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Micromechanics of root development in soil

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Abstract

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

Main text

Current knowledge of the biomechanics of plant root growth in soil is largely based on the extensive work of plant biophysicists from the second half of the 20th century [1-3]. The view was that both
roots and soil must be considered as continua so that the description of root soil interactions can be achieved with continuous mathematical functions of macroscopic variables such as Young’s modulus of root tissue, soil penetration stress, and pore water pressure [4]. Classical concepts from mechanics and physiology then provide a suitable framework to understand factors controlling tissue growth in its natural environment. The energy required to deform the root and surrounding soil, which originates from the photosynthetic chemical energy accumulated within the tissues, is converted into turgor pressure and mechanical energy [5]. Turgor pressure then overcome the resistance from cell wall to stretching, the resistance to movement of water across membranes, and the resistance to the displacement of the soil around the root [6].

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

To establish a biomechanical framework that accounts for the complexity of root interactions with the granular medium, one must capture the microscopic nature of particle forces and the collective action they have on root tissues (Figure 1A). Kolb, et al. [11] proposed to categorise the nature of root mechanical responses to soil based on the scale of the soil heterogeneities. When the medium is
composed of small particles, individual variations in the force required to move them are not perceived by the root. The behaviour of roots and soil can be homogenised, and classical continuum mechanics usually applies (Box 1A) [12]. Soils also contain objects that are too large and or too rigid for a root to deform and displace, for example when roots grow in contact with stones, in cracks or pores [13,14]. Growth forces cannot displace the obstacle and the root usually combines tropic responses and mechanical buckling to avoid the obstacle (Box 1B) [15]. The behaviour of roots growing in soils with particles of intermediate sizes is more challenging to understand. A root can displace individual particles from the soil, but the forces exerted by each of the particles can also influence the course of root development (Box 1C). Although such growth environments are common for fine roots or due to the presence of aggregate and sand particles, growth patterns in such conditions are not well understood. How frequently does a root deflect from their growth trajectory? What are the magnitude of deflections? How does the distribution of particle forces modify the growth trajectory?

Understanding the forces acting on a root during the elongation requires detailed knowledge of the physics of granular media. Granular media are assemblages of particles held by frictional and repulsive forces from adjacent particles. The forces holding particles together form chain-like networks that propagate at the contact points between neighbouring particles [16]. Because particles are disordered or have various sizes and shapes, large variations in magnitude and direction of particle forces arise [16,17]. Early theoretical work based on dry and static monodisperse particles showed that distribution of contact forces vary greatly and the overall force distribution follows an exponential decline [18,19]. Particles dynamics is better understood too. Contact forces in granular media propagate through complex waves [20] with appearance of macroscopic phenomenon such as clogging and arching, where particles spontaneously organise as vaults [21]. Solid, liquid and even gaseous phases may be observed in granular media depending on the external forces applied upon them [22]. Indeed, powerful techniques and hardware are available to examine theories in conditions that are nearly identical to experiments. 3D templates of the pore geometry together with description of the root and anatomical details can be obtained [23,24], and there are efficient computational
techniques that exploit the power of Graphical Processing Unit to simulate roots and soil at the particle
and cell resolution. Discrete Element Modelling (DEM) for example uses Newton’s second law to
describe the motion of millions of interacting particles [25,26]. The models reproduce closely
experimental observations, even in the case of biologically complex systems with detailed
quantification of the force distribution surrounding growing roots [27,28].

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

Adaptation to mechanical forces are also well characterised, including the changes in cell division
patterns, growth direction, cell differentiation and gene expression [32]

A main difficulty, however, is to understand the nature of the mechanical signals perceived from the
soil particles surrounding plant roots. It is central to develop capabilities to study not only the forces
and displacement produced in the root soil system, but also the biological responses due to
mechanical interactions with soil particles. Unfortunately, experimenting with natural soils is
challenging because of its opacity. Rhizotron systems have been an extremely powerful tool to study
root growth [33,34], glass interfaces introduce strong border effects and observations of
biomechanical processes are often biased. X-ray imaging allows visualisation of interactions between
roots and soil particles in situ in high resolution [35]. The technique allows time-lapse imaging for
several weeks of growth. Improved images can be obtained with the application of contrasting agents.
For example, iodine perfused into plant leaves revealed the vascular structures of the roots and rhizobial nodules [36]. Root hairs can be resolved using synchrotron sources with resolution of up to 5µm and at temporal resolution sufficient for tracking particle movement due to root growth [37]. However, X-ray is an ionising radiation that affects biological processes especially meristematic regions where high cell division rates occurs [38], and despite the increase in resolutions, details of the inner cellular processes and biochemical activity have remained invisible [39].

Optics and microscopy in the visible range have thus remained the preferred approach to make observation of the biology and mechanics of the root. Confocal Laser Scanning Microscopes (CLSM) have provided the first live images of root-particle interaction in high resolution with details available on contact with particle surface, anatomical features at cell resolution and gene expression [40-42]. FRET imaging now allows tension sensors to record molecular forces at the piconewton scale [43]. However, CLSM has proved limited for long observations due to photo toxicity and photo bleaching. Because of the confined environment of the microscope, it has also remained limited to small plant samples. The field is now turning to different types of microscopes. Light Sheet Microscopy (LSM), in particular, has drastically reduced the light doses to the samples [44]. Illumination of the sample is planar and achieved orthogonal to the detection so that 2D images are generated instantaneously often using the new generation of scientific-CMOS cameras. By taking a whole 2D section in one “shot”, volume scanning is accelerated, enabling small and fast developmental events to be tracked during development. The technique has considerably advanced our ability to observe living organisms both live and in situ with, for example, the ability to track cell growth, movement and divisions of entire embryos [45] or capturing the beating of a living heart [46]. Because axial resolution in light sheet systems is not dependent upon high numerical aperture imaging objectives, they allow larger fields of view and can easily accommodate microcosms and instruments for maintaining healthy growth conditions [47]. Details of the morphology and anatomy of tissues can be obtained without the use of markers [48,49] and recently dynamic light scattering (biospeckle) has been used to
enhance image contrast [50]. Light sheet imaging has also been used in granular matter physics for a long time, although its application to root and soil is just emerging [50-52].

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

Hydrogels can also be combined with fluorescent dyes and light sheet imaging to reconstruct interparticle forces within the granular medium [60].

Techniques for mimicking soil physical conditions under a microscope are also emerging rapidly. Transparent artificial media based on fluoropolymers that can mimic soil properties have been developed [42]. The media reproduces the physical and chemical properties of soil through control of the distribution of sizes and surface chemistry of the particles (Figure 1D). Because the particles are made of fluoropolymers that have refractive index close to water, only small adjustment of refractive indices, usually by adding a colloid to the nutrient solution, allows light to travel without refraction through the substrate. Microfluidics techniques have also progressed significantly and are becoming suitable to live and high resolution microscopy of roots and microbes [61,62]. Microfluidics allows precise and repeatable control of liquids and this could be used, for example, to control water tension and particle cohesion in soil during live experiments. The range of materials and fabrication techniques has been considerably expanded with the use of 3D printing [63], photo lithography [64], etching
technics [65] and the use of optically controlled fluidics [66,67]. It has been possible, for example, to produce chambers with physical heterogeneity, physical barriers and chemical gradients, with direct applications to root and soil studies [62,64,68].

The scientific community is better equipped than ever to make observations on the micromechanics of root development in soil. Experimental systems provide soil-like growth conditions and allow for observations, measurements and data generation with precision, accuracy and resolution. How then to transform the amount of information available to us into scientific breakthrough? The complexity of the root-particle interactions is a major challenge. At each growth step, a root is in contact with a new arrangement of particles that apply forces of varying magnitudes and orientations. Because there are countless numbers of possible arrangements, the forces applied on roots cannot be experimentally controlled. Measurements of granular forces in situ is required (Figure 2.1), and granular media physicist have achieved such measurements. There are now great opportunities to combine current knowledge of soil micromechanics with mechanobiology and propose a mechanistic framework that account for sensing and response to micro-scale heterogeneity (Figure 2.2). New theories must be developed to embrace stochasticity and explain responses to multiple mechanical stimuli (Figure 2.3-4). Major challenges remain, but a recent look at the literature indicates our thinking is evolving in the right way.
Figure 1: Growing roots interact mechanically with soil particles during growth. These interactions influence the morphology of the root, and the dynamics of development of the root system. A) Irregular growth of cortex cells is observed in hard or compacted soil [left, 69]. Resistance from the soil particles causes root diameter to increase and the root tip to buckle and bend towards the path of least resistance (middle, lentils roots grown at 2MPa confining pressure). At the scale of the root system, interactions causes growth trajectories to be stochastic as observed here on _Anthyllis vulneraria_ grown on landslide soils (image courtesy Loïc Pagès). Technological developments now allow precise characterisation of mechanical interactions between a root and the growth substrate. These include for example, B) photoelastic discs for measurement of growth forces in soil pores [56] (images courtesy Evelyne Kolb), C) root growing on a cantilever sensor for measuring growth forces [59], D) transparent soil substrates that provide the physical structure of soil with the ability to carry out 3D live imaging [50], E) Dual flow microfluidic systems with microscale both physical and nutrient heterogeneity [68] and F) discrete element modelling for testing root responses to interactions with granular media [28] (image courtesy Mahmoud Fakih).
Box 1: Root primary growth is a local process where elongation of tissues is taking place at the root tip. Soil heterogeneity influences strongly how the tissue elongates and deforms (top), and local interactions taking place at the tip can have drastic effects on the morphology and development of the whole root system, and the resources available to the plant (bottom). Mathematical modelling provides a useful framework to explain how heterogeneity can affect the morphology of the root system.

(A) When roots grow in soil particles which representative volume is small compared to the diameter of the roots, the action of the particles can be averaged (top). In such conditions, it is unlikely for a plant to perceive the fluctuations of forces from individual particles. If the mechanical resistance of
the soil is not limiting, root trajectories follow smooth streamlines (bottom). Mathematically, this
phenomenon has been described as the convection of root tips (density \( \rho \)) [70]. The growth velocity
\( E \) (cm.d\(^{-1}\)) and the rate of change in root angle due to gravitropism \( g \) (d\(^{-1}\)) define the growth of the root
system:

\[
\partial_t \rho + \nabla \cdot \mathbf{F} + \partial_\theta g \rho = 0,
\]

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
case, growth and resource acquisition is optimal.

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

\[
\partial_n \rho = 0.
\]

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

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

\[
\partial_t \rho + \nabla \cdot \mathbf{F} + \partial_\theta (g \rho + D \partial_\theta \rho) = 0,
\]
\[ g \rho + D \partial_\theta \rho \] the angular flux of roots. The parameter \( D \) is the angular diffusion coefficient. Because \( D \) relates to the probability of roots to be deflected by a particle, and the magnitude of such deflection, there is a direct link between micro-mechanics of root particle interactions and the morphology of the root system. Diffusive growth makes root trajectories irregular, and limits the expansion of the root system, even when the elongation rate is not affected. Mathematical analysis of equation 3 reveals the conditions for which transitions from convective growth to diffusive growth occur, i.e. for Peclet number \( P_e = \frac{g}{D} \ll 1 \).
Figure 2: Dissecting the complexity of root particles mechanical interactions requires an elaborate research strategy. (1) First step is to better understand the nature of the forces applied to a root. This can be achieved, using photo elastic beads, imaging, or developing artificial roots equipped with sensors [71], but also by revisiting older techniques, for example by analysing micro penetrometer test and exploit force fluctuations [72]. (2) In the second step, it is essential to characterise how these forces (orange arrows) are perceived by plant roots. This could be achieved using e.g. modern LSM microscopes, artificial soils, calcium or FRET tension sensors to inform on the perception of forces induced by heterogeneous media [73]. (3) Finally, the mechanism of response to complex distribution of forces must be characterised. In this case, responses can be studied on simplified systems where position and magnitude of forces can be controlled accurately, using lab-on-chip device and more traditional developmental biology approaches. Experiments and data can then be used to formulate and test new concepts and biomechanical theories (black arrows). Computational models can test
biomechanical theories in most realistic conditions using latest technologies, e.g. particle based simulations and computer hardware (4) and influence the design of new experiments (grey arrows).
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