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Multi-element accumulation near *Rumex crispus* roots under wetland and dryland conditions

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Abstract

Rumex crispus was grown under wet and dry conditions in two-chamber columns such that the roots were confined to one chamber by a 21 μ m nylon mesh, thus creating a soil–root interface ('rhizoplane'). Element concentrations at 3 mm intervals below the 'rhizoplane' were measured. The hypothesis was that metals accumulate near plant roots more under wetland than dryland conditions. Patterns in element distribution were different between the treatments. Under dryland conditions Al, Ba, Cu, Cr, Fe, K, La, Mg, Na, Sr, V, Y and Zn accumulated in soil closest to the roots, above the 'rhizoplane' only. Under wetland conditions Al, Fe, Cr, K, V and Zn accumulated above as well as 3 mm below the 'rhizoplane' whereas La, Sr and Y accumulated 3 mm below the 'rhizoplane' only. Plants on average produced 1.5 times more biomass and element uptake was 2.5 times greater under wetland compared to dryland conditions.

Keywords

Metal accumulation; Multi-element analysis; Rhizosphere; Wetland plants

1. Introduction

In contrast to 'dryland' plants, many wetland plants display constitutive tolerance to elevated metal concentrations in the soil, meaning that they are tolerant to metals regardless of the metal concentrations at their location of origin (Matthews et al., 2004; McCabe et al., 2001; McNaughton et al., 1974; Ye et al., 1997a). Otte and co-workers (McCabe et al., 2001; Otte et al., 2004) suggested that the development of metal tolerance in wetland plants may be attributed to the biogeochemistry of wetland substrates. They proposed that the formation of Fe plaque deposits in the vicinity of wetland plant roots contributes to higher metal mobility and thus greater metal accumulation near plant roots. As a consequence wetland plants have been exposed to higher concentrations than dryland plants over the course of evolution, which favored selection for constitutive metal tolerance.

The bioavailability and mobility of chemical elements are influenced by changes in soil properties surrounding living plant roots including pH, organic content, cation exchange

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Patterns of element accumulation near the roots of plants differ between dryland and wetland conditions.

capacity, redox potential (Eh), moisture status and temperature (Alloway, 1995; Davies, 1994; Jacob and Otte, 2003). Plant roots influence the environment directly adjacent to them in order to obtain access to nutrients, in particular the essential macro- and micro-nutrients (Inderjit and Weston, 2003; Jungk, 2002; Marschner et al., 1986; Mehra and Farago, 1994; Neumann and Romheld, 2002). Wetland plants can modify redox conditions, pH and organic matter of the soil or sediment and thus affect the mobility (Wright and Otte, 1999) and chemical speciation of metals in waterlogged environments (Jacob and Otte, 2003). Knowledge of the biogeochemistry of metals and the processes affecting their mobility and trophic transfer is important, (1) because of their potential ecotoxicological effects, (2) because recent research has shown that less-studied elements such as the rare earth elements may be beneficial to plant growth (Chang, 1991; Hong, 2002), and (3) because of the increasing demand for less-studied metals, such as the rare earth elements, for development of new technologies and their subsequent potential environmental impacts.

Hinsinger and Courchesne (2008) emphasize that rhizosphere studies play a key role in research on the biogeochemistry of elements. Most rhizosphere studies have used dryland plants such as *Brassica napus* (rape) (Kuchenbuch and Jungk, 1982), *Hordeum vulgare* L. var. Dorirumugi (barley) (Youssef and Chino, 1989b, 1991) and *Glycine max* (soybean) (Youssef and Chino, 1991). The few studies using wetland plants include *Oryza sativa* L. (rice) (Begg et al., 1994; Kirk and Bajita, 1995), *Halimione portulacoides* (sea purslane), *Spartina townsendii* (cordgrass) (Doyle and Otte, 1997), *Spartina anglica* (common cordgrass) (Otte et al., 1995) and *Typha latifolia* (narrow leaf cattail) (Jacob and Otte, 2004). But none of these studies have compared element concentrations across the rhizosphere under flooded (wetland) and non-flooded (dryland) conditions. The aim of this study was to investigate the hypothesis that metals accumulate in the direction of plant roots in flooded soil more than in non-flooded soil and that this would lead to greater uptake of those metals in plants.

2. Materials and methods

2.1. Seed collection and soil preparation

Mature *Rumex crispus* fruits were collected in West Fargo, North Dakota (N 46° 52' 30.7", W 96° 58' 08.7") in October, 2006, and stored at 5 °C (Baskin and Baskin, 1978) for 5 months. The seeds were washed in distilled water after sepal removal and germinated on moistened sterile sand for 2 weeks in an incubator (14 h photoperiod, 25 °C). The seedlings were planted in 5 cm potting soil (Sun Gro Sunshine LG3 germinating mix with vermiculite) and allowed to develop roots for about 6 weeks in a greenhouse (16 h photoperiod, 3.03 log lumen m⁻² (mean day time), 20–30 °C).

Local farmland soil was obtained from near Casselton in Cass County, North Dakota (N 46° 50' 51.4", W 97° 09' 20.0", 280 m). This soil was selected because it was more representative of natural conditions compared to substrates such as potting soil or sand. The soil was determined to be a silty clay with 4.1% organic matter, bulk density of 1.04 g cm⁻³ and particle size 5.8% Sand, 47.9% Silt and 46.3% Clay (North Dakota State University Soil Testing Lab). The soil was oven-dried (60 °C) to constant weight, crushed and passed

through a 2 mm screen. The soil was amended with sterile sand (Quikrete Premium Play Sand) at a ratio of 3:1 soil to sand (by weight) to aid root penetration in the clay-rich soil.

2.2. Column apparatus assembly

The columns consisted of 2 sections of soil which were separated by 21 μ m nylon mesh (Nylon 21/17, Miami Aqua-culture, Inc.). The mesh restricted root growth to the upper section of the column while allowing diffusion of nutrients and water throughout the soil. The mesh was considered the rhizoplane because it separated the roots from the soil in the lower section of the column. This design enabled soil sampling in two different regions of the soil column; 1) above the rhizoplane (upper section of column) and 2) below the rhizoplane at distinct distances (lower section of column) (Fig. 1).

The columns consisted of PVC pipe (9 cm diameter) cut into two sections measuring 10 cm (lower section) and 6 cm (upper section). The mesh was attached to one end of the 6 cm section. The two-chamber column was assembled by securing the 10 cm section to the 6 cm section with 5-cm wide waterproof Duct Tape (Nashua® Tape) with the nylon mesh in between. Both sections were filled with the prepared, homogenized soil/sand mixture, the lower section with 600 g to which 300 ml of distilled water was added evenly and the upper section with 300 g to which 150 ml of distilled water was also added evenly. To prevent soil loss from the column and still allow water movement, a 2 mm mesh was secured to the bottom end. The lower section of the column was inverted, filled with soil and secured with the 2 mm mesh before soil was added to the upper section to ensure contact between the bottom soil and the rhizoplane. The seedlings were removed from the potting soil, washed gently with distilled water and planted in the upper section of the column (1 seedling per column).

2.3. Soil flooding and monitoring moisture

This experiment was carried out in a greenhouse and the treatments arranged using complete randomized design. The columns were placed into 2 L containers. The plants were allowed to establish for two weeks prior to beginning the moisture treatments. The flooded treatment ($n = 10$) consisted of adding distilled water to the containers such that the surface of the soil was below 5 mm of water. The non-flooded treatment consisted of columns ($n = 10$) that received water as needed according to their wilting point weights (see below).

Both treatments were monitored daily to determine when water addition was necessary. Sterilized cotton wicks with one end in sealed bottles of distilled water and the other end inserted into the soil above the rhizoplane were used continuously to maintain saturation of the flooded treatment. The same approach was used for the non-flooded treatment when water addition was necessary. The wicks were inserted in the soil in the upper section of the column and spread between the rhizoplane and the soil above the rhizoplane with any exposed portion of the wick wrapped securely with plastic. Water levels for the flooded treatment were restored when necessary to the marked lines of the initial water level (water was added to the larger container outside the column). The weights and plant height of the non-flooded treatments were monitored daily to determine if they were within 1 g of the wilting point weight. The wilting point had been determined previously by saturating the soil

of four *R. crispus* plants growing in columns and then allowing the soil to dry. The weights of the columns containing plant and soil when the plant showed signs of wilting were determined. These weights were used to calculate an estimate of the weight of a column containing wilted plant and soil. *R. crispus* plants grown for 8 weeks were assessed for their height and weight which was used to obtain a linear equation with which to make adjustments when calculating the soil weight in the columns.

2.4. Soil sampling – pH and redox potential measurements

After 13 weeks, soil samples were collected from columns selected in random order. Each column was cut carefully to separate the upper and lower sections. The plant and soil in the upper section of the column were removed and soil was shaken from the roots. The soil remaining on the roots was collected and considered 'above rhizoplane' soil. The soil immediately below the nylon mesh (rhizoplane) was sampled using 60 ml syringes with the tips removed so they became small soil corers (2.5 cm diameter). The column was inverted and 3 syringes were inserted into the soil at the center, away from the column edges. The soil was extruded from each syringe in 3 mm intervals, sliced carefully and retained for analysis. Seven samples were collected from each syringe at 3, 6, 9, 12, 15, 18 and 21 mm intervals. Samples from the three syringes were pooled for each increment to obtain enough soil (at least 3 g) for analysis.

Immediately upon obtaining a sample, pH and Eh were measured using a VWR Symphony SP90M5 Handheld Multimeter. Approximately 1 g of fresh soil sample was used to determine the soil pH in a 1:2 soil:water ratio (Gavlak et al., 2003). A soil paste (about 500 mg fresh soil sample and 3 ml water) was used to measure the Eh (Patrick et al., 1996).

2.5. Ferrous iron (Fe^{2+}) concentration and multi-element analysis of soil and plants

Fe^{2+} concentration was determined using a method modified from Roden and Wetzel (1996). Fe^{2+} standards were prepared from a stock solution containing 100 mg L^{-1} $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ in 1% (v/v) 6 M HCl. A fresh soil sample of known weight (about 0.5 g), was immediately transferred to 5 ml of 0.5 M HCl and extraction allowed overnight. The extraction was then filtered (0.45 μm pressure filter, Pall Corporation Supor[®]-450), diluted (flooded samples – 1:40 dilution, non-flooded samples – 1:10 dilution) and 0.25 ml of the diluted sample or of standard was added to 1.25 ml of FerroZine solution (1% wt/wt FerroZine in 50 mM HEPES buffer). After about 5 min, the absorbance was measured using a Helios Gamma UV–Vis Spectrophotometer at $\lambda = 562$.

The remaining soil was oven-dried (60 °C) until constant weight, crushed using mortar and pestle and homogenized. The samples were then analyzed for multiple elements (37 elements) via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) by a commercial laboratory (Activation Laboratories, Ltd, Analysis by Aqua Regia Extraction with ICP/OES finish). Method detection limits in mg kg^{-1} were as follows; Ag 0.2; Al 100; As 2; B 10; Ba 10; Be 0.5; Bi 2; Ca 100; Cd 0.5; Co 1; Cr 1; Cu 1; Fe 100; Ga 10; Hg 1; K 100; La 10; Mg 100; Mn 5; Mo 1; Na 10; Ni 6; P 10; Pb 7; S 100; Sb 2; Sc 1; Sr 1; Te 1; Ti 100; Tl 2; U 10; V 1; W 10; Y 1; Zn 2 and Zr 1 (Accredited Laboratory; ISO/IEC 17025:2005).

The plants were washed gently in distilled water, separated into aboveground and belowground material, oven-dried (60 °C) until constant weight, crushed and homogenized. A known amount of this plant material (approximately 250 mg) was digested in 5 ml HNO₃ and 5 ml distilled water in a MARS Xpress Microwave Digester (1600 W, 100% Power, ramped to 200 °C). The digested samples were cooled and analyzed in our laboratory for multiple elements with a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP). A continuing control verification (CCV) was done after every 10 samples to check that variability was within 10% for Al and Ca while all other elements were monitored. These samples were also diluted (1:100 in 5% HNO₃) and analyzed for the same 32 elements using the ICP-OES. Method detection limits in mg/kg for these elements were as follows: Ag 0.03; Al 22; As 1; B 0.5; Ba 0.006; Be 0.005; Ca 0; Cd 0.13; Ce 0.3; Co 0.14; Cr 0.1; Cu 0.1; Fe 0.1; K 0; Li 0.06; Mg 0.9; Mn 0.02; Mo 0.3; Na 0.2; Ni 0.4; P 2; Pb 0.5; S 1.7; Sb 0.2; Si 0.07; Sn 1.4; Sr 0.06; Ti 0.02; Tl 1.2; V 0.4; Zn 0.2; Zr 0.002.

2.6. Statistical analysis

Data for concentrations were log₁₀ transformed before statistical analysis to obtain normal distribution and homogeneity of variance. Significance of differences (probability) was determined by a General Linear Model (two-way ANOVA, $P < 0.05$) and Multiple Comparison tests by the Tukey Method ($P < 0.01$) using Minitab statistical software (Minitab® 15 ©2006 Minitab Inc.). Data for soil pH, Eh and element concentrations were analyzed for the following significant differences: 1) between moisture treatments regardless of sampling interval, 2) between sampling intervals regardless of moisture treatment, 3) between moisture treatments for the equivalent sampling intervals, 4) between sampling intervals of the flooded treatment, and 5) between sampling intervals of the non-flooded treatment. To test for relationships between element concentrations, pH and Eh, Pearson correlations and P values were calculated using Minitab. Here we consider only correlations with $r = 0.707$, that is, those correlations explaining 50% or more of the variation (McClave and Sincich, 2006).

3. Results

3.1. Soil

The results for pH, Eh and element concentrations in the soil focus on four sampling intervals; above the rhizoplane (ARZ), the 3, 6 and 9–21 mm intervals. Within each moisture treatment, concentrations at the sampling intervals 9, 12, 15, 18 and 21 mm were not significantly different from each other for any of the elements and so were pooled for statistical comparisons.

3.1.1. Soil pH—There were significant differences in soil pH between moisture treatments and between sampling intervals (Table 1). There were also significant interactions for pH between the moisture treatments and the sampling intervals, indicating that patterns in pH across intervals were not the same in flooded compared to non-flooded treatments. The soil pH was significantly higher in the non-flooded treatment compared to the flooded treatment for all sampling intervals (Fig. 2). For each moisture treatment separately, the non-flooded

soil showed no significant variations in pH above or below the rhizoplane but the flooded soil showed significantly lower pH above the rhizoplane compared to sampling intervals below.

3.1.2. Redox potential and Fe oxidation—Significant differences for Eh and Fe^{2+} occurred between moisture treatments and between sampling intervals (Table 1). There were also significant interactions for Eh and Fe^{2+} between the moisture treatments and the sampling intervals. Eh was significantly higher in the non-flooded treatment compared to the flooded treatment for all sampling intervals except for the soil above the rhizoplane (Fig. 3). In the non-flooded treatment, the Eh above the rhizoplane was significantly lower than soil 9–21 mm below the rhizoplane. The flooded treatment showed significantly higher Eh above the rhizoplane than the sampling intervals below the rhizoplane. For these intervals below the rhizoplane, the soil Eh showed no significant differences.

Concentrations of Fe^{2+} were significantly higher in the flooded treatment compared to the non-flooded treatment for all the sampling intervals (Fig. 4). The non-flooded treatment showed no significant differences in the Fe^{2+} concentrations between sampling intervals. However, in the flooded treatment the Fe^{2+} concentrations were significantly lower above the rhizoplane than the sampling intervals below the rhizoplane which did not vary significantly from each other. In the non-flooded treatment, no precipitation of Fe-oxyhydroxide (reddish-brown precipitate) was observed anywhere in the soil column. However, in the flooded treatment a reddish-brown precipitate was clearly visible on the plant roots, rhizoplane and soil above the rhizoplane.

3.1.3. Multiple element analysis—Element concentrations were at or below detection limits for Ag, As, B, Be, Cd, Mo, Bi, Ga, Hg, Pb, Sb, S, Sc, Te, Ti, Tl, U, W and Zr. A few other elements were easily detectable but showed no significant variation – for these the mean element concentrations $\pm$ standard deviation in $\mu\text{mol g}^{-1}$ dry soil, averaged for all samples were calculated, as follows: Ca (465 ± 38), Co (0.21 ± 0.02), Mn (13.1 ± 2.8), Ni (0.6 ± 0.1), and P (14.1 ± 1.5). These elements will not be discussed further.

Significant variation was observed for Al, Ba, Cr, Cu, Fe, K, La, Mg, Na, Sr, V, Y and Zn concentrations between sampling intervals and (except for Mg) between moisture treatments (Table 1). In addition, significant interactions were found between the moisture treatments and sampling intervals. The non-flooded compared to the flooded treatment had significantly higher Al, Cr, Cu, K, Sr, V and Y concentrations above the rhizoplane and significantly higher Al, Cr, Fe, K, Sr, V and Zn concentrations at the 9–21 mm interval (Table 2). Na concentrations were significantly higher in the flooded compared to the non-flooded treatment at the 3, 6 and 9–21 mm intervals.

In the non-flooded treatment, Al, Cr, Fe, K, La, Mg, Na, Sr, and V concentrations were significantly higher above the rhizoplane than below the rhizoplane (Table 2, Figs. 4 and 5). Concentrations of Ba, Cu, Y and Zn were significantly higher above the rhizoplane compared to the 6 and 9–21 mm interval. Na concentrations at the 9–21 mm interval were significantly lower than above the rhizoplane but higher than the 3 mm interval.

The flooded treatment showed significantly higher concentrations of Al, Fe, K, and V above the rhizoplane compared to the 6 mm and 9–21 mm intervals (Table 2, Fig. 4). Above the rhizoplane Cr concentrations were significantly higher than the 6 mm interval whereas Zn concentrations were significantly higher than the 9–21 mm interval (Fig. 5). Concentrations of Al, Fe, Cr, K, La, Sr, V and Zn were significantly higher at the 3 mm interval than at the 6 mm interval. Ba, Mg and Na showed no significant variation between intervals of the flooded treatment.

Correlation analysis of the data was carried out to ascertain possible underlying patterns regardless of the treatments. Some elements that varied significantly between the sampling intervals for the moisture treatments (Al, Cr, Fe, K, La, Sr, V, Y and Zn) correlated significantly with each other (Table 3). Ba correlated significantly with Al, K and V. Fe^{2+} correlated significantly with Eh and pH. Covariate analysis showed Fe to be a significant covariate with other elements (Table 4).

3.2. Plants

In both treatments, *R. crispus* formed a dense mat of roots on the surface of the mesh, the rhizoplane. Root growth in the flooded treatment was also densely distributed throughout the soil, but in the non-flooded treatment the roots were not as widespread. Plant biomass (mean $\pm$ standard deviation) was significantly higher ($P < 0.001$) for the flooded treatment (289 ± 39 mg live aboveground biomass, 1569 ± 236 mg belowground biomass) compared to the non-flooded treatment (99 ± 33 mg live aboveground biomass, 1067 ± 155 mg belowground biomass). Differences in dead above-ground biomass (120 ± 33 mg) were not significant between treatments. For the element analysis of the 32 elements in the plant material, not all yielded results suitable for statistical analysis. Element concentrations in some plant material were at or below the detection limits (Ag, As, Be, Cd, Ce, Co, Mo, Pb, Sb, Sn, Tl, V, Zr) or showed no significant variation. For the latter, the mean element concentrations $\pm$ standard deviation in $\mu\text{mol g}^{-1}$ aboveground or belowground tissue, averaged for all samples were as follows: Cr (0.03 ± 0.01 $\mu\text{mol g}^{-1}$ aboveground, 0.08 ± 0.07 $\mu\text{mol g}^{-1}$ below-ground), Zn (0.5 ± 0.2 $\mu\text{mol g}^{-1}$ aboveground, 0.4 ± 0.2 $\mu\text{mol g}^{-1}$ belowground). The flooded treatment had higher concentrations of Ca, K, Mn and P per gram of aboveground tissue and higher concentrations of Cu, Fe, K and Mn per gram of belowground tissue compared to the non-flooded treatment (Table 5). The non-flooded treatment had higher concentrations of Al, Fe and Si concentrations per gram of aboveground tissue compared to the flooded treatment. The plants of the flooded treatment showed significantly higher element content of Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, S, Si, Sr and Ti compared to plants of the non-flooded treatment.

4. Discussion

Despite significant differences in leaf and root biomass, both flooded and non-flooded plants formed a dense mat of roots covering a similar surface area at the rhizoplane. Surface area plays an important role in the transport of materials and plants usually increase root surface area to volume ratios to facilitate efficient nutrient uptake (Jungk, 2002). However, by

forcing the plant roots to grow along the mesh in this experiment, it was ensured that the effective surface area was similar in both the flooded and non-flooded treatments.

Some studies have shown that living plant roots have the ability to influence the soil chemistry (Hinsinger and Courchesne, 2008; Jacob and Otte, 2003; Kirk and Bajita, 1995; Neumann and Romheld, 2002; Wright and Otte, 1999; Youssef and Chino, 1989b). This study assumes that observed changes in the soil chemistry are plant-induced in addition to the activity of microbes and inorganic processes in the soil (Neumann and Romheld, 2002). Soil pH in the vicinity of roots can be influenced by nutrient availability, uptake ratio of anions and cations (Gerendas and Ratcliffe, 2002; Neumann and Romheld, 2002; Tinker and Nye, 2000), iron oxidation (Begg et al., 1994; Kirk and Bajita, 1995; Tinker and Nye, 2000), soil moisture and aeration, CO₂ production by roots (Neumann and Romheld, 2002) and by root exudation (Begg et al., 1994; Neumann and Romheld, 2002). The difference in pH between moisture treatments may be due to differences in CO₂ dissolution in response to flooding (Ponnamperuma, 1972). In turn, changes in soil pH influence the mobility, solubility and availability of micro-nutrients (Jacob and Otte, 2003; Kirk and Bajita, 1995; Luo et al., 2000; Mendelsohn, 1993; Youssef and Chino, 1991). Acidification associated with low pH enhances the plant's ability to accumulate metals near the roots (Kirk and Bajita, 1995; Van der Welle et al., 2007). In the flooded treatment in the study reported here the change in pH near the roots was observed in the same zone of change in element concentrations (between the soil above the rhizoplane and the 6 mm interval). However, soil pH did not significantly correlate with element concentrations observed in the soil, probably because the range of pH overall was narrow. Youssef and Chino (1989a) observed small changes in soils of similar pH (8.4) which was attributed to a high buffering capacity (Tao et al., 2004). Our observations are consistent with typical pH ranges (7.4–8.4) reported by the USDA for the soil used in this study (Fargo-Hegne) (Soil Survey Staff, 2008).

The Eh of the non-flooded treatment indicated that the soil was oxidized throughout the soil column whereas in the flooded treatment the soil was reduced except for above the rhizoplane, which was as oxidized as the non-flooded treatment. In anaerobic, chemically reduced environments, Eh tends to increase towards plant roots due to radial oxygen loss (ROL) and oxidation of ferrous iron (Davies, 1994; Flessa and Fischer, 1992). This in turn can lead to an influx of metals that have an affinity for Fe plaque (Otte et al., 1995) in the direction of plant roots and subsequent accumulation in the rhizosphere (Jacob and Otte, 2003; Wright and Otte, 1999).

With the exception of Ba, Fe was a significant covariate with the elements that showed variation in the soil columns (i.e. Al, Cr, Cu, K, La, Mg, Na, Sr, V, Y and Zn). This suggests an important role of Fe in the underlying mechanisms of mobility of elements in the soil – Fe colloidal oxides are known to act as carriers of other metals (Shuman, 2005). Movement of iron as Fe(II) from the reduced soil layer to the oxidized soil above the rhizoplane most likely followed a concentration gradient caused by changes in Eh (De Laune et al., 1981; Neumann and Romheld, 2002; Van der Welle et al., 2007), while other elements behaved similarly because they are redox sensitive (Kirk, 2004) and/or they have a high affinity to co-precipitate or form complexes with secondary minerals of Fe (Kabata-Pendias and Pendias, 2001; Kirk, 2004; Mathys, 1980; Otte et al., 1991). In contrast, Ba has chemical properties

similar to Ca and Sr (Suarez, 1996) and is usually associated with K in geochemical processes (Kabata-Pendias and Pendias, 2001). It typically has no strong geochemical relationship with Fe and so does not follow a similar pattern. Al appears to be a dominant element in this clay-rich soil and it too correlated with other elements that showed variation in the soil columns (Ba, Cr, Fe, K, La, Sr, V, Y and Zn). This suggests that these elements may also be associated with the colloidal surfaces of clay minerals in this soil or hydrous oxides of Al (Kabata-Pendias and Pendias, 2001; McBride, 1994; Shuman, 2005).

Patterns in Eh coincided with those of Fe^{2+} concentrations in both treatments. The low and non-variable Fe^{2+} concentrations detected in the non-flooded treatment indicated that this soil was homogeneously oxidized. In the flooded treatment the presence of high soluble iron (Fe^{2+}) concentrations below the rhizoplane is indicative of reducing conditions (Justin and Armstrong, 1987). The Fe plaque is visible evidence of oxidation in the vicinity of plant roots (Armstrong, 1967). In the flooded treatment, low Fe^{2+} concentrations above the rhizoplane and high Fe^{2+} concentrations below the rhizoplane showed that the soil above the rhizoplane was oxidized compared to the soil below, implying an oxidized-reduced boundary layer. This boundary layer of soil in the flooded treatment facilitates conditions for the oxidation of Fe^{2+} to Fe^{3+} (Mendelssohn, 1993), resulting in decreased Fe^{2+} concentrations (Otte et al., 1995) and Fe oxide precipitation near plant roots (Mendelssohn, 1993; Sadana and Claassen, 1996).

Element concentrations in the non-flooded treatment may be expected to be similar throughout the soil column (McGrath et al., 1997; Lorenz et al., 1997; Luo et al., 2000; Youssef and Chino, 1989b), but this treatment showed element (Al, Ba, Cr, Cu, Fe, K, La, Mg, Na, Sr, Y, V and Zn) accumulation in the soil near the roots, above the rhizoplane. This may have been due to the release of chelators, protons or other exudates from roots and microbial activity (Marschner et al., 1986; Parker et al., 2005; Youssef and Chino, 1989b; Zhang et al., 1991). Another possible explanation may be transport of solutes to the roots via mass flow exceeding uptake by plants, resulting in accumulation of elements near the roots (Hinsinger and Courchesne, 2008). The flooded treatment showed a different pattern of accumulation compared to the non-flooded treatment. Higher element accumulations above the rhizoplane as well as at the 3 mm interval below the rhizoplane compared to sampling intervals below were observed in the flooded treatment. Higher metal concentrations in the rhizosphere of wetland plants were also observed by Begg et al. (1994), Doyle and Otte (1997), Kirk and Bajita (1995), Otte et al. (1991, 1995), and Wright and Otte (1999).

A possible explanation for why the concentrations in the zone above the rhizoplane were lower in the flooded treatment compared to the non-flooded treatment is as follows. Fe plaque serves as a sink for metals accumulating around wetland plant roots (Armstrong, 1978; Gambrell and Patrick, 1978; Howeler, 1973; Taylor and Crowder, 1983). Some studies have shown that Fe plaque does not necessarily reduce metal uptake and may indeed enhance it (Liu et al., 2008; Otte et al., 1989; Ye et al., 1997b, 1998, 2001). Zn and As become adsorbed to Fe plaque, but through root exudation can still be made available for uptake by plants (McCabe et al., 2001; Otte et al., 1989, 1991; Zhang et al., 1998). This may explain the lower concentrations of elements above the rhizoplane in the flooded compared

to the non-flooded treatment. Adsorption to Fe oxides at the rhizoplane as well as plant uptake may have lowered the concentrations in the soil immediately surrounding the roots.

Concentrations of elements in plants are not good measures of element uptake, because differences in biomass affect concentrations due to dilution effects. Small plants usually have higher concentrations than large plants under otherwise comparable conditions (Ernst, 1995). Element uptake in plants can therefore only be accurately measured as the total amount of element per plant, particularly when significant differences in biomass occur between the groups being compared. In this experiment, plant growth was significantly affected by the treatments. Element uptake expressed as amount per plant was higher under flooded conditions compared to non-flooded conditions.

It would be ideal if we were able to calculate a mass balance to show that the amounts of element taken up in the plants explained the lower concentrations in the 'above rhizoplane' compartment of the flooded treatment compared to the non-flooded treatment, or to quantify the zones of accumulation in both treatments, but this is not possible with the experimental set-up used here. Soil analyzed as the 'above rhizoplane' soil was not a representative sample of that entire compartment, because it was taken from soil adhering to the roots. Soil in that same compartment a few millimeters away from the roots was not analyzed. It is therefore not possible to calculate the amount of element present in that compartment.

5. Conclusions

What this research has shown is (1) that patterns in element distribution in the soil as affected by the roots vary significantly between wetland and dryland conditions, (2) that the zones of accumulation differ in size between wetland and dryland conditions, and (3) that when the same plant species is grown under wetland and dryland conditions, the plants grown under wetland conditions take up more element per plant than those grown under dryland conditions.

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Fig. 1. Column apparatus for *Rumex crispus* experiment showing column components on the left and sampling locations on the right.

Fig. 2.

Mean soil pH in the moisture treatments (flooded; non-flooded) for the different sampling intervals (above rhizoplane (ARZ) and below rhizoplane) under *Rumex crispus* (RZ = rhizoplane, $n = 10$, except for 9–21 mm interval; $n = 50$). Different letters within each moisture treatment indicates significant variation between sampling intervals (ARZ, 3, 6 and 9–21 mm) at $P < 0.01$. The results of the comparison between moisture treatments for each interval appears to the right of the graph (the small black and white shapes at the top right of the graph indicate this comparison (e.g. ■:□ means filled squares compared to open squares); + indicates significant differences and – indicates no significant differences between moisture treatments, $P < 0.01$).

Fig. 3.
Mean Eh in the moisture treatments and statistical differences as in Fig. 2.

Fig. 4.

Mean Fe and Fe^{2+} concentrations ($\mu\text{mol g}^{-1}$ dry soil) in the moisture treatments and statistical differences as in Fig. 2.

Fig. 5.

Mean Cu, Cr and Zn concentrations ($\mu\text{mol g}^{-1}$ dry soil) in the moisture treatments and statistical differences as in Fig. 2. Where there is a box containing a plus sign (+) to the right of a point marking an interval in the non-flooded treatment indicates significant differences ($P < 0.01$) between that interval and the corresponding interval in the flooded treatment (no boxes indicate no significant differences). The shaded regions of the graph represent zones of element accumulation.

Table 1

Significance of differences (probability) in soil pH, Eh and element concentrations between the moisture treatments (flooded and non-flooded) and between sampling intervals (above rhizoplane (ARZ), 3, 6 and 9–21 mm below rhizoplane) as determined by Two-Way ANOVA (NS indicates non-significance; $P < 0.05$, $n = 10$).

Source of variation			
	A. Moisture treatments	B. Sampling intervals	Interaction (A × B)
	Probability		
pH	0.000	0.000	0.001
Eh	0.000	0.000	0.000
Al	0.01	0.000	0.000
Ba	0.044	0.000	0.021
Cr	0.001	0.000	0.000
Cu	0.014	0.000	0.003
Fe	0.004	0.000	0.000
Fe ²⁺	0.000	0.000	0.000
K	0.002	0.000	0.000
La	0.005	0.000	0.020
Mg	NS	0.000	0.001
Na	0.000	0.000	0.000
Sr	0.000	0.000	0.000
V	0.003	0.000	0.000
Y	0.001	0.000	0.007
Zn	0.012	0.000	0.001

Table 2

Mean element concentrations ($\mu\text{mol g}^{-1}$ dry soil) in the moisture treatments for the different sampling intervals (mean $\pm$ standard deviation, $n = 10$, except for $*n = 50$, different letters within each moisture treatment indicates significant variation between sampling intervals at $P < 0.01$, significant differences between moisture treatments for equivalent intervals is marked by † before the significantly higher value for a particular interval).

Element	Element concentrations						
	Flooded			Non-flooded			
	ARZ	3 mm	6 mm	*9–21 mm	ARZ	3 mm	6 mm
Al	922 $\pm$ 36 a	954 $\pm$ 76 a	797 $\pm$ 61 b	812 $\pm$ 51 b	† 1033 $\pm$ 65 x	867 $\pm$ 53 y	841 $\pm$ 31 y
Ba	1.28 $\pm$ 0.08 a	1.33 $\pm$ 0.10 a	1.20 $\pm$ 0.16 a	1.18 $\pm$ 0.11 a	1.47 $\pm$ 0.16 x	1.27 $\pm$ 0.20 xy	1.21 $\pm$ 0.08 y
K	104 $\pm$ 5 a	111 $\pm$ 8 a	92 $\pm$ 9 b	95 $\pm$ 7 b	† 119 $\pm$ 7.9 x	102 $\pm$ 7.1 y	98.2 $\pm$ 5.0 y
La	0.17 $\pm$ 0.02 ab	0.17 $\pm$ 0.01 a	0.15 $\pm$ 0.01 b	0.16 $\pm$ 0.01 b	0.19 $\pm$ 0.01 x	0.17 $\pm$ 0.01 y	0.16 $\pm$ 0.01 y
Mg	543 $\pm$ 19 a	568 $\pm$ 30 a	538 $\pm$ 39 a	537 $\pm$ 34 a	587 $\pm$ 40 x	537 $\pm$ 18 y	517 $\pm$ 23 y
Na	27.0 $\pm$ 3.4 a	† 25.2 $\pm$ 3.4 a	† 23.5 $\pm$ 2.3 a	† 24.7 $\pm$ 2.6 a	25.7 $\pm$ 1.4 x	16.5 $\pm$ 1.8 y	19.1 $\pm$ 2.3 yz
Sr	0.50 $\pm$ 0.02 ab	0.53 $\pm$ 0.03 a	0.48 $\pm$ 0.05 b	0.49 $\pm$ 0.03 b	† 0.56 $\pm$ 0.05 x	0.51 $\pm$ 0.02 y	0.50 $\pm$ 0.02 y
V	1.93 $\pm$ 0.06 a	1.99 $\pm$ 0.12 a	1.70 $\pm$ 0.15 b	1.75 $\pm$ 0.12 b	† 2.14 $\pm$ 0.10 x	1.85 $\pm$ 0.11 y	1.79 $\pm$ 0.07 y
Y	0.14 $\pm$ 0.01 ab	0.15 $\pm$ 0.01 a	0.14 $\pm$ 0.01 ab	0.13 $\pm$ 0.01 b	† 0.16 $\pm$ 0.01 x	0.15 $\pm$ 0.01 xy	0.14 $\pm$ 0.01 y

Table 3

Pearson correlations for pH, Eh and element concentrations in soil below *Rumex crispus*. Correlations with $r = 0.707$ (that explain 50% or more of variation) are shown ($P < 0.001$).

	Al	Cr	Fe	Fe2+	K	La	Sr	V	Y
pH				-0.726					
Eh				-0.964					
Ba	0.715				0.722			0.772	
Cr	0.900								
Fe	0.883	0.828							
K	0.966	0.907	0.900						
La	0.766	0.705	0.738		0.762				
Sr	0.861	0.841	0.812		0.886	0.727			
V	0.935	0.915	0.907		0.952	0.745	0.840		
Y	0.832	0.783	0.795		0.830	0.786	0.796	0.794	
Zn	0.901	0.837	0.846		0.888	0.734	0.788	0.859	0.864

Table 4

Analysis of covariance for element concentrations with moisture treatments, sampling intervals and interaction between the two as fixed variables and Fe as a covariate (NS indicates non-significance; $P < 0.05$).

Element	Source of variation			
	Fe (covariate)	A. Moisture treatments	B. Sampling intervals	Interaction (A × B)
	<i>P</i>			
Al	0.000	NS	0.000	0.007
Ba	0.000	NS	NS	NS
Cr	0.000	NS	0.002	0.046
Cu	0.000	NS	NS	0.035
K	0.000	NS	0.004	0.050
La	0.000	NS	NS	NS
Mg	0.000	NS	NS	0.014
Na	0.000	0.000	0.000	0.001
Sr	0.000	0.031	NS	0.008
V	0.000	NS	0.000	0.018
Y	0.000	NS	0.000	NS
Zn	0.000	NS	0.000	NS

Table 5

Mean element concentration of shoot and root (mean $\pm$ standard deviation, $n = 10$, different letters within each plant compartment indicates significant variation between moisture treatments at $P < 0.05$).

	Shoot		Root		Whole plant	
	Flooded	Non-flooded	Flooded	Non-flooded	Flooded	Non-flooded
Al	12 $\pm$ 8 a	30 $\pm$ 14 b	95 $\pm$ 52 d	54 $\pm$ 18 d	157 $\pm$ 92 x	63 $\pm$ 25 y
B	4.0 $\pm$ 0.6 a	5.8 $\pm$ 1.6 a	1.3 $\pm$ 0.2 d	1.6 $\pm$ 1.1 d	3.2 $\pm$ 0.5 x	2.3 $\pm$ 1.0 y
Ba	0.42 $\pm$ 0.05 a	0.54 $\pm$ 0.14 a	0.4 $\pm$ 0.1 d	0.3 $\pm$ 0.1 d	0.7 $\pm$ 0.2 x	0.3 $\pm$ 0.1 y
Ca	715 $\pm$ 79 a	530 $\pm$ 126 b	219 $\pm$ 28 d	220 $\pm$ 84 d	551 $\pm$ 92 x	283 $\pm$ 78 y
Cu	0.2 $\pm$ 0.02 a	0.2 $\pm$ 0.04 a	0.2 $\pm$ 0.03 d	0.1 $\pm$ 0.03 e	0.35 $\pm$ 0.06 x	0.15 $\pm$ 0.02 y
Fe	4 $\pm$ 2 a	10 $\pm$ 4 b	53 $\pm$ 20 d	28 $\pm$ 35 e	85 $\pm$ 36 x	29 $\pm$ 29 y
K	533 $\pm$ 71 a	416 $\pm$ 75 b	336 $\pm$ 55 d	236 $\pm$ 25 e	679 $\pm$ 124 x	292 $\pm$ 42 y
Li	0.7 $\pm$ 0.3 a	1.0 $\pm$ 0.4 a	0.4 $\pm$ 0.2 d	0.4 $\pm$ 0.3 d	0.9 $\pm$ 0.3 x	0.5 $\pm$ 0.3 y
Mg	432 $\pm$ 39 a	463 $\pm$ 102 a	125 $\pm$ 20 d	164 $\pm$ 81 d	323 $\pm$ 66 x	219 $\pm$ 78 y
Mn	5.6 $\pm$ 1.4 a	0.6 $\pm$ 0.1 b	6.2 $\pm$ 3.3 d	0.8 $\pm$ 1.1 e	11 $\pm$ 5.8 x	0.8 $\pm$ 0.9 y
Na	113 $\pm$ 72 a	233 $\pm$ 173 a	58 $\pm$ 7.6 d	45 $\pm$ 43 d	121 $\pm$ 21 x	71 $\pm$ 44 y
Ni	0.07 $\pm$ 0.03 a	0.08 $\pm$ 0.07 a	0.13 $\pm$ 0.09 d	0.08 $\pm$ 0.05 d	0.2 $\pm$ 0.1 x	0.09 $\pm$ 0.06 y
P	98 $\pm$ 16 a	64 $\pm$ 22 b	67 $\pm$ 6.6 d	72 $\pm$ 15 d	133 $\pm$ 20 x	83 $\pm$ 20 y
S	92 $\pm$ 33 a	114 $\pm$ 57 a	46 $\pm$ 4.3 d	45 $\pm$ 10 d	98 $\pm$ 14 x	60 $\pm$ 14 y
Si	59 $\pm$ 9 a	84 $\pm$ 18 b	94 $\pm$ 15 d	80 $\pm$ 18 d	167 $\pm$ 38 x	95 $\pm$ 30 y
Sr	0.7 $\pm$ 0.1 a	0.6 $\pm$ 0.1 a	0.40 $\pm$ 0.03 d	0.37 $\pm$ 0.05 d	0.82 $\pm$ 0.09 x	0.46 $\pm$ 0.06 y
Ti	0.2 $\pm$ 0.1 a	0.4 $\pm$ 0.1 a	1.2 $\pm$ 0.6 d	1.0 $\pm$ 1.2 d	2.0 $\pm$ 1.1 x	1.1 $\pm$ 0.97 y