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

15

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24

25 **Keywords**

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

27

28 **Abstract**

29 We review methods and models that help to assess how root activity changes soil properties and  
30 affects the fluxes of matter in the soil. Subsections discuss (i) experimental systems including plant  
31 treatments in artificial media, studying the interaction of model root and microbial exudates with soil  
32 constituents, and microcosms to distinguish between soil compartments differing in root influence, (ii)  
33 the sampling and characterization of rhizosphere soil and solution, focusing on the separation of soil at  
34 different distances from roots and the spatially resolved sampling of soil solution, (iii) cutting-edge  
35 methodologies to study chemical effects in soil, including the estimation of bioavailable element or  
36 ion contents (biosensors, diffusive gradients in thin-films), studying the ultrastructure of soil  
37 components, localizing elements and determining their chemical form (microscopy, diffractometry,  
38 spectrometry), tracing the compartmentalization of substances in soils (isotope probing,  
39 autoradiography), and imaging gradients in-situ with micro electrodes or gels or filter papers  
40 containing dye indicators, (iv) spectroscopic and geophysical methods to study the plants influence on  
41 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-

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

88

## 89 **Experimental systems**

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

103

### 104 *Artificial substrates*

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

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

123

#### 124 *Testing root influence on specific soil materials*

125 An effective way of investigating the influence of root activity on the structure or reactivity of soil  
126 components like clay minerals or oxides is to study their interaction with isolated root exudates or  
127 model compounds (e.g., carboxylates, siderophores) in the absence of plants (Ochs et al. 1993;  
128 Reichard et al. 2005). Data on sorption of organic compounds by soil materials can give clues about  
129 their migration potential in soils (Jones and Brassington 1998). The compilation of Martell and Smith  
130 (1974-1989) provides thermodynamic data on equilibria between exudates as ligands and dissolved  
131 metal ions. The behavior of carboxylate anions in soils was reviewed by Jones (1998), that of  
132 phytosiderophores by Kraemer et al. (2006). An elegant way to test the effect of individual compounds  
133 on the bioavailability of nutrients was presented by Ström et al. (2002). They grew maize seedlings in  
134 “rhizotubes”, added a solution with carboxylate anions to a <sup>33</sup>P labeled patch of soil, and measured the  
135 <sup>33</sup>P uptake.

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

140

141 *Laboratory systems to assess gradients in soil*

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

150

151 *Microcosms in which roots are in direct contact with soil*

152 Pot and column studies belong into this category. Differences between bulk and rhizosphere soil can  
153 be assessed by separating rhizosphere from bulk soil by shaking or washing (Liu et al. 2004), by resin  
154 impregnation followed by microscopic or spectroscopic inspection of thin sections, or by non-invasive  
155 3D tomography (Pierret et al. 2003). Both repacked soil (aggregate structure destroyed) and soil  
156 monoliths can be studied.

157 Flat boxes, in which quasi 2D root systems are formed in a narrow slit filled with soil come in various  
158 dimensions. The so-called “Hohenheim” box is inclined to force the root system to develop  
159 preferentially along the lower cover plate (Dinkelaker and Marschner 1992). This type of microcosms  
160 is usually filled with repacked soil or artificial substrates, which may be arranged in zones of different  
161 properties (Hodge et al. 1999). Often the boxes are at least partly transparent to allow the visual  
162 observation of root development. Rhizosphere gradients can be assessed by sampling the soil in  
163 different distances from the root. More importantly, such microcosms are ideal for the application of  
164 non-invasive methods for *in-situ* characterization of gradients. Soil solution can be sampled in defined  
165 distances from given root segments as described below. The advantage of having roots in direct  
166 contact with soil is contrasted by the difficulties of detecting small effects by individual roots.

167



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

169 Membranes, usually made of poly-amide, are used to separate microcosms into different  
170 compartments. Membranes with a mesh size of 20-30  $\mu\text{m}$  can be penetrated by fungal hyphae and root  
171 hairs, but not roots. Membranes with a mesh size of 0.45  $\mu\text{m}$  allow exchange of soil solution and gases  
172 but neither hyphae nor roots can penetrate.

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

177 In other systems dense root mats are formed which are in contact with the soil via the membrane (Fig.  
178 2). The root mat itself can be in contact with soil or an artificial substrate (Gahoonia and Nielsen  
179 1991), or it is formed in an air-filled compartment (Wenzel et al. 2001). Such systems are ideal for  
180 assessing chemical rhizosphere gradients by sampling the soil or the soil solution in the root-free  
181 compartment in defined distances from the membrane. The root mat approach has the advantage of  
182 amplifying the root influence, and thus to enable the detection also of otherwise small effects.

183 However, the results may not be representative for field conditions with less dense root systems. Also,  
184 the exchange of water and ions between root and soil can be affected by the membrane (Fitz et al.  
185 2006).

186

187 *Field systems*

188 Lysimeters are large 3D, usually cylindrical, and often weighable structures to study water, element  
189 and ion fluxes in larger soil volumes under field conditions (not to be confused with tension or  
190 tension-free lysimeters which are soil solution collection devices). Lysimeters either contain a soil  
191 monolith or are refilled with loose soil material. While refilled lysimeters allow to establish  
192 experimental setups with several treatments under the same soil conditions (Luster et al. 2008),  
193 monolith lysimeters provide a controlled access to naturally structured soil (Bergström and Stenström  
194 1998). Rhizosphere in a microscopic sense cannot be studied unless coupled to observation tools such

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

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

201

## 202 **Sampling and characterization of rhizosphere soil and soil solution**

203 Dependent on soil texture and structure, plant species and observed parameter, root induced changes  
204 of most soil properties can be observed up to a distance of a few  $\mu\text{m}$  to about 7 mm from the surface  
205 of an active root segment or a root mat (Jungk and Claassen 1997; Jones et al. 2003). Sampling  
206 procedures for rhizosphere soil and solution have to cope with this demand for spatial resolution.  
207 However, rhizosphere effects may also reach beyond this range when considering highly mobile  
208 compounds like water or  $\text{CO}_2$  (Gregory 2006, Hinsinger et al. 2005) or when including the effects of  
209 fungal hyphae extending from mycorrhizal root segments (“mycorrhizosphere”, e.g. Agerer 2001).

210

### 211 *Sampling rhizosphere soil*

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

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

224

#### 225 *Characterization of rhizosphere soil*

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

230 There are two major groups of methods for chemical soil properties. The first deals with the total  
231 analysis of the soil solid phase, which is generally of little interest to rhizosphere research. The  
232 exception is total C and N analysis which is well applicable because of the small amounts of sample  
233 required by modern elemental analyzers. The second group comprises a large variety of extraction  
234 procedures to characterize different fractions of soil bound molecules or ions. Extractions for organic  
235 compounds (root and microbial exudates, contaminants) usually aim at complete recovery. Volatile  
236 organic compounds with a boiling point < 200 °C are purged from a heated soil suspension in water or  
237 methanol by an inert gas and trapped on suitable sorbents, while less volatile compounds are extracted  
238 using suitable solvents and applying different techniques (Sawhney 1996). By contrast, extractants for  
239 elements, inorganic ions and inorganic or organometallic compounds are often chosen to obtain a  
240 bioavailable fraction. An overview of commonly used extractants for this purpose is given in Table 1.  
241 Note that fractions are defined mainly operationally, and thus results obtained with different methods  
242 may not be easily compared. Nevertheless, depending on extractant, element and plant species there  
243 may be good correlations between extractable element concentration and plant uptake (citations in  
244 Sparks 1996 or Pansu and Gautheyrou 2006). A comprehensive characterization of soil-bound  
245 elements can be achieved by sequential extractions. There are protocols defining several fractions for  
246 organic nitrogen and carbon (Stevenson 1996; VonLützwow et al. 2007), phosphorus (Psenner et al.  
247 1988; Kuo 1996) and trace metals (Tessier et al. 1979; Zeien and Brümmer 1989). Since extraction  
248 methods have been developed without sample volume restrictions, the often limited sample amount

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

258 Isotopic exchange is another method for determining bioavailable contents applicable to ions of a few  
259 elements with radioactive isotopes ( $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{K}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ) (Frossard and Sinaj 1997). A small  
260 amount of isotopic tracer is added to a soil suspension and the dilution of the label by homoionic  
261 exchange with the non-labeled ions at the soil solid phase is characterized. Either so-called E-values  
262 (contents in the soil solid phase that are exchanged within a defined incubation time), or kinetic  
263 parameters of the exchange are determined.

264

#### 265 *Collection of soil solution*

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

277 with the soil matrix, and by texture and actual moisture of the soil. Secondly, analytes may be sorbed  
278 by or released from the sampling system (Rais et al. 2006), which asks for thorough testing of a  
279 particular system for a given problem. Nevertheless, the method has been applied successfully to  
280 assess rhizosphere gradients for major inorganic cations and anions (Wang et al. 2001), organic acid  
281 anions (Dessureault-Rompré et al. 2006) and trace metals (Shen and Hoffland 2007).

282 Alternatively, soil solution can be trapped by the application of filter papers, cellulose acetate filters or  
283 blotting membranes onto roots exposed in flat rhizoboxes, a method which has been used mainly for  
284 the collection of root exudates or root-secretory enzymes (Neumann 2006).

285

#### 286 *Analysis of small volumes of aqueous solution*

287 The miniaturization of sampling devices also minimizes the sample volume available for analysis. In  
288 principle all common analytical methods like ICP-OES, AAS, HPLC (high performance liquid  
289 chromatography), IC (ion chromatography), or colorimetry (manual or automatic as in flow-injection  
290 and auto analyzers) can be used, because except for flame AAS and standard ICP applications the  
291 sample amount needed for the measurement itself is not very high. The main task in adapting  
292 analytical methods to small sample volumes often is to optimize the autosampling system (Table 2).  
293 There are techniques available that significantly reduce the sample consumption of ICP-OES (Mermet  
294 and Todoli 2004) or ICP-MS (Prabhu et al. 1993; Lofthouse et al. 1997), which is normally in the  
295 range of several milliliters. Capillary electrophoresis (CE) offers the possibility to analyze samples as  
296 small as one droplet. Göttlein and Blasek (1996) optimized CE for the analysis of major cations and  
297 anions in soil solutions. Because CE is a true ion-analytical method it offers the possibility to detect  
298 the potentially phytotoxic  $Al^{3+}$  ion, which is of particular interest for studies of acidic soils (Göttlein  
299 1998). Combining the analysis of labile species by CE or miniaturized voltammetric systems (Tercier-  
300 Waeber et al. 2002) with total analysis by graphite furnace AAS or micro-injection ICP methods  
301 (Göttlein 2006) allows metal speciation in rhizosphere solutions. ISFET-sensors enable pH  
302 measurements in one to two droplets (Göttlein and Blasek 1996), and afterwards the sample can be  
303 used for other analyses, because the sensors do not contaminate the sample like standard pH  
304 electrodes. Dissolved organic carbon (DOC) in small sample volumes can be measured using TC

305 analyzers with a direct sample injection option, or, taking the UV absorption as an indirect measure,  
306 using an HPLC system with a UV-detector but without separation column (Göttlein and Blasek 1996).  
307 Employing the microanalytical methods described above, a comprehensive characterization of soil  
308 solution including metal speciation is possible with a sample volume of about 250  $\mu\text{l}$ . If only pH  
309 measurement and CE analysis of cations and anions are done, 30 to 50  $\mu\text{l}$  are sufficient. Very small  
310 liquid sample volumes may also be analyzed by scanning electron microscopy coupled with energy-  
311 dispersive X-ray analysis, however after sophisticated sample preparation (Bächmann and Steigerwald  
312 1993).  
313 Since for small solution samples the risk of contamination or adsorption losses is particularly high, the  
314 proper preconditioning and cleaning of all devices and containers that the sample comes in contact  
315 with are pivotal to reliable results (for recommended methods see Nollet 2007). Furthermore,  
316 evaporation losses during sampling should be minimised (Göttlein et al. 1996). Some natural water  
317 standard reference materials ([www.nist.gov/srm](http://www.nist.gov/srm); [www.erm-crm.org](http://www.erm-crm.org)) can be used for total analysis.  
318 For speciation, quality assurance must rely on internal references.

319

### 320 *Sampling and analysis of soil gases*

321 Measuring the total efflux of  $\text{CO}_2$  *in-situ* from a given, usually circular surface area of soil using  
322 infrared gas analysers is a well established and routinely used method. The contribution of rhizosphere  
323 respiration has been estimated either by comparing total soil respiration with respiration measured  
324 after terminating autotrophic respiration by detopping of plants (Andersen and Scagel 1997), girdling  
325 (Ekberg et al. 2007) or trenching (Sulzmann et al. 2005), or by applying suitable modeling to the soil  
326 respiration data (Raich and Mora 2005). Alternatively, rhizosphere respiration can be assessed by  
327 coupling  $^{13}\text{C}$  labeling of the plant shoots with sampling of the soil  $\text{CO}_2$  efflux and analysing its  $\delta^{13}\text{C}$   
328 using isotope-ratio mass spectrometry (Yevdokimov et al. 2007).

329 Membrane probes allow the diffusive sampling of soil gases like  $\text{CO}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{CH}_4$  or  $\text{H}_2$  at various soil  
330 depths in the field or in microcosms (Rothfuss and Conrad 1994; Yu and DeLaune 2006), and are  
331 sometimes coupled with on-line analysis (Panikov et al. 2007). It should be tested whether gradients in  
332 the partial pressure of gases from the rhizosphere to the bulk soil can be assessed with this technique.

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

335

### 336 **Cutting-edge methods for studying plant effects on rhizosphere soil**

#### 337 *In-situ assessment of soil solution*

338 *In-situ* measurements of chemical variables in the rhizosphere involve both the characterization of the  
339 solid and the solution phase. Impregnating rooted soil “profiles” in microcosms with dye indicators  
340 dissolved in agarose gel has been used for assessing root induced changes in pH (Fig. 4) and the  
341 exudation of aluminum complexing ligands or Fe(III) reducing agents (Engels et al. 2000; Neumann  
342 2006). Root-induced Mn reduction and the excretion of acid phosphatases can be detected by applying  
343 specially impregnated filter papers to the rooted soil “profiles” (Dinkelaker and Marschner 1992;  
344 Dinkelaker et al. 1993). While such staining methods can be used to monitor pH changes in the  
345 rhizosphere with time in artificial systems composed of agarose gel (Plassard et al. 1999), they can  
346 hardly be used for a continuous monitoring in real soil. Recently, a novel non-invasive method was  
347 presented by Blossfeld and Gansert (2007) for the visualisation of rhizosphere pH dynamics in  
348 waterlogged soils using a pH-sensitive fluorescent indicator dye in a proton permeable polymer matrix  
349 (pH planar optode). However, the applicability of this method to non-saturated soils has still to be  
350 proven. In aerated soils, antimony micro-electrodes allow high resolution monitoring of root induced  
351 changes of pH in the rhizosphere (Häussling et al. 1985; Fischer et al. 1989; Zhang and Pang 1999).  
352 Measuring soil redox potential with Pt micro-electrodes dates back to Lemon and Erickson (1952) and  
353 has seen improvements to date (Hui and Tian 1998; VanBochove et al. 2002; Cornu et al. 2006). In  
354 particular, they were used in microcosms to monitor redox gradients in the rhizosphere of rice in order  
355 to study the formation of iron plaque on roots (Bravin et al. 2008). Except for a single application of  
356 Na<sup>+</sup> ion selective electrodes by Hamza and Aylmore (1991), this methodology has not been applied to  
357 other chemical parameters due to the lack of suitable electrodes that can be operated reliably in soil.  
358 The DGT-technique (diffusive gradients in thin-films, Zhang et al. 1998) has been developed to  
359 evaluate the phytoavailable pool of metals and phosphorus. A DGT device consists of a gel-embedded  
360 resin layer acting as a sink for the species of interest, overlaid by another gel layer and a filter through

361 which the molecules or ions have to diffuse to reach the resin. Element and ion contents in soil  
362 extracted by DGT correlate well with contents in plants (Zhang et al. 2001). Up to now, DGT devices  
363 have been applied mostly to moist pastes of separated soil samples. However, they are particularly  
364 promising tools for direct application to the surface of rooted soil “profiles” in rhizoboxes (Fitz et al.  
365 2003b; Nowack et al. 2004). Spatially resolved maps of DGT extractable species can be obtained by  
366 slicing the resin gel prior to analysis (Zhang et al. 2001) or by measuring the metal in the resin gel by  
367 laser ablation ICP-MS (Warnken et al. 2004).

368

### 369 *Biosensors*

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



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

392

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

395 This subsection is restricted to studies of the soil solid phase, while the characterization of roots is  
396 addressed in Neumann et al. (2008). Standard techniques for two-dimensional element mapping are  
397 scanning electron microscopy (SEM) and transmission EM (TEM) coupled with energy dispersive X-  
398 ray microanalysis (EDX). Energy filtered TEM (EFTEM) offers a higher resolution and better  
399 detection limit (about 10 nm and 1-10  $\mu\text{g g}^{-1}$ , respectively). Other tools for two-dimensional element  
400 mapping include synchrotron-based micro X-ray fluorescence ( $\mu\text{SXRF}$ ), micro-particle induced X-ray  
401 emission ( $\mu\text{PIXE}$ ), secondary ion mass spectrometry (SIMS) and laser ablation (LA)- ICP-MS. SIMS  
402 and LA-ICP-MS have been coupled with stable isotope probing (SIP) to image the distribution of C  
403 isotopes in the soil at a sub- $\mu\text{m}$  (nanoSIMS) and sub-mm (LA-ICP-MS) resolution (Bruneau et al.  
404 2002; DeRito et al. 2005). Three-dimensional images of soil porosity can be obtained non-invasively  
405 by X-ray computed tomography (CT) (Mooney et al. 2006a), a method also used to study root  
406 architecture *in-situ* (Hodge et al. 2008). Alternatively, Moran et al. (2000) used X-ray absorption and  
407 phase contrast imaging to study the relation between roots and soil structure, and Mooney et al.  
408 (2006b) investigated the relation between the structure of a mineral landfill cap and root penetration  
409 by polarising microscopy.

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

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

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

422 Mineral weathering and formation of secondary minerals have been studied intensively by EM  
423 techniques, particularly by SEM-EDX (Gadd 2007) and TEM-EDX (Hinsinger et al. 1993). Observing  
424 the size and shape of minerals and estimating their composition allow to predict the nature of the  
425 minerals present. X-ray diffraction (XRD) allows a direct identification of minerals. Standard powder  
426 diffractometers are limited by the amount of sample required (1 g), but recent instruments require only  
427 a few tens of mg. Using EM and XRD, various precipitates and products of mineral weathering were  
428 detected in the vicinity of fungi and roots (Hinsinger et al. 1993; April and Keller 2005; Gadd 2007).

429 However, the weak sensitivity of XRD for minor phases remains a major limitation. It can be partly  
430 overcome by micro-XRD ( $\mu$ XRD) using laboratory or synchrotron X-ray sources, or by separation  
431 prior to XRD analysis. Furthermore, XRD on oriented clays, which requires only a few mg of  
432 particles, is suited to trace changes in clay mineralogy occurring in the rhizosphere, as shown in  
433 artificial substrates (Hinsinger et al. 1993) and in soils (Kodama et al. 1994). Recently, Barré et al.  
434 (2007) proposed a more quantitative approach for studying changes in the composition of the clay  
435 fraction in the rhizosphere.

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

441 Bulk XAS provides information on major metal species. This technique was combined with  $\mu$ XRF  
442 (Voegelin et al. 2007) and X-ray fluorescence microtomography (Hansel et al. 2001; Blute et al.  
443 2004) to study the distribution and speciation of heavy metals in the root plaque of plants growing in  
444 flooded environments. These studies revealed a heterogeneous composition of Fe(III) and Fe(II)

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

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

466  $^{13}\text{C}$ ,  $^{31}\text{P}$ ,  $^{15}\text{N}$  and  $^1\text{H}$  solid and liquid state nuclear magnetic resonance (NMR) spectroscopies are  
467 classical tools for the characterization of molecular structures and functional groups in soil organic  
468 matter (SOM) and for the identification of low molecular weight molecules (Fan et al. 1997).  
469 Advanced techniques such as high-resolution magic-angle spinning and 2D NMR open new  
470 possibilities (Kelleher et al. 2006). The large sample size required for solid state NMR (0.5 to 1 g of  
471 isolated SOM compared to a few tens of mg for liquid state NMR), limits its use for rhizosphere  
472 applications. Fourier transformed infrared (FTIR) spectroscopy is another classical tool for the

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

479

#### 480 *Labelling with and tracing / imaging of stable and radioactive isotopes*

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

488 Laterally resolved information on the distribution of an isotope can be obtained in different ways.

489 Gradients around roots can be determined using microcosms of the root mat type and analyzing slices  
490 of soil at various distances from the root mat (Kuzyakov et al. 2003). Microcosms of the “Hohenheim”  
491 type allowed to assess the equilibration of stable isotope labels for Mg, K and Ca between rhizosphere  
492 soil and solution (Göttlein et al. 2005). Autoradiography on flat microcosms provides non-invasive 2D  
493 imaging of the distribution of radioactive isotopes. Images were classically obtained on films or  
494 photographic emulsions, then on phosphor storage screens, and more recently by electronic  
495 autoradiography (Fig. 6; Rosling et al. 2004). Apart from following C fluxes, this versatile method can  
496 be used to characterize the spatial distribution and its change over time of added radioactive P  
497 (Hendriks et al. 1981; Hübel and Beck 1992; Lindahl et al. 2001),  $\text{SO}_4^{2-}$  (Jungk and Claassen 1997) or  
498 Zn and Cd (Whiting et al. 2000).

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

501

502 **Mapping the plants influence on soil moisture**

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

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

509 Some of the methods to image root systems in microcosms are sensitive also to differences in  
510 substrate moisture and can therefore be used to assess the plants influence on soil moisture  
511 distribution. Light transmission imaging (Garrigues et al. 2006) is a rather inexpensive method with  
512 which large quasi 2D microcosms (e.g. 1000 x 500 x 4 mm) can be studied at a resolution of  $\geq 1500$   
513  $\mu\text{m}$ . With magnetic resonance imaging (MRI; Chudek and Hunter 1997; Herrmann et al. 2002), which  
514 depends on the accessibility to a medical imager or an NMR spectrometer with a suitable accessory,  
515 3D images can be obtained from boxes (up to 70 x 70 x 20 mm) or cylinders (diameters up to 60 mm  
516 and heights up to 200 mm) at a resolution between 10 and several hundred  $\mu\text{m}$ . Considering the high  
517 spatial resolution, these methods are able to assess plant effects on soil moisture on the scale of a  
518 single-root. However, their applicability to real soil is limited by inherent incompatibilities. Light-  
519 transmission is restricted to translucent sand with addition of small amounts of clay and MRI to soils  
520 with low iron contents. By contrast, X-ray computed tomography allows to map root effects on  
521 structure and moisture distribution in real soils at a resolution of 100  $\mu\text{m}$  to 1 mm for typically  
522 cylindrical samples with a diameter of a few cm (Hamza and Aylmore 1992; Gregory and Hinsinger  
523 1999). The sensitivity to soil water content, however, is comparatively weak. Recently, Oswald et al.  
524 (2008) demonstrated the high sensitivity of Neutron radiography to differences in soil water content  
525 and could show variable water uptake by different parts of root systems growing in flat microcosms  
526 (170 x 150 x 13 mm) made of aluminum at a spatial resolution of  $\geq 100 \mu\text{m}$ . Although the contrast is  
527 highest in quartz sand, the method can also be applied to natural soil (Menon et al. 2007).

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

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

543

#### 544 **Rhizosphere Modeling**

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

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

555

556 *Macroscopic models*

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

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

575 *Biogeochemical ecosystem models:* These models are used to identify the governing parameters in  
576 ecosystems in order to understand element or nutrient cycles or to predict ecosystem dynamics.

577 Examples include the DNDC model which simulates soil carbon and nitrogen biogeochemistry (Li et  
578 al. 1994). A plant growth submodel is used to calculate root respiration, N uptake and plant growth  
579 and these processes are linked to climate and soil status. Biogeochemical models pay more attention to  
580 soil processes than crop models. Complexation, cation exchange, precipitation, and adsorption can be  
581 included in various degrees of complexity (Cosby et al. 1985; Alewell and Manderscheid 1998).

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

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

592 *Whole root system scale:* Several root architecture models are available that simulate the growth of  
593 whole root systems at high spatial resolution to generate two or three-dimensional representations of  
594 root systems, e.g. ROOTMAP (Diggle 1988), SimRoot (Lynch et al. 1997) or Root Typ (Pagès et al.  
595 2004). An example of a modeled root system is shown in Fig. 8a. Doussan et al. (2006) extended a  
596 whole root-system model to include water transport in soils with full coupling of water transport in the  
597 root system and the influence of aging on the hydraulic conductivity of root segments and thus on  
598 water uptake. The linking of such models to the underlying biology is not yet strongly advanced  
599 (Darrah et al. 2006). However, several models have been developed that take into account interactions  
600 between root systems, water and nutrients in the environment (Dunbabin et al. 2002). Wu et al. (2007)  
601 recently presented a dynamic simulation model that is multi-dimensional, operates on a field scale, is  
602 weather driven and models C and N cycling between plants, soil and microbes.

603

#### 604 *Microscopic models*

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



612 *Semi-empirical models on the single root scale:* Semi-empirical root models simulate the uptake of  
613 nutrients by an isolated root segment. The classical rhizosphere model is that of Nye and Tinker  
614 (1977) and (Barber 1995). It supposes a cylindrical root surrounded by an infinite amount of soil, with  
615 convection and diffusion of nutrients through the soil and uptake through Michaelis-Menten type  
616 kinetics at the root surface. The non-linearity of the model requires a numerical solution but recently  
617 an analytical solution of the equations was obtained (Roose et al. 2001). This model has also been  
618 extended to describe P or metal uptake in microcosms of the root mat type (Kirk 1999; Puschenreiter  
619 et al. 2005b). Most of these models are based on a rather simplified description of soil chemistry and  
620 the effects of plant roots. The actions exerted by roots on their rhizosphere are generally limited to  
621 element uptake, and the chemical interactions between dissolved elements and the soil are reduced to a  
622 buffer power or Freundlich adsorption isotherm (Barber 1995; Kirk 1999). Fig. 8b shows as an  
623 example the influence of citrate exudation on phosphate solubilization. The effect of exudation has  
624 been incorporated into the basic modeling concept, and conditional models parameterized for different  
625 soils have been formulated, e.g. to model the effect of organic acid exudation on phosphate  
626 mobilization (Gerke et al. 2000ab). The application of certain rhizosphere models requires to write a  
627 new computer program or to change existing software. Schnepf et al. (2002) have shown that pde-  
628 solvers are useful in rhizosphere modeling because they make it easy to create, reproduce or link  
629 models from the known constituting equations.

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

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

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

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

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

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

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

672

### 673 **Challenges ahead**

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

693

694 *Methodological improvements for investigations at the micro scale*

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

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

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

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

722 damages by X-ray, electron or particle beams. Another challenge is to link the molecular- and  
723 microscopic-scale information obtained by these techniques to information obtained at higher scale.  
724

### 725 *Upscaling*

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

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

759

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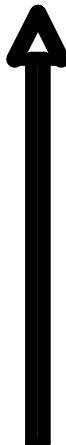
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- 1243



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 <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O	H <sub>2</sub> O
	hot H <sub>2</sub> O; NH <sub>4</sub> <sup>+</sup> , NO <sub>3</sub> <sup>-</sup> in salt extracts (KCl, CaCl <sub>2</sub> ..)	Ca-lactate; NH <sub>4</sub> -lactate; Citrate	NH <sub>4</sub> Cl <sup>a</sup> ; BaCl <sub>2</sub> <sup>a</sup>	NH <sub>4</sub> Cl <sup>a</sup> ; BaCl <sub>2</sub> <sup>a</sup>	NaNO <sub>3</sub> ; NH <sub>4</sub> Cl <sup>a</sup> ; BaCl <sub>2</sub> <sup>a</sup> ; NH <sub>4</sub> -acetate
		Ca acetate/ lactate; NaHCO <sub>3</sub> ; NH <sub>4</sub> F/HCl	HNO <sub>3</sub> ; HCl	EDTA; NH <sub>4</sub> -oxalate	NH <sub>4</sub> -EDTA; NH <sub>4</sub> -oxalate
		H <sub>2</sub> SO <sub>4</sub>	HCl / HNO <sub>3</sub>	Na-dithionite; HCl/HNO <sub>3</sub>	HNO <sub>3</sub> ; HCl/HNO <sub>3</sub>

1251 <sup>a</sup>methods 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 <sub>4</sub> )	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 <sub>3</sub> , N <sub>tot</sub> )	common, intermediate	autosampler and sample injection limiting; direct injection option reduces sample demand to 50 µl
ion chromatography (inorganic anions, organic acids, NH <sub>4</sub> )	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 <sub>4</sub> )	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

1259 **Figure captions**

1260 Fig. 1. Rhizosphere as 3-phase system with soil solid phase (SP), soil solution (SS), and soil gas phase  
1261 (SG); spatial heterogeneity along and perpendicular to root growth added by a developing root system  
1262 is emphasised and is overlaid by temporal variability: root growth (A), turnover of roots and fungal  
1263 hyphae (B), diurnal or seasonal changes in the activity of roots (exudation, uptake; C), or associated  
1264 organisms (D).

1265

1266 Fig. 2. Example of a root mat type microcosm. It is composed of a lower part containing a thin soil  
1267 layer (1-3 mm thick; or, alternatively, a soil cylinder of greater height if aiming at studying  
1268 rhizosphere gradients), and of an upper part containing the root mat, separated by a polyamide  
1269 membrane. For pregrowth, the upper part is immersed in aerated nutrient solution (adapted from  
1270 Guivarch et al. 1999, Figure 1; with kind permission from Springer Science+Business Media); for  
1271 further explanations see Chaignon and Hinsinger (2003).

1272

1273 Fig. 3. Studying the influence of a growing oak root on soil solution chemistry using a micro suction  
1274 cup array installed in a “Hohenheim” type microcosm (adapted from Göttlein et al. 1999; with kind  
1275 permission from Springer Science+Business Media)

1276

1277 Fig. 4 Effect of soil-buffering capacity ( $\text{CaCO}_3$  content) on the extension of root-induced rhizosphere  
1278 acidification of chickpea (*Cicer arietinum* L.) seedlings 12 DAS, detected in “Hohenheim” type  
1279 microcosms by soil impregnation with pH-indicator (bromocresol purple) agar (from Römheld 1986;  
1280 courtesy of the International Potash Institute, Switzerland)

1281

1282 Fig. 5: Zn K-edge bulk EXAFS spectra of a Zn-contaminated sediment (control), treated with mineral  
1283 amendments and planted with *Agrostis tenuis*, and distribution of Zn species determined from the  
1284 analysis of these data and  $\mu$ EXAFS spectra. The amendments induce a significant oxidation of ZnS  
1285 and the formation of secondary species. These effects are strongly enhanced in the presence of *A.*

1286 *tenuis*, with an almost complete removal of ZnS (adapted from Panfili et al. 2005; Copyright Elsevier  
1287 (2005)).

1288

1289 Fig. 6: Peat microcosm containing *Pinus sylvestris* seedlings colonised by *Hebeloma crustuliniforme*  
1290 and pure mineral patches of either K feldspar (K) or quartz (Q). Fifteen weeks after introducing  
1291 mineral patches at the growing mycelial front (a), the shoots were pulse labelled with  $^{14}\text{CO}_2$ . Greater  
1292 amounts of labelled carbon are allocated to root tips and mycelia associated with patches of F feldspar  
1293 compared to patches of quartz (b). CPM: counts per min. (adapted from Rosling et al. 2004; with kind  
1294 permission from the New Phytologist Trust).

1295

1296 Fig. 7: Changes in soil moisture in a profile during drying shown as difference between the inverted  
1297 electrical resistivity at about 8 days after irrigation and immediately after irrigation. Root zones of  
1298 corn rows (R1 to R8) show as dark zones that dry out quickly (adapted from Michot et al. 2003;  
1299 Reproduced/modified by permission of American Geophysical Union)

1300

1301 Figure 8: Examples of different rhizosphere models. a) Macroscopic model, whole root system scale:  
1302 modeled root system of *Lupinus albus* (from Doussan et al. 2006; with kind permission from Springer  
1303 Science+Business Media). b) Microscopic, mechanistic single root model of citrate exudation and its  
1304 influence on phosphate solubilization (dots: experimental; black line: modeled P in soil; dotted line: P  
1305 in solution; dashed line: citrate in soil) (from Kirk 1999; with kind permission from Blackwell  
1306 Publishing). c) Microscopic single root model, molecular scale: influence of citrate on phosphate  
1307 mobilization (P in solution in the absence and presence of citrate exudation) (from Geelhoed et al.  
1308 1999; with kind permission from Blackwell Publishing).

Figure 1  
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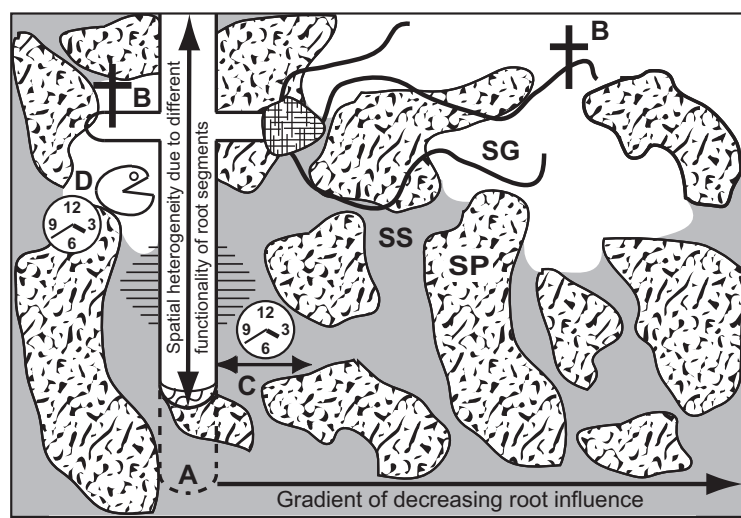
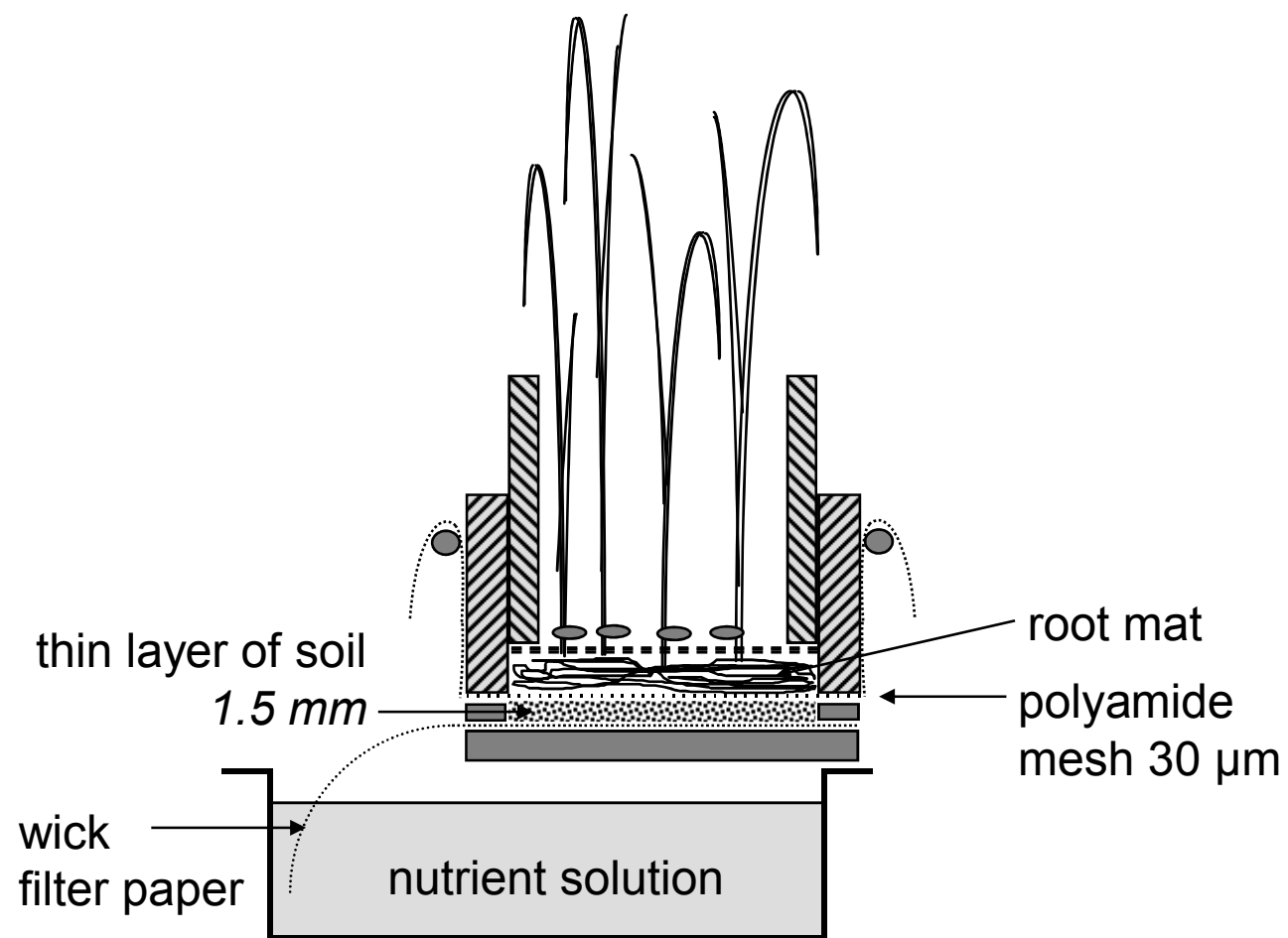


Figure 2

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**Figure 3**

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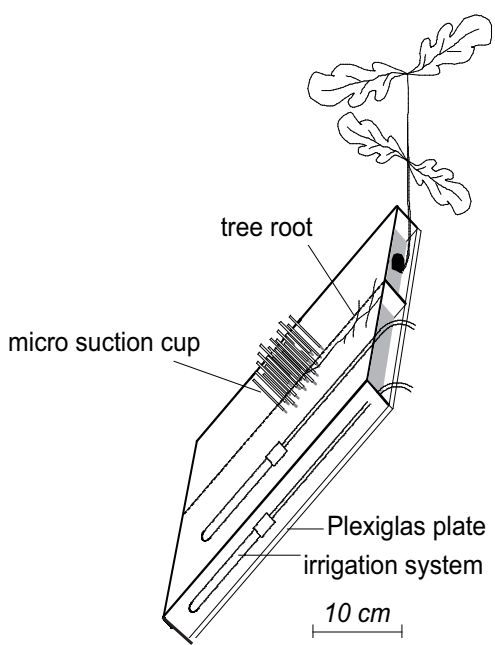




Figure 4  
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Extension: 1-2 mm

< 1mm

0 mm

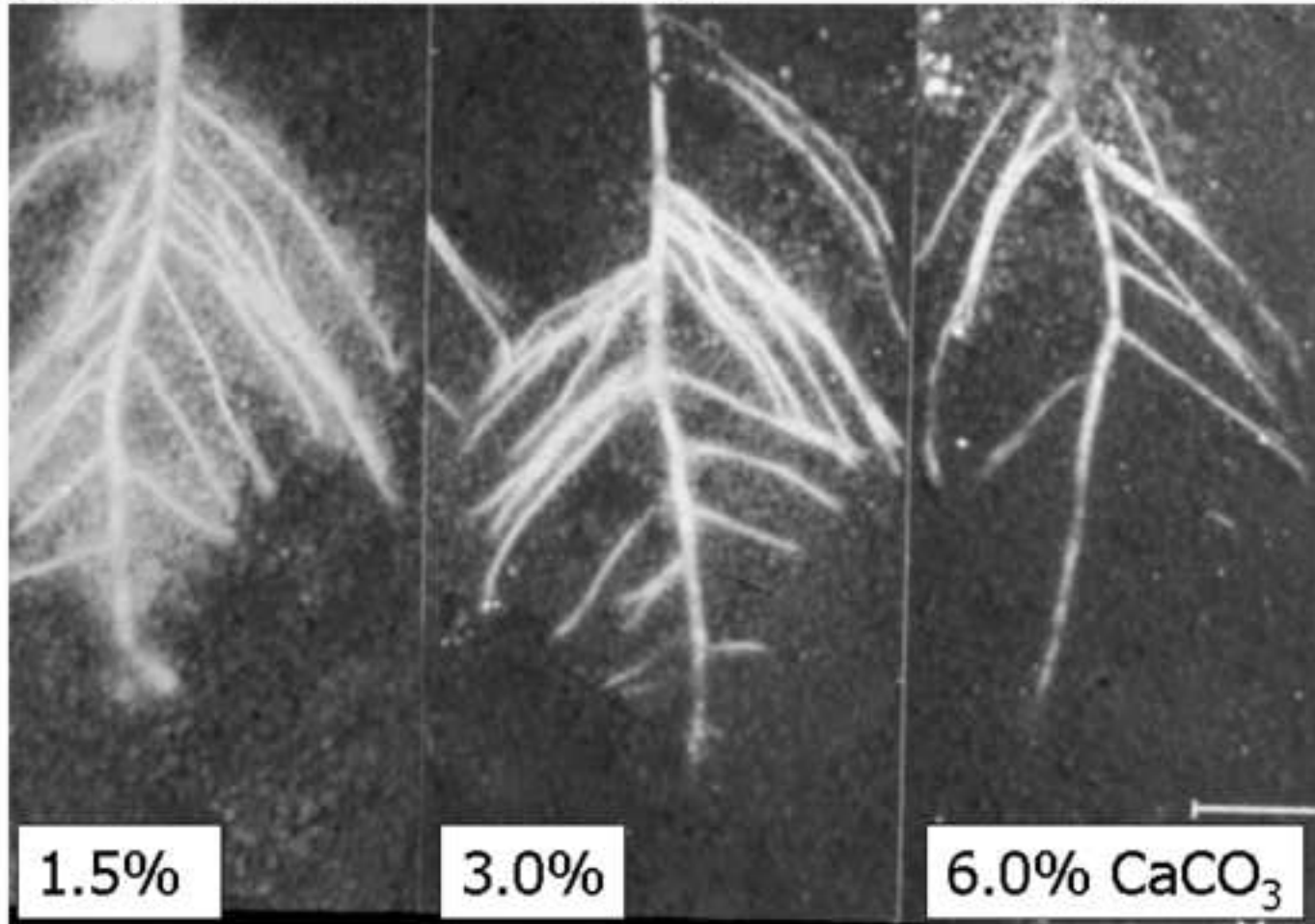


Figure 5

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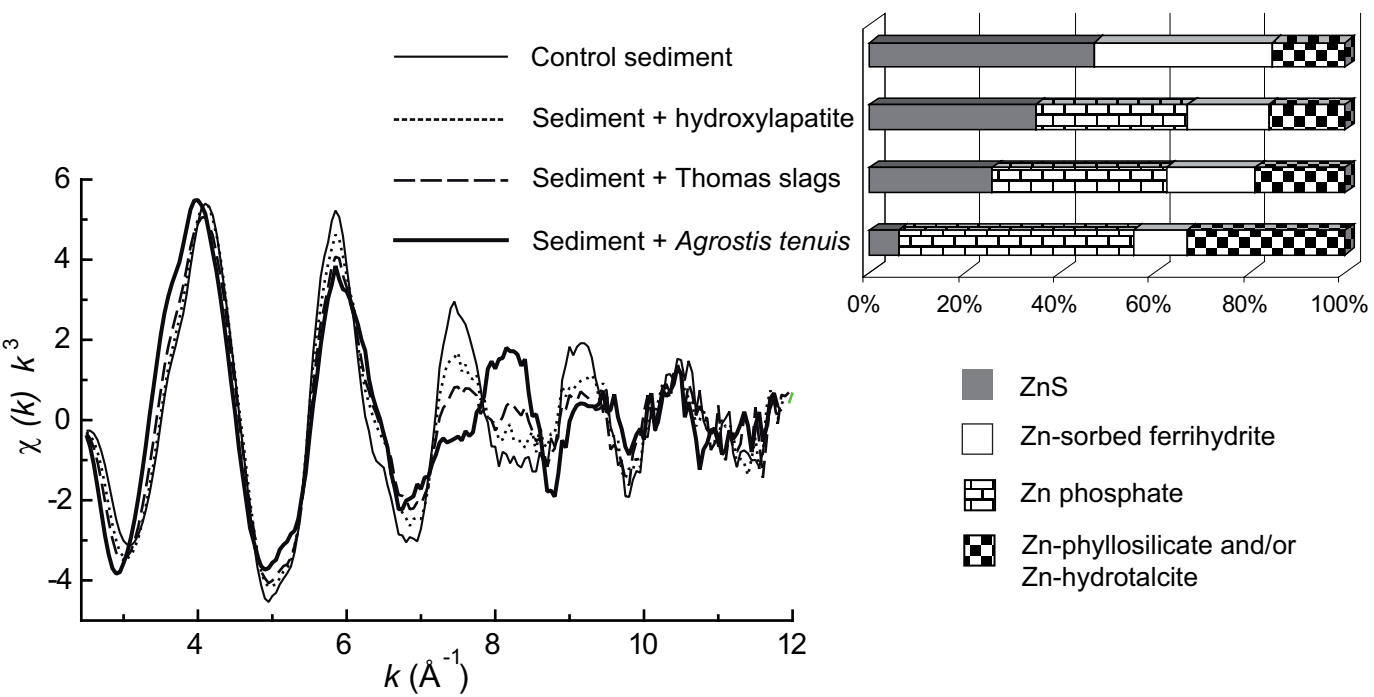


Figure 6  
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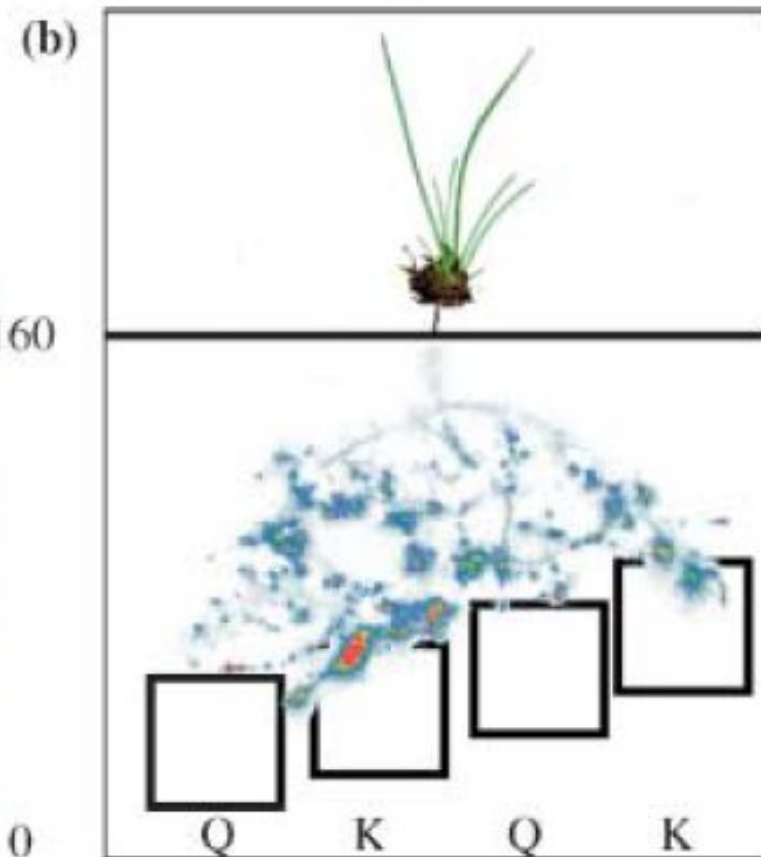
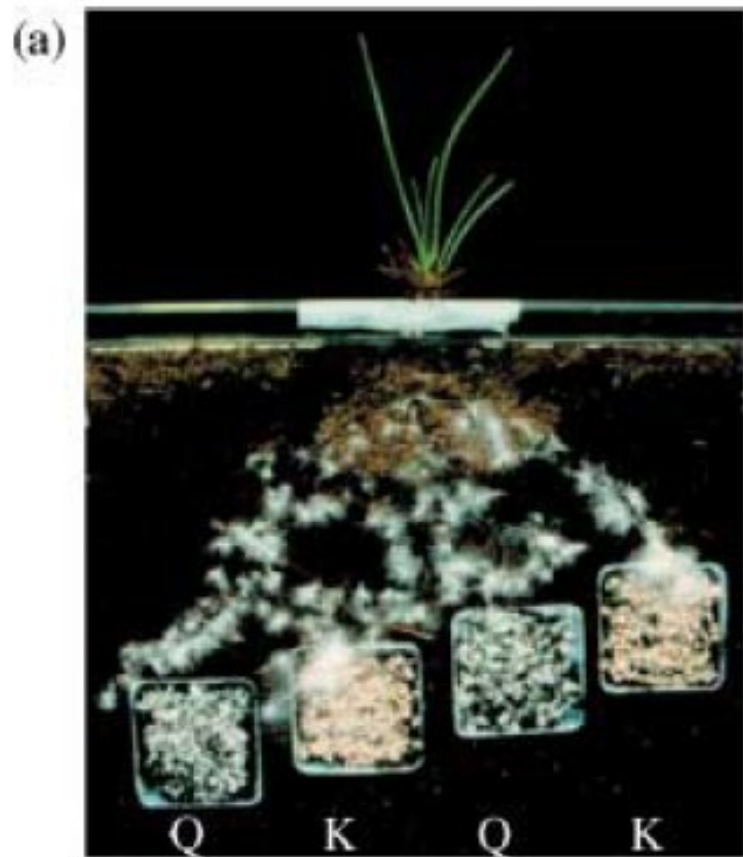


Figure 7

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