Rhizosphere pH dynamics in trace-metal-contaminated soils, monitored with planar pH optodes

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Received: 8 June 2009 / Accepted: 1 October 2009 / Published online: 17 October 2009

Abstract The present study presents new insights into pH dynamics in the rhizosphere of alpine pennycress (*Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey), maize (*Zea mays* L.) and ryegrass (*Lolium perenne* L.), when growing on three soils contaminated by trace metals with initial pH values varying from 5.6 to 7.4. The pH dynamics were recorded, using a recently developed 2D imaging technique based on planar pH optodes. This showed that alpine pennycress and ryegrass alkalinized their rhizosphere by up to 1.7 and 1.5 pH units, respectively, whereas maize acidified its rhizosphere by up to −0.7 pH units. The alkalinization by the roots of alpine pennycress and ryegrass was permanent and not restricted to specific root zones, whereas the acidification along the maize roots was restricted to the elongation zone and thus only temporary. Calculations showed that such pH changes should have noticeable effects on the solubility of the trace metal in the rhizosphere, and therefore on their uptake by the plants. As a result, it is suggested that models for trace metal uptake should include precise knowledge of rhizospheric pH conditions.

Keywords Maize · Alpine pennycress · Ryegrass · Cadmium · Alkalinization · Acidification

Responsible Editor: Philippe Hinsinger.

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Introduction

The pH value of soils is known to be a heterogeneously distributed parameter (Fischer et al. 1989; Hinsinger et al. 2005; Jaillard et al. 1996). This variability can be caused by several abiotic physico-chemical reactions in the soils, *e.g.* the dissolution of CO_2, the reduction of iron or manganese hydroxides or the hydrolysis of Al in the soil pore water (Hinsinger et al. 2003; Kirk 2004; Scheffer and Schachtschabel 2002). Besides abiotic reactions, biotic activities can also influence the soil pH values. For example plant roots actively alter the rhizospheric pH, to extents varying with those of the diffusion processes (Kim and Silk 1999). This

can happen for instance due to proton (H^+) excretion, uptake or hydroxide (OH^-) excretion at the root surface, which is known to happen during either ammonium or nitrate uptake by roots (Marschner 1995; Marschner and Römheld 1983). It has also been shown that plant roots change the rhizospheric availability of nutrients like Fe and P by altering the pH value (Kirk and Bajita 1995; Kirk and Kronzucker 2005; Marschner 1995; Walter et al. 2000).

On the other hand, pH is a factor which can significantly affect the speciation and the solubility of trace metals in soils (Bruemmer et al. 1986; Sauvé et al. 2000) and therefore their availability to plants. As a consequence, the change in the rhizospheric pH might influence the living conditions and the composition of plants growing in soils contaminated with elements such as Cd, Ni, Pb or Zn. An increase in trace metal availability, due to a root mediated pH shift towards acidic conditions, leads to an increased availability of those trace metals for plant uptake (Christensen 1984; Scheffer and Schachtschabel 2002). This increased availability can cause severe injury and even death to trace-metal sensitive species or at least enhance the concentration of potentially toxic elements in edible plant organs. On the other hand, an increase in soil pH around the roots, by reducing the availability of toxic trace elements would be a way to reduce the exposure of plants to potentially toxic trace metals (Bravin et al. 2009a, b). While some studies have suggested that there is no correlation between rhizospheric pH value and the uptake of trace metals (Luo et al. 2000; McGrath et al. 1997), other works have described positive correlations between the trace metal concentration in the plants and the rhizospheric pH value (Loosemore et al. 2004; Monsant et al. 2008). However, none of these studies investigated pH changes at the mm-scale resolution along the root surfaces, but used mixtures of several grams of soil samples, which might have diluted the possible effect of root induced pH changes. Recently, a rhizosphere pH gradient at the millimeter scale was assessed, using glass electrodes to measure the pH in the solution extracted from thin slices of an acidic soil, previously in contact with a root mat (Bravin et al. 2009b). Nevertheless, a quantification of the rhizosphere pH of soil-grown single roots or root systems with high spatial and temporal resolution was still lacking.

Therefore, to quantify the dynamics of pH at the rhizosphere scale of soil roots grown in trace-metal-contaminated soils, a newly developed method for the non-invasive 2D imaging of pH, based on planar pH optodes (Blossfeld and Gansert 2007) was used and adapted to this specific question. The results of this approach applied to three plant species grown in three unsaturated soils contaminated with Cd, Pb and Zn are presented in this article.

Material and methods

Soils

Three soils were used (soils A, B & C). Soils A and soil B were strongly contaminated with Cd, Pb and Zn by the atmospheric emissions of two lead and zinc smelters (Sterckeman et al. 2002). They were characterized in a previous study (Sterckeman et al. 2005) as a sandy-clayey-loamy soil with a pH(H_2O) of 6.2 and as a silty-loamy-sandy soil with a pH(H_2O) of 8.1, respectively (Table 1). Besides their different pH values, the soils differ in the bioavailability of trace metals as can be seen from the *E* values for Cd and Zn, which are much higher in soil A than in soils B and C (Sterckeman et al. 2005). Soil C was chosen because it was loamy and showed a similar pH(H_2O) to soil A but no (or very low) contamination with Pb and Zn. Indeed, this soil enabled the measurement of pH in the rhizosphere of ryegrass on a slightly acid soil, as in pot cultivation this plant only rarely grew on soil A, possibly because of the toxicity of a highly available Zn content. The soils were sieved at 2 mm before being placed into the rhizoboxes. The pH ($CaCl_2$) of each soil was measured according to ISO 10390:2005. Fertilizers were not added to the soils as the major nutrient status showed no deficiencies (Table 1).

Plant species and cultivation

Three species with contrasting abilities to accumulate trace metals were investigated. One of the selected species was alpine pennycress (*Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey also known as *Thlaspi caerulescens* J. & C. PRESL., Viviez population), which is a well-known Cd and Zn hyperaccumulator (Reeves et al. 2001). The second species chosen, ryegrass (*Lolium perenne* L., cv Prana), can be specified as a trace metal "excluder" as the metal is

Table 1 Characteristics of the soils used during the experimentation. Data compiled from Sterckeman et al. (2004), Sterckeman et al. (2005) and Gérard (2000) except pH $CaCl_2$, for which our own measurements were carried out according to ISO 10390:2005. *E* values were measured through the isotope dilution technique and represent the pool of labile elements, *i.e.* the ions in solution together with the sorbed ones in equilibrium with those in solution

		Soil A	Soil B	Soil C
Particle size distribution g kg^{-1}	Clay	208	162	204
	Silt	469	602	454
	Sand	323	236	342
pH (H_2O)		6.2	8.1	6.3
pH ($CaCl_2$)		5.7	7.4	5.9
$CaCO_3$ g kg^{-1}		0	13	<1
Organic C g kg^{-1}		26.38	16.9	15.7
P Olsen g P_2O_5 kg^{-1}		0.021	0.156	0.025
C/N ratio		16.8	12.9	8.9
CEC cmol+ kg^{-1}		11.8	12.0	11.0
Exchangeable cations cmol+ kg^{-1}	Ca^{2+}	7.1	12.6	9.0
	Mg^{2+}	1.2	0.4	3.5
	K^{+}	0.6	0.5	0.3
Total Cd mg kg^{-1}		19.9	19.5	6.32
Total Zn mg kg^{-1}		3,362	1,538	52
E_{Cd} mg kg^{-1}		14.7	6.7	4.2
E_{Zn} mg kg^{-1}		1,654	145	ND

generally less concentrated in its shoots than in the soil (Sterckeman et al. 2005). Previous work showed that it was able to grow on soil A, although this was potentially phytotoxic as a consequence of the high availability of trace metals. Maize (*Zea mays* L. cv INRA MB862) was also investigated, due to the fact that this species is a standard model plant, for which some data on rhizospheric pH are available (Fan and Neumann 2004; Peters 2004; Taylor and Bloom 1998). This species can accumulate the metal in its shoots, although it is known to be sensitive to trace metals (Page et al. 1981) and shows higher concentrations in roots than in shoots (Perriguey et al. 2008).

The selected plant species were transplanted to or sown in PVC rhizoboxes (height 300 mm, width 150 mm, depth 50 mm) that were filled with one of the three soil types described above. The front plates of the rhizoboxes were cut from conventional glass of 2 mm in thickness and fixed to the rhizobox by use of six removable metal clamps (Fig. 1). Alpine pennycress was first sown on compost and grown there for 10 to 14 days before transplantation. Maize was germinated on moistened filter paper for two to 3 days before transplantation while ryegrass was directly sown onto the soil in the rhizoboxes. Three seedlings of maize and alpine pennycress where transplanted in each rhizobox, while rye grass seedlings were thinned after germination to about 10 plants per rhizobox.

During cultivation, the rhizoboxes were placed on a rack with an inclination of 45° to force the roots to grow along the front plates. By this arrangement, the roots were also protected from light. The plants were grown and investigated in a growth chamber with a 16 h/8 h day–night cycle (350 µmol photons m^{-2} s^{-1}

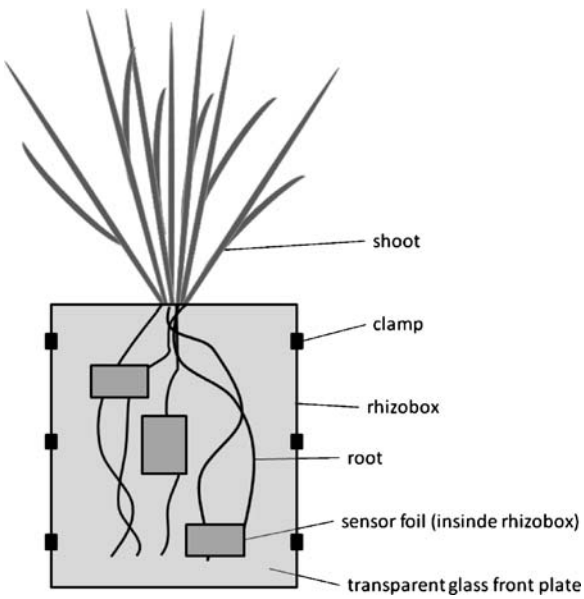

Fig. 1 Design of the rhizoboxes used: Clamps hold a glass front plate, planar pH optode sensor foils are placed inside, in direct contact with the roots and the glass front plate

during daytime). Average day and night temperature were 24°C and 18°C, respectively. From the outset, the water content of each rhizobox was set to 70% to 80% of the water holding capacity, controlled by weighing, and was kept constantly at this level during the experiments. The duration of the cultivation of the three species in the rhizoboxes prior to the measurements was different due to the species specific root growth; i.e. 6–8 weeks for alpine pennycress, 8–9 days for maize and 4–5 weeks for ryegrass.

pH measurement

The pH measurements were made using a non-invasive optical technique, described by Blossfeld and Gansert (2007). This technique uses planar pH optodes, based on the measurement of the fluorescence decay time of pH sensitive indicator dyes (Gansert and Blossfeld 2008; Gansert et al. 2006; Huber et al. 2001; Klimant et al. 2001). These indicator dyes are dispersed and immobilized on an inert supporting film, creating a thin sensor foil of 10 µm in thickness. The measurement itself was carried out via an optical glass fiber from outside the rhizobox, which was connected to a light source and measuring device (pH-1 mini, PreSens GmbH, Regensburg, Germany). The glass fiber was moved automatically by a x-y stepper motor device, connected to and operated by a conventional personal computer. By this, the pH value could be measured in a line scanning mode from outside the rhizobox. This decoupling of sensor and detector, allows a non-invasive investigation of the pH dynamics in the soil-rhizosphere-root network, using light as the carrier of information (Blossfeld and Gansert 2007, Fig. 2).

According to the root growth and the position of individual roots and root networks during the cultivation, the glass front plate was removed and one to three planar pH optodes (PreSens GmbH; maximal dimensions: 20 mm×40 mm) were fixed to the inner surface of the front plate using a thin layer of silicon grease as adhesive. Afterwards, the front plate was fixed to the rhizobox again, to ensure that the roots and the soil were in direct contact with the planar pH optodes (i.e. sensor foils in Figs. 1 & 2).

The initial objective was to measure the soil pH (i) at the tip of a single root, (ii) on a single root as far as possible from its apex and (iii) in densely rooted zone. This was possible in the case of ryegrass and alpine pennycress. However, maize roots did not form a root network until the main roots reached the bottom of the rhizoboxes. As this could have altered the whole root system's functioning, measurement of pH in a densely rooted zone was not carried out for this species. Alternatively, the tips of the main roots and aged parts of these main roots were investigated.

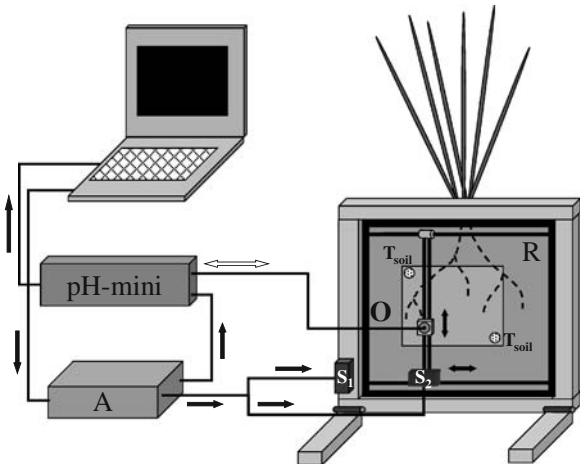

Fig. 2 Diagram of the hardware components used for the optical non-invasive pH measurements. The planar optode (F) is scanned from outside via an optical fiber (O), which is moved by two stepper motors (S_1, S_2). A specific interface controls the stepper motors and triggers the fiber-optic detection device (pH-mini). The data transfer to the computer (C) and the trigger impulses are indicated as *black arrows*. The optical data transfer is indicated by a *double-headed white arrow*. The soil temperature is measured at two positions (T_{soil}) in the rhizobox (R). Reproduced from Blossfeld and Gansert (2007) with kind permission from Plant, Cell & Environment (Wiley-Blackwell)

Depending on the position and the number of roots in contact with the sensor foils, variable sections of these foils were scanned by the fiber in 2 to 3 mm steps and 4 s intervals. The pH value was mapped using SigmaPlot software (Systat Software, Inc., San Jose, CA, USA). The resulting 2D color contour plots represented the measured pH value of the selected sections of the planar optodes. For further technical details, calibration procedure and data processing, see Blossfeld and Gansert (2007).

In the case of ryegrass grown on soil B, the measurement was carried out on three rhizoboxes. As there was no significant difference between the three rhizoboxes (data not shown), the measurements for the other plant species were carried out in only one rhizobox for each of the three soils.

Results

pH measurements

In Fig. 3, the dynamics of the rhizospheric pH of ryegrass, growing in soil B, is exemplarily shown. During the course of 1 week (D1–D7) five roots grew across the investigated section of the planar pH sensor foil. All five roots were indicated by red dots on the photograph in the lower right image in Fig. 3. This photograph was taken at the end of the experiment, *i.e.* 6 days after D7, when the front plate of the rhizobox was removed, showing other visible roots in this image that were grown after D7. The alkalinization of two roots (indicated with roman numerals I, II) was detectable at the end of the light cycle of day one of the experiment (indicated as D1 at 19:00). Twenty four hours later (D2 at 19:00) root II had already grown across the entire surface of the investigated section (*i.e.* more than 20 mm) and root I had grown about 9 mm during the same time. During the next 24 h (D3 at 19:00), a third root appeared (indicated as III) and the alkalinization of the roots I and II was greater than the previous days (up to 0.4 pH units). The rhizosphere pH of root I was more alkaline (up to pH 8.4) than the rhizosphere of root II or root III (both up to pH 8.0). Until day seven of the experiment (D7 at 11:00), all three roots grew out of, and a fourth and a fifth root started to grow across, the investigated section of the sensor foil. The 2D-imaging technique also revealed that the radius of proximate alkalinization around single roots could reach up to three millimeters of the root surface. The maximum alkalinization by the ryegrass roots compared to the non-rooted bulk soil (soil B) was 1.2 pH units, as the pH of bulk soil was 7.2.

In the dense root networks, the soil pH map was highly complex and formed a mosaic like pattern. Figure 4 illustrates such a pattern for a root network of ryegrass growing in soil B. In this case, due to the root activity even the pH of non-rooted zones is alkalinized; only a few spots with the initial pH value were left compared to the situation shown in Fig. 3.

The values given in Table 2 represent the average of the measured pH values from ten sampling points from the root surfaces and the bulk soil of the last measurement of each experiment (when the root tip of the single root fully crossed the sensor foil). They show that all investigated plant species affect the rhizospheric pH in all investigated soil types.

As shown above, ryegrass roots alkalinized their rhizosphere in soil B, as they also did when growing in soil C (Table 2). However, in soil C the rhizospheric pH value was not affected by single roots of ryegrass, whereas an alkalinization within root networks increased the rhizospheric pH to pH 7.7 (Table 2). Due to an increased root curvature and thus a reduced contact between sensor foil and roots, the number of sampling points was reduced in the case of single root investigations in soil C.

For the roots of alpine pennycress, a clear alkalinization of the rhizosphere was also found (Fig. 5). In soil A, the roots of alpine pennycress alkalinized their rhizosphere up to an average pH of 7.0 within root networks (Table 2). This corresponds to an alkalinization of 1.4 pH units compared to the average pH of the bulk soil (pH=5.6). For soil B, a rhizosphere alkalinization by the roots of alpine pennycress of up to a maximum of 1.2 pH units along single roots was detectable (Table 2). In soil C the fully-grown individuals of alpine pennycress did not form a dense root network, due to reduced root growth of the individuals, but again a rhizosphere alkalinization compared to the bulk soil was clearly detectable along the single roots (up to 1.7 pH units; Table 2).

Contrarily to ryegrass and alpine pennycress, young maize roots did not alkalinize, but acidified the rhizosphere (Table 2; Fig. 6). Compared to the pH of the bulk soil, the rhizosphere of maize showed a pH value by up to −0.7 pH units in the case of soil A, corresponding to a rhizospheric pH value of pH 4.9 (average pH bulk soil: 5.6). In the case of soil B, the rhizospheric acidification was in a lower range (−0.3 pH units). In soil C, only a small amount of data points were available, due to an increased root curvature and a consequently reduced contact between sensor foil and roots. Within this soil, the results show no significant pH change in the rhizosphere of young single roots (Table 2).

The investigation of aged parts of the maize roots showed contrasting effects of the roots on rhizospheric pH values, within a quantitative change of −0.1 (soil A), +0.1 (soil B) to +0.3 pH units (soil C). This indicates that a stronger acidification is locally restricted to the root tip and the elongation zone of the roots (Table 2). Furthermore, when growing in soil B and especially in soil C, an alkalinization along the basal parts of the single maize roots could be observed.

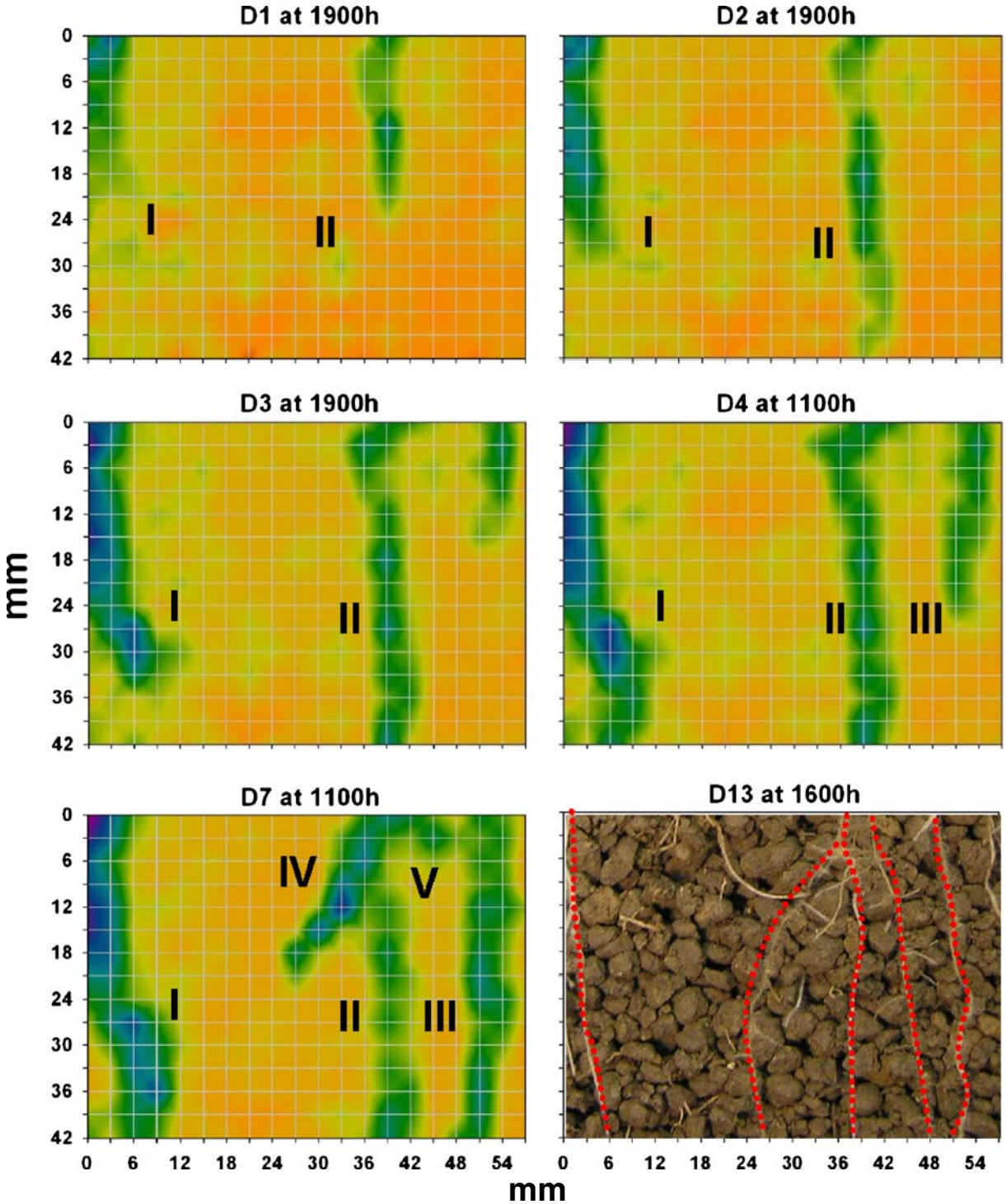

Fig. 3 Series of 2D images of the pH pattern in the rhizosphere of young ryegrass roots growing in soil B. Roman numerals indicate different roots, abscissa and ordinate in mm scale. The photograph was taken at the end of the experiment. *Red dotted lines* in the photograph indicate location of the indexed roots (I–V)

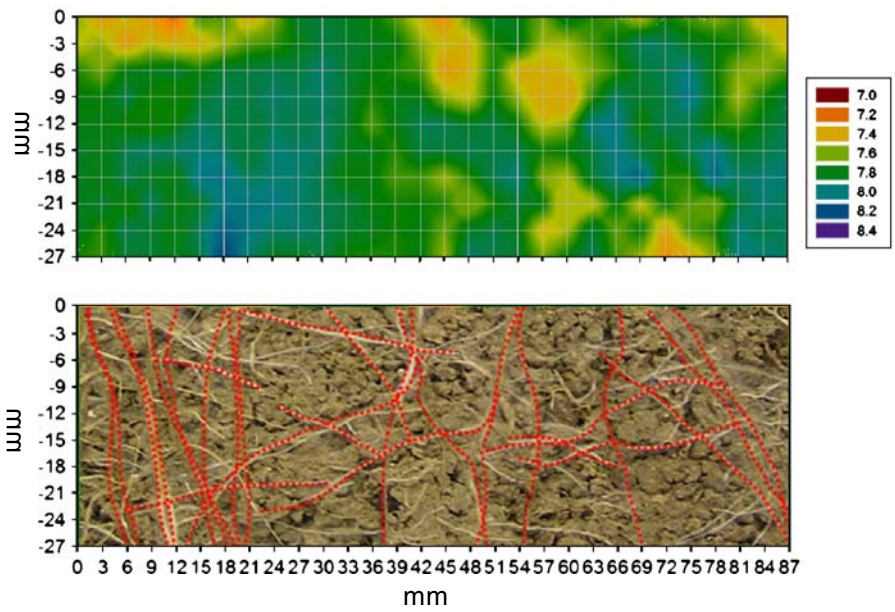

Fig. 4 Series of 2D images of the pH pattern in the rhizosphere of a network of ryegrass roots growing in soil B. The photograph was taken at the end of the experiment. *Red dotted lines* in the photograph indicate location of main roots

Finally, it can be noticed that the pH of the bulk soil measured by the planar optodes was similar to that measured in $CaCl_2$ 0.01 M soil suspension and generally lower than that measured in the water suspension (Tables 1 & 2).

Discussion

Planar optodes allow a continuous and cartographic monitoring of soil or rhizosphere pH, at a millimetric scale. This technique was initially validated for the measurement of the rhizosphere pH of a plant species (*Juncus effusus* L.) that grows in water-saturated soils (Blossfeld and Gansert 2007). Proving the suitability of this technique under even moderate soil moisture conditions had still to be done (Hinsinger et al. 2009; Luster et al. 2009). Our study confirms the use of planar pH optodes for a wide range of rhizospheric research, from moderate to waterlogged soil moisture conditions. Moreover, our measurements with this technique revealed remarkable results with regard to contrasting rhizospheric pH dynamics of three plant species.

The species investigated show different influences of their roots on rhizospheric pH patterns. The roots of ryegrass and alpine pennycress alkalinize the rhizosphere, whereas the roots of maize acidify it. The acidification along the roots of maize found in our study conforms to previous findings (Fan and Neumann 2004; Peters 2004; Taylor and Bloom 1998). The observed restriction of the acidification

Table 2 Rhizospheric and bulk soil pH measured non-invasively with planar pH optodes

	Ryegrass			Alpine pennycress			Maize		
Soil type	A	B	C	A	B	C	A	B	C
Average pH along single root	–	7.8±0.1***	6.5±0.3* (n=4)	6.8±0.1***	8.4±0.2***	7.8±0.3***	4.9±0.2***	7.1±0.2*** (n=9)	6.0±0.1 n.s. (n=4)
Average pH in root network/ along aged roots	–	7.9±0.1***	7.7±0.2***	7.0±0.2***	7.9±0.3***	n.d.	5.5±0.1***	7.5±0.1***	6.5±0.2*
pH bulk soil	–	7.4±<0.1	6.2±0.3	5.6±0.1	7.5±0.1	6.1±0.1	5.6±<0.1	7.4±0.1	6.2±0.3

Asterisks indicate significant differences between rhizospheric and bulk soil pH of the selected species and soil type

t-test, n=10 unless stated differently

*p<0.05 **p<0.01 ***p<0.001

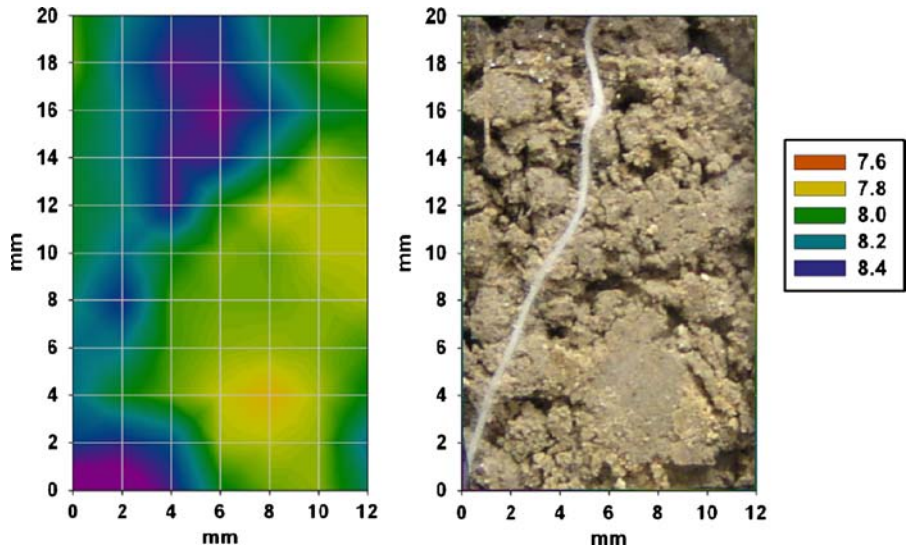

Fig. 5 2D image of a typical rhizospheric pH pattern of young alpine pennycress roots growing in soil B. The photograph was taken at the end of the experiment. Color codes for pH-values differ from those used in Figs. 3 and 4

zone to the root tip and the elongation zone was also reported by others (Fan and Neumann 2004; Peters 2004). This acidification might be caused by a locally restricted uptake of positively charged ions, such as potassium or ammonium, that are necessary for plant nutrition. This effect is well-known to cause an acidification along the roots (Bravin et al. 2009a; Marschner 1995; Miller and Cramer 2004). However, as reported earlier (Colmer and Bloom 1998; Taylor and Bloom 1998), the uptake of ammonium does not seem to be limited to a specific zone along the root surface of maize.

On the other hand, several studies clearly related the locally restricted acidification along the roots of maize to the acid-growth mechanism (Peters 2004; Pilet et al. 1983; Versel and Mayor 1985; Versel and Pilet 1986). These studies linked a local acidification 2–4 mm behind the root apex of maize roots to the

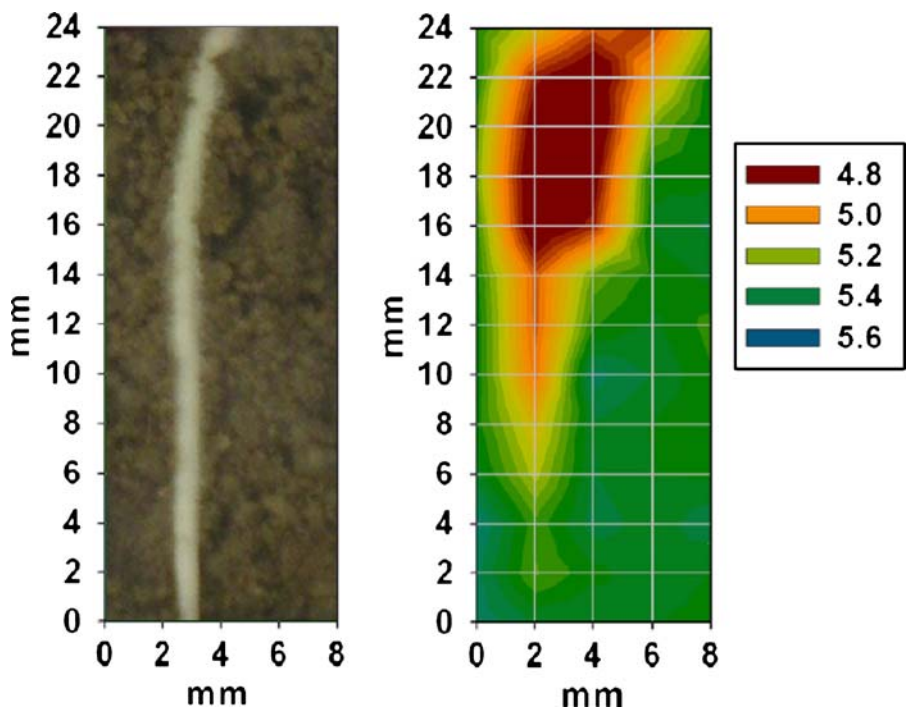

Fig. 6 2D image of a typical rhizospheric pH pattern of young maize roots growing in soil A. The photograph was taken at the end of the experiment. Color codes for pH-values differ from those used in Figs. 3 and 4

Table 3 Calculated Cd concentration and partitioning coefficient of cadmium (K_d^{Cd}) in the rhizosphere compared to bulk soil conditions. Data are based on the pH data from Table 2, previously described cadmium concentrations in the bulk soil (Gérard 2000; Perriguey 2006) and the Eqs. (1) and (2)

	Rhizospheric soil									Bulk soil		
	Ryegrass			Alpine pennycress			Maize					
Soil type	A	B	C	A	B	C	A	B	C	A	B	C
Cd (nmol L^{-1})	–	33.4	475	69.5	8.4	6.0	5,524	167	1,502	1,102	78	1,015
K_d^{Cd} (L kg^{-1})	–	1,667	385	540	3,281	1,667	63	757	219	139	1,098	265
[Cd] bulk soil/[Cd] rhizosphere	–	2	2.1	16	9	171	0.20	0.47	0.68			
K_d^{Cd} bulk soil/ K_d^{Cd} rhizosphere	–	0.7	0.7	0.26	0.33	0.16	2.21	1.45	1.21			

highest level of root growth of this particular region. Since the other processes mentioned above do not explain the locally restricted acidification in our case, the acid-growth mechanism might apply to our experiments as well. However, this has to be clarified through further investigation.

In contrast, the rhizosphere alkalinization by the roots of ryegrass and alpine pennycress is obviously permanent and the entire root surface of these species modifies the rhizospheric pH value towards alkaline conditions. For ryegrass, other studies have also reported an alkalinization in the rhizosphere (see for instance Gahoonia et al. 1992; Pinel et al. 2003), through the measurement of the pH of a soil suspension at the end of the experiments. To our knowledge there are no other data available in the literature about the pH pattern in the rhizosphere of alpine pennycress.

The underlying process of this alkalinization by these two species is yet unknown and has to be identified by further investigations. One possible explanation might be the uptake of negatively charged ions necessary for plant nutrition like nitrate (NO_3^-) (Bravin et al. 2009a; Marschner 1995; McClure et al. 1990; Miller and Cramer 2004; Rausch and Bucher 2002; Taylor and Bloom 1998), i.e. the reversed process as discussed above concerning the acidification of maize rhizosphere. This would lead to the assumption that ryegrass and alpine pennycress show a preference towards for instance NO_3^-, while maize would preferentially absorb NH_4^+.

Moreover, plants have been found to have a developmental program for the control of shoot metal concentrations, causing a seasonally-varying pattern of phytoaccumulation over a large range of metal availabilities in the soil (Silk et al. 2006). The resulting variation in trace metal root uptake could also cause rhizosphere pH variations. On the other hand, it is also possible that microbial activities associated to the plant roots are responsible for these different rhizospheric pH patterns. Depending on the plant species, different microbial communities might have been established (Costa et al. 2006; Wieland et al. 2001) and therefore affected rhizospheric pH due to specific proton generating-reactions *e.g.* nitrification or iron oxidation. However, the microbial activities were neither controlled nor quantified during our study in order to verify this assumption. Since no data are yet present to verify these assumptions, further studies of the ion fluxes and role of microbial communities along the root surfaces of ryegrass and alpine pennycress are needed.

Furthermore, small-scaled rhizospheric pH changes as demonstrated above are generally not taken into account in mechanistic models that simulate the uptake of trace metals by roots (Barber 1995; Roose and Kirk 2009; Sterckeman et al. 2004; Tinker and Nye 2000). However, the concentration of the solute in the soil solution (C_l), which is a key parameter in the soil-to-plant transfer (Sterckeman et al. 2004) is highly dependent on the pH (Bruemmer et al. 1986; Sauvé et al. 2000). In some recent works, rhizosphere pH gradients were successfully modeled (Bravin et al. 2009b; Loosemore et al. 2004). This approach could be coupled to a reactive transport model describing trace element root uptake, as previously done for phosphorus by Kirk and Saleque (1995). The use of the optode technology presented here would then help to parameterize or validate such a model.

In our study, measured alkalinization along the roots of ryegrass and alpine pennycress above pH 7

(up to pH 8.6) should strongly decrease the availability of trace-metals in the soil solution due to an increase of the sorption capacity of the soil (Bravin et al. 2009a; Christensen 1984; Loosemore et al. 2004; Ma and Lindsay 1995; Sauvé et al. 2000; Scheffer and Schachtschabel 2002). It is well-known that the logarithm of the amount of soluble metals like Cd^{2+} linearly decreases as pH increases. Depending on the specific soil conditions, slopes of this relationship can vary between −0.6 and −2.0 log units (Ma and Lindsay 1995; Salam and Helmke 1998). In $NaNO_3$ extracts of 120 French cultivated soil samples, Sterckeman et al. (2000) found a mean slope of −1, which gave a correlation between pH and Cd as follows:

$$\log(\text{Cd NaNO}_3) = b - 1.0\text{pH}, \quad (1)$$

b being a constant depending on the soil.

$NaNO_3$ extracts can be regarded as a reliable method for assessing the metal concentrations in soil solutions (Gupta and Aten 1993; Lebourg et al. 1998). Therefore, this relationship might serve to estimate and highlight the effect of the recorded pH changes in the rhizosphere of the three selected plants on the solubility of Cd.

The Cd concentration in the bulk soil is 1,102 nmol L^{-1} and 78.3 nmol L^{-1} for soils A and B (Gérard 2000) and 1,015 nmol L^{-1} for soil C (Perriguey 2006). Using these concentrations and mean bulk pH in Eq. (1), the soil-dependent constant b will be 8.6, 9.3 and 9.2 for the soils A, B and C, respectively. Thus, C_l in the rhizosphere can be estimated using the average pH values along a single root (Table 2).

The impact of rhizospheric pH changes on the availability of Cd was also assessed using the relationship between K_d^{Cd} and pH as described by Sauvé et al. (2000) from 830 data points:

$$\log K_d^{Cd} = 0.49\text{pH} - 0.6, \quad (2)$$

where K_d^{Cd} (L kg^{-1}) is the partitioning coefficient of Cd, *i.e.* the ratio between soil total and dissolved metal.

According to these calculations, the recorded alkalinization of the rhizosphere by the roots of ryegrass and alpine pennycress should have decreased the Cd concentration in the soil solution up to more than two orders of magnitude, compared to the bulk soil conditions (Table 3). On the other hand, the maximal acidification of the rhizosphere by the roots of maize should have strongly increased the concentration of Cd. Similarly, the K_d^{Cd} values should clearly increase in the alkalinized rhizosphere of ryegrass and alpine pennycress compared to those in the bulk soil, reflecting the sorption of the metal on the solid phase with pH increase (Table 3). As expected, K_d^{Cd} should decrease in the rhizosphere of maize (or at least in the acidified part of it), thanks to the dissolution of part of the metal from the solid phase.

In conclusion, depending on plant species, soil type, and even on the location along the root in the case of maize, the availability of Cd is clearly different in the rhizosphere than in the bulk soil. As suggested by sensitivity analysis carried out on mechanistic modeling (Sterckeman et al. 2004), such differences should have important consequences on the uptake of Cd by the plants. Finally, the use of planar optodes shows a great potential for the monitoring of pH dynamics in a variety of soils and in the rhizosphere of various plant species.

Acknowledgements This work was supported by the "Deutscher Akademischer Austausch Dienst" (DAAD) and the "Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche" within the framework of the PROCOPE programme in 2008.

The authors would like to express their thanks to Bernard Colin (INPL(ENSAIA)/INRA), W. Seidel, A. Lanzinger, M. Laug and W. Müller (University Düsseldorf) for their technical support and PreSens GmbH for the supply of planar optodes.

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