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► **To cite this version:**

Lx Dupuy, M Mimault, D Patko, Vincent Ladmiral, B. Ameduri, et al.. Micromechanics of root development in soil. *Current Opinion in Genetics and Development*, 2018, 51, pp.18 - 25. 10.1016/j.gde.2018.03.007 . hal-01898743

HAL Id: hal-01898743

<https://hal.science/hal-01898743v1>

Submitted on 25 Apr 2019

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1 **Micromechanics of root development in soil**

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10 Keywords: plant roots, particle, granular media, biomechanics

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12

13 **Abstract**

14 Our understanding of how root develop in soil may be at the eve of significant transformations. The
15 formidable expansion of imaging technologies enables live observations of the rhizosphere micro-pore
16 architecture at unprecedented resolution. Granular matter physics provides ways to understand the
17 microscopic fluctuations of forces in soils, and the increasing knowledge of plant mechanobiology may
18 shed new lights on how roots perceive soil heterogeneity. This opinion paper exposes how recent
19 scientific achievements may contribute to design a new theory for root growth in heterogeneous
20 environments.

21 **Main text**

22 Current knowledge of the biomechanics of plant root growth in soil is largely based on the extensive
23 work of plant biophysicists from the second half of the 20th century [1-3] . The view was that both

24 roots and soil must be considered as continua so that the description of root soil interactions can be
25 achieved with continuous mathematical functions of macroscopic variables such as Young's modulus
26 of root tissue, soil penetration stress, and pore water pressure [4]. Classical concepts from mechanics
27 and physiology then provide a suitable framework to understand factors controlling tissue growth in
28 its natural environment. The energy required to deform the root and surrounding soil, which
29 originates from the photosynthetic chemical energy accumulated within the tissues, is converted into
30 turgor pressure and mechanical energy [5]. Turgor pressure then overcome the resistance from cell
31 wall to stretching, the resistance to movement of water across membranes, and the resistance to the
32 displacement of the soil around the root [6].

33 This classical view of root-soil biomechanics has been central to identify the biophysical factors limiting
34 growth in soil, but it is now challenged to predict morphologies and developmental patterns observed
35 in natural conditions (Figure 1). If roots were to experience homogeneous mechanical stress from the
36 soil, one would expect turgor pressure and Lockhart equation [1] to predict accurately growth arrest
37 in soil. This is not the case and large discrepancies remain between measured turgor pressure (in the
38 order of 1MPa [7]) and the levels of mechanical stresses at which growth is arrested (>5MPa [8]).
39 Classical mechanics of continua is ill-equipped to explain the links between soil heterogeneity and
40 stochasticity of plant development. The root tissue itself is heterogeneous and cell types have
41 different roles in facilitating growth and penetration. Anchoring the base of the root for example, is
42 necessary for cell elongation to produce apical movement and deformation of the soil [9]. The root
43 cap and its associated border cells have also a fundamental role in reducing friction from the bulk soil.
44 It was shown recently that wheat genotypes with sharper root tips are more efficient at soil
45 penetration [10].

46 To establish a biomechanical framework that accounts for the complexity of root interactions with the
47 granular medium, one must capture the microscopic nature of particle forces and the collective action
48 they have on root tissues (Figure 1A). Kolb, et al. [11] proposed to categorise the nature of root
49 mechanical responses to soil based on the scale of the soil heterogeneities. When the medium is

50 composed of small particles, individual variations in the force required to move them are not
51 perceived by the root. The behaviour of roots and soil can be homogenised, and classical continuum
52 mechanics usually applies (Box 1A) [12]. Soils also contain objects that are too large and or too rigid
53 for a root to deform and displace, for example when roots grow in contact with stones, in cracks or
54 pores [13,14]. Growth forces cannot displace the obstacle and the root usually combines tropic
55 responses and mechanical buckling to avoid the obstacle (Box 1B) [15]. The behaviour of roots growing
56 in soils with particles of intermediate sizes is more challenging to understand. A root can displace
57 individual particles from the soil, but the forces exerted by each of the particles can also influence the
58 course of root development (Box 1C). Although such growth environments are common for fine roots
59 or due to the presence of aggregate and sand particles, growth patterns in such conditions are not
60 well understood. How frequently does a root deflect from their growth trajectory? What are the
61 magnitude of deflections? How does the distribution of particle forces modify the growth trajectory?
62 Understanding the forces acting on a root during the elongation requires detailed knowledge of the
63 physics of granular media. Granular media are assemblages of particles held by frictional and repulsive
64 forces from adjacent particles. The forces holding particles together form chain-like networks that
65 propagate at the contact points between neighbouring particles [16]. Because particles are disordered
66 or have various sizes and shapes, large variations in magnitude and direction of particle forces arise
67 [16,17]. Early theoretical work based on dry and static monodisperse particles showed that
68 distribution of contact forces vary greatly and the overall force distribution follows an exponential
69 decline [18,19]. Particles dynamics is better understood too. Contact forces in granular media
70 propagate through complex waves [20] with appearance of macroscopic phenomenon such as
71 clogging and arching, where particles spontaneously organise as vaults [21]. Solid, liquid and even
72 gaseous phases may be observed in granular media depending on the external forces applied upon
73 them [22]. Indeed, powerful techniques and hardware are available to examine theories in conditions
74 that are nearly identical to experiments. 3D templates of the pore geometry together with description
75 of the root and anatomical details can be obtained [23,24], and there are efficient computational

76 techniques that exploit the power of Graphical Processing Unit to simulate roots and soil at the particle
77 and cell resolution. Discrete Element Modelling (DEM) for example uses Newton's second law to
78 describe the motion of millions of interacting particles [25,26]. The models reproduce closely
79 experimental observations, even in the case of biologically complex systems with detailed
80 quantification of the force distribution surrounding growing roots [27,28].

81 Despite recent experimental and theoretical breakthroughs, granular matter physics has not
82 transformed our understanding of the mechanics of root growth. Many current limitations are due to
83 our lack of understanding of how roots respond to complex mechanical signals, and particularly how
84 competition between multiple mechanical stimuli affects root responses. Cellular mechanisms
85 involved in the response to physical obstacles have not been fully characterised, but a growing number
86 of studies are now revealing the signalling and regulatory mechanisms involved in plant responses to
87 mechanical force. Research in animal sciences have identified a multitude of proteins which binding
88 domains are modified by mechanical forces [29] and their discovery in plants may follow. Large
89 families of mechanosensitive ion channels have been identify in plants [30], with for example MCA
90 calcium mechanosensitive channels being linked to growth response to hard gel layers [31].
91 Adaptation to mechanical forces are also well characterised, including the changes in cell division
92 patterns, growth direction, cell differentiation and gene expression [32]

93 A main difficulty, however, is to understand the nature of the mechanical signals perceived from the
94 soil particles surrounding plant roots. It is central to develop capabilities to study not only the forces
95 and displacement produced in the root soil system, but also the biological responses due to
96 mechanical interactions with soil particles. Unfortunately, experimenting with natural soils is
97 challenging because of its opacity. Rhizotron systems have been an extremely powerful tool to study
98 root growth [33,34], glass interfaces introduce strong border effects and observations of
99 biomechanical processes are often biased. X-ray imaging allows visualisation of interactions between
100 roots and soil particles *in situ* in high resolution [35]. The technique allows time-lapse imaging for
101 several weeks of growth. Improved images can be obtained with the application of contrasting agents.

102 For example, iodine perfused into plant leaves revealed the vascular structures of the roots and
103 rhizobial nodules [36]. Root hairs can be resolved using synchrotron sources with resolution of up to
104 5 μ m and at temporal resolution sufficient for tracking particle movement due to root growth [37].
105 However, X-ray is an ionising radiation that affects biological processes especially meristematic
106 regions where high cell division rates occurs [38], and despite the increase in resolutions, details of
107 the inner cellular processes and biochemical activity have remained invisible [39].

108 Optics and microscopy in the visible range have thus remained the preferred approach to make
109 observation of the biology and mechanics of the root. Confocal Laser Scanning Microscopes (CLSM)
110 have provided the first live images of root-particle interaction in high resolution with details available
111 on contact with particle surface, anatomical features at cell resolution and gene expression [40-42].
112 FRET imaging now allows tension sensors to record molecular forces at the piconewton scale [43].
113 However, CLSM has proved limited for long observations due to photo toxicity and photo bleaching.
114 Because of the confined environment of the microscope, it has also remained limited to small plant
115 samples. The field is now turning to different types of microscopes. Light Sheet Microscopy (LSM), in
116 particular, has drastically reduced the light doses to the samples [44]. Illumination of the sample is
117 planar and achieved orthogonal to the detection so that 2D images are generated instantaneously
118 often using the new generation of scientific-CMOS cameras. By taking a whole 2D section in one
119 “shot”, volume scanning is accelerated, enabling small and fast developmental events to be tracked
120 during development. The technique has considerably advanced our ability to observe living organisms
121 both live and *in situ* with, for example, the ability to track cell growth, movement and divisions of
122 entire embryos [45] or capturing the beating of a living heart [46]. Because axial resolution in light
123 sheet systems is not dependent upon high numerical aperture imaging objectives, they allow larger
124 fields of view and can easily accommodate microcosms and instruments for maintaining healthy
125 growth conditions [47]. Details of the morphology and anatomy of tissues can be obtained without
126 the use of markers [48,49] and recently dynamic light scattering (biospeckle) has been used to

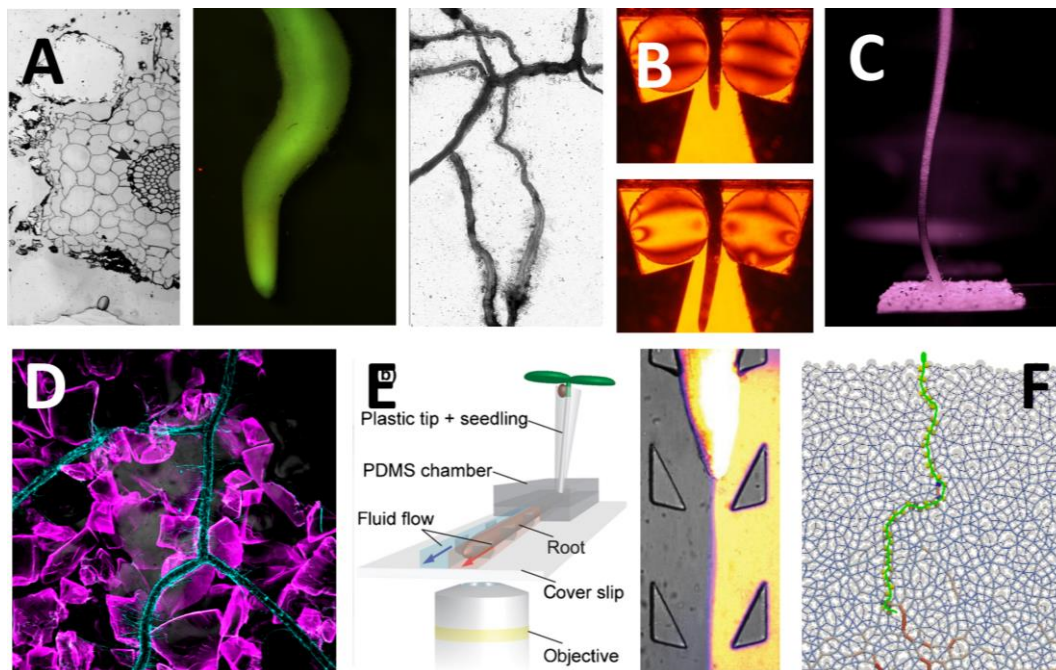
127 enhance image contrast [50]. Light sheet imaging has also been used in granular matter physics for a
128 long time, although its application to root and soil is just emerging [50-52].

129 Optics and microscopy also provides many ways to control and measure mechanical forces. Laser
130 ablation for example, has long been used to understand the distribution of forces within a tissue [53],
131 whilst optical trapping has been used to apply small localised forces [54]. Photoelastic materials have
132 been central to establishing the nature of the chains of forces and how they propagate within a
133 granular medium [55]. Kolb, et al. [56] used photoelasticity to characterise the forces created by root
134 growth within a pore, and Wendell, et al. [57] have successfully created a granular medium using a
135 photo elastic media where maximum growth forces and avoidance mechanisms could be observed
136 (Figure 1B). New cantilever-based optical sensors [58,59] have also been developed to measure
137 simultaneously growth forces generated by a root and three-dimensional strain rate in responses to
138 changes in external forces applied to the root. Bizet, et al. [59] for example, obtained stereoscopic
139 data to decompose root response to axial mechanical forces into different phases (Figure 1C).
140 Hydrogels can also be combined with fluorescent dyes and light sheet imaging to reconstruct
141 interparticle forces within the granular medium [60].

142 Techniques for mimicking soil physical conditions under a microscope are also emerging rapidly.
143 Transparent artificial media based on fluoropolymers that can mimic soil properties have been
144 developed [42]. The media reproduces the physical and chemical properties of soil through control of
145 the distribution of sizes and surface chemistry of the particles (Figure 1D). Because the particles are
146 made of fluoropolymers that have refractive index close to water, only small adjustment of refractive
147 indices, usually by adding a colloid to the nutrient solution, allows light to travel without refraction
148 through the substrate. Microfluidics techniques have also progressed significantly and are becoming
149 suitable to live and high resolution microscopy of roots and microbes [61,62]. Microfluidics allows
150 precise and repeatable control of liquids and this could be used, for example, to control water tension
151 and particle cohesion in soil during live experiments. The range of materials and fabrication techniques
152 has been considerably expanded with the use of 3D printing [63], photo lithography [64], etching

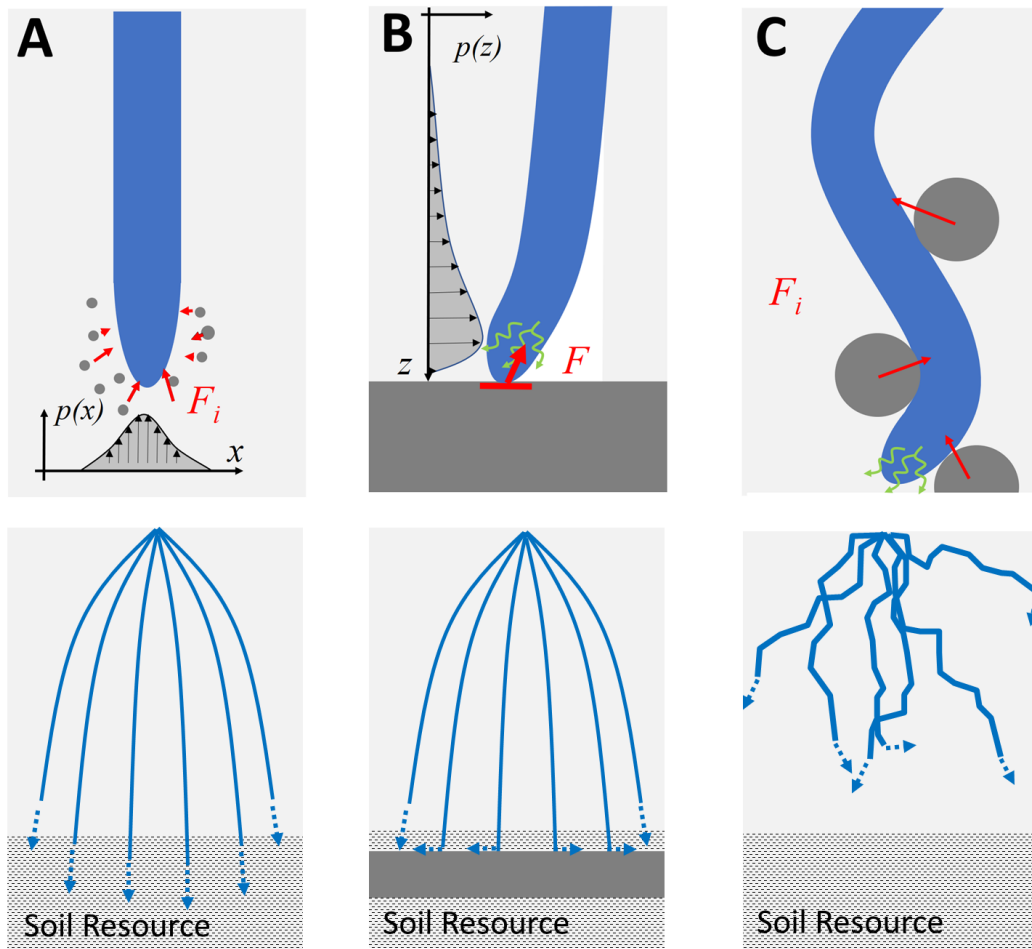
153 technics [65] and the use of optically controlled fluidics [66,67]. It has been possible, for example, to
154 produce chambers with physical heterogeneity, physical barriers and chemical gradients, with direct
155 applications to root and soil studies [62,64,68] .

156 The scientific community is better equipped than ever to make observations on the micromechanics
157 of root development in soil. Experimental systems provide soil-like growth conditions and allow for
158 observations, measurements and data generation with precision, accuracy and resolution. How then
159 to transform the amount of information available to us into scientific breakthrough? The complexity
160 of the root-particle interactions is a major challenge. At each growth step, a root is in contact with a
161 new arrangement of particles that apply forces of varying magnitudes and orientations. Because there
162 are countless numbers of possible arrangements, the forces applied on roots cannot be
163 experimentally controlled. Measurements of granular forces *in situ* is required (Figure 2.1), and
164 granular media physicist have achieved such measurements. There are now great opportunities to
165 combine current knowledge of soil micromechanics with mechanobiology and propose a mechanistic
166 framework that account for sensing and response to micro-scale heterogeneity (Figure 2.2). New
167 theories must be developed to embrace stochasticity and explain responses to multiple mechanical
168 stimuli (Figure 2.3-4). Major challenges remain, but a recent look at the literature indicates our
169 thinking is evolving in the right way.



171

172 Figure 1: Growing roots interact mechanically with soil particles during growth. These interactions
 173 influence the morphology of the root, and the dynamics of development of the root system. A)
 174 Irregular growth of cortex cells is observed in hard or compacted soil [left, 69]. Resistance from the
 175 soil particles causes root diameter to increase and the root tip to buckle and bend towards the path
 176 of least resistance (middle, lentils roots grown at 2MPa confining pressure). At the scale of the root
 177 system, interactions causes growth trajectories to be stochastic as observed here on *Anthyllis*
 178 *vulneraria* grown on landslide soils (image courtesy Loïc Pagès). Technological developments now
 179 allow precise characterisation of mechanical interactions between a root and the growth substrate.
 180 These include for example, B) photoelastic discs for measurement of growth forces in soil pores [56]
 181 (images courtesy Evelyne Kolb), C) root growing on a cantilever sensor for measuring growth forces
 182 [59], D) transparent soil substrates that provide the physical structure of soil with the ability to carry
 183 out 3D live imaging [50], E) Dual flow microfluidic systems with microscale both physical and nutrient
 184 heterogeneity [68] and F) discrete element modelling for testing root responses to interactions with
 185 granular media [28] (image courtesy Mahmoud Fakh).



187

188

189 *Box 1: Root primary growth is a local process where elongation of tissues is taking place at the root*
 190 *tip. Soil heterogeneity influences strongly how the tissue elongates and deforms (top), and local*
 191 *interactions taking place at the tip can have drastic effects on the morphology and development of*
 192 *the whole root system, and the resources available to the plant (bottom). Mathematical modelling*
 193 *provides a useful framework to explain how heterogeneity can affect the morphology of the root*
 194 *system.*

195 *(A) When roots grow in soil particles which representative volume is small compared to the diameter*
 196 *of the roots, the action of the particles can be averaged (top). In such conditions, it is unlikely for a*
 197 *plant to perceive the fluctuations of forces from individual particles. If the mechanical resistance of*

198 *the soil is not limiting, root trajectories follow smooth streamlines (bottom). Mathematically, this*
 199 *phenomenon has been described as the convection of root tips (density ρ) [70]. The growth velocity*
 200 *E ($\text{cm}\cdot\text{d}^{-1}$) and the rate of change in root angle due to gravitropism g (d^{-1}) define the growth of the root*
 201 *system:*

$$202 \quad \partial_t \rho + \nabla \cdot \mathbf{F} + \partial_\theta g \rho = 0,$$

203 *with $\mathbf{F} = \rho E (\cos(\theta) + \sin(\theta))$ is the spatial flux of root tips and $g \rho$ is the angular flux of roots. In this*
 204 *case, growth and resource acquisition is optimal.*

205 *(B) When soil elements cannot be displaced, in the case of stones and rock for example, the root*
 206 *adopts avoidance behaviours. Optimal growth is not affected and remains similar to (A), until the*
 207 *obstacle is reached. Heterogeneities in this case define the boundaries within which convective*
 208 *growth is taking place. Using the same mathematical framework, presence of such boundaries can be*
 209 *modelled through boundary conditions, for example*

$$210 \quad \partial_n \rho = 0.$$

211 *Large scale soil heterogeneities can be problematic because they may restrain access to pools of*
 212 *resources, e.g. deep water, even though the root growth in most parts of the soil domain is unaffected.*
 213 *They may also forms paths of least growth resistance, for example in the case of pores and cracks.*

214 *(C) Intermediate cases are more problematic to analyse. Roots are in contact with particles which*
 215 *apply forces of varying magnitudes and orientation. Although the root may overcome these forces, a*
 216 *single particle may be able to deflect the growth trajectory. Since particles have inhomogeneous*
 217 *distribution, root deflection occurs is stochastic. Mathematically, the phenomenon can be described*
 218 *by a convection, where the growth velocity $e < E$ ($\text{cm}\cdot\text{d}^{-1}$) and the rate of change in root angle due to*
 219 *gravitropism g (d^{-1}) and random fluctuations define the dynamics:*

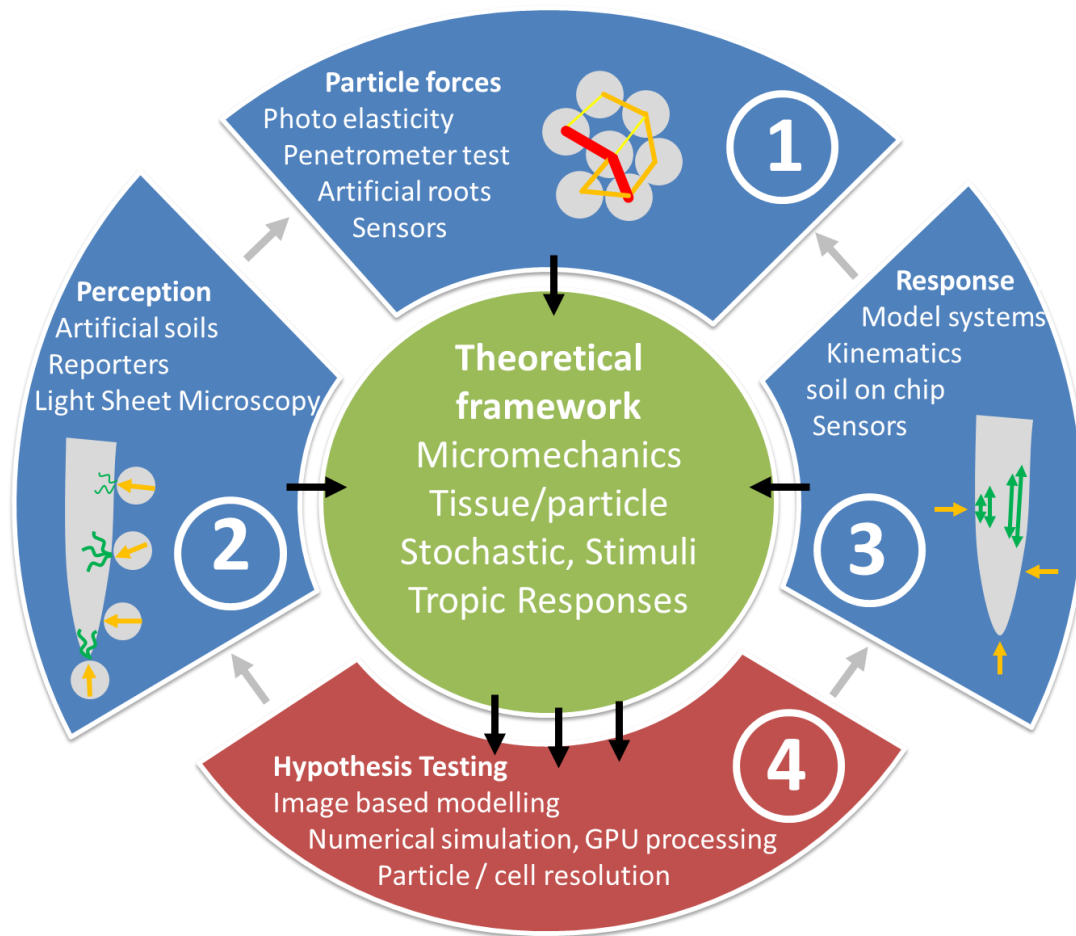
$$220 \quad \partial_t \rho + \nabla \cdot \mathbf{F} + \partial_\theta (g \rho + D \partial_\theta \rho) = 0,$$

221 $g\rho + D\partial_{\theta}\rho$ the angular flux of roots. The parameter D is the angular diffusion coefficient. Because D
222 relates to the probability of roots to be deflected by a particle, and the magnitude of such deflection,
223 there is a direct link between micro-mechanics of root particle interactions and the morphology of the
224 root system. Diffusive growth makes root trajectories irregular, and limits the expansion of the root
225 system, even when the elongation rate is not affected. Mathematical analysis of equation 3 reveals
226 the conditions for which transitions from convective growth to diffusive growth occur, i.e. for Peclet
227 number $P_e = \frac{g}{D} \ll 1$.

228

229

230



231

232 Figure 2: Dissecting the complexity of root particles mechanical interactions requires an elaborate
 233 research strategy. (1) First step is to better understand the nature of the forces applied to a root. This
 234 can be achieved, using photo elastic beads, imaging, or developing artificial roots equipped with
 235 sensors [71], but also by revisiting older techniques, for example by analysing micro penetrometer
 236 test and exploit force fluctuations [72]. (2) In the second step, it is essential to characterise how these
 237 forces (orange arrows) are perceived by plant roots. This could be achieved using e.g. modern LSM
 238 microscopes, artificial soils, calcium or FRET tension sensors to inform on the perception of forces
 239 induced by heterogeneous media [73]. (3) Finally, the mechanism of response to complex distribution
 240 of forces must be characterised. In this case, responses can be studied on simplified systems where
 241 position and magnitude of forces can be controlled accurately, using lab-on-chip device and more
 242 traditional developmental biology approaches. Experiments and data can then be used to formulate
 243 and test new concepts and biomechanical theories (black arrows). Computational models can test

244 biomechanical theories in most realistic conditions using latest technologies, e.g. particle based
245 simulations and computer hardware (4) and influence the design of new experiments (grey arrows).
246

247 **Acknowledgements**

248 This work was supported by the consolidator fellowship from the European Research Council ERC
249 SENSOILS-647857. We also thank the colleagues from the RoSoM project for inspiring discussions on
250 root biomechanics and discrete element modelling (Agropolis Fondation ID 1202-073; LabexAgro:
251 ANR-10-LABX- 001-01).

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