticality which confirms the role of gravitropism in the formation of helical patterns.
 756 Simulations of trajectory with random deflection ($q_2=0.5$, red) leads to large deviation
 757 from verticality and do not form dominant helical waveforms. When the sense of
 758 rotation is fixed ($q_2=1$, green), helical patterns are formed but deviations from
 759 verticality are observed. When root deflection is gravitropic (equation 11, blue) helices
 760 are formed with switches from clockwise to anticlockwise rotations. Plain lines were
 761 obtained by linear regression and shaded areas indicate the prediction intervals.