The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots

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Highlight: The holistic rhizosphere framework unifies rhizosphere terminology and integrates the diverse processes in the rhizosphere. This review demonstrates how interdisciplinary methodologies and collaborations will increase understanding of the holistic rhizosphere.

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Abstract

Despite often being conceptualized as a thin layer of soil around roots, the rhizosphere is actually a dynamic system of interacting processes. Hiltner originally defined the rhizosphere as the soil influenced by plant roots. However, soil physicists, chemists, microbiologists, and plant physiologists have studied the rhizosphere independently, and therefore conceptualized the rhizosphere in different ways and using contrasting terminology. Rather than research-specific conceptions of the rhizosphere, the authors propose a holistic rhizosphere encapsulating the following components: microbial community gradients, macroorganisms, mucigel, volumes of soil structure modification, and depletion or accumulation zones of nutrients, water, root exudates, volatiles, and gases. These rhizosphere components are the result of dynamic processes and understanding the integration of these processes will be necessary for future contributions to rhizosphere science based upon interdisciplinary collaborations. In this review, current knowledge of the rhizosphere is synthesized using this holistic perspective with a focus on integrating traditionally separated rhizosphere studies. The temporal dynamics of rhizosphere activities will also be considered, from annual fine root turnover to diurnal fluctuations of water and nutrient uptake. The latest empirical and computational methods are discussed in the context of rhizosphere integration. Clarification of rhizosphere semantics, a holistic model of the rhizosphere, examples of integration of rhizosphere studies across disciplines, and review of the latest rhizosphere methods will empower rhizosphere scientists from different disciplines to engage in the interdisciplinary collaborations needed to break new ground in truly understanding the rhizosphere and to apply this knowledge for practical guidance.

Introduction

Holistic - *Characterized by comprehension of the parts of something as intimately interconnected and explicable only by reference to the whole* (Oxford English Dictionary, 2015).

The rhizosphere is a complex space

The rhizosphere is often conceptualized as a small volume of soil clinging to short root segments, but the rhizosphere extends past the physical association of root and soil particles to a more complex volume of overlapping and functionally integrated zones. Within the rhizosphere, roots forage for soil-based resources, nutrients flux between organic and inorganic pools, mediated by the soil microbial community, and animals graze across trophic levels. The rhizosphere has major implications for climate and environment change with regards to greenhouse gas emissions and carbon sequestration, soil fertility management, and food security. The most succinct and clear definition of 'rhizosphere' is arguably the original definition of Hiltner (1904): *soil influenced by roots*. Since that time, many developments have augmented the understanding of roots and the soil in which they live, and along the way different researchers in distinct disciplines have coined new words and changed definitions to suit their needs. Reviewing the broad literature on the rhizosphere, highlighting knowledge gaps, and identifying future research are necessary to advance our understanding of the interactions between roots and soil. Central to this consideration will be the adoption of systematic definitions and conceptual models that will allow greater synthesis of rhizosphere concepts and facilitate interdisciplinary collaboration.

A brief history of the rhizosphere

The study of plant nutrition and its relation to soil fertility is ancient. Cado the Elder promoted manuring grain land around 160 B.C. in *De Agri Cultura* and Varro documented the use of green manures around 27 B.C. (Cato and Varro, 1913). Petrus de Crescentiis compiled Roman literature on agriculture into the *Ruralia Commoda* in 1309, which included the use of manure to increase soil fertility (Nortcliff and Gregory, 2013), so that farmers and philosophers from the European Middle Ages understood that plant roots gained nutrition from soil is implicit. Many simply assumed plant roots ate soil particles directly (Moore and Clark, 1995), until an elegant experiment demonstrated no change in soil mass even as a tree grew large after 5 years (van Helmont, 1662; but see Hershey, 2003 for why van Helmont may not have been the first). However, van Helmont erroneously interpreted these results to mean only water was necessary for plant growth because researchers had not yet discovered photosynthesis as the means by which plants accumulate mass. Woodward (1699) demonstrated pure water was not sufficient for plant growth, rather the water must contain 'impurities' arising from Earth. Early research on the relation of soil fertility with agricultural productivity led to many of the fundamental ideas of plant science (Thomas, 1930). However, Hiltner (1904) first proposed the idea that plants are not only influenced by soil, but are active participants through roots creating the rhizosphere, and since that time development of rhizosphere theory has been constant.

The holistic rhizosphere

Problems with 'rhizosemantics'

Since Hiltner coined the term ‘rhizosphere’, the use of the Latin prefix *rhizo* became popular, and at times the creation of new words appeared to take precedence over advancing clear concepts. Subsequent use of these terms led to accumulated ambiguity, usually in relation to the experimental practices employed to sample various spatially-defined regions. The *rhizoplane* was introduced by Clark (1949) and defined as, “external surfaces of plant roots together with any closely adhering particles of soil or debris.” However, subsequent research has ambiguously used this term, often driven by the limitations of experimental approaches. At times, when roots are excavated from soil or other media, only the soil adhering to the roots is considered the *rhizosphere*, and the washed root epidermis free of soil particles is deemed the *rhizoplane* (Cook and Lochhead, 1959; Wieland *et al.*, 2001; Bulgarelli *et al.*, 2012). However, this usage contradicts the original definition of rhizoplane, decreases the spatial extent of the rhizosphere greatly (in the sense that volumes of soil which would have been under the influence of roots when *in situ* would not be included in such samples), and redundantly refers to the root epidermis as the rhizoplane, so should be eliminated in favour of Clark’s original definition.

The term *endorhizosphere* refers to the root cortex when colonized by bacteria (Balandreau and Knowles, 1978). However, the term is misleading because the rhizosphere is defined as external to the root, such that no aspect of the rhizosphere may be within the root, with several other substantial issues discussed by Kloepper *et al.* (1992). Anatomical terms already exist to describe internal root anatomy, and so the authors agree with Kloepper *et al.* (1992) that the term *endorhizosphere* should be eliminated from usage, along with the associated *ectorhizosphere*, which simply refers to the rhizosphere. However, the idea that there is a continuum of soil solution with chemical and microorganismal contents between the rhizosphere and the root cortex remains an important concept. The unique environment of the internal colonized root has also been referred to as the root endosphere (Compant *et al.*, 2010), and we suggest this term is more appropriate when needed.

Sheaths composed of adhering soil particles surrounding the roots of desert grasses were described in the 19th century (Volkens, 1887), and were deemed *rhizosheaths* by Wullstein *et al.* (1979). Rhizosheaths are generally described in wild grasses and cereal crops, especially in dry conditions (Price, 1911; Wullstein *et al.*, 1979; Watt *et al.*, 1994; Young, 1995). However, nothing about its definition limits the rhizosheath to plants of the family Poaceae. Though rhizosheaths are associated with drying soils, that their formation occurs in wet soils is not disputed, however rhizosheaths may be further induced while soil dries (Watt *et al.*, 1994). Mucigel surrounds roots (Jenny and Grossenbacher, 1963) and is composed of mucilaginous compounds derived from the focal plant and associated microorganisms. Mucigel, along with root hairs and fungal hyphae (Moreno-Espíndola *et al.*, 2007), is responsible for the agglutination of soil particles observed in rhizosheaths. Observations that roots from wet soil have smaller rhizosheaths may be partially explained by the decreased integrity of hydrated mucilage such that the rhizosheath is more likely to be lost when loosening roots from soil (also discussed by Ghezzehei and Albalasmeh, 2015). Therefore, the authors propose that the use of *rhizosheath* more broadly as agglutinated soil particles surrounding roots from any plant species is appropriate and consistent with the original usage, for example, as measured by Sprent (1975) in drying soils with soybean (*Glycine max*) and by Moreno-Espíndola *et al.* (2007) in sunflower (*Helianthus*

annuus). Referring to rhizosheaths as either hydrated (wet) or desiccated (dry) allows discussion of the particular conditions (Read *et al.*, 1999).

Experimentally, the rhizosphere has been sampled in various ways that have led to different functional definitions being used in soil science, microbial ecology, and plant biology. The authors have outlined the problems with 'rhizosemantics' above and encourage researchers to be more consistent with their terminology by referring to the root surface as the root epidermis, when appropriate, the adhering soil and binding materials, such as mucigel, as the rhizosheath, and the combination of the epidermis and rhizosheath as the rhizoplane (Fig 1.), which is one component of the holistic rhizosphere in agreement with Puente *et al.* (2004). This synthesis of the terms allows a new exploration of a holistic rhizosphere composed of overlapping and integrated zones. The rhizosphere is holistic because the structure and function of rhizosphere components can only be understood by reference to the entire rhizosphere construct and the relations between components.

Components of the holistic rhizosphere

The rhizosphere can be conceived as the culmination of a myriad of influences that roots exert on the surrounding soil. Most research has only considered one of these influences at a time, and generally defined the rhizosphere in the context of that influence. However, understanding the multiple components as parts of a holistic rhizosphere is more useful conceptually, especially for understanding the components as the results of interacting processes. The authors will restrict the definition of rhizosphere to the soil 'currently' being influenced by roots, because over extended timescales arguably most vegetated soil has been influenced by roots. Such an inclusive definition ceases to be useful. An overview of the zones in the holistic rhizosphere is given in Table 1 and Fig. 2, where the authors propose a new, clearer taxonomy of rhizosphere components based on the existing literature.

Abiotic rhizosphere zones

The abiotic rhizosphere zones are those in which roots influence the non-living aspects of soil. Depletion zones surrounding roots form due to the uptake of soil resources, primarily mineral nutrients and water. Accumulation zones occur from root exudation and from movement of molecules to the root surface that are not taken up by the root. Roots also influence soil structure through compression and by influencing the process of soil aggregation. These zones influence the biology and chemistry of the rhizosphere greatly.

Water travels by mass flow while the plant is transpiring. The water flow is driven by a gradient in water potential between the roots and the soil. Soil has little influence on root water uptake when wet, because soil hydraulic conductivity is much greater than that of the roots. However, as the soil dries, its conductivity decreases several orders of magnitude and, ultimately, limits root water uptake (Passioura, 1980; Draye *et al.*, 2010). The pioneering work of Gardner (1960) showed that significant gradients in volumetric soil water content ($m^3\ m^{-3}$) (i.e. depletion zones) and soil water potential (MPa) can form around the roots at very negative water potentials (0.1-0.2 MPa). Below these negative water potentials, the profile of soil water potential and soil water content are expected to decrease towards the roots, with the slope of the profiles becoming steeper closer to the root surface. The gradients of the soil water content and

soil water potential are affected by soil properties and water fluxes. In near-saturated soils, water is extracted from larger pore spaces first and flux is dominated by capillary forces, but as water content decreases, especially at higher matric potentials, water flows along and is held within thin films around soil particles (Or and Tuller, 1999). When soil hydraulic conductivity is not great enough to sustain root water uptake, water depletion zones are expected to form around the roots. The decreasing water contents towards the roots correspond in a non-linear way to gradients in soil water potential driving water to the root surface (Fig. 3). The lesser the soil hydraulic conductivity, the greater the potential gradients needed to sustain root water uptake (Carminati *et al.*, 2011). The extent of the water depletion zone around a root could be enhanced by root hairs, as shown by Segal (2008) who combined magnetic resonance imaging (MRI) and numerical modelling of root water uptake. On the other hand, an increased water holding capacity of the soil near the roots may counteract any water depletion around the roots. A higher water content in the rhizosphere was observed by Young (1995), Carminati (2010), and Moradi (2011), and was interpreted as the effect of mucilage exuded by roots (Fig. 3, 4). Increased soil density (and decreased porosity) around the roots due to soil structure modification would also increase the water content near the root surface at negative water potentials (Aravena *et al.*, 2014). Conversely, the presence of surfactants in the mucilage can decrease the water content near the roots (Read *et al.*, 2003; Dunbabin *et al.*, 2006). Finally, while small scale (a few mm) local water depletion zones around the roots are expected only in dry soils as affected by the specific hydraulic properties of the rhizosphere, larger scale water depletion zones will occur at the scale of the root system (1-10 cm) due to the comparably high water uptake in soil regions with a high density of active roots (Doussan *et al.*, 2006).

Bray (1954) postulated nutrient ‘sorption’ zones around roots that depended on the mobility of the respective nutrient in soil. Further work demonstrated that nutrients travel to the root surface by diffusion and mass flow (Fig. 5; Barber, 1962). The effective diffusion rate of a nutrient will be a function of the chemical gradient, the ionic exchange capacity and saturation level of the soil, nutrient concentration, and the electric charge of the nutrient. Nutrients that interact strongly with soil are said to be diffusion limited, and the depletion zones will have small radii (mm scale). Mass flow is the movement of nutrients to the root surface dissolved in the water that is eventually transpired. Depletion zones with large radii (cm scale) are created when the uptake of a nutrient or chemical exceeds mass flow to the root (Barber, 1962).

If the uptake of chemicals traveling to the root surface does not exceed the supply from mass flow, then those chemicals will increase in concentration surrounding the root and create accumulation zones. Extreme examples have been observed where crystalline calcium (calcrete) forms around roots that is clearly visible when excavated (Barber and Ozanne, 1970). Accumulation zones may also be formed by the exudation of ions, especially protons, by plant roots (reviewed in Hinsinger *et al.*, 2003).

Roots also affect the physical structure of the soil, creating a zone of soil structure modification (SSM). As the growing tip of a roots burrows through soil, particles are displaced that can form a zone of higher density soil around roots. The SSM zone concept was supported by earlier work investigating soil deformations using radially expanding tubes (Dexter and Tanner, 1972), and by subsequent measurements around roots grown in field soil (Bruand *et al.*, 1996). Braunack and Freebairn (1988) found

a reduction in porosity immediately adjacent to the root using radiographic methods which they argued was due to soil compression as the root expanded. Aravena *et al.* (2011, 2014) showed root induced soil compaction can increase root-soil contact which has key implications for hydrological behaviour in this zone that they demonstrated using modelling approaches. Thus, soil porosity is generally believed to decrease at the root-soil interface. However, other research showed a general increase in porosity in the presence of roots even over timescales of a few weeks (Feeney *et al.*, 2006). Most studies have used different species and soil types, so the generality of how roots affect soil structure is not known. Beyond this SSM zone immediately at the root-soil interface, roots and root exudates stabilize soil aggregates at several spatial scales (Tisdall and Oades, 1982).

Biotic rhizosphere zones

The biotic zones of the rhizosphere essentially comprise microbial and faunal communities, and concentration gradients of biochemicals, which are all primarily determined by rhizodeposition. Rhizodeposition (originating with Shamoot *et al.*, 1968) was experimentally deduced by measuring the increased concentration of carbon compounds in soils supporting plant growth after experimental removal of all the roots. Rhizodeposits include sloughed-off border cells and a wide range of organic exudates, such as sugars, organic acids, amino compounds, and polysaccharide and glycoproteinaceous mucilages (Jones *et al.*, 2009). Mucilage exudation may increase due to increased mechanical impedance (Boeuf-Tremblay *et al.*, 1995), which demonstrates a potential direct linkage with mucilage facilitating root penetration of soil via lubrication.

The availability of energy in rhizodeposits as a carbon source is widely believed to drive changes in the microbial community in the rhizosphere (Paterson, 2003; Denef *et al.*, 2009), especially in the rhizoplane (i.e. root epidermis and rhizosheath together). In the rhizoplane, microbial biodiversity and numbers tend to be substantially greater than in bulk soil, though this is not always the case (Fig 6). However, as well as providing a basic supply of energy, plants may exert more subtle and specific controls upon microbial community structure and activity through chemical signalling (Paterson, 2003; Weston and Mathesius, 2013) and allelopathic mechanisms (Bertin *et al.*, 2003; Zhou *et al.*, 2013). Recently, genetic variation was discovered that directly influenced associations with a rhizosphere bacteria, which in turn determined the relative fitness of plant genotypes (Haney *et al.*, 2015). There is typically a successional colonisation of the rhizoplane as a root extends and grows into new soil zones, with bacteria proliferating in the first instance, the inocula being sourced from the immediate contact in the vicinity of the adjacent soil. If sufficient moisture is present, motile bacteria then migrate to the root surface, following carbon-source concentration gradients which arise as a result of exudation. Saprophytic fungal hyphae also follow carbon-source gradients while foraging, and after encountering the root, they extend rapidly along the longitudinal root epidermis. Parasitic fungal hyphae will penetrate susceptible hosts and proliferate intra-radically. A trophic cascade then develops, when secondary and tertiary colonisers such as protozoa and nematodes subsequently arrive and feeding relationships between the various groups develop (Moore *et al.*, 2007), resulting in elevated rates of nutrient cycling (Bonkowski and Clarholm, 2012). These communities remain active while energy inputs prevail, driven first by exudates and sloughed cells, and eventually by senescing tissues. Distinct

successional series within the primary colonising bacteria have recently been demonstrated to be dependent upon the plant type interacting with soil type (Tkacz *et al.*, 2015).

Mycorrhizae are mutualistic associations between plant roots and fungi (Fig. 7), although the fungi themselves are often erroneously referred to as mycorrhizae *per se.* This association is essentially the norm for most families of plants growing in soil with a few exceptions such as the Brassicacae (Smith and Read, 1997). There are four major types of mycorrhizal association that differ anatomically, physiologically, and by host range, namely arbuscular (AM), ecto- (ECM), ericaceous and orchidaceous mycorrhizae. The distribution of fungal biomass with respect to the root varies greatly between these groups, and this variety of structural form further complicates concepts of the natural rhizosphere. However, all fungal forms involve networks of extra-radical hyphae which permeate the surrounding soil pore networks, exploring for nutrients and water, akin to their botanical hosts. This leads to the analogous concept of the 'hyphosphere', i.e. the zone of influence in the vicinity of fungal hyphae (Tarafdar and Marschner, 1994), generated by mechanisms not dissimilar to those of the rhizosphere but at much smaller spatial scales; and then the 'mycorrhizosphere' (Kraigher *et al.*, 2013) which is a literal concatenation of these two spheres for mycorrhizal forms. The nature of the mycorrhizosphere in arbuscular, ericaceous and orchidaceous types is diffuse, where the extraradical hyphae are highly dispersed, versus that for ECM types where the fungus forms both a dense mantle around the root such that the outer cortex is entirely masked from the surrounding soil, and is connected to exploratory extra-radical hyphae. In total, the biotic zones of the holistic rhizosphere represent a complex space with substantial biodiversity.

Combining rhizosphere zones

The abiotic and biotic zones discussed above do not exist in isolation, but rather interactively form the holistic rhizosphere. While progress has been made by reducing the rhizosphere to these components for experimentation, future research will benefit from understanding the rhizosphere as a holistic whole. Most experiments have quantified these zones at limited time points and distances from the roots. However, the extent of these zones and their interactions must be considered as the results of dynamic process, which are discussed next.

The dynamic rhizosphere

Plant communities are dynamic systems, experiencing changing conditions ranging over annual, seasonal, daily, and hourly time scales. On a yearly scale, fine roots turn over and soil acidity can be modified. Indeed, most topsoil is eventually influenced by roots, so the rhizosphere must be considered as an active rhizosphere around current roots, as in the distinction between an 'active' rhizosphere and 'relic' rhizosphere, or that soil which is left altered after the death of roots which modified it (Jones *et al.*, 2004). Roots may preferentially grow in the biopores left after previous roots decay (Han *et al.*, 2015). Watt *et al.* (2006) took into account spatial and temporal scales in order to make predictions about rhizosphere development, especially with regards to root elongation rates, diffusivities of exudates, and microbial growth rates. The development of diffusion and accumulation zones also occurs over the period of days, while development and decay of a rhizosphere occupies intermediate time scales.

Many important rhizosphere processes fluctuate on an hourly basis. For example, decreasing root water uptake during the afternoon was recently predicted to avoid excessive dehydration of the rhizosphere and its potentially catastrophic effects on water (and nutrient) influx (Caldeira *et al.*, 2014). Circadian regulation of gene expression and/or activity of root water channels (termed aquaporins) could provide an adaptive mechanism to vary water flow during the day/night cycle. Intriguingly, the PIP class of aquaporin channel in both *Arabidopsis* and maize roots exhibit a circadian pattern of expression (Takase *et al.*, 2011; Caldeira *et al.*, 2014) peaking at dawn and lowest at the end of day, consistent with such a regulatory mechanism. Furthermore, magnetic resonance imaging measurements have revealed that the water content of Arabidopsis roots grown on agar plates varies diurnally, peaking at night and lowest at midday, a pattern that was disrupted in the circadian mutant *elf3* (Takase *et al.*, 2011). Nevertheless, whether this diurnal pattern of aquaporin expression also occurs in soil and impacts the daily flux of water from the rhizosphere remains unclear but, if proven, this novel adaptive response would have major implications for our current understanding of root water uptake.

Diurnal fluctuations in the uptake of nutrients have also been observed (Hanson and Biddulph, 1953). Most of these experiments could not uncouple uptake driven by fluctuating transpiration and uptake driven by fluctuations in the capacity of active transport at the root epidermis. However, a study of nitrate, potassium, and water uptake in tomato showed that although the highest peak of nutrient uptake occurred with the peak of highest transpiration, another peak occurred at night with 40% of uptake occurring during the night (Le Bot and Kirkby, 1992). Photosynthesis may be required to drive nitrate assimilation, during which mineral nitrate is converted to more readily used organic forms and decreased in cytoplasmic solution. Nitrate assimilation, in turn, may be required to maintain an ionic balance conducive to nitrate uptake. These processes may explain why diurnal variation in nitrate assimilation predicts nitrate acquisition (Cardenas-Navarro *et al.*, 1998). Possibly, internal nitrate concentrations drive transcript abundance, which drives the number of transporters and uptake capacity (Ono *et al.*, 2000). These oscillations in nutrient uptake by the plant have not been investigated for corollary changes in the rhizosphere depletion and accumulation zones. However, diurnal changes observed in rhizosphere pH extending up to 2 mm from the root epidermis in sand culture demonstrate measuring dynamic rhizosphere processes is possible (Rudolph *et al.*, 2013).

At even finer temporal resolutions, induction of nitrate transporters takes as little as 30 minutes following exposure of nitrate starved roots to nutrient solution (Quaggiotti *et al.*, 2003). Induction of transporters may explain the increases in per unit root length uptake of nitrate observed in several studies following exposure to higher nitrate concentrations to local sections of the root system (Robinson *et al.*, 1994; van Vuuren *et al.*, 1996). Transient changes in uptake kinetics may be an important adaptive strategy for plants to forage in nutrient patches before growth responses increase root density in the patches (Hodge, 2004).

The rhizosphere is not a static place, but rather a dynamic system of processes. Increasing the spatiotemporal resolution of rhizosphere measurements will lead to new insights about how these components are created, interact with one another, and dismantle.

Genetic basis of the rhizosphere

The dynamic nature of the rhizosphere created by a root arguably allows it to be considered as an extended phenotype (Dawkins, 1982), or an external manifestation of a plant's genetics. The genetics of this complex phenotype are not well-studied, and it is influenced by other soil organisms, but there are some examples of how the rhizosphere is partially determined by plant genetics. Specific rhizosheath weight, where the mass of rhizosheath soil is divided by dry weight of roots, gives an index of rhizosheath size and was measured in a mapping population of barley (*Hordeum vulgare*) in the field (George *et al.*, 2014). Specific rhizosheath weight had substantial heritability, and was positively correlated with both root hair length and phosphorus (P) acquisition. In common bean (*Phaseolous vulgaris*), total acid and proton exudation were measured in solution culture in a mapping population and were found to have heritabilities greater than 85% with several quantitative trait loci (QTL) discovered (Yan *et al.*, 2004). The genetics of exudation were reviewed by Rengel (2002), but little progress has been made. The biosynthesis, transport, and exudation processes are complex, and differ among the multitude of exudates (Weston *et al.*, 2012). Little is known about the development and genetics of root mucilage, although the chemical components of mucilage and involvement of the Golgi apparatus are known (Guinel and McCully, 1986). The biology of seed coat mucilage is better understood and may serve as a basis for further work on root mucilage exudation (reviewed in Haughn and Chaudhury, 2005). QTL for allelopathic effects of rice (*Oryza sativa*) roots were identified, yet the actual exuded compounds were not quantified (Ebana *et al.*, 2001). Clearly, the genetics controlling this extended phenotype are important to understand the development of the rhizosphere, and indeed genetic relations may explain other observed rhizosphere interactions.

Methods for studying the holistic rhizosphere

Empirical

The challenges associated with studying the rhizosphere are substantial because soil is opaque to visible wavelengths of light and generally fragile. Direct observation of the rhizosphere can be achieved with laborious soil micromorphological techniques adapted to preserve biological tissues (Ritz, 2011). The study of root system architecture and its relation to soil properties has been greatly advanced in recent years primarily through the interdisciplinary application of imaging techniques previously utilized by the medical and material sciences including X-ray computed tomography (CT) (Mooney *et al.*, 2011; Mairhofer *et al.*, 2013), MRI (Schulz *et al.*, 2013), and neutron radiography (Carminati, 2010) to non-destructively image living roots in soil. Many of the following rhizosphere methods were recently reviewed in greater detail by Oburger and Schmidt (2016).

The influence of compaction on root growth has been assessed in several species (Tracy *et al.*, 2012*a*,*b*). Tracy *et al.* (2015) recently developed X-ray CT for analysing water distribution within soil pores along a range of matric water potentials to measure hydraulic conductivity, and confirmed the results with reconstructed pore geometry in simulation modelling of water flow. Combining these methods suggested that rhizosphere soil had less saturated hydraulic conductivity than bulk soil (Daly *et al.*, 2015), however the definition of rhizosphere in this study was broad because planted and non-planted pots were compared. Other work using both X-ray CT and simulations demonstrated increased water flow through root modified soil in low density aggregated soils (Aravena *et al.*, 2014). Synchrotron radiation X-ray

tomographic microscopy was used to image root hairs in soil then root morphology and soil particle data were used in a simulation model of phosphorus uptake, which indicated that root hairs and root epidermis contributed equally to uptake, contrary to contemporary thinking (Keyes *et al.*, 2013).

Neutron radiography is an imaging technique which is complementary to X-ray imaging because of its high sensitivity to hydrogen-rich materials, such as water. Carminati (2010) and Moradi (2011) used neutron radiography to image the water content distribution near roots in two and three dimensions. They found that during a drying period, the water content increased towards the roots of lupines growing in small containers filled with sandy soil. The increasing water content towards the roots was interpreted as the effect of mucilage exuded by roots. The gradients around the roots extended over a distance of 1-2 mm from the root surface. Neutron radiography was also used to trace the transport of deuterated water across the root-soil interface. Lupines were grown in rhizoboxes containing capillary barriers of coarse sand used to separate zones of soil injected with deuterated water (Zarebanadkouki *et al.*, 2014).

MRI is more sensitive to hydrogen and less sensitive to the density of materials relative to X-ray CT, and has been previously used to study root and water relationships (MacFall *et al.*, 1990; Pohlmeier *et al.*, 2008; Segal *et al.*, 2008). In loblolly pine (*Pinus taeda*), MRI demonstrated water uptake around the taproot, lateral roots, and mycorrhizal roots, and strongly suggested that water uptake occurred along the suberized portion of the taproot (MacFall *et al.*, 1990). Advancements in MRI technology that increased resolution allowed Segal *et al.* (2008) to quantify water content as a function of distance from the root surface. Water depletion zones at a root system level were demonstrated to coincide with regions of greater root density using MRI and image processing (Pohlmeier *et al.*, 2008).

Rhizoboxes are constructed by filling soil or media between two large flat panels with one being transparent and positioned at an angle such that roots grow along the windows for ease of observation. GLO-Roots is an observatory platform where *Arabidopsis* is grown in a thin rhizobox using luciferase-based luminescent reporters and an imaging system to co-visualize roots, gene expression, and water content of the soil (Rellán-Álvarez *et al.*, 2015). Soil zymography is an *in situ* method where thin agarose gels with appropriate substrates are affixed to open faces of soil from root rhizoboxes in order to localize and quantify the activity of exuded plant and microbial enzymes (Spohn *et al.*, 2013). The activities of amylase, cellulase, chitinase, phosphatase, and protease have been reported using soil *in situ* zymography (Spohn and Kuzyakov, 2013, 2014; Spohn *et al.*, 2013).

Measurements of solutes in soil solution have traditionally been accomplished by withdrawing soil solution or soil samples and measuring using photospectrometry, gas chromatography, elemental analysis, and related techniques. The nature of these methods make increased spatial and temporal resolution difficult. However, the advent of imaging optodes (the optical equivalent of an electrode, originally 'optrode,' Klimant *et al.*, 1995) for rhizosphere measurements (briefly reviewed in Blossfeld, 2013) is a promising technological advance. Single optodes are often fibre optic and rely on an indicator dye that changes fluorescent properties depending on the concentration of the target analyte such that when the dye is excited by appropriate wavelengths, the corresponding released light may be captured by various imaging sensors, including consumer cameras. Single optodes have been embedded in a variety

of samples, similar to how water sensors are used in field and greenhouse studies. Planar optodes (Glud *et al.*, 1996) rely on the same principles as single optodes, yet use a thin membrane embedded with the indicator dye that is pressed onto a surface such that the analyte may diffuse into the membrane and the changes in fluorescence measured. Planar optodes yield a two dimensional array of analyte concentrations that may also be measured over time. Optodes have been used to measure oxygen, carbon dioxide, methane (Elberling *et al.*, 2011), pH (Faget *et al.*, 2013), phosphate (Warwick *et al.*, 2013), and ammonium (Strömberg, 2008; Delin and Strömberg, 2011) in soil. Extending planar optode measurements to nitrate will be an important advance. Similar to planar optodes, the diffusive gradients in thin films (DGT) technique relies on a thin film allowing an analyte to diffuse across and bind to a resin backing, followed by desorbing the analyte and measuring using an appropriate technology (Davison and Zhang, 1994). The DGT method was applied for local and temporal measurements of phosphorus around roots by pressing the film to an exposed soil surface and demonstrated P influx and efflux around the roots and allowed measurements of depletion volume (Santner *et al.*, 2012).

Microbes and animals in the rhizosphere influence the soil and roots directly, so must be considered in the holistic rhizosphere. The mapping of microbes in soil has identified microbial hotspots in the rhizosphere (Kuzyakov and Blagodatskaya, 2015), and the hyphosphere (Eickhorst and Tippkötter 2008), even to the level of individual cells (Schmidt *et al.*, 2012). These techniques could potentially be combined with X-ray CT as most studies to date appear to show X-rays do not harm microbes significantly at doses commonly used (e.g. Schmidt *et al.*, 2015), although older research suggests that various forms of radiation and dose can influence microbial populations (reviewed in Zappala *et al.*, 2013). Soil fauna are also known to influence the rhizosphere, principally via direct effects upon roots by parasitism (nematodes) or grazing. Earthworms create biopores and transform soil organic matter (Lamandé *et al.*, 2003). Roots are known to preferentially grow in such macropores (reviewed in Logsdon and Linden, 1992) and the transformation of organic matter by earthworms influences the microbial population and soil chemistry in burrows (Devliegher and Verstraete, 1997; Tiunov and Scheu, 1999). In studies of root herbivory by insects, on average, 63% of roots are lost resulting in a 13% reduction in shoot mass (Zvereva and Kozlov, 2012). Understanding the impact of rhizosphere microbes and fauna on other rhizosphere processes, and vice versa, will benefit research on crop disease and nutrient management.

Most direct measurements of rhizosphere processes have occurred in laboratory settings, so confirmation of these processes is needed in the field. Methods that require the addition of artificial substrates such as zymography will require analysis as to how those materials influence rhizosphere processes, if at all. Techniques such as time-domain reflectometry for water measurements (Dalton and Van Genuchten, 1986) and the use of resin bags for binding nutrients (Binkley, 1984) have spatial scales that are too coarse for rhizospheric studies. Microtensiometers measure soil matric potential and commonly have a diameter around 1.3 mm (Vetterlein and Jahn, 2004), however the response time required for equilibrium can be up to 30 minutes. Although both the spatial and temporal resolution can be increased with pliable tip microtensiometers that use a geotextile wick to make contact with the soil (Segal *et al.*, 2008). Ceramic micro suction cups operate at the same millimeter scale and allow extraction of small

amount of soil solution for collection and subsequent analysis of dissolved molecules with appropriate technology (Göttlein *et al.*, 1996).

Microdialysis relies on a continuous flow of a solution (the perfusate) through a tube with a section being enclosed with a semi-permeable membrane, with diameters less than 1 mm and the exposed membrane between 1 and 10 mm. The membrane is placed in an area to be sampled and the analyte allowed to diffuses across the membrane to the perfusate which flows to be quantified (Miró and Frenzel, 2005). Microdialysis is less invasive than taking soil cores or extracting soil solution, and allows determination of absolute concentrations and fluxes with proper calibration, with possible spatial and temporal resolution in natural soils of less than 0.5 mm and 30 minutes, respectively (Inselsbacher *et al.*, 2011). Interestingly, microdialysis measurements indicate that available amino acid contributions are comparable to inorganic nitrogen sources in soil, which is generally not true with traditional soil extractions (Inselsbacher *et al.*, 2011; Shaw *et al.*, 2014). As microdialysis allows measurement of actual concentrations in soil solution, rather than what might be 'bioavailable,' it is likely to contribute greatly to future research of root uptake capacity and nutrient fluxes in the field (Brackin *et al.*, 2015).

Methods for measuring chemical, physical, and biological properties of the rhizosphere in space and time continue to evolve. Combining these methods at the greatest possible resolutions will advance our understanding of the holistic rhizosphere.

Rhizosphere models and computer simulations

Rhizosphere modelling is not common, and has focused mostly at millimetre scales with little upscaling. In contrast, modelling of root systems with water and nutrient uptake has advanced significantly (six such models are reviewed in Dunbabin *et al.*, 2013), yet soil is typically modelled entirely as bulk soil with no influence of the roots on soil properties. However, rhizosphere models can be informative, and likely have profound impacts on larger scale systems. For example, a rhizosphere model of a growing root demonstrated stable changes in soil pH occurring within 6 hours with a 1 mm accumulation zone, and that measurements using agar overestimated the size of the accumulation zone due to increased diffusion (Kim *et al.*, 1999). A single root simulation of exuded mucilage and water uptake demonstrated greater benefits at greater water uptake rate potential and when mucilage didn't diffuse as far (Ghezzehei and Albalasmeh, 2015). Another model of water uptake extended the Tardieu-Davies model to include circadian rhythms of stomatal and root hydraulic conductance based on the rhythm of ABA concentrations, and this model could be combined with both genetic regulatory models and whole plant or population models (Tardieu *et al.*, 2015). Clearly, considering the rhizosphere is necessary in root structural-functional simulations.

More robust soil models including the dynamics of microorganisms will be especially important in future research of the rhizosphere. A growth model of AM fungi adequately predicted hyphal length as a function of distance from the root and could be used to influence the nutrient sink terms of current root system models (Schnepf *et al.*, 2008), similar to the modelling of root hairs (Itoh and Barber, 1983). Rhizosphere carbon flow modelling including rhizodeposition and microbial population dynamics was reviewed by Toal *et al.* (2000). Sensitivity analysis revealed the importance of

the rhizodeposition rate and quality in controlling the whole system and rhizosphere scientists were tasked to report rhizodeposition in standard units and conditions (Toal *et al.*, 2000). The relationship between rhizodeposition and plant nutrient status is highlighted by the rhizosphere priming effect where N mineralization is increased near roots due to microbial activity (Kuzyakova *et al.*, 2000). Game theory modelling, where the strategy of one organism depended on the strategies of others, demonstrated that rhizosphere priming could develop as a mutualism between plants and microbes in some limited ecological conditions (Cheng *et al.*, 2014). However, none of these simulations have been coupled with root system scale models.

To our knowledge, the only work to upscale from a rhizosphere model to an entire root system is that of Dunbabin *et al.* (2006). Based on earlier empirical work demonstrating the influence of exuded surfactants on water and phosphorus dynamics in the soil (Read *et al.*, 2003), a rhizosphere volume of soil was parametrized in the RSA simulation ROOTMAP where the exudate decreased hydraulic conductivity yet decreased P adsorption to soil and so increased P concentration in soil solution (Dunbabin *et al.*, 2006). Relative to a single root segment finite grid model, the architectural model predicted greater P uptake which highlights the importance of considering rhizosphere processes at greater scales.

Linking root system simulation models with rhizosphere processes is complicated, but not impossible. Since most root system models have a spatially explicit soil grid (Dunbabin *et al.*, 2013) and because most rhizosphere influences have known effects on soil properties, simulations can readily be adapted to have basic rhizospheres by simply registering soil near roots and updating the soil properties of those points. For example, if soil elements contain both adsorbed phosphate and phosphate in solution, then acid exudation from the roots would force phosphate to desorb thus being more available. Linking such models will probably require inclusion of submodels of specific processes, such as nitrogen mineralization as influenced by microbial activity and carbon sources from roots. While upscaling single rhizosphere process models is necessary, the even greater challenge will be integrating all rhizosphere processes into a single model. Integrating plant models across scales and processes was recently discussed by Zhu *et al.* (2015). Making these models even more computationally intensive is the tradeoff, but as access to supercomputers and cluster computers increase in biology this tradeoff will be partially mitigated. Increasing the details of root and soil models to include rhizosphere processes will allow experimentation that would be impossible to do in the lab or the field and provide invaluable guidance for understanding the rhizosphere.

Integration of rhizosphere processes, methods, and models to uncover new mechanistic insights

Better understanding of interactions between roots and rhizosphere processes promise to lead to new knowledge and mechanistic insights. Table 2 shows pairwise interactions of selected zones and demonstrates little is known about how zones integrate; imagining three and four way interactions is even more difficult. The range of scales involved are enormous, from the gene to rhizosphere to field, so multi-scale simulation and empirical research is required (Hill *et al.*, 2013). Interactions between rhizosphere processes and root system architecture (RSA) are also expected because RSA will determine the extent of overlap among proximate individual root rhizospheres (York *et al.*, 2013). Coupling of experimental work with simulation

modelling is being employed in rhizosphere research, such as in work with rhizosphere restructuring affecting soil hydraulic properties (Daly *et al.*, 2015; Tracy *et al.*, 2015), the interaction of root hair and soil geometry for phosphorus uptake (Keyes *et al.*, 2013), and the uptake of water by roots (Zarebanadkouki *et al.*, 2014). Combinatorial *in situ* and *in silico* research promises to continue to improve our understanding of rhizosphere processes and mechanisms.

A wide range of experimental approaches have also been combined to enhance understanding of rhizosphere-related processes. For example, positron emission tomography (PET), which relies on positron-emitting radioactive tracers by detecting gamma rays, has been used in conjunction with MRI to localize and quantify assimilated ^{11}C in three dimensions (Jahnke *et al.*, 2009). Positron emission imaging has also been used to detect uptake and translocation of ^{15}O-labeled water (Nakanishi *et al.*, 2003) and ^{13}N-labeled ammonia (Kiyomiya *et al.*, 2001), but not yet in 3D. MRI and X-ray CT were demonstrated to be complementary in their abilities to segment root systems at various soil moistures and soil types, with X-ray CT having higher resolution but MRI having greater contrast between roots and soil (Metzner *et al.*, 2015). Fluorescent and neutron imaging approaches were combined to simultaneously monitor root growth, exudation, pH, oxygen, and soil water content (Rudolph-Mohr *et al.*, 2014). Soil zymography and autoradiography were combined to determine the relative contributions of plants and microbes to phosphatase activity (Spohn and Kuzyakov, 2013), while roots transformed to express fluorescent proteins were used in conjunction with pH planar optodes to study the effect of roots from different species on soil acidification (Faget *et al.*, 2013). It is clear that combinatorial imaging coupled with modelling will advance our understanding of rhizosphere processes in the near future.

In contrast, new mechanistic understanding about important rhizosphere-related processes, such as root exudation, has been surprisingly limited from genetic models such as *Arabidopsis.* Instead, most studies of root exudates have occurred in wild and crop plants most probably because *Arabidopsis* root growth and development is generally studied using agar plates. Despite agar plates obvious limitations, adaptive root mechanisms such as hydropatterning (Bao *et al.*, 2014) and hydrotropism (Moriwaki *et al.*, 2013) reflecting growth and developmental responses to local variation in air and water content within the rhizosphere, have been successfully discovered and/or studied using *Arabidopsis* on agar plates, respectively. Hence, imaginative agar-based screens replicating specific soil micro-environmental conditions represent promising routes to characterize the mechanistic basis of important rhizosphere processes. In parallel, recent advances in *Arabidopsis* root imaging such as GLO-Roots coupled to technologies like zymography and optodes could increase our understanding of root adaptive responses to rhizosphere conditions.

Many process in the rhizosphere lead to interactions been roots, microbes, water, and nutrients. For example, plant roots and microbes compete for nitrogen, and most likely other nutrients (Kuzyakov and Xu, 2013). Root exudates can increase mineralization from soil organic matter as much as 20% (Kuzyakov *et al.*, 2007), yet the implications for competition between roots and microbes are not well understood. Mucilage contains phospholipid surfactants that decrease capillary forces, preventing P adsorption by soil particles, and increasing P in solution by as much as 10% (Read *et al.*, 2003), which could presumably benefit plants (and microbes). Using simulation

modelling, Dunbabin *et al.* (2006) demonstrated a potential 3-4% increase in P availability due to these rhizosphere processes. However, little is known about how mucilage affects nutrient uptake, even though progress is being made in understand the effects of mucilage on water. Water content of soil is linked to nutrient availability both through diffusion and mass flow. The radius of P depletion zones has been reported to decrease from 0.2 cm to 0.1 cm when water content was decreased from 20% to 14%, respectively (Gahoonia *et al.*, 1994). Given the number of nutrients, species, and soil types of the world, research addressing interactions of rhizosphere processes is in its infancy, but is set to explode in the next decade.

An example of such integrative rhizospheric research would be identifying how mucilage, nitrate uptake, and bacterial communities interact. Screening a maize population might reveal a range of related genotypes that differ in mucilage composition and exudation rate. Several genotypes covering the range of mucilage exudation could be grown in rhizoboxes of sieved field soil with natural microbial populations, or in the same soil that had been autoclaved and sterilized. After the root systems were established, $^{15}NO_3^-$ could be injected in the vicinities of roots. After several days, plants, rhizosphere soil, and bulk soil could be tested for ^{15}N content, which acts as a tracer. At the same time the microbial community in the rhizosphere and bulk soil could be tested for ^{15}N, diversity, and abundance. Such a system could identify effects of mucilage on both N uptake and microbial abundance, while simultaneously measuring the effects of microbial abundance on plant N uptake, and possibly uncover important interactions that cannot be predicted.

The above examples illustrate how multiple rhizosphere processes can interact to create complex, non-linear outcomes, necessitating the use of modelling approaches. For example, numerical modelling of microbial populations, exudation, oxygen, and carbon dioxide demonstrated oscillations with multiple chaotic and nonchaotic attractors (Faybishenko and Molz, 2013). The reciprocal nature of rhizosphere interactions can be abstracted as a system of differential equations modelling dynamics in time and space:

$$\frac{\partial \vec{S}}{\partial t} = \vec{r}\left(\vec{S}, \theta, \vec{P}, \vec{E}, \vec{N}\right) \qquad \textit{Equation 1}$$

$$\frac{\partial \theta}{\partial t} = \vec{\vec{h}}\left(\vec{S}, \theta, \vec{P}, \vec{E}, \vec{N}\right) \qquad \textit{Equation 2}$$

$$\frac{\partial \vec{P}}{\partial t} = \vec{\imath}\left(\vec{S}, \theta, \vec{P}, \vec{E}, \vec{N}\right) \qquad \textit{Equation 3}$$

$$\frac{\partial \vec{E}}{\partial t} = \vec{z}\left(\vec{S}, \theta, \vec{P}, \vec{E}, \vec{N}\right) \qquad \textit{Equation 4}$$

$$\frac{\partial \vec{N}}{\partial t} = \vec{o}\left(\vec{S}, \theta, \vec{P}, \vec{E}, \vec{N}\right) \qquad \textit{Equation 5}$$

The abundances of microbial species (S), soil water content (θ), soil properties (P) (such as pore size distribution and pore connectivity), exudate composition and concentrations (E), and nutrient composition and concentrations (N) are each functions of all the others in a reciprocal fashion, such that changes in one have the potential to influence all the others. For simplicity of display, microbes, soil properties, nutrients, and exudates are depicted as vectors denoted by the arrow (→), meaning several types

are included in each and each type has its own function (denoted by function vectors *r, h, i, z,* and *o*). The exact mathematical relations are implicit, but include root uptake kinetics, exudation rates, diffusion coefficients, etc. These equations highlight the holistic rhizosphere as being a system of processes, where spatial boundaries only arise for moments in time when steady states might be reached. Such boundaries can only be arbitrarily defined as locations where the rhizosphere values reach some threshold of the values in bulk soil. Despite this apparent simplicity, the strength of the model is providing a conceptual framework for holistic rhizosphere science. Conceptual models using differential equations of soil formation and ecosystem properties were partly popularized by Jenny (1941) and proved to be very successful in promoting rigorous thought about the diverse and interacting processes involved. In the case of the rhizosphere, while more explicit mathematical models of a few rhizosphere processes exist, none capture the extraordinary complexity of the rhizosphere as in the model proposed here. The dynamics of the holistic rhizosphere are defined by the integration of these individual processes.

Conclusions

The rhizosphere has been defined in terms of the effects of roots on soil microorganisms (Toal *et al.*, 2000), the depletion of water (Segal *et al.*, 2008), changes in pH (Kim *et al.*, 1999), adhering soil (Bulgarelli *et al.*, 2012), and so on. Hiltner (1904) defined the rhizosphere as the soil influenced by roots, so though reductionist research led to more narrow conceptions and to a greater understanding of individual processes, the interdisciplinary research of the future must acknowledge a dynamic region of interacting processes: the holistic rhizosphere. However, in acknowledging the rhizosphere as a 'whole in reciprocal interaction with its own parts' (Levins and Lewontin, 1980), that the rhizosphere itself is but a part of a greater soil system must also be realized. By using integrative methods including non-destructive imaging, next-generation chemical assays with substantial spatiotemporal resolution, and simulation modelling, the secrets of the dynamic rhizosphere will be revealed. Holistic rhizosphere science has the potential to substantially increase understanding of plant-soil systems and provide guidance for pressing issues of the 21st century, such as agricultural sustainability and environmental change.

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Tables

Table 1. A list of rhizosphere components, generally accepted definitions, and their spatial extent (size). Depletion or accumulation zones of all mineral nutrients exist, but only P and N are listed here as examples of relatively immobile and mobile nutrients, respectively. Size is generally measured from the root epidermis.

Component	**Size**	**Definition**
Rhizosphere	~cm	Soil influenced by roots
Rhizoplane	1 mm	Root epidermis, mucigel, and adhering soil
Rhizosheath	1 mm	Soil adhered by root hairs and mucilage
P depletion zone	3 mm	Concentration gradient of P in soil solution due to uptake
N depletion zone	2 cm	Concentration gradient of N in soil solution due to uptake
Accumulation zone	1 mm	Calcium from mass flow but not adsorbed
Soil structure modification	1 cm	Changes in soil porosity, soil architecture modification
Oxygen depletion	3 mm	Oxygen uptake due to root and microbial respiration
CO_2 Accumulation	3 mm	Respired carbon dioxide from roots and microbes
Exudation zone	2 mm	Sugars, mucilage, acids, allelochemicals released by roots
Microbe	µm - m	Fungal mycelia transcend 6 orders of magnitude in scale

Table 2. A cross table of selected rhizosphere zones. SSM is an abbreviation for soil structure modification. Intersections show possible interactions and shaded areas show the areas of least knowledge.

	Sugars	**Acids**	**Mucilage**	**Nutrient**	**Water**	**Microbes**	**SSM**
Sugars						consume	
Acids				release		consume	
Mucilage					retain/repel	facilitate	
Nutrient		release			availability	competition	
Water			retain/repel	availability			
Microbes	consume	consume	facilitate	competition			facilitate
SSM						facilitate	

Figure captions

Figure 1. A barley root sampled from the field is depicted with its rhizosheath, soil particles bound by root hairs, and mucigel. The rhizoplane includes both the root epidermis and the rhizosheath, while the rhizosphere may extend beyond the boundaries of the rhizosheath. Micrograph kindly provided by Margaret McCully.

Figure 2. A few components of the holistic rhizosphere. A barley root system was scanned using X-ray computed tomography. Approximate boundaries of rhizosphere zones were digitally added depicting exudate accumulation and bacterial community changes, phosphate depletion, nitrate depletion, and water depletion, only a few components of the holistic rhizosphere (see legend).

Figure 3. Root acquisition of water reduces soil water content (blue) and increases air-filled pore space (white) in the surrounding soil, while remaining water tightly adheres to soil particles as capillary bridges and thin films. As the water content decreases, the hydraulic conductivity decreases and the root may be unable to acquire water at the required rate, or the root may even lose contact with the water completely. However, exudation of mucilage may allow the root to form a hydraulic bridge between the epidermis and the surrounding soil particles. In this case, water content may be higher near the root epidermis due to the water holding capacity of mucilage.

Figure 4. Neutron radiography of roots of a 3-weeks old lupin growing in sandy soil. The picture was taken 30 minutes after irrigation of the sample from the bottom. The image shows the water high water content around the root tips in the deeper soil layers, probably caused by mucilage rehydration, and the low water content around the upper roots, caused by water repellency in the rhizosphere. The sample was 30 cm high and 15 cm wide. Adapted from Carminati (2013).

Figure 5. Nutrients arrive at the root surface where they are absorbed through diffusion and mass flow. Effective diffusion in soil is influenced by charge interactions between nutrient ions and particle surfaces, moisture content, and tortuosity of the path. Nutrients may diffuse from solution to the root (D1), from particle to root (D2), between exchange sites on the particles (D3), and replenishing between solution and exchange sites. Mass flow (MF) is the movement of nutrients with water. Contemporary interpretation of Fig. 1 from Barber (1962).

Figure 6. The species abundance and population sizes are generally increased in the rhizosphere relative to the bulk soil. The loss of root border cells and mucilage exudation at the root tip create another specialized rhizosphere region. In this case, arbuscular mycorrhizal fungi have infected the root and their hyphae extend into the soil creating a larger ‘mycorrhizosphere.’

Figure 7. A connected system. Macro photograph via dissecting microscope of roots of *Plantago lanceolata* growing in grassland mineral soil, enmeshed by anonymous fungal mycelia, likely both mycorrhizal and saprotrophic. Mucilage films are also visible, and water films on aggregate surfaces. Scale bar 1 cm. From Ritz (2011).

Figures

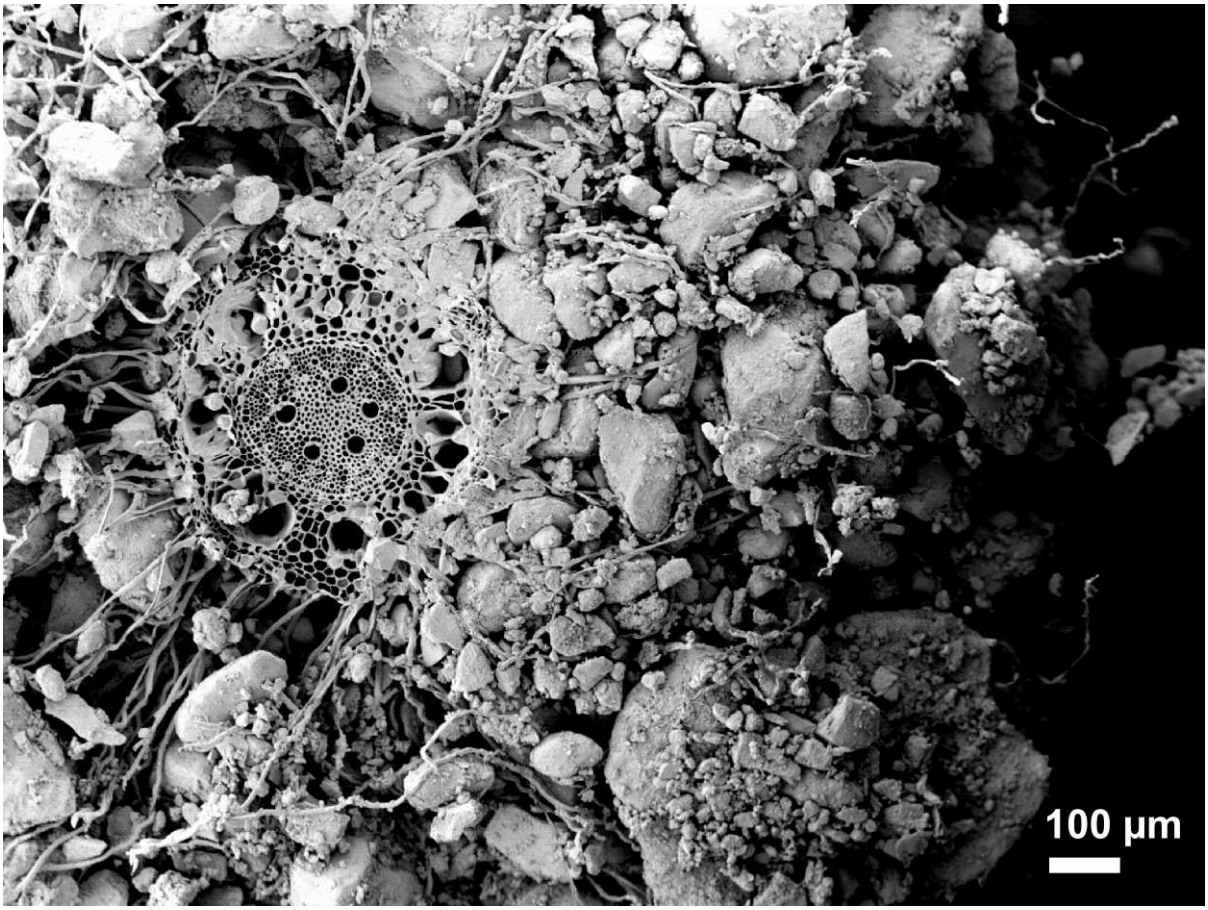


Figure 1.

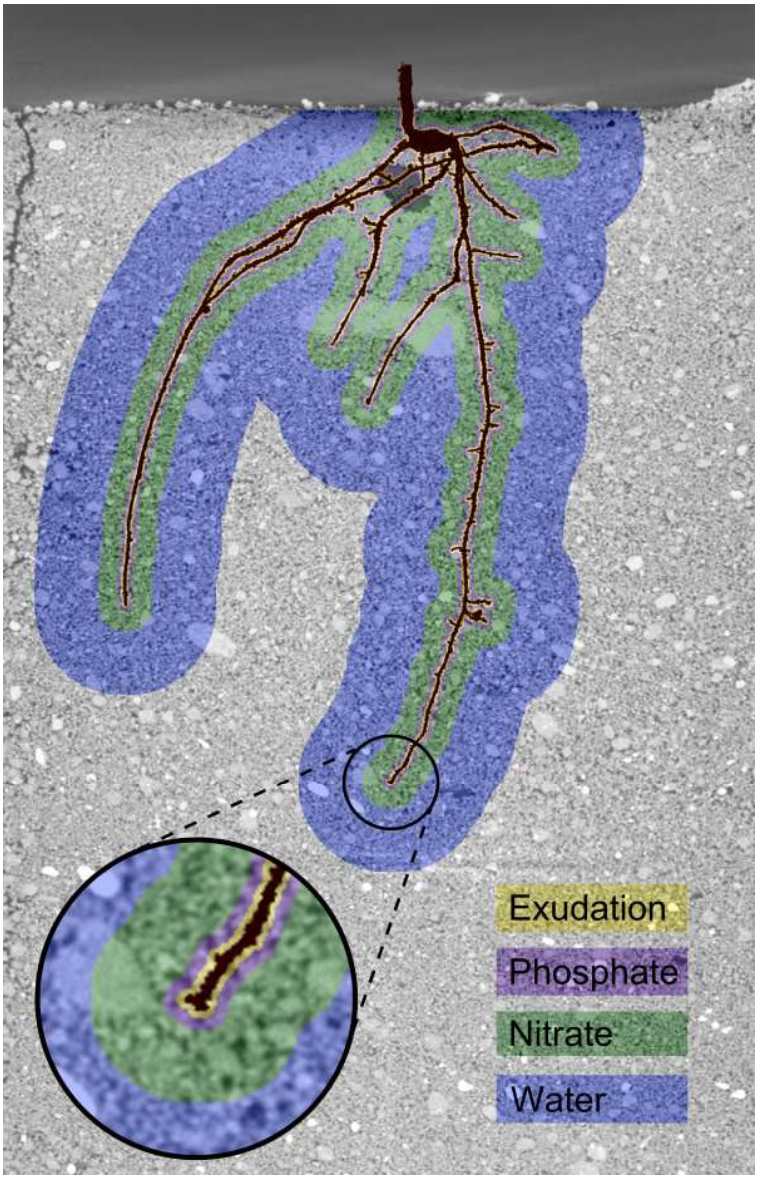
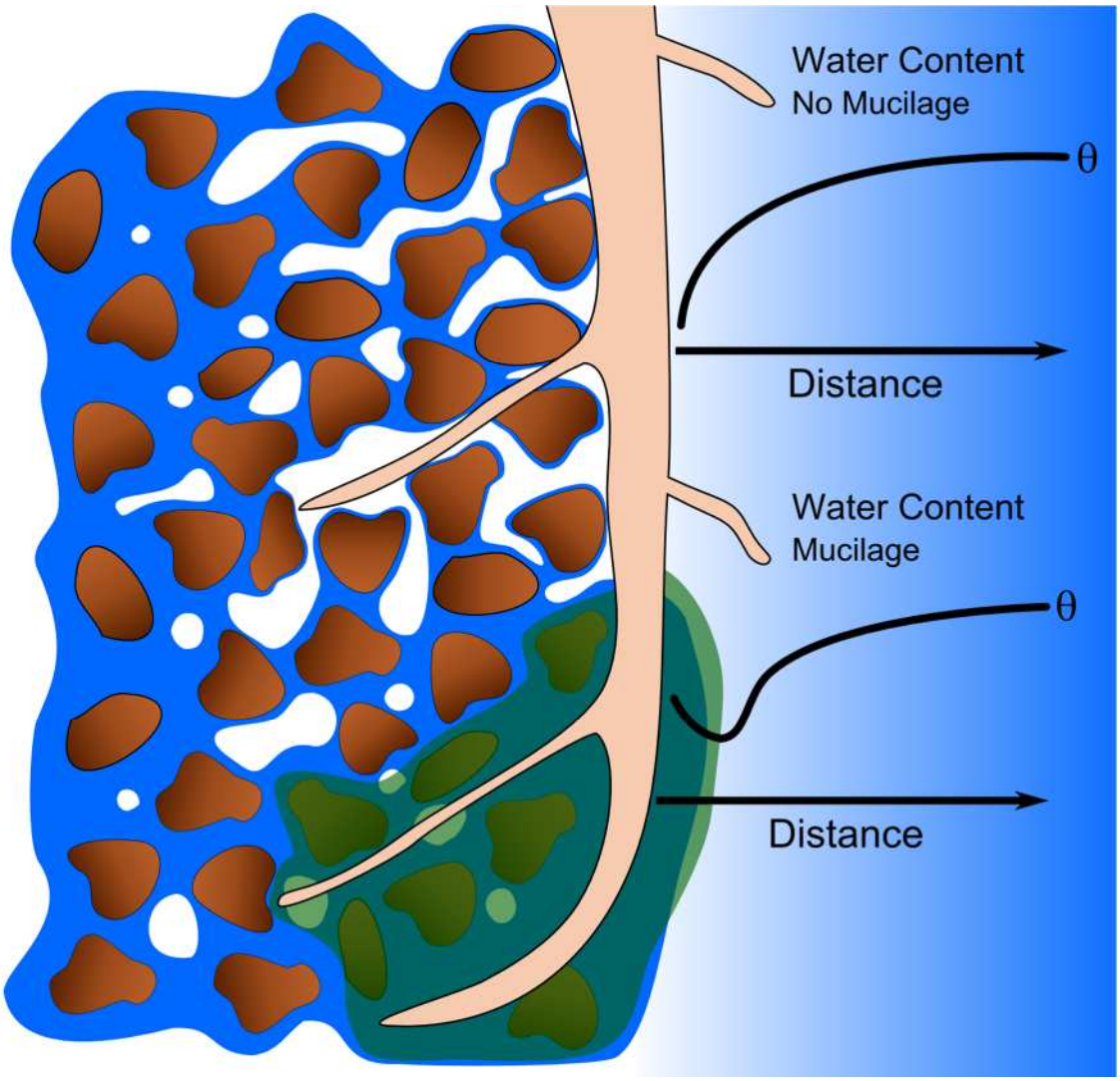


Figure 2.


Water Content
No Mucilage
θ
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Water Content
Mucilage
θ
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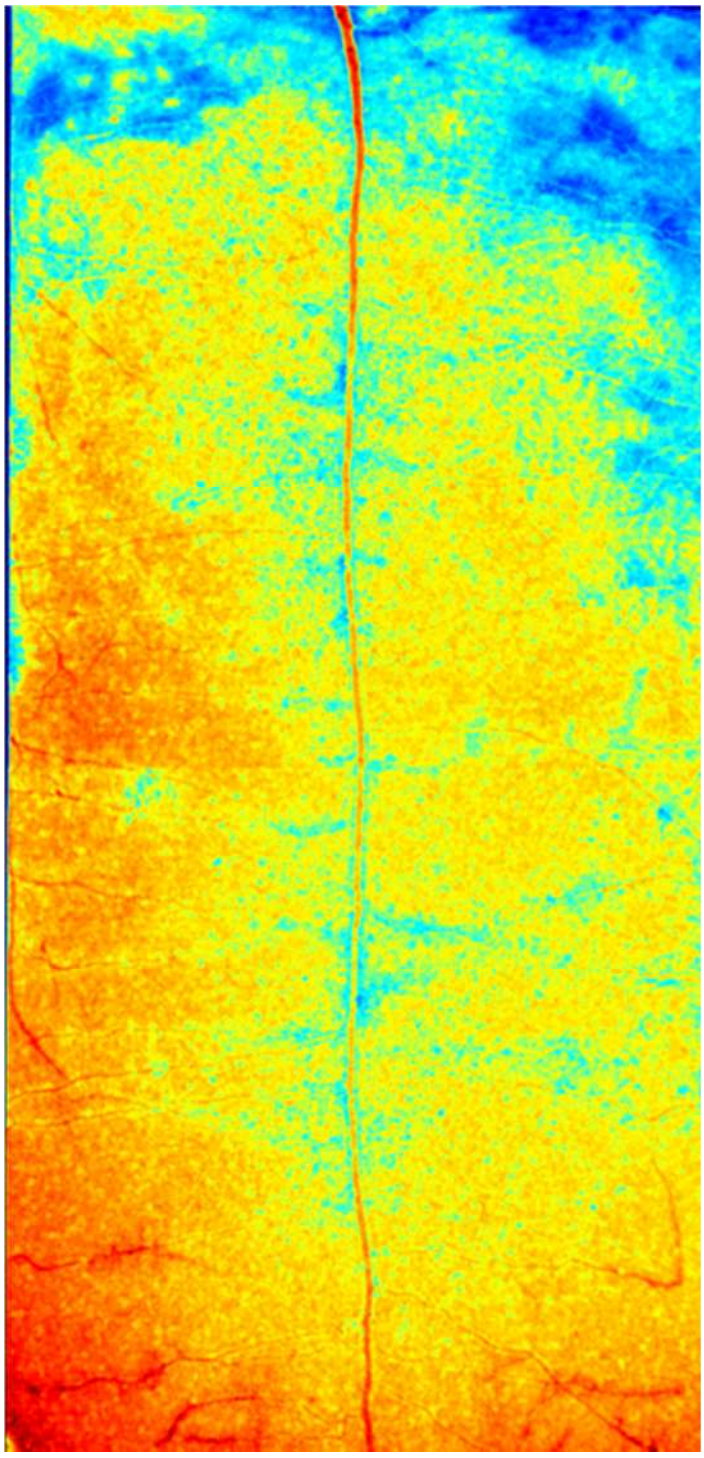
Figure 3.



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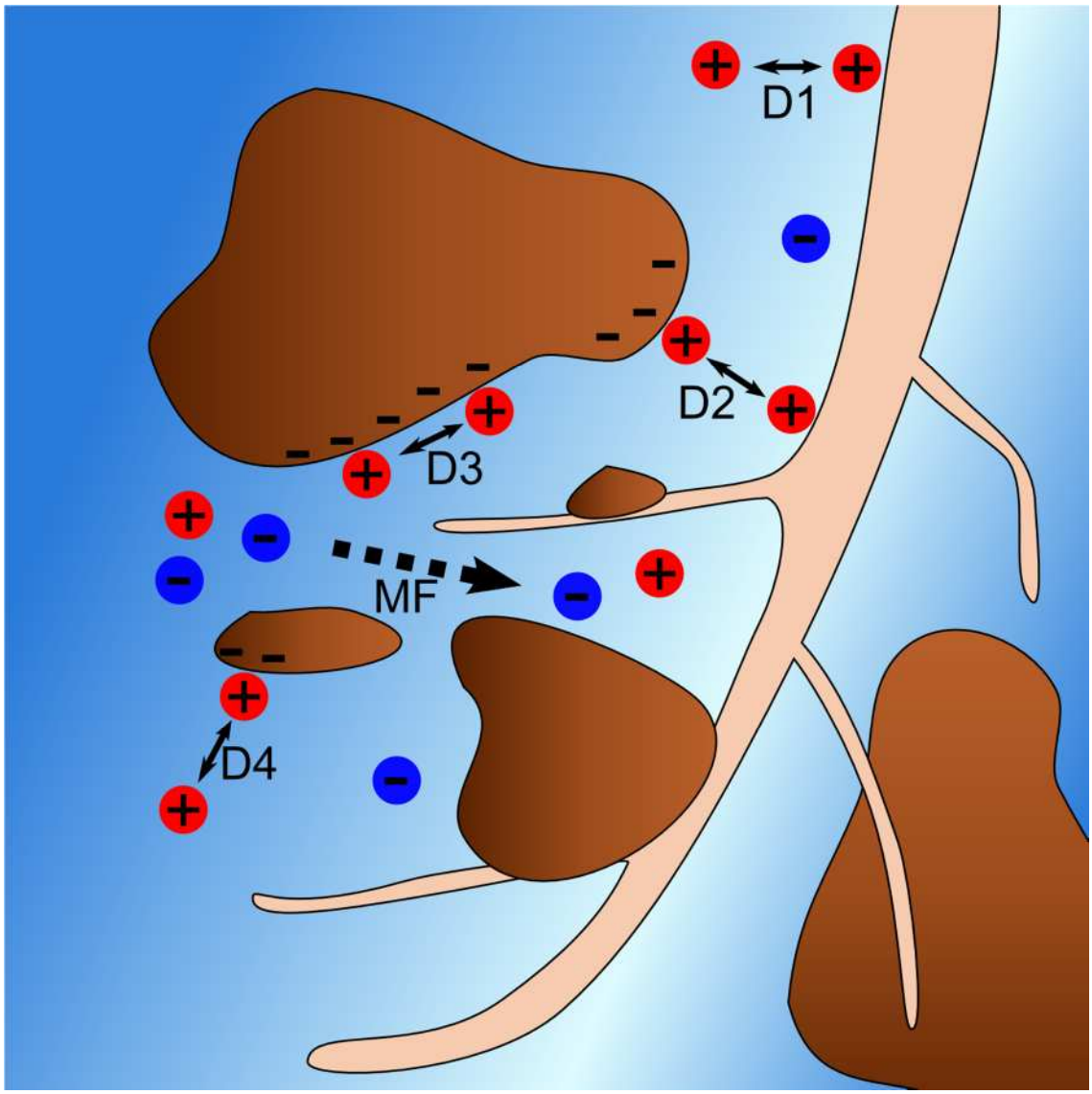

Figure 5.

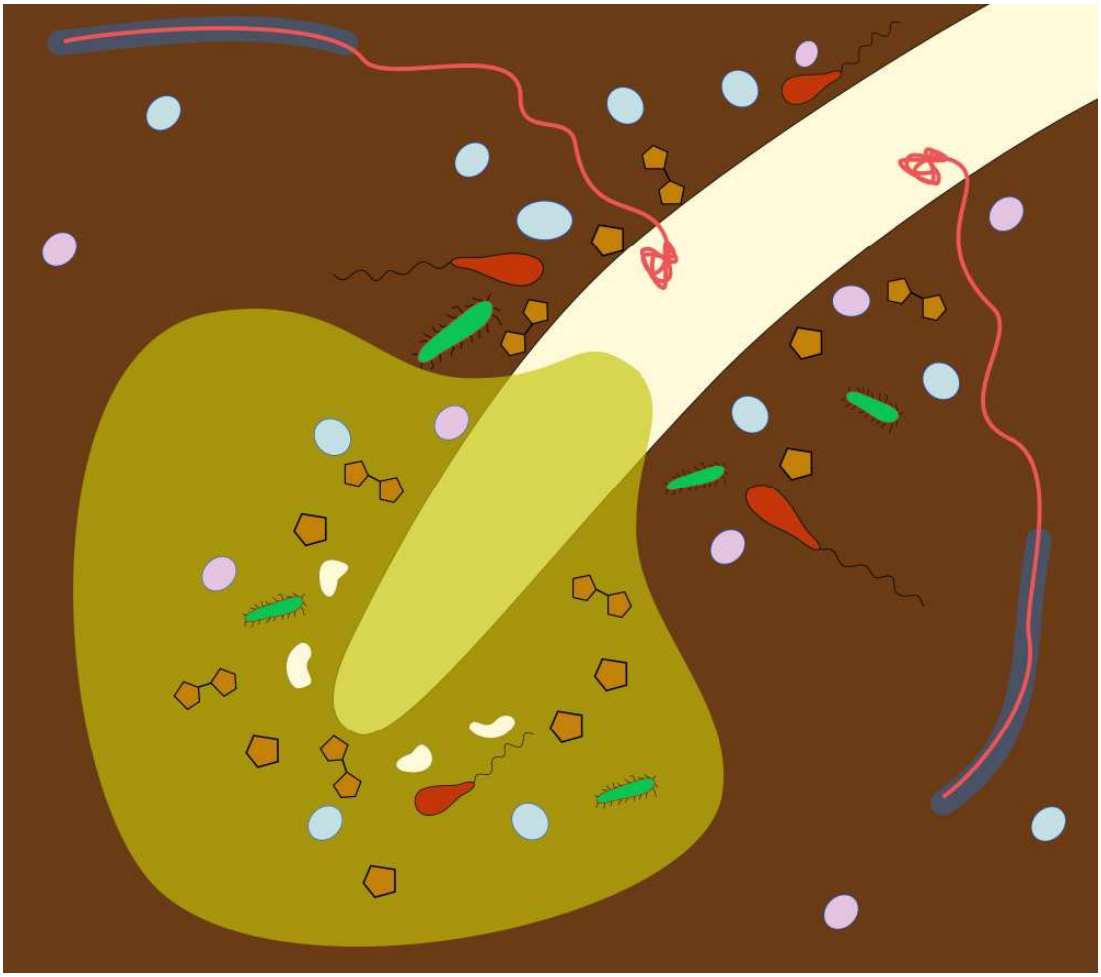

Figure 6.

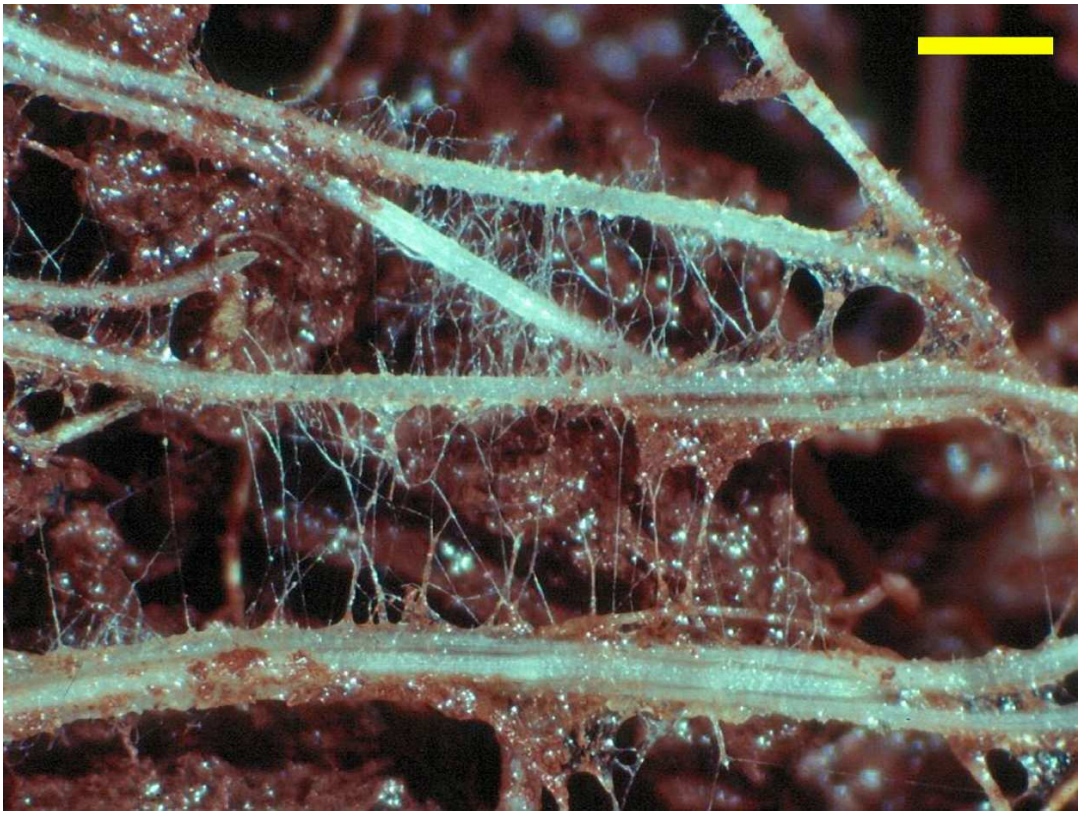

Figure 7.