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Sampling, defining, characterising and modeling the rhizosphere – The soil science tool box

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geophysics, imaging, isotope probing, microcosms, soil solution, spectroscopy

Abstract

We review methods and models that help to assess how root activity changes soil properties and affects the fluxes of matter in the soil. Subsections discuss (i) experimental systems including plant treatments in artificial media, studying the interaction of model root and microbial exudates with soil constituents, and microcosms to distinguish between soil compartments differing in root influence, (ii) the sampling and characterization of rhizosphere soil and solution, focusing on the separation of soil at different distances from roots and the spatially resolved sampling of soil solution, (iii) cutting-edge methodologies to study chemical effects in soil, including the estimation of bioavailable element or ion contents (biosensors, diffusive gradients in thin-films), studying the ultrastructure of soil components, localizing elements and determining their chemical form (microscopy, diffractometry, spectrometry), tracing the compartmentalization of substances in soils (isotope probing, autoradiography), and imaging gradients in-situ with micro electrodes or gels or filter papers containing dye indicators, (iv) spectroscopic and geophysical methods to study the plants influence on the distribution of water in soils, and (v) the modeling of rhizosphere processes. Macroscopic models

42 with a rudimentary depiction of rhizosphere processes are used to predict water or nutrient
43 requirements by crops and forests, to estimate biogeochemical element cycles, to calculate soil water
44 transport on a profile scale, or to simulate the development of root systems. Microscopic or
45 explanatory models are based on mechanistic or empirical relations that describe processes on a single
46 root or root system scale and / or chemical reactions in soil solution.

47 We conclude that in general we have the tools at hand to assess individual processes on the microscale
48 under rather artificial conditions. Microscopic, spectroscopic and tracer methods to look at processes
49 in small „aliquots“ of naturally structured soil seem to step out of their infancy and have become
50 promising tools to better understand the complex interactions between plant roots, soil and
51 microorganisms. On the field scale, while there are promising first results on using non-invasive
52 geophysical methods to assess the plant's influence on soil moisture, there are no such tools in the
53 pipeline to assess the spatial heterogeneity of chemical properties and processes in the field. Here,
54 macroscopic models have to be used, or model results on the microscopic level have to be scaled up to
55 the whole plant or plot scale. Upscaling is recognized as a major challenge.

56

57 **Introduction**

58 There are two basic questions involved with this part of rhizosphere research. (i) How are physical and
59 chemical soil properties and related functional parameters (e.g. structural stability, availability of
60 water, nutrients or toxic substances) affected by root growth, root physiological processes involved in
61 nutrient acquisition and uptake and related root-microbe interactions, and how far do these effects
62 extend from the root (Hinsinger et al. 2005)? (ii) How do these root-related processes affect the fluxes
63 of water, elements and ions in the soil, and thus biogeochemical cycles? On principle all methods for
64 the analysis and modeling of the properties of the respective soil phases apply and can be looked up in
65 standard textbooks such as Weaver et al. (1994; biochemical and isotopic methods), Sparks (1996;
66 chemical methods), Dane and Topp (2002; physical methods), Pansu and Gautheyrou (2006;
67 mineralogical and chemical methods) and Nollet (2007; water analysis with implications for soil
68 solution analysis). The critical issue, which is the red-line of this chapter, is to separate, define or
69 identify the rhizosphere. In a first section, the various degrees of simplifying real soil and
70 experimental systems to study the interaction of model root and microbial exudates with soil
71 constituents are discussed. Laboratory and field systems are presented that allow a distinction of soil
72 compartments in terms of root influence, that facilitate the sampling of rhizosphere soil or soil
73 solution, or that enable the *in-situ* analysis of the root's influence on soil properties. In the second
74 section, methods to separate rhizosphere from bulk soil and to sample rhizosphere solution and gas are
75 presented together with a brief overview of analytical methods for their characterization. Soil
76 biological methods are described by Sørensen et al. (2008). The third section is devoted to cutting-
77 edge methodologies to study chemical effects in soils. This includes techniques to assess bioavailable
78 contents, to trace the compartmentalization of organic carbon, and to map the distribution of elements
79 and species *in-situ*. In the fourth section, the prospects of spectroscopic and geophysical methods to
80 image non-invasively the plant influence on soil moisture distribution in the laboratory and field are
81 discussed. Modeling, the topic of the fifth section, is an important tool to understand and predict plant
82 influence on soil properties, and vice versa, how to manage the soil to fulfill plant water and nutrient
83 requirements. In addition, models are useful to estimate how plant activity affects terrestrial element
84 cycles, and vice versa, how plants react to climatic changes. Scaling model results up from the single-

root level to the whole-plant, plot or catchment level is one of the most demanding current research issues. In a sixth and last section we discuss this and other challenges ahead. An alternative treatment of aspects dealt with in this paper can be found in Luster and Finlay (2006).

Experimental systems

Field soil is a complex three-phase system with varying degrees of spatial and temporal heterogeneity of physical and chemical properties. Soil fauna, microorganisms and growing plant roots increase this heterogeneity. In particular, growing plant roots add spatial gradients in two directions (Fig. 1). Along the growth direction, root segments differ in their functionality in terms of uptake (water, nutrients) or exudation, causing a variability of root-induced changes in the properties of the surrounding soil. This root influence decreases with increasing distance from the root surface leading to gradients from the rhizosphere to the bulk soil. In addition, there is a temporal variation in root influence due to diurnal, seasonal or age related changes in the physiological activity of root segments. Dead parts of the root system first become local sources of organic matter, and after their degradation macropores can be created which can have a strong impact on the soils transport properties. The goal of rhizosphere research being to assess these plant influences, minimising the heterogeneity of the soil itself is an important consideration. The degree of simplification in terms of substrate properties and / or system geometry must be adequate for the problem and allow a correct interpretation of the data.

Artificial substrates

The nature of artificial growth media relates to the fact that root activity generally needs water as medium. They either contain no solid phase at all (hydroponics) or employ a solid phase with low chemical reactivity suspended in or irrigated with nutrient or treatment solution. Artificial solid substrates are often easier to sterilize than soil material. Sterilization of soils can alter their chemical and physical properties (Wolf and Skipper 1994) and it is difficult to maintain sterility during longer experiments. As such artificial substrates are excellent tools to study plant physiological reactions (Neumann et al. 2008), but also potential plant effects on soil solution can be investigated.

In hydroponic culture the composition of root exudates can be studied without adsorption losses to a solid phase, whereas the effect of mechanical impedance experienced by roots growing in soil on exudation is neglected (Neumann and Römheld 2001). The in- or efflux of ions from root segments can be measured in hydroponics using micro electrodes (Plassard et al. 2002), or in gelatinized solutions by visualizing gradients with dye indicators and quantification with videodensitometry (Plassard et al. 1999). In order to add mechanical impedance to growing roots, while maintaining the advantage of controlled soil solution composition, glass beads (Hodge et al. 1996) or sand mixtures (Tang and Young 1982) have been used as growth media for the collection of root exudates. The chemical inertness of these media, however, is limited (Sandnes and Eldhuset 2003). Volcanic glasses like perlite or clays like vermiculite are excellent preculture media, but are of limited use to assess root exudation or chemical gradients around roots (Heim et al. 2003).

Testing root influence on specific soil materials

An effective way of investigating the influence of root activity on the structure or reactivity of soil components like clay minerals or oxides is to study their interaction with isolated root exudates or model compounds (e.g., carboxylates, siderophores) in the absence of plants (Ochs et al. 1993; Reichard et al. 2005). Data on sorption of organic compounds by soil materials can give clues about their migration potential in soils (Jones and Brassington 1998). The compilation of Martell and Smith (1974-1989) provides thermodynamic data on equilibria between exudates as ligands and dissolved metal ions. The behavior of carboxylate anions in soils was reviewed by Jones (1998), that of phytosiderophores by Kraemer et al. (2006). An elegant way to test the effect of individual compounds on the bioavailability of nutrients was presented by Ström et al. (2002). They grew maize seedlings in “rhizotubes”, added a solution with carboxylate anions to a ^{33}P labeled patch of soil, and measured the ^{33}P uptake.

Alternatively, minerals can be mixed into an inert substrate and the effect of a growing root system with or without microbial inoculation on weathering can be assessed (Leyval and Berthelin 1991). The spatial extent of root exudation on weathering can be studied effectively using root mat systems as described below (Hinsinger and Gilkes 1997).

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Laboratory systems to assess gradients in soil

When studying root influence on soil, simplifications with respect to soil structure and root system geometry are usually involved, and / or compartments with a high root density separated from root-free soil. Depending on the system, destructive methods for the collection of rhizosphere soil can be applied, rhizosphere soil solution can be sampled, or gradients can be assessed by non-invasive tools. There is no unambiguous nomenclature for such systems. For example, rhizotrones and rhizoboxes are often used for similar types of flat growth systems in which plants form quasi 2D root systems. In the following we will use the term “microcosm” and differentiate between types by the way how roots interact with the soil and how rhizosphere is defined.

Microcosms in which roots are in direct contact with soil

Pot and column studies belong into this category. Differences between bulk and rhizosphere soil can be assessed by separating rhizosphere from bulk soil by shaking or washing (Liu et al. 2004), by resin impregnation followed by microscopic or spectroscopic inspection of thin sections, or by non-invasive 3D tomography (Pierret et al. 2003). Both repacked soil (aggregate structure destroyed) and soil monoliths can be studied. Flat boxes, in which quasi 2D root systems are formed in a narrow slit filled with soil come in various dimensions. The so-called “Hohenheim” box is inclined to force the root system to develop preferentially along the lower cover plate (Dinkelaker and Marschner 1992). This type of microcosms is usually filled with repacked soil or artificial substrates, which may be arranged in zones of different properties (Hodge et al. 1999). Often the boxes are at least partly transparent to allow the visual observation of root development. Rhizosphere gradients can be assessed by sampling the soil in different distances from the root. More importantly, such microcosms are ideal for the application of non-invasive methods for *in-situ* characterization of gradients. Soil solution can be sampled in defined distances from given root segments as described below. The advantage of having roots in direct contact with soil is contrasted by the difficulties of detecting small effects by individual roots.

Microcosms in which membranes are used to separate compartments or root mats

Membranes, usually made of poly-amide, are used to separate microcosms into different compartments. Membranes with a mesh size of 20-30 μm can be penetrated by fungal hyphae and root hairs, but not roots. Membranes with a mesh size of 0.45 μm allow exchange of soil solution and gases but neither hyphae nor roots can penetrate.

Compartment systems are devices, in which membranes are used to separate “root zone”, “fungal hyphae zone” and root / hyphae free soil. Often the properties of the different compartments are compared as a whole. If root density in the root compartment is large, rhizosphere gradients may be observed in an adjacent soil compartment (Corgié et al. 2003; Vetterlein and Jahn 2004).

In other systems dense root mats are formed which are in contact with the soil via the membrane (Fig. 2). The root mat itself can be in contact with soil or an artificial substrate (Gahoonia and Nielsen 1991), or it is formed in an air-filled compartment (Wenzel et al. 2001). Such systems are ideal for assessing chemical rhizosphere gradients by sampling the soil or the soil solution in the root-free compartment in defined distances from the membrane. The root mat approach has the advantage of amplifying the root influence, and thus to enable the detection also of otherwise small effects.

However, the results may not be representative for field conditions with less dense root systems. Also, the exchange of water and ions between root and soil can be affected by the membrane (Fitz et al. 2006).

Field systems

Lysimeters are large 3D, usually cylindrical, and often weighable structures to study water, element and ion fluxes in larger soil volumes under field conditions (not to be confused with tension or tension-free lysimeters which are soil solution collection devices). Lysimeters either contain a soil monolith or are refilled with loose soil material. While refilled lysimeters allow to establish experimental setups with several treatments under the same soil conditions (Luster et al. 2008), monolith lysimeters provide a controlled access to naturally structured soil (Bergström and Stenström 1998). Rhizosphere in a microscopic sense cannot be studied unless coupled to observation tools such

as mini-rhizotrons (Majdi 1996). However, plant effects on soil can be studied by comparing planted and plant-free lysimeters.

There are several designs of root windows described in the literature (Polomski and Kuhn 2002). The most common type consists of glass- or plexiglass plates pressed onto a soil profile and can be combined with sampling and observation methods similar to microcosms of the “flat box” type (Dieffenbach and Matzner 2000).

Sampling and characterization of rhizosphere soil and soil solution

Dependent on soil texture and structure, plant species and observed parameter, root induced changes of most soil properties can be observed up to a distance of a few μm to about 7 mm from the surface of an active root segment or a root mat (Jungk and Claassen 1997; Jones et al. 2003). Sampling procedures for rhizosphere soil and solution have to cope with this demand for spatial resolution. However, rhizosphere effects may also reach beyond this range when considering highly mobile compounds like water or CO_2 (Gregory 2006, Hinsinger et al. 2005) or when including the effects of fungal hyphae extending from mycorrhizal root segments (“mycorrhizosphere”, e.g. Agerer 2001).

Sampling rhizosphere soil

For the separation of rhizosphere soil from so-called bulk soil several procedures based on shaking or washing-off soil particles adhering to roots have been proposed. First, the root system, together with adhering soil is carefully removed from the soil. Then Naim (1965) obtained rhizosphere soil by shaking the root system for 5 minutes in water. Turpault (2006) defined bulk soil, rhizosphere soil (detaches spontaneously when drying the root system) and rhizosphere interface (falls off when shaking the dried root system). Others define the soil falling off when shaking the root system as bulk soil and only the soil that is removed by subsequent brushing as rhizosphere soil (Yanai et al. 2003). Because soil texture and actual soil moisture strongly influence the amount adhering to the root system, results from different experiments should be compared with caution.

Slicing techniques require root mat type microcosms. Gahoonia and Nielsen (1991) sliced the frozen soil with a microtome in different distances to the root mat. Because freezing the soil may alter its chemical properties, Fitz et al. (2003a) developed a device that allows thin-slicing without freezing.

Characterization of rhizosphere soil

For the characterization of separated rhizosphere soil in principle all soil analytical methods published in text books (see introduction) or recommended by organizations such as Deutsches Institut für Normung (www.din.de), United States Environmental Protection Agency (www.epa.gov) or United Nations Economic Commission for Europe (www.unece.org) may be used.

There are two major groups of methods for chemical soil properties. The first deals with the total analysis of the soil solid phase, which is generally of little interest to rhizosphere research. The exception is total C and N analysis which is well applicable because of the small amounts of sample required by modern elemental analyzers. The second group comprises a large variety of extraction procedures to characterize different fractions of soil bound molecules or ions. Extractions for organic compounds (root and microbial exudates, contaminants) usually aim at complete recovery. Volatile organic compounds with a boiling point < 200 °C are purged from a heated soil suspension in water or methanol by an inert gas and trapped on suitable sorbents, while less volatile compounds are extracted using suitable solvents and applying different techniques (Sawhney 1996). By contrast, extractants for elements, inorganic ions and inorganic or organometallic compounds are often chosen to obtain a bioavailable fraction. An overview of commonly used extractants for this purpose is given in Table 1. Note that fractions are defined mainly operationally, and thus results obtained with different methods may not be easily compared. Nevertheless, depending on extractant, element and plant species there may be good correlations between extractable element concentration and plant uptake (citations in Sparks 1996 or Pansu and Gautheyrou 2006). A comprehensive characterization of soil-bound elements can be achieved by sequential extractions. There are protocols defining several fractions for organic nitrogen and carbon (Stevenson 1996; VonLützow et al. 2007), phosphorus (Psenner et al. 1988; Kuo 1996) and trace metals (Tessier et al. 1979; Zeien and Brümmer 1989). Since extraction methods have been developed without sample volume restrictions, the often limited sample amount

may hamper their application in rhizosphere research, depending on analyte content in the soil and on the sensitivity of the analytical method. Generally extracts can be analysed by commonly available analytical equipment such as potentiometry, molecular absorption spectrometry, gas and liquid chromatography, atomic absorption spectrometry (AAS) or inductively-coupled plasma optical emission spectrometry (ICP-OES). Only the detection of less-abundant analytes asks for more specialised equipment involving mass-spectrometric detection. Because the availability of standard reference materials for extractable contents in soils is limited (www.nist.gov/srm; www.erm-crm.org), most extraction methods require the use of internal references and the traceability of instrument calibration to certified standards.

Isotopic exchange is another method for determining bioavailable contents applicable to ions of a few elements with radioactive isotopes (PO_4^{3-} , SO_4^{2-} , K^+ , Zn^{2+} , Cd^{2+}) (Frossard and Sinaj 1997). A small amount of isotopic tracer is added to a soil suspension and the dilution of the label by homoionic exchange with the non-labeled ions at the soil solid phase is characterized. Either so-called E-values (contents in the soil solid phase that are exchanged within a defined incubation time), or kinetic parameters of the exchange are determined.

Collection of soil solution

Göttlein et.al. (1996) presented a system for the microscale collection of soil solution based on micro suction cups made of ceramic capillaries with an outer diameter of 1mm. Their system was used successfully to detect gradients in the rhizosphere (Göttlein et.al. 1999). Matrices of micro suction cups placed in front of a developing root system allowed to monitor the changes in soil solution chemistry when the root system passed through (Fig. 3; Dieffenbach et.al. 1997). This micro suction cup system was slightly modified by Dessureault-Rompré et al. (2006) to allow for localized collection of carboxylate anions and by Shen and Hoffland (2007) who introduced polyethersulfone as porous cup material. Puschenreiter et al. (2005a) presented a suction cup with a different geometry based on a nylon membrane (diameter 3mm) suitable for sampling soil solution in a defined distance to root mats. Sampling soil solution with micro suction cups faces the same problems and restrictions as with ordinary suction cups, just on a smaller scale. Firstly, sampling is influenced by the contact

with the soil matrix, and by texture and actual moisture of the soil. Secondly, analytes may be sorbed by or released from the sampling system (Rais et al. 2006), which asks for thorough testing of a particular system for a given problem. Nevertheless, the method has been applied successfully to assess rhizosphere gradients for major inorganic cations and anions (Wang et al. 2001), organic acid anions (Dessureault-Rompré et al. 2006) and trace metals (Shen and Hoffland 2007). Alternatively, soil solution can be trapped by the application of filter papers, cellulose acetate filters or blotting membranes onto roots exposed in flat rhizoboxes, a method which has been used mainly for the collection of root exudates or root-secretory enzymes (Neumann 2006).

Analysis of small volumes of aqueous solution

The miniaturization of sampling devices also minimizes the sample volume available for analysis. In principle all common analytical methods like ICP-OES, AAS, HPLC (high performance liquid chromatography), IC (ion chromatography), or colorimetry (manual or automatic as in flow-injection and auto analyzers) can be used, because except for flame AAS and standard ICP applications the sample amount needed for the measurement itself is not very high. The main task in adapting analytical methods to small sample volumes often is to optimize the autosampling system (Table 2). There are techniques available that significantly reduce the sample consumption of ICP-OES (Mermet and Todoli 2004) or ICP-MS (Prabhu et al. 1993; Lofthouse et al. 1997), which is normally in the range of several milliliters. Capillary electrophoresis (CE) offers the possibility to analyze samples as small as one droplet. Göttlein and Blasek (1996) optimized CE for the analysis of major cations and anions in soil solutions. Because CE is a true ion-analytical method it offers the possibility to detect the potentially phytotoxic Al^{3+} ion, which is of particular interest for studies of acidic soils (Göttlein 1998). Combining the analysis of labile species by CE or miniaturized voltammetric systems (Tercier-Waeber et al. 2002) with total analysis by graphite furnace AAS or micro-injection ICP methods (Göttlein 2006) allows metal speciation in rhizosphere solutions. ISFET-sensors enable pH measurements in one to two droplets (Göttlein and Blasek 1996), and afterwards the sample can be used for other analyses, because the sensors do not contaminate the sample like standard pH electrodes. Dissolved organic carbon (DOC) in small sample volumes can be measured using TC

analyzers with a direct sample injection option, or, taking the UV absorption as an indirect measure, using an HPLC system with a UV-detector but without separation column (Göttlein and Blasek 1996). Employing the microanalytical methods described above, a comprehensive characterization of soil solution including metal speciation is possible with a sample volume of about 250 µl. If only pH measurement and CE analysis of cations and anions are done, 30 to 50 µl are sufficient. Very small liquid sample volumes may also be analyzed by scanning electron microscopy coupled with energy-dispersive X-ray analysis, however after sophisticated sample preparation (Bächmann and Steigerwald 1993). Since for small solution samples the risk of contamination or adsorption losses is particularly high, the proper preconditioning and cleaning of all devices and containers that the sample comes in contact with are pivotal to reliable results (for recommended methods see Nollet 2007). Furthermore, evaporation losses during sampling should be minimised (Göttlein et al. 1996). Some natural water standard reference materials (www.nist.gov/srm; www.erm-crm.org) can be used for total analysis. For speciation, quality assurance must rely on internal references.

Sampling and analysis of soil gases

Measuring the total efflux of CO₂ *in-situ* from a given, usually circular surface area of soil using infrared gas analysers is a well established and routinely used method. The contribution of rhizosphere respiration has been estimated either by comparing total soil respiration with respiration measured after terminating autotrophic respiration by detopping of plants (Andersen and Scagel 1997), girdling (Ekberg et al. 2007) or trenching (Sulzmann et al. 2005), or by applying suitable modeling to the soil respiration data (Raich and Mora 2005). Alternatively, rhizosphere respiration can be assessed by coupling ¹³C labeling of the plant shoots with sampling of the soil CO₂ efflux and analysing its δ¹³C using isotope-ratio mass spectrometry (Yevdokimov et al. 2007). Membrane probes allow the diffusive sampling of soil gases like CO₂, N₂O, CH₄ or H₂ at various soil depths in the field or in microcosms (Rothfuss and Conrad 1994; Yu and DeLaune 2006), and are sometimes coupled with on-line analysis (Panikov et al. 2007). It should be tested whether gradients in the partial pressure of gases from the rhizosphere to the bulk soil can be assessed with this technique.

The oxygen concentration in soil can be measured with microelectrodes in high spatial resolution (Rappoldt 1995).

Cutting-edge methods for studying plant effects on rhizosphere soil

In-situ assessment of soil solution

In-situ measurements of chemical variables in the rhizosphere involve both the characterization of the solid and the solution phase. Impregnating rooted soil “profiles” in microcosms with dye indicators dissolved in agarose gel has been used for assessing root induced changes in pH (Fig. 4) and the exudation of aluminum complexing ligands or Fe(III) reducing agents (Engels et al. 2000; Neumann 2006). Root-induced Mn reduction and the excretion of acid phosphatases can be detected by applying specially impregnated filter papers to the rooted soil “profiles” (Dinkelaker and Marschner 1992; Dinkelaker et al. 1993). While such staining methods can be used to monitor pH changes in the rhizosphere with time in artificial systems composed of agarose gel (Plassard et al. 1999), they can hardly be used for a continuous monitoring in real soil. Recently, a novel non-invasive method was presented by Blossfeld and Gansert (2007) for the visualisation of rhizosphere pH dynamics in waterlogged soils using a pH-sensitive fluorescent indicator dye in a proton permeable polymer matrix (pH planar optode). However, the applicability of this method to non-saturated soils has still to be proven. In aerated soils, antimony micro-electrodes allow high resolution monitoring of root induced changes of pH in the rhizosphere (Häussling et al. 1985; Fischer et al. 1989; Zhang and Pang 1999). Measuring soil redox potential with Pt micro-electrodes dates back to Lemon and Erickson (1952) and has seen improvements to date (Hui and Tian 1998; VanBochove et al. 2002; Cornu et al. 2006). In particular, they were used in microcosms to monitor redox gradients in the rhizosphere of rice in order to study the formation of iron plaque on roots (Bravin et al. 2008). Except for a single application of Na⁺ ion selective electrodes by Hamza and Aylmore (1991), this methodology has not been applied to other chemical parameters due to the lack of suitable electrodes that can be operated reliably in soil. The DGT-technique (diffusive gradients in thin-films, Zhang et al. 1998) has been developed to evaluate the phytoavailable pool of metals and phosphorus. A DGT device consists of a gel-embedded resin layer acting as a sink for the species of interest, overlaid by another gel layer and a filter through

which the molecules or ions have to diffuse to reach the resin. Element and ion contents in soil extracted by DGT correlate well with contents in plants (Zhang et al. 2001). Up to now, DGT devices have been applied mostly to moist pastes of separated soil samples. However, they are particularly promising tools for direct application to the surface of rooted soil “profiles” in rhizoboxes (Fitz et al. 2003b; Nowack et al. 2004). Spatially resolved maps of DGT extractable species can be obtained by slicing the resin gel prior to analysis (Zhang et al. 2001) or by measuring the metal in the resin gel by laser ablation ICP-MS (Warnken et al. 2004).

Biosensors

Whole-cell bacterial biosensors are constructed by insertion of a gene coding for an autofluorescent protein, the most common one being the *lux* gene for the green fluorescent protein (GFP) (Killham and Yeomans 2001). Three types have been developed, differing by the physiological process the expression of bioluminescence is related to. Firstly, in non-specific biosensors, bioluminescence is related to the basal metabolism. They can be used to detect C rhizodeposition (strains with a broad range of substrates should be chosen to account for all exudates) and rhizosphere bacterial colonization. In semi-specific biosensors, luminescence is linked to a generic process such as oxidative stress. In specific biosensors, lighting reports on the expression of a specific pathway such as the utilisation of a particular exudate compound, the degradation of or resistance to a given contaminant. A number of biosensors have been developed to estimate the bioavailability of organic and inorganic contaminants (Hansen and Sørensen 2001). While the simplicity and rapidity of the measurement, and the possibility to monitor *in situ* various substances over time make biosensors attractive, their application to real-world environmental samples is still a challenge (Rodriguez-Mozaz et al. 2006). They cannot be applied directly to soils because soil particles absorb part of the emitted light, and some soil constituents are autofluorescent. Usually, either the biosensor is inoculated and then extracted from the soil before analysis, or the biosensor is applied to a solution after an extraction stage. Several parameters should be considered carefully during the analysis such as the colonization of the medium, the survival of the organisms over time, and possible matrix effects due to the presence of organic matter, other contaminants, etc. The distribution of compounds can be visualised by

combining biosensors with imaging by a CCD camera, as shown for root exudates in sand microcosms (Paterson et al. 2006). In most cases, the measured signals are used to compare different conditions, but not to determine the actual concentration of a compound.

Characterization of ultrastructure and element mapping using microscopic, diffractometric and spectroscopic techniques

This subsection is restricted to studies of the soil solid phase, while the characterization of roots is addressed in Neumann et al. (2008). Standard techniques for two-dimensional element mapping are scanning electron microscopy (SEM) and transmission EM (TEM) coupled with energy dispersive X-ray microanalysis (EDX). Energy filtered TEM (EFTEM) offers a higher resolution and better detection limit (about 10 nm and 1-10 $\mu\text{g g}^{-1}$, respectively). Other tools for two-dimensional element mapping include synchrotron-based micro X-ray fluorescence (μSXRF), micro-particle induced X-ray emission (μPIXE), secondary ion mass spectrometry (SIMS) and laser ablation (LA)– ICP-MS. SIMS and LA-ICP-MS have been coupled with stable isotope probing (SIP) to image the distribution of C isotopes in the soil at a sub- μm (nanoSIMS) and sub-mm (LA-ICP-MS) resolution (Bruneau et al. 2002; DeRito et al. 2005). Three-dimensional images of soil porosity can be obtained non-invasively by X-ray computed tomography (CT) (Mooney et al. 2006a), a method also used to study root architecture *in-situ* (Hodge et al. 2008). Alternatively, Moran et al. (2000) used X-ray absorption and phase contrast imaging to study the relation between roots and soil structure, and Mooney et al. (2006b) investigated the relation between the structure of a mineral landfill cap and root penetration by polarising microscopy.

The various microscopic techniques listed above can be used on any growth system (artificial, microcosm or field soil) after appropriate sample preparation. This sample preparation is a critical step for rhizosphere samples because they contain living and hydrated components. Classical procedures involving dehydration, chemical fixation, resin embedding and staining are progressively replaced by cryo fixation. The latter enables the measurement of hydrated samples with techniques such as SEM, TEM, μXRF and μPIXE , thus limiting possible artefacts related to dehydration and keeping the systems in a more natural state (Fomina et al. 2005). Environmental SEM (ESEM) also enables

observation and analysis of hydrated root and soil samples with minimal perturbation (e.g. Houghton and Donald 2008), however at a limited resolution.

Despite recent advances in data acquisition time each analysis by a microscopic technique implies a compromise between resolution and size of the sample. Therefore, the representativeness of the samples should be evaluated, possibly by upscaling from high resolution to coarser observation scales.

Mineral weathering and formation of secondary minerals have been studied intensively by EM techniques, particularly by SEM-EDX (Gadd 2007) and TEM-EDX (Hinsinger et al. 1993). Observing the size and shape of minerals and estimating their composition allow to predict the nature of the minerals present. X-ray diffraction (XRD) allows a direct identification of minerals. Standard powder diffractometers are limited by the amount of sample required (1 g), but recent instruments require only a few tens of mg. Using EM and XRD, various precipitates and products of mineral weathering were detected in the vicinity of fungi and roots (Hinsinger et al. 1993; April and Keller 2005; Gadd 2007). However, the weak sensitivity of XRD for minor phases remains a major limitation. It can be partly overcome by micro-XRD (μ XRD) using laboratory or synchrotron X-ray sources, or by separation prior to XRD analysis. Furthermore, XRD on oriented clays, which requires only a few mg of particles, is suited to trace changes in clay mineralogy occurring in the rhizosphere, as shown in artificial substrates (Hinsinger et al. 1993) and in soils (Kodama et al. 1994). Recently, Barré et al. (2007) proposed a more quantitative approach for studying changes in the composition of the clay fraction in the rhizosphere.

The local chemical environment of metals can be assessed by X-ray absorption spectroscopy (XAS), including X-ray absorption near edge structure (XANES, also called NEXAFS for near-edge X-ray absorption fine structure) and extended X-ray absorption fine structure (EXAFS) spectroscopy. Major advantages of these techniques include element specificity, sensitivity to amorphous and weakly crystalline species, and detection limits of 100 to 300 mg kg⁻¹ depending on target element and matrix.

Bulk XAS provides information on major metal species. This technique was combined with μ XRF (Voegelin et al. 2007) and X-ray fluorescence microtomography (Hansel et al. 2001; Blute et al. 2004) to study the distribution and speciation of heavy metals in the root plaque of plants growing in flooded environments. These studies revealed a heterogeneous composition of Fe(III) and Fe(II)

phases with associated trace element species including As(V) and Zn(II), whereas Pb(II) was complexed by organic functional groups possibly belonging to bacterial biofilms. Micro-XAS (μ XAS), generally combined with bulk XAS and μ XRF, provides information on the chemical form of metals with a lateral resolution of a few μm^2 to a few hundreds of nm^2 (Manceau et al. 2002). These tools were used to study the impacts of remediation treatments on metal speciation in contaminated substrates (Fig. 5; Nachtegaal et al. 2005; Panfili et al. 2005, Manceau et al., 2008). Micro XRD, available as additional tool on some spectrometers, allows the simultaneous identification of crystalline metal bearing phases. These tools can be applied to any growth system (artificial, microcosm or field soil) after homogenizing and grinding (for bulk XAS), or after resin impregnation followed by thin sectioning (for μ XRF/ μ XAS/ μ XRD). A major limitation of these synchrotron-based techniques (and of state-of-the art microscopic facilities in general) is their restricted access due to the small number of beamlines and microscopes worldwide.

The speciation of light elements including carbon, nitrogen, sulfur and phosphorus can be studied by bulk XANES and by scanning transmission X-ray microscopy (STXM, including μ XRF and μ XANES) using soft X-rays (Myneni 2002). The X-ray spot sizes are generally $< 1 \mu\text{m}$ and can be as small as few tens nm. Working with wet systems is also possible in some spectrometers. These techniques have been used to study soil colloids (Schumacher et al. 2005) and bacterial biomineralization (Benzerara et al. 2004) at the single-particle and single-cell scale, respectively. Electron energy loss spectrometry (EELS) is a more exotic technique for speciating elements. Main advantages are the coupling with TEM imaging and the very good lateral resolution of around 10 nm (Watteau and Villemin 2001).

^{13}C , ^{31}P , ^{15}N and ^1H solid and liquid state nuclear magnetic resonance (NMR) spectroscopies are classical tools for the characterization of molecular structures and functional groups in soil organic matter (SOM) and for the identification of low molecular weight molecules (Fan et al. 1997). Advanced techniques such as high-resolution magic-angle spinning and 2D NMR open new possibilities (Kelleher et al. 2006). The large sample size required for solid state NMR (0.5 to 1 g of isolated SOM compared to a few tens of mg for liquid state NMR), limits its use for rhizosphere applications. Fourier transformed infrared (FTIR) spectroscopy is another classical tool for the

characterization of molecular structures in SOM. Attenuated total reflectance (ATR)-FTIR allows the study of wet systems, and FTIR microscopy enables 2D mapping with a resolution of a few micrometers (Raab and Vogel 2004). Electron paramagnetic resonance (EPR) has been used to quantify free radicals in organic molecules, and to study the interaction of paramagnetic metals with SOM in terms of oxidation state, ligand types and coordination geometry (Senesi 1996). For EPR, the same sample size restrictions apply as for solid state NMR.

Labelling with and tracing / imaging of stable and radioactive isotopes

Carbon fluxes in the rhizosphere can be assessed by $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ pulse-labelling the atmosphere of a plant soil system, and measuring the radioactivity or the $\delta^{13}\text{C}$ value in the compartment of interest (soil, isolated DOC, microbial biomass, roots, etc.) by liquid scintillation or isotope ratio mass spectrometry (IRMS), respectively (Killham and Yeomans 2001, Rangel Castro et al. 2005). Gas chromatography may be coupled with IRMS in order to probe a specific molecule or family of molecules (Derrien et al. 2005). A more exotic method is the labelling with ^{11}C (Minchin and McNaughton 1984).

Laterally resolved information on the distribution of an isotope can be obtained in different ways. Gradients around roots can be determined using microcosms of the root mat type and analyzing slices of soil at various distances from the root mat (Kuzyakov et al. 2003). Microcosms of the “Hohenheim” type allowed to assess the equilibration of stable isotope labels for Mg, K and Ca between rhizosphere soil and solution (Göttlein et al. 2005). Autoradiography on flat microcosms provides non-invasive 2D imaging of the distribution of radioactive isotopes. Images were classically obtained on films or photographic emulsions, then on phosphor storage screens, and more recently by electronic autoradiography (Fig. 6; Rosling et al. 2004). Apart from following C fluxes, this versatile method can be used to characterize the spatial distribution and its change over time of added radioactive P (Hendriks et al. 1981; Hübel and Beck 1992; Lindahl et al. 2001), SO_4^{2-} (Jungk and Claassen 1997) or Zn and Cd (Whiting et al. 2000).

The use of stable isotope probing (SIP) to assess microbial activity in the rhizosphere is treated by Sørensen et al. (2008).

Mapping the plants influence on soil moisture

Using micro-tensiometers and small time-domain reflectometry sensors installed in rhizoboxes and compartment systems, one-dimensional rhizosphere gradients in soil moisture and differences between root and root-free compartments could be shown (Göttlein et al. 1996; Vetterlein and Jahn 2004).

Recently, microorganisms have been genetically altered to indicate changes in soil moisture by varying the expression of the green fluorescent protein as detected by epifluorescence microscopy (Cardon and Cage 2006).

Some of the methods to image root systems in microcosms are sensitive also to differences in substrate moisture and can therefore be used to assess the plants influence on soil moisture distribution. Light transmission imaging (Garrigues et al. 2006) is a rather inexpensive method with which large quasi 2D microcosms (e.g. 1000 x 500 x 4 mm) can be studied at a resolution of ≥ 1500 μm . With magnetic resonance imaging (MRI; Chudek and Hunter 1997; Herrmann et al. 2002), which depends on the accessibility to a medical imager or an NMR spectrometer with a suitable accessory, 3D images can be obtained from boxes (up to 70 x 70 x 20 mm) or cylinders (diameters up to 60 mm and heights up to 200 mm) at a resolution between 10 and several hundred μm . Considering the high spatial resolution, these methods are able to assess plant effects on soil moisture on the scale of a single-root. However, their applicability to real soil is limited by inherent incompatibilities. Light-transmission is restricted to translucent sand with addition of small amounts of clay and MRI to soils with low iron contents. By contrast, X-ray computed tomography allows to map root effects on structure and moisture distribution in real soils at a resolution of 100 μm to 1 mm for typically cylindrical samples with a diameter of a few cm (Hamza and Aylmore 1992; Gregory and Hinsinger 1999). The sensitivity to soil water content, however, is comparatively weak. Recently, Oswald et al. (2008) demonstrated the high sensitivity of Neutron radiography to differences in soil water content and could show variable water uptake by different parts of root systems growing in flat microcosms (170 x 150 x 13 mm) made of aluminum at a spatial resolution of ≥ 100 μm . Although the contrast is highest in quartz sand, the method can also be applied to natural soil (Menon et al. 2007).

Electrical resistivity tomography (ERT) and ground penetrating radar (GPR) are non-invasive

geophysical methods increasingly used in hydrological studies of the vadose zone. ERT is a comparatively inexpensive method exploiting the spatial variability in the electrical conductivity of the soil (Benderitter and Schott 1999). Among other applications the method can be used to monitor changes in soil water content in the field indirectly via inverse modelling of resistivity and the use of petrophysical relationships. Large stone contents make application of ERT difficult and spatial resolution for true non-invasive surface applications decreases strongly with soil depth. GPR velocity tomography can be used for the same purpose, because the water content influences the soils permittivity to radar waves (Annan 2005). The method, however, is ineffective in soils with clay. A few studies have made the attempt to use ERT and / or GPR tomography to examine spatial variability or temporal changes in soil moisture content caused by plant water uptake on the scale of the whole root system (Fig. 7; Michot et al. 2003; AlHagrey 2007). Theoretically, depending on the electrode spacing or the antenna frequency, the spatial resolution of ERT and GPR can be increased to the cm range. However, feasibility and applicability to map root-soil water interactions in the field on a smaller scale than the whole root system remain to be shown.

Rhizosphere Modeling

The nature of concentration gradients in the soil caused by plant activity depends mainly on two sets of factors that modeling needs to take into account. These are (i) physical and biological factors such as geometry, morphology and symbiotic status of the root system, rates of growth, uptake and exudation by roots, and diffusion properties of the soil around roots, and (ii) chemical factors such as the distribution and speciation of chemical elements in the soil.

There are two main approaches to model rhizosphere processes. The first category of models follows a macroscopic, empirical approach and operates on a whole plant or even field scale. Here the root system is treated as a single unit without considering the effect of individual roots. The second category deals with a single root or a root system and follows a microscopic approach. Table 3 gives an overview of the categories and the scales discussed in this chapter.

Macroscopic models

Macroscopic models are descriptive and explanatory and help to understand the dynamic and complex interactions occurring adjacent to roots (Darrah et al. 2006). These models can have several layers of complexity, ranging from simple single-root models to sophisticated whole-root system models.

Crop / forest models: Although many models predicting the flow of nutrients between soil and plants have been developed, few of these deal in detail with root processes. Such models often use a simplified approximation of rhizosphere processes and verification is at scales larger than the individual plant. Such models have been used intensively as a tool to analyze the performance of cropping systems under variable climate (Wang and Smith 2004) or forest growth affected by different environmental variables (Pinjuv et al. 2006). They typically involve many subprocesses and satisfactory verification does not guarantee that the rhizosphere subprocesses have been modeled accurately (Darrah et al. 2006). Root water uptake is normally treated in a highly simplified submodel, usually with the root system acting as a zero-sink for nutrients, with uptake controlled by soil water potential and transpiration rate or by diffusion flux rate (Darrah 1993). These models can be used to investigate the relative impact of integrated rhizosphere processes on plant and crop scales. They normally incorporate numerical schemes for deducing nutrient concentrations at root surfaces from bulk soil parameters, but do not represent the rhizosphere as a volume of soil with properties different from the bulk soil (Dunbabin et al. 2006). Some models also incorporate the influence of exudation or microorganisms on uptake (Siegel et al. 2003).

Biogeochemical ecosystem models: These models are used to identify the governing parameters in ecosystems in order to understand element or nutrient cycles or to predict ecosystem dynamics. Examples include the DNDC model which simulates soil carbon and nitrogen biogeochemistry (Li et al. 1994). A plant growth submodel is used to calculate root respiration, N uptake and plant growth and these processes are linked to climate and soil status. Biogeochemical models pay more attention to soil processes than crop models. Complexation, cation exchange, precipitation, and adsorption can be included in various degrees of complexity (Cosby et al. 1985; Alewell and Manderscheid 1998).

Soil profile scale: Soil physical models describing water transport in soils also include a root water uptake term, usually a pressure head dependant sink term that is introduced into the soil water balance (Hopmans and Bristow 2002). There has been a tendency to describe the root water uptake analogous

to Darcy's equation, assuming that the rate of uptake is proportional to soil hydraulic conductivity and the difference between the total pressure head at the root-soil interface and the corresponding pressure head in the soil. This approach is useful to understand the root water extraction process, but it is difficult to use for the interpretation of field data. Water transport models have been extended to include solute uptake. In one example a three-dimensional solute transport model including passive and active nutrient uptake by roots has been linked to a three-dimensional transient model for soil water flow and root growth (Somma et al. 1998).

Whole root system scale: Several root architecture models are available that simulate the growth of whole root systems at high spatial resolution to generate two or three-dimensional representations of root systems, e.g. ROOTMAP (Diggle 1988), SimRoot (Lynch et al. 1997) or Root Typ (Pagès et al. 2004). An example of a modeled root system is shown in Fig. 8a. Doussan et al. (2006) extended a whole root-system model to include water transport in soils with full coupling of water transport in the root system and the influence of aging on the hydraulic conductivity of root segments and thus on water uptake. The linking of such models to the underlying biology is not yet strongly advanced (Darrah et al. 2006). However, several models have been developed that take into account interactions between root systems, water and nutrients in the environment (Dunbabin et al. 2002). Wu et al. (2007) recently presented a dynamic simulation model that is multi-dimensional, operates on a field scale, is weather driven and models C and N cycling between plants, soil and microbes.

Microscopic models

Microscopic models, also called explanatory models, help to understand the complex and dynamic interactions in the rhizosphere and are based as far as possible on mechanistic relations derived from the laws of chemistry and physics and empirical relations (Kirk 2002). These models can be divided into two subgroups, the molecular and the semi-empirical models. The molecular models are based on the description of chemical processes by a suite of single reactions, e.g. speciation in solution or surface complexation. The semi-empirical models use a more simplified description of molecular processes, e.g. a buffer power to describe adsorption, desorption or precipitation/dissolution.

Semi-empirical models on the single root scale: Semi-empirical root models simulate the uptake of nutrients by an isolated root segment. The classical rhizosphere model is that of Nye and Tinker (1977) and (Barber 1995). It supposes a cylindrical root surrounded by an infinite amount of soil, with convection and diffusion of nutrients through the soil and uptake through Michaelis-Menten type kinetics at the root surface. The non-linearity of the model requires a numerical solution but recently an analytical solution of the equations was obtained (Roose et al. 2001). This model has also been extended to describe P or metal uptake in microcosms of the root mat type (Kirk 1999; Puschenreiter et al. 2005b). Most of these models are based on a rather simplified description of soil chemistry and the effects of plant roots. The actions exerted by roots on their rhizosphere are generally limited to element uptake, and the chemical interactions between dissolved elements and the soil are reduced to a buffer power or Freundlich adsorption isotherm (Barber 1995; Kirk 1999). Fig. 8b shows as an example the influence of citrate exudation on phosphate solubilization. The effect of exudation has been incorporated into the basic modeling concept, and conditional models parameterized for different soils have been formulated, e.g. to model the effect of organic acid exudation on phosphate mobilization (Gerke et al. 2000ab). The application of certain rhizosphere models requires to write a new computer program or to change existing software. Schnepf et al. (2002) have shown that pde-solvers are useful in rhizosphere modeling because they make it easy to create, reproduce or link models from the known constituting equations.

Semi-empirical models on the root system scale: An upscaling of single root models to the whole root system allows to predict plant uptake by integrating the flux on a unit segment basis over the total root length. The approach of Roose et al. (2001) allowed the direct incorporation of root branching structures and whole roots into plant uptake models, based on a mechanistic description of root uptake and soil processes (Roose and Fowler 2004ab).

Molecular soil solution models: In hydrogeochemistry, sophisticated computational tools have been developed to describe acid-base and redox reactions, complexation, ion exchange, adsorption and desorption, dissolution and precipitation of chemical species in soil environments using thermodynamic and kinetic relationships. Examples are PHREEQC (Parkhurst and Appelo 1999), ECOSAT (Keizer and VanRiemsdijk 1995) and ORCHESTRA (Meeussen 2003). Additionally there

are computer codes that are specialized in modeling three-dimensional transport in variably saturated media that include geo-chemical modeling, e.g. MIN3P (Mayer et al. 2002). Applications of some of these models to rhizosphere research is described in the forthcoming paragraphs.

In some of the semi-empirical models mentioned above, soil solution speciation was included as input parameter. Calba et al. (2004) modeled the effect of protons, solid phase dissolution and adsorption on aluminum speciation in the rhizosphere, and Puschenreiter et al. (2005b) considered Ni speciation in soil solution when looking at Ni uptake by a hyperaccumulator. Zhao et al. (2007) used speciation modeling to elucidate the effect of plant roots on metal mobilization and speciation in soils. However, in these last two examples speciation was considered static and not to be affected by root activity. In particular the feedback loops between exudation, soil and element uptake are not considered implicitly in single root models, although many authors have demonstrated their importance in the plant availability of mineral elements (Parker and Pedler 1997).

Molecular models at the single root scale: The full coupling of single-root models with speciation calculations is still in its infancy. An example of the inclusion of solution and surface speciation into rhizosphere models is the modeling of the effect of citrate exudation on phosphate uptake (Geelhoed et al. 1999). The model calculations showed that citrate exudation from roots increases the plant availability of sorbed phosphate (Fig. 8c). Recently a simple rhizosphere model was described in which the uptake into a single root was linked to three geochemical computational tools (ORCHESTRA, MIN3P, and PHREEQC) (Nowack et al. 2006). The first step in this approach was an accuracy analysis of the different solution strategies by comparing the numerical results to the analytical solution of solute uptake by a single cylindrical root. All models were able to reproduce the concentration profiles as well as the uptake flux. The strength of this new approach is that it can also be used to investigate more complex and coupled biogeochemical processes in the rhizosphere. This was shown exemplarily with simulations involving both exudation and the simultaneous uptake of solute and water.

Molecular models at the soil profile scale: The coupling of root uptake, speciation modeling and water transport in soils is even less advanced than on the single root scale. In order to describe metal uptake in the presence of ligands, Seuntjens et al. (2004) developed a model coupling processes under steady-

state flow conditions with rhizosphere processes and speciation modeling. The simulations showed that exudation of ligands does not necessarily increase the solubility and bioavailability of metals, but that bioavailability may actually be reduced by formation of ternary surface complexes or reduction of the free metal concentration. The model can be easily extended to include further processes.

Challenges ahead

Our review on current methodology to study the effects of root and microbial activity on soil properties in the rhizosphere has shown that – although there is a need for improvements in certain aspects as outlined below - in general we have the tools at hand to assess individual processes on the microscale under rather artificial conditions. This is true mainly for looking at soil chemical properties and processes, while due to still large methodological limitations our understanding of the biophysics of the rhizosphere is comparatively limited (Gregory and Hinsinger 1999), despite major recent advances (Pierret et al. 2007, Hinsinger et al. 2008). Microscopic, spectroscopic and tracer methods to look at individual and coupled chemical processes in small „aliquots“ of naturally structured soil seem to step out of their infancy and have become promising tools to better understand the complex interactions between roots, soil and microorganisms. On the field scale, however, while there are promising first results on using non-invasive geophysical methods to assess the plant’s influence on soil moisture, there are no tools in the pipeline to assess the spatial heterogeneity of chemical properties and processes in the field. For the time being, the use of macroscopic models or the upscaling of model results from the single root to the whole plant or plot scale is the only solution to this problem. However, upscaling itself is a major issue as outlined below. An optimal feedback between different developments requires a good communication between the various disciplines involved in rhizosphere research, in particular between experimental and modeling works. Both, early incorporation of new insights gained experimentally at the micro scale into explanatory models and involving models in experimental design could accelerate progress.

Methodological improvements for investigations at the micro scale

While most studies on root and microbial exudation limit their analysis to more abundant substances like sugars, carboxylates, amino acids and siderophores, the fate and role of many compounds like sterols or lactones that are exuded for signalling or as allelochemicals (Bertin et al. 2003) still need to be evaluated. Coupling of advanced chromatographic or electrophoretic separation methods with mass spectrometry allows to identify such compounds, e.g. in extracts of bacterial isolates (Frommberger et al. 2004). However, they cannot be detected in real soil solution with current methodologies.

Another challenge is to identify the source of a particular compound measured in soil solution, i.e. whether it has been exuded by plant roots, fungal hyphae or bacteria, or is the product of SOM degradation. Further advancements in compound specific isotopic analysis are needed in order to be able to trace ^{13}C labels to individual compounds. Currently, isotopic ratios can be determined for total DOC in small volumes of soil solution (Glaser 2005), while for individual compounds, even for more abundant ones, this will require drastic improvements in the detection limit of the coupled chromatography – IRMS instrumentation.

Considering the large potential of biosensors to assess the spatial heterogeneity of bioavailable molecules or ions, their *in-situ* application to microcosms containing real soil would be highly desirable. The difficulty to discriminate between the signals from biosensors and autofluorescent soil components must be overcome, and good correction factors for the reabsorption of the biosensor signal by soil particles must be determined. Furthermore, the development of multi-reporter gene biosensors, or the combined use of several biosensors in a given system, might help to control the influence of external factors (nutrient conditions, competition, inhibition factors, etc.), and thus to get more quantitative results in soils.

There have been great efforts to use microscopic and spectroscopic methods to assess the properties of soil and their components on the microscopic and molecular scale. The techniques are slowly getting sufficiently spatially resolved to separate components that are intimately associated. Apart from improving the capabilities of the instruments (flux and size of the incident beam, efficiency of detector systems) to get better sensitivity and resolution, efforts should focus on limiting the perturbation of the systems, e.g. by preserving their hydrated state, and better assessing or controlling the radiation

damages by X-ray, electron or particle beams. Another challenge is to link the molecular- and microscopic-scale information obtained by these techniques to information obtained at higher scale.

Upscaling

On the microscale, plant physiology and soil microbiology have developed a detailed understanding of plant water and nutrient uptake, root respiration, root release of organic carbon and interactions between roots and soil microorganisms. However, there is a lack of understanding as to how the multiple complex interactions in the rhizosphere affect ecosystem functions on the macroscale (soil profile, plot, catchment). There is an urgent need to improve the mechanistic bases of models aimed at crop growth, forest production or biogeochemical element cycling by including rhizosphere processes. Closing the gaps between the different scales, or in other words making explanatory or predictive models on the macro scale more process-based, is a major challenge in biogeochemical research. At present, most of the available upscaling approaches for soil water processes ignore the effects of vegetation or use an extremely simplified approach. There is a need to develop upscaling approaches that explicitly account for the effects of growing plants under field conditions (Vereecken et al. 2007). A step into this direction is BIOCHEM-ORCHESTRA, a modeling tool that integrates ecotoxicological transfer functions with speciation and transport modeling (Vink and Meeussen 2007). The plant module, however, is still very simple and uses only empirical parameters such as the relevant rooting zone and a time-dependent uptake behavior. Root architecture models such as Root Typ (Pagès et al. 2004) have a great potential to be linked with other model approaches and could thus contribute significantly to the integration at higher scales. On the opposite end of the scale spectrum, there is an urgent need for new modeling approaches that combine the molecular description of chemical processes in soils with pore-scale transport and root uptake. Up to now, molecular scale analytical tools and modeling approaches have developed rather independently. The coupling of 3-dimensional root growth modeling, root uptake, speciation modeling and water transport in soils presents challenges both on the computational and on the conceptual level. An example of a first step into this direction is the modeling of the effects of phospholipid surfactants on nutrient and water uptake by whole root systems (Dunbabin et al. 2006).

One key problem in the upscaling of rhizosphere processes is to assess correctly the distribution of active root segments in the soil. Non-invasive methods like X-ray computed tomography and MRI can, under certain conditions, produce well-resolved 3D images of the root system, but they are restricted to small laboratory systems. First results have demonstrated the potential of ERT and GPR to provide coarse images of root systems non-invasively and *in-situ* in the field via their imprint on soil moisture distribution. With GPR reflection it was even possible to resolve larger single roots in a silty sand (AlHagrey 2007). This warrants further exploration of geophysical methods in terms of delineating response from roots and soil structural heterogeneities, of improving spatial resolution (ERT), and of application to soils with higher clay contents (GPR).

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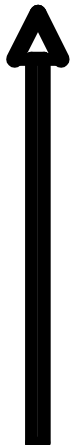
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1243

1244 Table 1: Common extractants for elements and ions grouped approximately in decreasing order of
 1245 plant availability as compiled from standard method collections. For most extractants there are several
 1246 slightly different protocols in terms of extractant concentration, extraction time, etc.. Also, there can
 1247 be large differences in the extractive power of a given extractant depending on soil properties such as
 1248 pH or soil organic matter content (e.g. some extractants can only be used either for calcareous or
 1249 acidic soils).
 1250

Phytoavailability of extracted species	N	P	K, Ca, Mg	Fe, Al	Trace metals
	H ₂ O	H ₂ O	H ₂ O	H ₂ O	H ₂ O
	hot H ₂ O; NH ₄ ⁺ , NO ₃ ⁻ in salt extracts (KCl, CaCl ₂ ..)	Ca-lactate; NH ₄ -lactate; Citrate	NH ₄ Cl ^a ; BaCl ₂ ^a	NH ₄ Cl ^a ; BaCl ₂ ^a	NaNO ₃ ; NH ₄ Cl ^a ; BaCl ₂ ^a ; NH ₄ -acetate
		Ca acetate/ lactate; NaHCO ₃ ; NH ₄ F/HCl	HNO ₃ ; HCl	EDTA; NH ₄ -oxalate	NH ₄ -EDTA; NH ₄ -oxalate
		H ₂ SO ₄	HCl / HNO ₃	Na-dithionite; HCl/HNO ₃	HNO ₃ ; HCl/HNO ₃

1251 ^amethods to determine exchangeable cation contents; from the sum of all major cations the
 cation exchange capacity of the soil can be calculated

1252 Tab.2: Techniques for analyzing main parameters of aqueous solutions and their applicability to
 1253 rhizosphere research

Technique (analytes)	Availability, costs	suitability for / adaptation to rhizosphere research (limited sample amount)
potentiometry (pH)	common, low	ISFET instead of glass electrodes
flow injection analysis (NH ₄)	common, low	autosampler and sample loop limiting
Voltammetry (labile metal cations)	special, low	micro-sensors necessary, however sample demand still in ml-range
TC/TN analyser (DOC, CO ₃ , N _{tot})	common, intermediate	autosampler and sample injection limiting; direct injection option reduces sample demand to 50 µl
ion chromatography (inorganic anions, organic acids, NH ₄)	common, intermediate	autosampler and sample loop limiting; microbore systems allow reduction of sample demand to the sub-µl-range
HPLC (organic acids, sugars, etc.)	common, intermediate	as for ion chromatography
Flame AAS (total metal conc.)	common, intermediate	hardly possible because of high sample demand
Graphite furnace AAS (total metal conc.)	special, intermediate	suitable, sample demand of 20 to 50 µl for single element analysis
capillary electrophoresis (inorganic anions, organic acids, free metal cations, NH ₄)	special, intermediate	with a demand of 20 nL suitable for the analysis of minimal sample amounts
ICP-OES	common, expensive	special nebulizers for lowering sample demand to about 100µl for multielement analysis
ICP-MS	special, expensive	as for ICP-OES

1254

1255

1256 Table 3: Approaches and scales in rhizosphere modeling

Model type		Model scale	Main model targets	Examples
Macroscopic (empirical)		Agricultural field / forest	Plant yield, forest growth	Pinjuv et al. (2006); Siegel et al. (2003); Cosby et al. (1985)
		Ecosystem	Element and nutrient cycles	Li et al. (1994)
		Soil profile	Water transport	Somma et al. (1998)
		Whole root system	Root growth	Diggle (1988); Doussan et al. (2006); Dunbabin et al. (2002); Lynch et al. (1997)
Microscopic (explanatory)	semi-empirical	Single root	Root processes	Nye and Tinker (1977); Barber (1995); Kirk (1999); Roose et al. (2001)
		Root system	Root system development	Roose and Fowler (2004ab)
	molecular	Soil solution	Speciation in solution	Calba et al. (2004); Puschenreiter et al. (2005b)
		Single root	Integration of chemical reactions	Geelhoed et al. (1999); Nowack et al. (2006)
		Soil profile	Integration of all mechanisms	Seuntjens et al. (2004)

1257

1258

Figure captions

Fig. 1. Rhizosphere as 3-phase system with soil solid phase (SP), soil solution (SS), and soil gas phase (SG); spatial heterogeneity along and perpendicular to root growth added by a developing root system is emphasised and is overlaid by temporal variability: root growth (A), turnover of roots and fungal hyphae (B), diurnal or seasonal changes in the activity of roots (exudation, uptake; C), or associated organisms (D).

Fig. 2. Example of a root mat type microcosm. It is composed of a lower part containing a thin soil layer (1-3 mm thick; or, alternatively, a soil cylinder of greater height if aiming at studying rhizosphere gradients), and of an upper part containing the root mat, separated by a polyamide membrane. For pregrowth, the upper part is immersed in aerated nutrient solution (adapted from Guivarch et al. 1999, Figure 1; with kind permission from Springer Science+Business Media); for further explanations see Chaignon and Hinsinger (2003).

Fig. 3. Studying the influence of a growing oak root on soil solution chemistry using a micro suction cup array installed in a “Hohenheim” type microcosm (adapted from Göttlein et al. 1999; with kind permission from Springer Science+Business Media)

Fig. 4 Effect of soil-buffering capacity (CaCO_3 content) on the extension of root-induced rhizosphere acidification of chickpea (*Cicer arietinum* L.) seedlings 12 DAS, detected in “Hohenheim” type microcosms by soil impregnation with pH-indicator (bromocresol purple) agar (from Römheld 1986; courtesy of the International Potash Institute, Switzerland)

Fig. 5: Zn K-edge bulk EXAFS spectra of a Zn-contaminated sediment (control), treated with mineral amendments and planted with *Agrostis tenuis*, and distribution of Zn species determined from the analysis of these data and μEXAFS spectra. The amendments induce a significant oxidation of ZnS and the formation of secondary species. These effects are strongly enhanced in the presence of *A.*

tenuis, with an almost complete removal of ZnS (adapted from Panfili et al. 2005; Copyright Elsevier (2005)).

Fig. 6: Peat microcosm containing *Pinus sylvestris* seedlings colonised by *Hebeloma crustuliniforme* and pure mineral patches of either K feldspar (K) or quartz (Q). Fifteen weeks after introducing mineral patches at the growing mycelial front (a), the shoots were pulse labelled with $^{14}\text{CO}_2$. Greater amounts of labelled carbon are allocated to root tips and mycelia associated with patches of F feldspar compared to patches of quartz (b). CPM: counts per min. (adapted from Rosling et al. 2004; with kind permission from the New Phytologist Trust).

Fig. 7: Changes in soil moisture in a profile during drying shown as difference between the inverted electrical resistivity at about 8 days after irrigation and immediately after irrigation. Root zones of corn rows (R1 to R8) show as dark zones that dry out quickly (adapted from Michot et al. 2003; Reproduced/modified by permission of American Geophysical Union)

Figure 8: Examples of different rhizosphere models. a) Macroscopic model, whole root system scale: modeled root system of *Lupinus albus* (from Doussan et al. 2006; with kind permission from Springer Science+Business Media). b) Microscopic, mechanistic single root model of citrate exudation and its influence on phosphate solubilization (dots: experimental; black line: modeled P in soil; dotted line: P in solution; dashed line: citrate in soil) (from Kirk 1999; with kind permission from Blackwell Publishing). c) Microscopic single root model, molecular scale: influence of citrate on phosphate mobilization (P in solution in the absence and presence of citrate exudation) (from Geelhoed et al. 1999; with kind permission from Blackwell Publishing).

Figure 1
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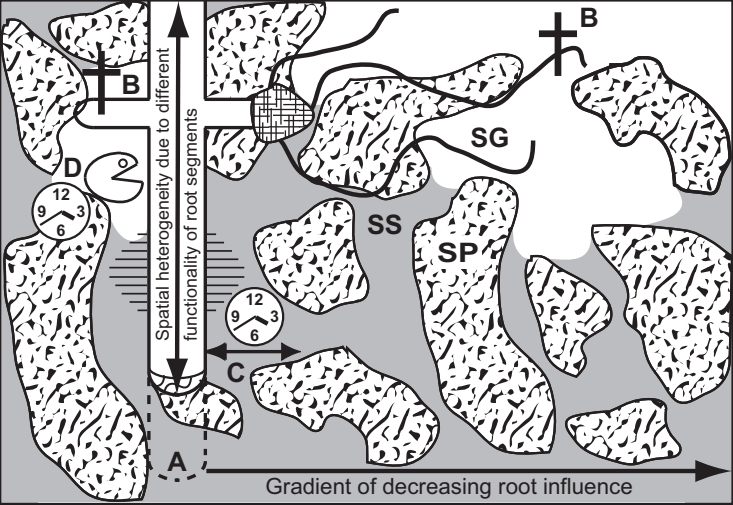


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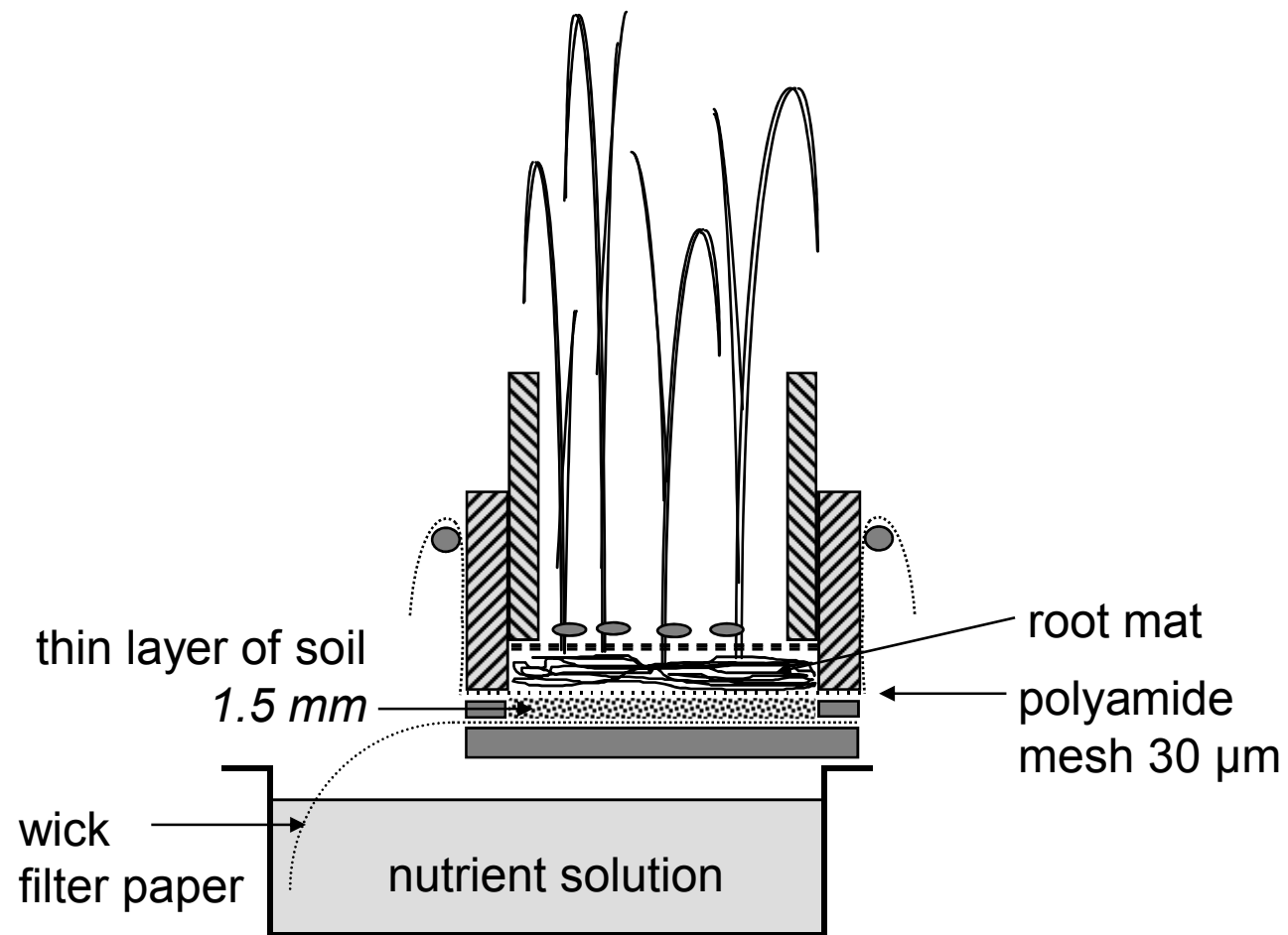


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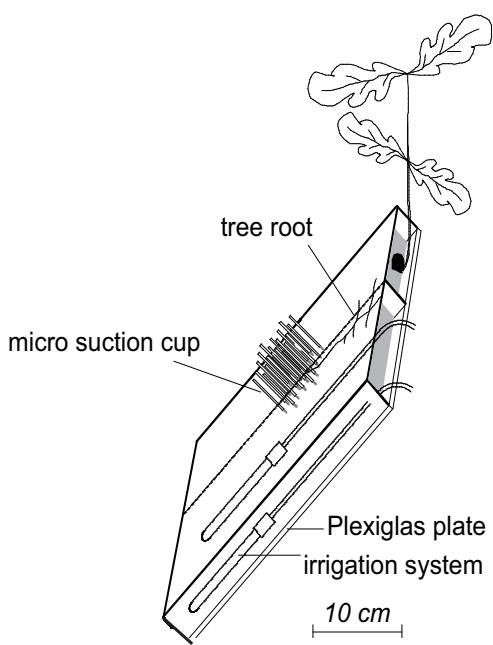


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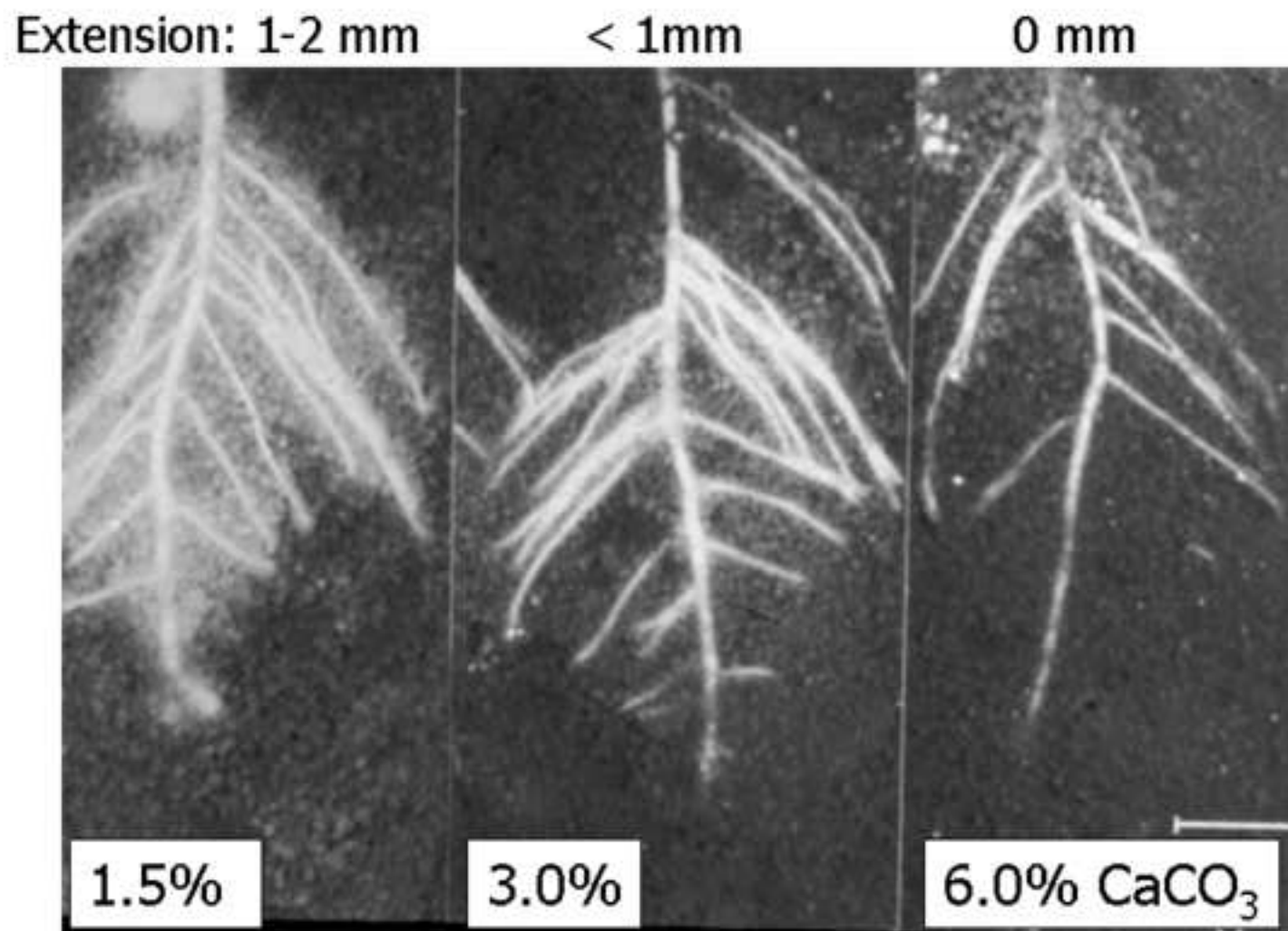


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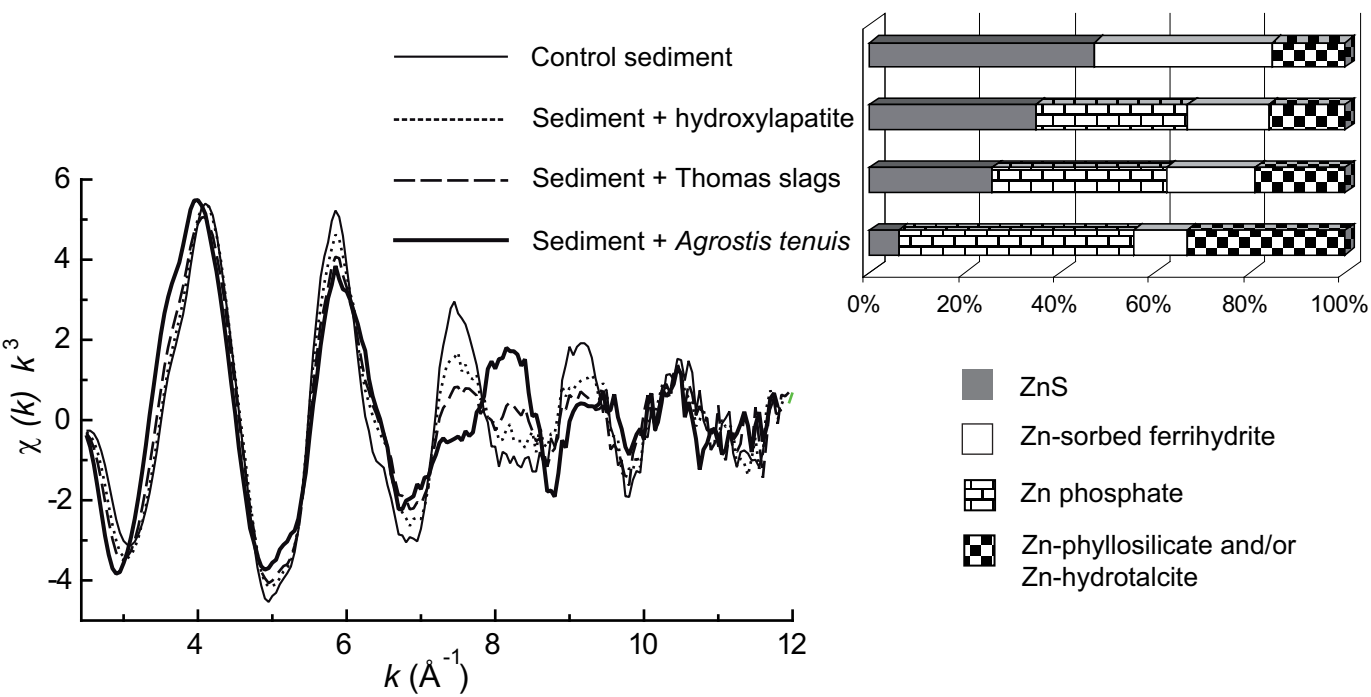


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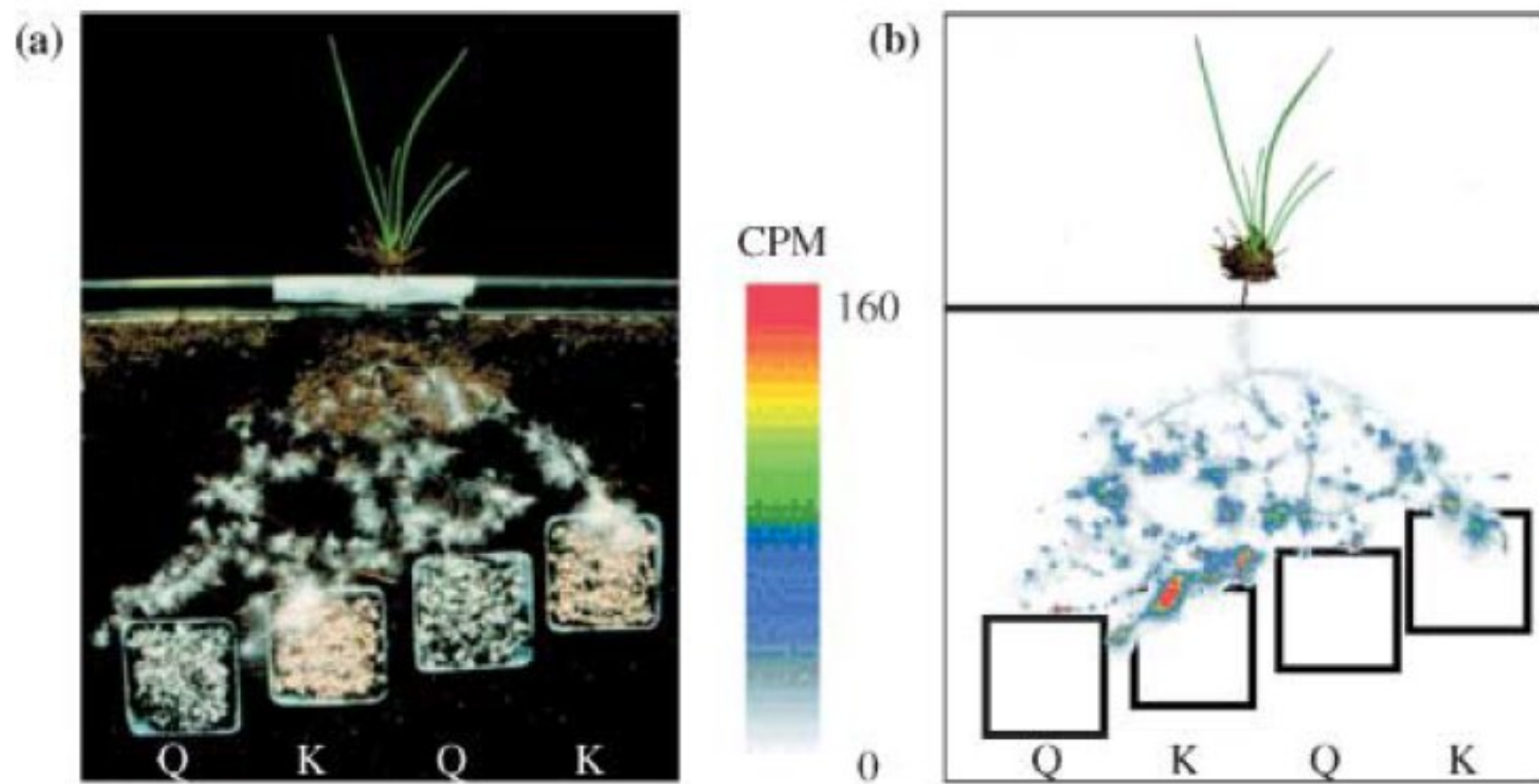


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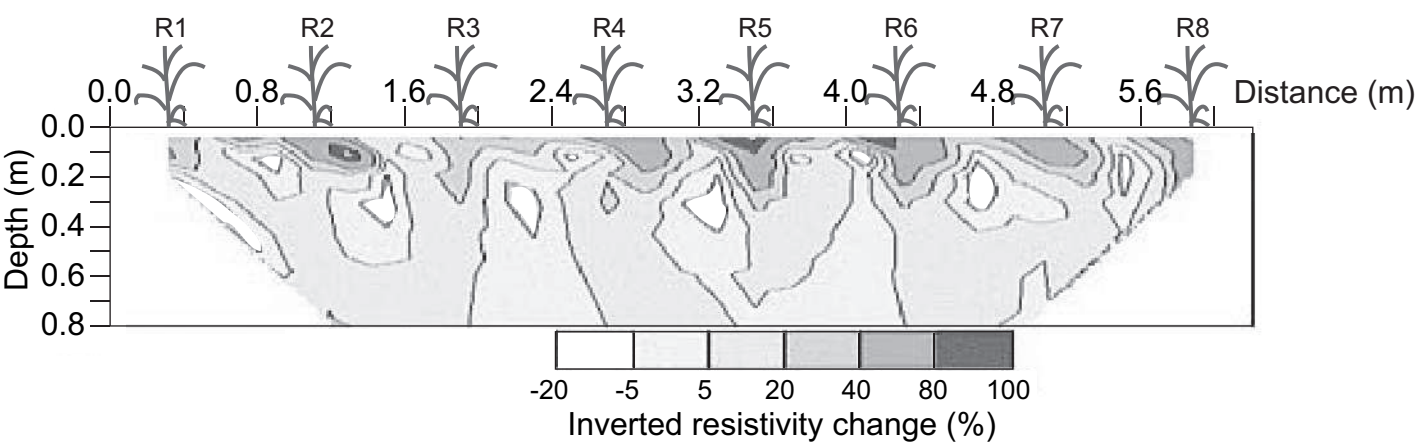


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