Chemical and biological rhizosphere interactions in low zinc soils

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Abstract

Soil provides ecosystem services critical for life. The availability of micronutrients, such as zinc (Zn), in soils is an essential factor for normal healthy growth and reproduction of plants. Zinc deficiency is, however, a global problem in crop production due to low Zn bioavailability in soils to plants. The bioavailable Zn fraction in soils is controlled by several factors and is not directly related to the total Zn content of soils. The main objective of this thesis was the determination of factors which control Zn bioavailability in soils to plants and to assess approaches to improve the prediction of Zn plant uptake.

Based on rhizobox experiments, *in situ* measurements in the rhizosphere as well as multisurface- and radial transport modeling approaches it was shown that the effect of root exuded citrate for increasing plant available Zn is soil specific and does not depend on a specific concentration of low molecular weight organic acids (e.g. citric acid) in the soil solution. Using various low Zn soils at the same time in an experimental setting improved the understanding of soil-responsiveness to root exuded citrate.

Another insight was that multisurface models, which are widely used to assess the potential ecotoxicological risk in metal-contaminated soils, are also accurate to predict the Zn activity in soils with low Zn levels. The predictions were validated with the soil column Donnan Membrane Technique by using various soils with low Zn levels. It was predicted that soil organic matter is the dominant Zn sorbent and controlled the Zn activity also at low soil organic matter levels. Examples were shown how this modeling approach can be used to assess management options to increase bioavailable Zn to plants.

Using soil extracted Zn fractions to directly predict the Zn plant uptake at low Zn levels was shown to be inaccurate. Using a stepwise approach where the steps of the uptake process were characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot made the prediction of Zn plant uptake more accurate. Root surface adsorbed Zn was shown to be a useful proxy for the bioavailable Zn.

The framework of experimental and modeling approaches which were developed and applied in this thesis can also be used to study the plant-availability of other micronutrients at low concentration levels and how that is affected by various root exuded ligands.

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Abbreviations

CE	Capillary Electrophoresis
CEC	Cation Exchange Capacity
CBD	Citrate-Bicarbonate-Dithionite
DMT	Donnan Membrane Technique
DAG	Days After Germination
DTPA	DiethyleneTriamine Penta-acetic Acid
HFO	Hydrous Ferric Oxide
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma – Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
LMWOA	Low Molecular Weight Organic Acid
SOC	Soil Organic Carbon
UPW	Ultra-Pure Water

1. General Introduction

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Soil provides ecosystem services critical for life. Soil acts as a water filter and a growing medium, provides habitat for numerous organisms and supplies most of the antibiotics used to fight diseases. Soil is therefore linked to everything around us and performs many important roles in sustaining life on earth. All soils support biomass production, whether it is natural vegetation or planted for agriculture and forestry. From the smallest seedling to the largest tree, all land-based vegetation depends on soil to provide it with nutrients, water and root support. In turn, this vegetation supports animal life on land. The productivity of soil is dependent upon its biological, physical and chemical conditions, as well as on climate. However, future needs (e.g. biofuel production, urban development) and restrictions (e.g. (salinization, erosion, pollution) may result in competition for high quality agricultural land resulting in further pressure on soil (Sposito 2008).

The availability of the essential micronutrients (Zn, Cl, Fe, B, Mn, Cu, Mo and Ni), macronutrients (N, K, Ca, Mg, P and S), water and oxygen in the soils is the main controlling factor for sustainable crop growth on soils which was already stated by Sprengel (1828). He published in the beginning of the 19th century several works about the role of essential resources on plant growth. He concluded that "when a plant needs 12 substances to develop, it will not grow if any one of these is not available in a sufficiently large amount as required by the nature of plants." Some years later, Justus Von Liebig re-elaborated Sprengel's pioneering ideas and articulated the "law of the minimum" (Liebig 1855). The law of the minimum states that plant growth is limited by a single resource at any one time. Only after the availability of that resource increases to the point of sufficiency can another resource enhance plant growth. In the following the main focus of the thesis are the factors, processes and constraints which control the availability of the micronutrient zinc (Zn) for plant growth.

1.1 Zinc as an essential element for organisms

Zinc is a transition metal of atomic number 30 and has five stable isotopes: ^{64}Zn (48.63%), ^{66}Zn (27.90%), ^{67}Zn (4.90%), and ^{68}Zn (18.75%) and ^{70}Zn (0.62%) (Broadley et al. 2007). Zinc makes up about 75 ppm (0.0075%) of the Earth's crust and is therefore the 24th most abundant element (Emsley 2003). Zinc content in soils varies from 5–770 mg kg^{-1}. The concentration in seawater (30 µg kg^{-1}) is orders of magnitude lower. The Zn concentration in the atmosphere is 0.1–4 µg m^{-3} (Emsley 2003; Rauch 2011).

Zinc is an essential micronutrient for the normal healthy growth and reproduction of plants, animals and humans. Zinc is constituent of more than 200 structural components of enzymes which play a significant role in metabolism of proteins, carbohydrates, lipids and nucleic acids. It takes part in synthesis of ribonucleic acids and chlorophyll (Alloway 2008; Broadley

Fig. 1.1 Crop cultivation in low Zn soils: Effect of foliar application of Zn (100 mg Zn m^{-2} as ZnSO$_4$.7H$_2$0) to barley grown on a Zn deficient soil. Effect of Zn application (green parts on the field) was distinct within 3 weeks (a, photo from Cakmak et al. (1996)). Corn treated with 3.4 kg Zn ha^{-1} (left) significantly outgrew corn planted with no additional zinc 44 days after planting (b, photo by Maurice C. Wolcott). Young rice plants showing chlorosis, which is a typical symptom of zinc deficiency (c, photo by International Plant Nutrition Institute).

et al. 2012). The chlorosis and insufficient growth of leaves are the most characteristic symptoms of Zn deficiency in plant species (Fig. 1.1) (Alloway 2008). The zinc concentration in terrestrial plants varies widely (20-300 mg kg^{-1} dry weight (Kabata-Pendias and Pendias 1984)) depending on the geochemical and biogeochemical conditions. Zinc toxicity is less widespread than Zn deficiency. An excessive intake and accumulation of zinc leads to toxic effects in all living organisms. Zinc toxicity occurs in polluted soils due to Zn mining, smelting or dumping of Zn. An excessive concentration of Zn in humans induces fibrosis of the pulmonary system, vomiting, nausea and pneumonia (Nriagu 2011).

Zinc deficiency is a global problem both in human populations and in crop production and it is estimated that about 50% of the cereal-cultivated soils worldwide are low in plant available Zn, leading to reductions in crop production and also nutritional quality of the harvested

grains (Sillanpää and Vlek 1985; Graham et al. 1992; Cakmak 2008). Widespread Zn deficiency in human population occurs not surprisingly mostly in regions where cultivated soils are low in plant available Zn and cereal-based foods are the major source of daily calorie intake (Fig. 1.2). Up to 75% of the daily intake of calories in the rural population of developing countries comes only from cereal-based foods with very low Zn concentrations.

1.2 Total zinc vs. bioavailable zinc in soils

The total Zn content of a soil can largely be derived from the geochemical composition of the (weathering rock) parent material on which the soil has developed (Rauch 2011). Low total Zn concentrations occur in strongly leached tropical soils (e.g. Ferrasols) developed on highly weathered parent materials in the humid tropics on the continental shields of South America and Africa and on easily weatherable rock in hot and humid climates. Sandy soils (e.g. Arenosols, Regosols) which developed on sandstones or sandy drift are in general also low in
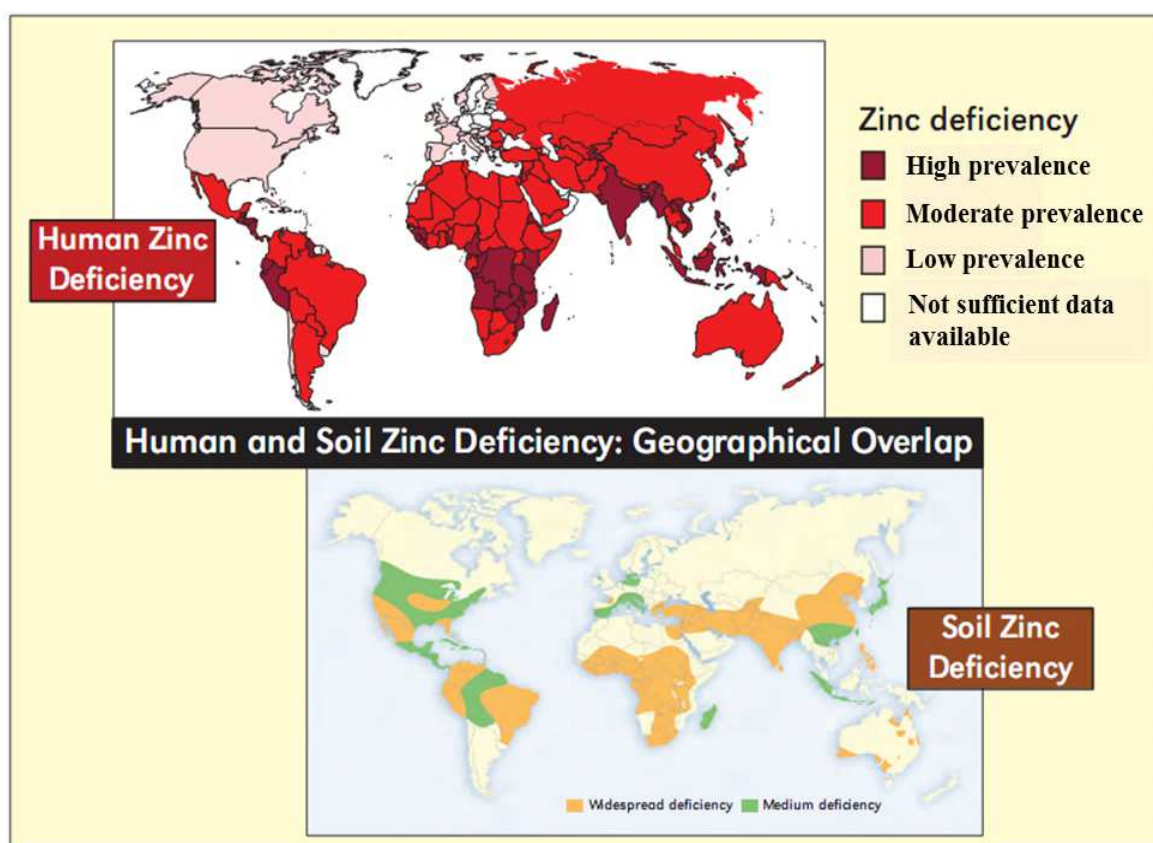

Fig. 1.2 Geographical overlap of human Zn deficiency (upper map) and soil Zn deficiency (lower map). (Sources: http://www.izinc.org; Alloway (2008))

Zn (Fig. 1.2). There is a significant discrepancy between the total Zn content of soils and the bioavailable Zn fraction in soils (Naidu et al. 2008; Temminghoff et al. 2008). The total soil Zn content can therefore often not be directly related to the amount of Zn available to plants and *vice versa*. There are several definitions of bioavailability, which were comprehensively reviewed by Naidu et al. (2008). In terms of plant uptake a chemical element can be defined as bioavailable if it is present as, or can be transformed readily to, the free ion species; if it can move to plant roots on a time scale that is relevant to plant growth and development and if once absorbed by the root it affects the life cycle of the plant (Sposito 2008). Plant available Zn forms are the free ions (Zn^{2+} and $ZnOH^+$) (Broadley et al. 2012) and soluble organic complexes such as Zn-phytosiderophores (Von Wirén et al. 1996), Zn-EDTA, Zn-NTA (Wang et al. 2009), Zn–histidine and Zn-citrate (Gramlich et al. 2013).

1.3 The rhizosphere

The bioavailability of Zn and other elements in the soil is directly influenced by the distance to the root surface of plants (Luster et al. 2009; Neumann et al. 2009). In 1904, Lorenz Hiltner first coined the term "rhizosphere" to describe the plant-root interface, a word originating in part from the Greek word "rhiza", meaning root (Hiltner 1904; Hartmann et al. 2008). Hiltner described the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced by the chemicals released from plant roots such as amino acids, low molecular weight organic acids (LMWOA`s) or phytosiderophores. The rhizosphere can in general be divided in three zones based on their relative proximity to the root. The endorhizosphere includes portions of the cortex and endodermis in which microbes and cations can occupy the "free space" between cells (apoplastic space). The rhizoplane is the medial zone directly adjacent to the root including the root epidermis and mucilage. The outermost zone is the ectorhizosphere which extends from the rhizoplane out into the bulk soil, i.e. the soil which is not penetrated or influenced by plant roots. The rhizosphere is not a region of definable size or shape. It consists of a gradient in chemical, biological and physical properties which change along the root (Luster et al. 2009; McNear Jr 2013).

1.4 Controlling factors of zinc bioavailability in soils

The transport and the speciation of different Zn forms in the bulk soil and the rhizosphere, and therefore the availability of Zn for plant uptake, is controlled by a range of factors. The main controlling soil factors of plant-available Zn forms are pH, sorption to the surface of the solid phase (soil organic matter, clay, iron (hydr)oxides), concentrations of other trace

elements (especially Fe, Cu and Ni) and macronutrients (especially N and P) and the concentration of (root exuded) organic ligands (Alloway 2009; Ivezić et al. 2012).

The soil pH is a main factor in controlling Zn bioavailability and affects adsorption-desorption processes, the solubility of metal hydroxide minerals and transport (Oste et al. 2001; Weng et al. 2001a). With increasing pH the adsorptive capacity of the soil's solid phase, the formation of hydrolyzed forms of Zn and co-precipitation in Fe oxides increases, and Zn bioavailability decreases. High pH values in soils are the result of a high $CaCO_3$ content (pedogenic or due to liming), high salt contents and reducing conditions (Lindsay 1972). The pH between the rhizosphere and the bulk soil can differ up to 2.5 pH units (Marschner et al. 1986; Shen et al. 2005; Bravin et al. 2012). Bravin et al. (2012) for instance showed that root-induced alkalinisation considerably altered Cu dynamic speciation in the acidic soils (pH < 5.5) by decreasing Cu^{2+} concentration and Cu lability. In the rhizosphere, soil pH influence almost disappeared as rhizosphere pH was neutral to slightly alkaline (7.3–7.5) whatever the initial bulk soil pH (4.8–7.5) was. Blossfeld et al. (2007; 2010) used planar pH optodes for the non-invasive 2D imaging of pH. They 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. Their calculations showed that such pH changes should have noticeable effects on the solubility of the trace metal in the rhizosphere. They suggested that studies and models dealing with trace metal uptake should include the effect of rhizospheric pH conditions, since up to now the bulk soil pH is often used to determine metal bioavailability to plants.

Soil organic matter (SOM) also controls the bioavailability of Zn. Especially in sandy soils organic matter is one of the most relevant solid phases that adsorbs Zn and other metals (Stevenson 1982; Weng et al. 2001a). The sorption of metals to SOM depends on pH and the origin and age of the organic material which determines the ratio of the functional groups (phenolic and carboxylic groups) and the nonreactive part (Milne et al. 2001). Weng et al. (2001a) and Bonten et al. (2008) used speciation and complexation models to estimate heavy metal sorption in soils and validated it with observed data from experiments. In both studies soil organic matter was the dominant sorbent for Zn. However, both studies were conducted at relatively high Zn levels. It is therefore not certain yet if that is also valid at low Zn levels.

Plant root exuded organic ligands such as phytosiderophores, citrate and other LMWOA`s are part of the dissolved soil organic matter. These ligands can form complexes with Zn which increase the mobility of Zn in the solution and the availability to plants (Von Wirén et al. 1996; Hoffland et al. 2006; Gramlich 2013). Hoffland et al. (2006) proposed that the citrate exudation capacity can be related to the tolerance of plants to Zn deficiency. Hajiboland et al. (2005) and Yang et al. (2003) showed that crops increased malate exudation in response to

higher bicarbonate levels in the root growth medium. Gao et al. (2009) also found increased malate concentrations in the rhizosphere at low Zn supply to plants. They detected malate concentrations of approximately 0.5 mM in the rhizosphere soil solution, but that concentration was not sufficient to significantly increase the Zn concentration in the soil solution. Getting a better understanding about the role of root exudates for increasing the bioavailability of Zn requires therefore new insights about the effects of root exudates in the soil solution over time and space (distance to the root surface).

Other trace elements in the soil (especially Fe, Cu and Ni) influence also the availability of Zn to plants. With increasing concentrations of other trace elements competition at biotic (Sunda and Huntsman 1998; Plette et al. 1999) and soil surfaces (Gao et al. 2010) is increasing. Kalis et al. (2006a) studied the effects of humic acid and competing cations on metal uptake by *Lolium perenne*. They concluded that the complexation of cations such as Cu, Pb, and Fe with high affinity for (dissolved) organic matter may lead to increased uptake of cations with low affinity for organic matter (Ni, Zn, and Cd) because of competition between cations at the root surface. Increasing concentrations of trace metals compete with Zn for the formation of complexes with root exuded ligands as described in the previous paragraph. Especially Fe^{3+} has higher formation constants than Zn^{2+} for ligands like phytosiderophores (Ptashnyk et al. 2011) and citrate. The significance of this competition effect needs to be assessed in more detail under rhizosphere conditions to quantify their effect on Zn bioavailability.

The concentrations of macronutrients in the soil solution can also influence the availability of Zn to plants. Especially phosphorus (P) has strong interaction with Zn. On the one hand, high P levels can induce Zn deficiency by changing either soil or plant factors (Broadley et al. 2012), such as the decrease of Zn solubility (Loneragan et al. 1979) and a decrease of the root colonization with arbuscular mycorrhiza (Ryan et al. 2008). Under low P conditions, on the other hand, plants are able to exude root exudates which are able to increase the mobility and availability of Zn (Dinkelaker et al. 1989; Hoffland et al. 2006).

1.5 Approaches to determine plant-available zinc

There are several methods to determine bioavailable Zn for plants, which can be divided in experimental and modeling approaches.

Most of the experimental approaches are based on determining a soil Zn fraction with a soil extraction solution. Several types of extraction solution can be distinguished: Complexing agents (such as the widely used DTPA (Lindsay and Norvell 1978) and EDTA (Sachdev et al. 1992) extractants), acid extractants (such as 0.1 M HCl (Wu and Hendershot 2009) or 0.43 M

HNO$_3$ (Römkens et al. 2009)) or salt extractants (such as 0.01 M CaCl$_2$ (Houba et al. 2000) and 0.1 M NaNO$_3$ (Gupta and Aten 1993)). However, due to wide variety of soil and plant properties there is no specific extractant which is accurate for all these different conditions (Menzies et al. 2007). Main reasons for this uncertainty are: (i) that the extracted fractions can often not be defined as specific plant available Zn species and (ii) how (directly or indirectly (see in the next section)) and to which plant parameter (dry weight, Zn content in grain or shoot) the extracted Zn fractions are related.

To measure a well-defined Zn or metal fraction, such as the free ion concentration, several speciation techniques are available. Within an extracted soil solution the free Zn ion concentration can be measured by Ion-Selective Electrodes (Bakker and Pretsch 2005), Cathodic Stripping Voltammetry (Xue and Sunda 1997), Permeation Liquid Membrane (Parthasarathy et al. 2001), Absence of Gradients and Nerstian Equilibrium Stripping (Chito et al. 2012) and the Donnan Membrane Technique (DMT) (Temminghoff et al. 2000). The advantage of the DMT is that several free metal ion concentrations can be measured at the same time in a single multicomponent solution sample (Kalis et al. 2007b). Additionally the DMT can be connected to soil columns to determine the free metal ion concentration which is continuously buffered by soil samples (Weng et al. 2001a). However, this approach was not tested yet for soils with low Zn or metal levels, because the analytical techniques were not sensitive enough for such low concentration levels.

Mechanistic computer modeling can be used as an approach to predict free metal ion concentrations or metal concentrations in the soil solution. Multisurface adsorption models which consider the interaction of metals with the solid phase and metal speciation in the solution can be useful tools for such predictions. Multisurface models were already applied in several studies to accurately predict the activity of metals in metal contaminated soils (Weng et al. 2001a; Dijkstra et al. 2004; Schröder 2005; Groenenberg et al. 2010), but this approach was not validated yet at low Zn levels. Since the speciation of metals changes with the distance to the root surface the accuracy of the Zn activity prediction could be improved by coupling a multisurface model with a radial transport (diffusion and convection) model.

1.6 Zinc uptake characteristics

The ion uptake of plants can in general be characterized by selectivity and accumulation (White 2012). Selectivity means that certain minerals are taken up at higher rates than others compared to their selective presence in the soil solution. Accumulation is another uptake characteristic which means that in the cell sap of the plants the concentration of a specific element can be much higher than in the soil solution. The Zn uptake by roots from nutrient

solutions can quantitatively be described by the Michaelis-Menten kinetics at Zn^{2+} concentrations $>0.5\ \mu M$ (Hacisalihoglu et al. 2001; Hart et al. 2002)

$$I\ (C) = \frac{I_{max}C}{K_m + C}$$ (1)

where I(C) is the rate of Zn accumulation in roots at a Zn^{2+} concentration C in the solution, I_{max} is the maximum rate of Zn accumulation, and K_m is the Zn^{2+} concentration at which the accumulation rate reaches half the maximum rate. However, at Zn^{2+} concentrations $<0.5\ \mu M$, which are often observed in low Zn soils, the Michaelis-Menten equation can be reduced to a linear relationship (Degryse et al. 2009).

Zinc concentrations which were determined in soil extracts or predicted by models can be related directly or stepwise to the shoot or grain Zn concentration (Fig. 1.3). Relating Zn concentrations directly to shoot Zn concentrations (Chojnacka et al. 2005; Feng et al. 2005) can be a source of inaccuracy, because the processes in the rhizosphere and at the root surface are not considered. Dividing the uptake process into a stepwise approach, where the steps of the uptake process are characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot may improve the prediction of plant Zn uptake. Kalis et al. (2007a) showed that considering the metal adsorption to the root surface in the stepwise approach improved the prediction of metal uptake by *Lolium perenne* grown in metal polluted soils. This approach may also improve the prediction of Zn uptake by crops grown under deficiency conditions, but was not considered yet in available studies.

1.7 Aim and outline of the thesis

Availability of metals in soils to plants has always been a complicated concept because many chemical, physical and biological processes are involved, such as metal speciation, solute flow to (metal) and from (exudates) the root surface and plant uptake. A full understanding of the concept and of determining factors needs an integrating approach using models to bridge the gap between spatial resolution of sampling techniques and the supposed scale of heterogeneity of rhizosphere processes.

The general aim of this thesis is to improve the understanding of processes which control metal, especially Zn, bioavailability to plants and to accurately predict plant Zn uptake (Fig. 1.3). This thesis will focus on the following three groups of objectives:

► The role of root exudates to increase Zn bioavailability to plants. The main questions are if citrate concentrations in the rhizosphere can significantly increase the Zn concentrations in the soil solution and how this effect is changing over time and distance to the root surface?

► Prediction of the Zn activity in the soil solution by multisurface models. The main questions are whether multisurface models, which were developed for heavy metal polluted soils, can be used to accurately predict the Zn activity also in soils with low Zn levels? Is it possible to experimentally validate such models in the low concentration range? Can multisurface models be used as tool to assess management options?

► Relating soil extracted Zn fractions to plant Zn uptake. Can the prediction of plant Zn uptake with $CaCl_2$-Zn, DTPA Zn or Zn^{+2} be improved when the interactions at the root surface are considered?
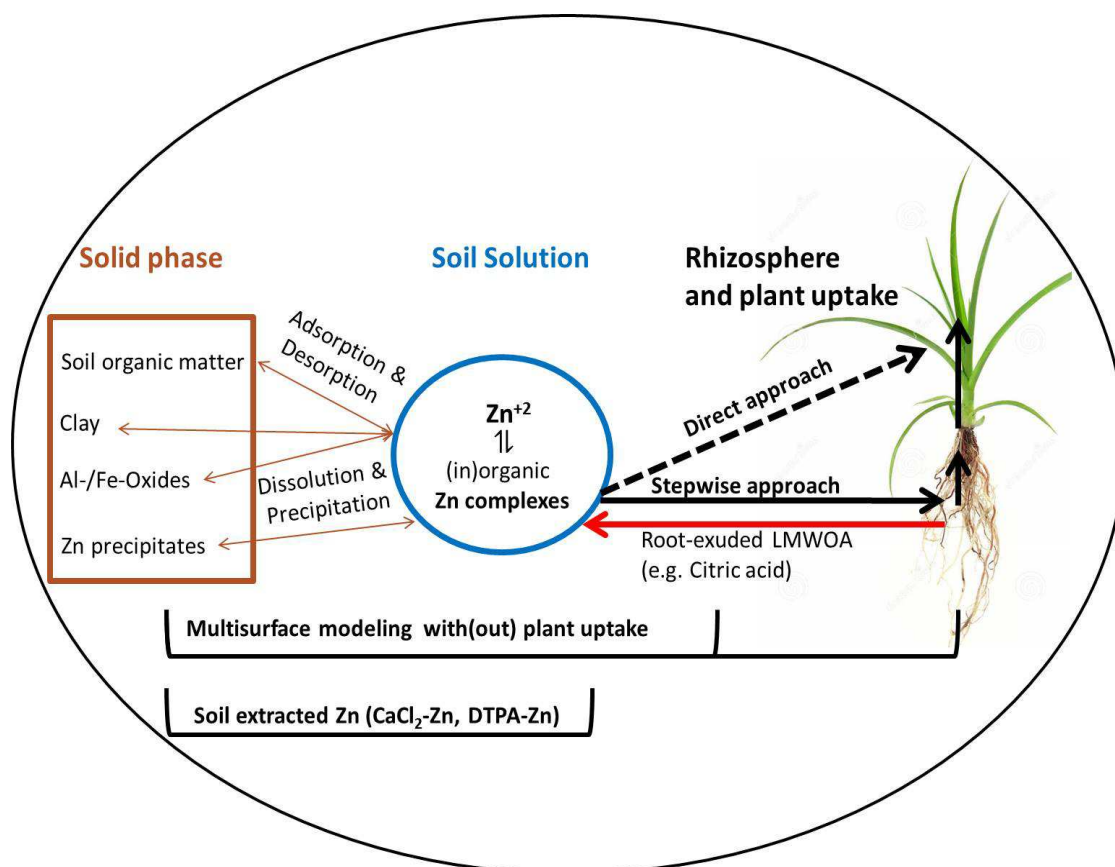

Fig. 1.3 Overview of the approaches (indicated by black arrows and brackets) and processes (indicated by brown and red arrows) which were studied in this thesis.

The following outline gives an overview on how these objectives where tackled. Figure 1.3 shows the object of investigation and gives an overview about the processes and approaches which were assessed in this thesis.

In a first step (**Chapter 2**) rhizobox experiments were conducted to determine *in situ* the chemical conditions (pH, metal concentrations, DOC, citrate concentrations, etc.) in the rhizosphere and in the bulk soil at low Zn levels. The exudation rate of the plants was determined with a nutrient solution experiment. Additionally a soil extraction experiment was conducted to determine the mobilization potential of citrate for Zn. These results were used to assess if root exuded citrate is able to increase Zn and P concentrations in the rhizosphere soil solution and the plant uptake of Zn and P.

Relating different soil extractable Zn fractions (DTPA-Zn, $CaCl_2$-Zn and Zn^{2+}) from low Zn soils directly with the plant Zn uptake is often inaccurate. Therefore a stepwise approach was tested (**Chapter 3**) where the steps of the uptake process are characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot. The aim of this study was to improve the prediction of the Zn bioavailability to wheat by integrating pH dependent nonlinear root surface adsorption. For doing this, wheat plants were grown in a wide range of low Zn soils to determine plant growth and Zn plant uptake and the Zn^{2+} concentration was determined experimentally with the Donnan Membrane Technique.

In **Chapter 4**, a multisurface model which is widely used to assess the potential ecotoxicological risk in metal contaminated soils was tested whether it is also able to accurately predict the Zn solid-solution distribution and the Zn^{2+} concentration in low Zn soils. The overall accuracy of the model was evaluated by comparing the predicted values with the values which were determined with a soil column Donnan Membrane Technique experiment. The multisurface model approach was also used to assess the influence of management options (pH changes, organic matter application and Zn fertilization) on Zn solid-solution distribution and Zn speciation.

In **Chapter 5** a combined multisurface- and radial transport model approach was used to study the effect of root exuded citrate on Zn speciation in the rhizosphere over time and distance to the root surface. Interactions of Zn, Fe and citrate with the sorbents goethite and soil organic matter, degradation of citrate and diffusion of Zn towards and citrate away from the root surface, respectively, were considered.

In the final chapter (**Chapter 6**) the main outcomes of the thesis were discussed in a general context considering the main research questions presented in the general introduction. In

addition implications of the results for practical management options and future research are described.

2. Bioavailability of zinc and phosphorus in calcareous soils as affected by citrate exudation

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2.1 Abstract

Zinc (Zn) and phosphorus (P) deficiency often occurs at the same time and limits crop production in many soils. It has been suggested that citrate root exudation is a response of plants to both deficiencies. We used white lupin (*Lupinus albus* L.) as a model plant to clarify if citrate exuded by roots could increase the bioavailability of Zn and P in calcareous soils. White lupin was grown in nutrient solution and in two calcareous soils in a rhizobox. Rhizosphere soil solution was sampled to determine citrate, metals and P. Based on the measured citrate concentrations, a soil extraction experiment with citrate as extractant was done. Absence of Zn triggered neither cluster root formation nor citrate exudation of white lupin grown in nutrient solution, whereas low P supply did. The maximum citrate concentration (~1.5 mM) found in the cluster rhizosphere soil solution of one soil mobilized P, but not Zn. In the other soil the highest citrate concentration (~0.5 mM) mobilized both elements. White lupin does not respond to low Zn bioavailability by increasing citrate exudation. Such a response was observed at low P supply only. Whether Zn and P can be mobilized by citrate is soil-dependent and the possible controlling mechanisms are discussed.

2.2 Introduction

Zinc (Zn) deficiency is limiting crop production in ±30 % of the world's soils (Sillanpää and Vlek 1985). In these areas, Zn deficiency is often caused not by low total soil Zn contents but by low bioavailability of Zn (Alloway 2009). Zinc deficiency often co-occurs with P deficiency, because the bioavailability of both elements decreases with increasing pH (Zhu et al. 2001). At neutral pH (pH<8.4) Zn solubility is mainly controlled by adsorption reactions (Catlett et al. 2002) and P can precipitate as Ca-phosphate (Weng et al. 2011b). Quijano-Guerta et al. (2002) studied the tolerance of rice (*Oryza sativa* L.) germplasm to Zn deficiency. They showed that genotypes tolerant to Zn deficiency also have at least a moderate tolerance to P deficiency. Hoffland et al. (2006) related these characteristics of multiple tolerance to citrate exudation. Mobilization of Zn and P by citrate is based on different mechanisms. The free Zn ion (Zn^{2+}) is considered as the main bioavailable species (Kalis et al. 2007a). Zinc mobilization is based on acidification due to the exudation of protons (Hinsinger et al. 2003) and on formation of soluble complexes of exuded citrate with Zn^{2+} in solution, which reduces the activity of Zn^{2+} in the soil solution. As a consequence Zn^{2+} desorbs from soil surfaces or dissolves from labile solid phases to replenish Zn^{2+} in solution (Lindsay and Norvell 1978). Phosphate mobilization from soils by citrate and proton exudation is attributed to (i) the enhanced dissolution of P minerals caused by acidification and complexation of cations such as calcium (in alkaline soils), aluminum and iron (in acid

soils) from phosphate minerals by citrate, and (ii) the competitive adsorption of phosphate and citrate on metal (hydr)oxides (Geelhoed et al. 1998). Although it is well documented that citrate (and protons) is able to mobilize P in soil (Hoffland 1992; Geelhoed et al. 1999; Kirk et al. 1999a; Hinsinger 2001; Oburger et al. 2011), there are contrasting reports on root exudation of citrate or other LMWOA`s under Zn deficiency and/or their potential to mobilize Zn in soil. On the one hand Rose et al. (2011) proposed that enhanced exudation of malate, but not citrate, is a response of Zn-efficient rice genotypes to Zn deficiency. Widodo et al. (2010) stated that the high tolerance of rice cultivars to Zn deficiency is most likely a result of malate exudation. Neither of these studies, however, proved Zn mobilization from soil by citrate. Degryse et al. (2008) showed that carboxylates exuded from roots of spinach (*Spinacia oleracea* L.) and tomato (*Solanum lycopersicum* L.) were able to mobilize Cu and Zn from a calcareous soil. On the other hand Gao et al. (2009) showed that malate concentrations observed in the rhizosphere of rice had a negligible effect on the concentration of Zn in soil solution of a low Zn soil.

We aimed to test the effectiveness of citrate exudation as response to low Zn and P in calcareous soils. White lupin (*Lupinus albus* L.) is a useful model plant to study mobilization, because it is known from non-calcareous soils that white lupin shows a strong and effective response to low P supply by the formation of cluster roots (bottlebrush-like clusters of rootlets (Purnell 1960)) and exudation of citrate and protons (Dinkelaker et al. 1995; Weisskopf et al. 2006). Citrate concentrations can reach mM levels in the rhizosphere solution of cluster roots (Dessureault-Rompré et al. 2008). Gardner et al. (1982) showed that white lupin grown on an alkaline soil also responds to iron stress by the development of a higher proportion of cluster roots and an increase in proton and reducing and chelating compound production. However, to our best knowledge, such a response was not yet shown for white lupin under Zn deficiency conditions. There are different approaches to determine a possible multiple stress response to low Zn and P bioavailability in the rhizosphere. One is the use of rhizoboxes in combination with micro-suction cups (Göttlein et al. 1996) which were already used with different plants and soils (Wenzel et al. 2001; Dessureault-Rompré et al. 2008; Gao et al. 2009). In the present study we determined in situ Zn and P mobilization by citrate in the rhizosphere of calcareous soils and linked potential Zn and P mobilization to plant uptake and the results of a nutrient solution and a soil extraction experiment.

2.3 Material and methods

2.3.1 Nutrient solution experiment

With a nutrient solution experiment we studied the formation of cluster roots and the exudation of citrate by white lupin in absence of Zn and P. White lupin (*Lupinus albus* L. var. Feodora) seeds were germinated in quartz sand and were transplanted after 5 days to 50 L containers filled with a continuously aerated nutrient solution. The composition of the nutrient solution was based on Jaitz et al. (2011). The pH of the nutrient solution was adjusted to 7.3 (±0.2). In addition to the control treatment containing all nutrients, there were two other treatments: either without P or without Zn. After 21 days of growth, each plant was put individually in a Petri dish with 30 mL 1 mM $CaCl_2$ solution to determine the citrate exudation rate (Neumann and Römheld 2007). Two hours later, a sample of 10 mL was taken from the solution. After filtration (0.45 μm), 50 μL of $CHCl_3$ was added to avoid microbial degradation and the samples were analyzed for citrate and nitrate using capillary electrophoresis (CE) (see below). The citrate exudation rate was determined for four individual plants per treatment (n=4). After collecting root exudates, roots were scanned on a root scanner followed by drying the roots at 70 °C for root dry weight determination.

2.3.2 Soils

Soil samples were collected in Hofuf (Saudi Arabia) and Anatolia (Turkey) from the top layer (0–20 cm). The soils are named after the location of collection. The sampling sites were used for crop cultivation and both soils were classified as Aridisols. In crops grown on Hofuf soil Fe chlorosis (Schenkeveld et al. 2008) and in crops grown on Anatolia soil Zn chlorosis (Cakmak et al. 1996) was manifested, respectively. The pre-treatment of the soil samples consisted of drying (40 °C), sieving (2 mm) and homogenizing. The soil characteristics are shown in Table 2.1. The pH and *aqua regia* extractable Zn fractions of the two soils are comparable. The DTPA extractable Zn (Lindsay and Norvell 1978) in the loamy Anatolia soil was five times lower than in the sandy Hofuf soil (Table 2.1). For quality assurance, reference samples from clay and sandy soils were analyzed (respectively ISE 989 and ISE 949, WEPAL, www.wepal.nl).

2.3.3 Rhizobox experiment

With a rhizobox experiment we investigated how the rhizosphere is affected by the response of white lupin to Zn and P deficiency in the two soils (Anatolia and Hofuf). The experiment was conducted in a climate chamber at Wageningen University (photoperiod of 15 h (6:00–21:00 h), light intensity 525 $\mu M\ m^{-2}\ s^{-1}$, day/night temperature 25/18 °C, rel. humidity 70 %). Rhizoboxes, so-called "Hohenheim" boxes (Luster et al. 2009) (33 cm long, 20 cm wide, and 1.5 cm deep) were filled with moistened soil to a dry bulk density of 1.1 g cm^{-3}. Treatments included a combination of two Zn and two P levels (i.e. –P/–Zn, +P/–Zn, –P/+Zn and +P/+Zn). Except for the –P and –Zn treatments the soil was fertilized with 100 mg N kg^{-1} (as $Ca(NO_3)_2\ 4H_2O$), 80 mg P kg^{-1} (as KH_2PO_4), 100 mg K kg^{-1} (as KCl), 10 mg Zn kg^{-1} (as $ZnCl_2$) and 1.5 mg Fe kg^{-1} (as Fe-HBED, (Chaney 1988)), respectively. In the –P and –Zn treatment, P or Zn, respectively was omitted. Each treatment was duplicated. The soil was adjusted to 60% of its water holding capacity every day. Seeds of white lupin (*Lupinus albus* L. var. Feodora) were soaked in 10 % H_2O_2 for 10 min. After a germination period of 4 days on moist (double-distilled water) paper tissue, the seedlings were transplanted to the rhizoboxes (two plants per rhizobox), which was defined as the first Day After Germination (DAG). The rhizoboxes were standing in racks at an angle of 30° to force the roots to grow along the Plexiglas plate side, which enabled us to observe and localize the roots. The Plexiglas was covered most of the time with a plastic sheet to exclude any light effect on root growth. The other side of the rhizobox consisted of a non-transparent PVC sheet with a 5×5 mm grid of holes (1.8 mm diameter) for insertion of micro-suction cups, which were used for *in situ* soil solution sampling. The design, pre-cleaning- and sampling procedure with the micro suction cups was done according to Shen and Hoffland (2007).

Soil solution was sampled in the rhizosphere of the cluster roots and in the bulk soil. Around the cluster roots 6–8 micro-suction cups were inserted per rhizobox at a distance of ±1 mm from the rootlet. We considered soil which was more than 5 cm away from the roots as bulk soil. Between the 1st and the 11th day after the emergence of the cluster root (17–27 days after germination (DAG)) the soil solution was sampled every second day at 14:00 h (Dessureault-Rompré et al. 2007) for 2.5 h, which yielded a volume of ±0.2 mL per micro-suction cup. To obtain enough soil solution for analysis, the samples collected by 6–8 micro-suction cups per cluster root were mixed. In the Hofuf soil only one replicate was analyzed 7 days (+P/–Zn treatment) and 9 days (–P/+Zn and –P/–Zn treatment) after cluster emergence, because the sample volume was too small to allow for replicates. The pH was determined immediately after sampling. The solution was subsequently divided into subsamples for the analysis of citrate and nitrate with Capillary Electrophoresis (see below) and of Zn, Fe and P with Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Elan 6000, Perkin Elmer). The detection limit for P and Zn with ICP MS was 1 μgL^{-1} and 0.3 μgL^{-1}, respectively.

Phosphorus analysis with ICP-MS includes the inorganic and the organic P fractions. Roots and shoots were harvested 27 DAG. Roots were washed thoroughly with distilled water to remove all soil particles and the number of cluster roots per plant was counted followed by washing the roots for 1 min in a 0.01M EDTA solution to remove the root surface adsorbed metals (Kalis et al. 2007a). Afterwards the roots and shoots were dried at 70 °C for 3 days to determine their dry weight and milled and digested according to the procedure described by Novozamsky et al. (1996). The Zn and P concentrations in digests were measured with ICP-MS. Plant uptake was calculated by summing the products of root and shoot dry weight and their respective tissue content of P and Zn, minus the product of seed dry weight and seed tissue content of P and Zn. The rhizobox experiment was done twice, yielding highly similar results. Results of one of the two experiments are shown.

2.3.4 Soil extraction experiment

The soil extraction experiment with the Anatolia and Hofuf soils aimed to determine the Zn and P mobilizing capacity of citrate. Air-dried soil (3 g) was added to a 50 mL centrifugation tube and 30 mL extraction solution was added (Houba et al. 2000). The extraction solution consisted of citric acid with a concentration range from 0 to 7 mM in 0.01M $CaCl_2$. Hundred μL of $CHCl_3$ was added to the extraction solution to avoid microbial degradation. The tubes were shaken horizontally for 24 h at 20 °C. The pH in the suspensions was measured immediately after shaking, then the suspensions were centrifuged at 3000×g for 10 min, and the supernatants were filtered (0.45 μm) before determining the concentrations of citrate (Capillary Electrophoresis, see below), Zn and P (ICP-MS). The experiment was done in duplicate.

Table 2.1 Soil characteristics

Soil	pH[a]	Clay	CaCO$_3$[b]	SOC[c]	CEC[d]	CBD[e]	0.005 M DTPA[f]		*Aqua regia*[g]
						Fe	Zn	Fe	Zn
		%	%	g kg^{-1}	cmol$^+$ kg^{-1}	g kg^{-1}	------------------ mg kg^{-1} --------------------		
Hofuf [#]	7.7	4	6.2	7.1	3.5	0.6	5.03	6.7	28
Anatolia	7.9	22	9.3	10	32.1	5.1	0.09	11.2	33

[#] Data of Hofuf soil are from Schenkeveld et al. (2008)

[a] 0.01 *M* CaCl$_2$, ISO/DIS 10390; [b] ISO 10693, [c] Soil organic carbon, Walinga et al. (1992) ; [d] Cation exchange capacity, ISO/DIS 11260; [e] Citrate Bicarbonate Dithionite, Holmgren (1967) ; [f] Lindsay and Norvell (1978); [g] ISO 11466

2.3.5 Citrate and nitrate analysis

Citrate and nitrate were analyzed with capillary electrophoresis (CE) in adaptation to the method of Westergaard et al. (1998). The instrument (Waters Corp., Milford, MA, USA) was equipped with a UV detector (indirect) and a 254 nm filter. All separations were performed in a fused-silica capillary (accu-Sep 75 µm x 60 cm capillary, Waters Corp., Milford, MA, USA). Between two samples the capillary was rinsed with deionized water for 0.5 min, with 0.1 M NaOH for 1 min and with deionized water for 0.5 min, respectively, after which it was preconditioned with the background electrolyte for 3 min. The background electrolyte was prepared with 1,2,4-benzenetri-carboxylic acid [trimellitic acid, (TMA)]. The electrolyte consisted of 3 mM TMA and 0.02% (v/v) diethylenetriamine (DETA) with the pH adjusted to 5.8 with NaOH. Cation interference (metal-organic anion complexes) was prevented by adding 50 µL of a 25 mM tetra-sodium-ethyleneditetraacetic (Na_4EDTA, pH >10) solution to each sample of 450 µL. The CE was run at 20°C and the high voltage was set to 25 kV. The detection limit was 10 µM.

2.3.6 Statistical analysis

Statistical analysis of data was performed with SPSS analytical software (SPSS Inc., Chicago, IL, USA; version 17). A two-way ANOVA was done to analyze main effects of the P and Zn treatments on shoot dry weight, on Zn and P plant uptake and on Zn, Fe and P concentrations in the soil solution. The variation among data was homogeneous (Levene's test). Bivariate correlation was used to determine the Pearson correlation coefficient (r) between the P or Zn contents in the shoot tissue and the number of cluster roots, respectively, with a two tailed test for significance (P<0.05).

2.4 Results

2.4.1 Nutrient solution experiment

Cluster root formation was observed neither on plants grown in the nutrient solution without Zn nor on those grown in the complete nutrient solution. In the solution without added P considerable cluster root formation was observed (Fig. 2.1). Citrate exudation by root systems grown in -Zn and in complete nutrient solution was below the detection limit (10 µM), which means that the exudation rate was below ~0.8 µmol g^{-1} h^{-1}. In the -P treatment, the exudation
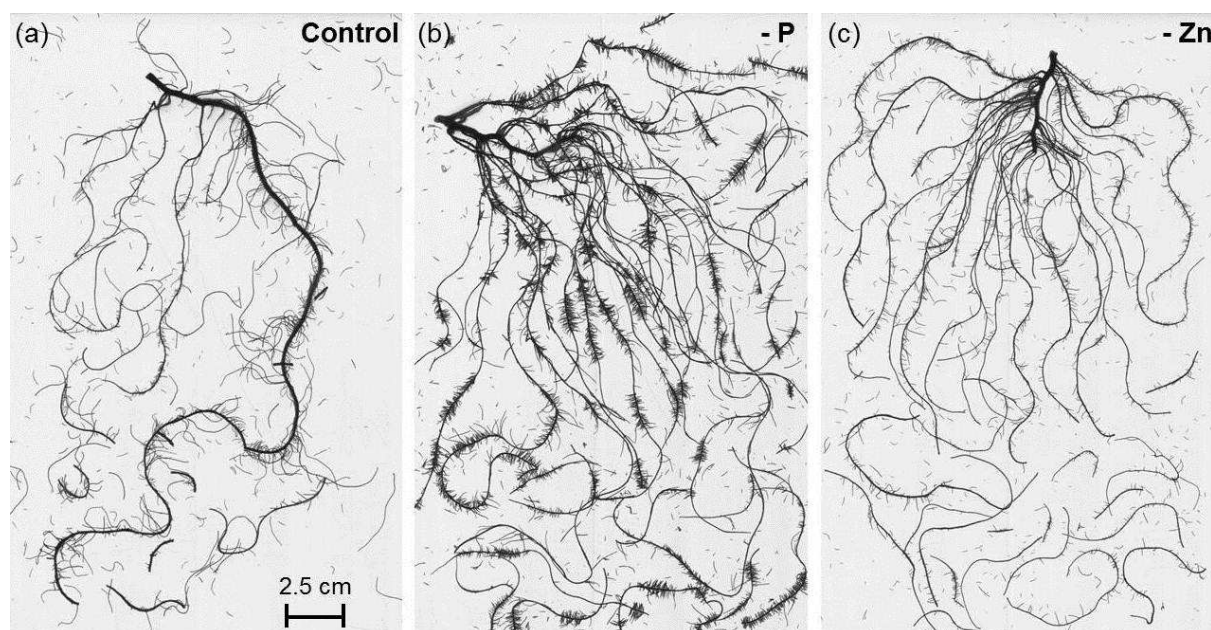
Fig. 2.1 Representative root system scans of white lupin plants grown in nutrient solution with all nutrients (a), without P (b), or without Zn (c). The bottle-brush-like clusters of rootlets in (b) are cluster roots.

rate was 3.9 µmol g^{-1} h^{-1}. The plants grown on the -Zn treatment showed Zn deficiency symptoms (chlorotic leaves with brown necrotic spots). Neither the plants grown in the nutrient solution without P nor those grown in the complete nutrient solution showed visible deficiency symptoms apart from growth reduction.

2.4.2 Rhizobox experiment

Plants of all treatments looked similar. No stress symptoms could be observed. Shoot dry weight was increased significantly (P=0.002) by P fertilization in the Anatolia soil, but not by Zn fertilization. Shoot dry weight of the plants grown in the Hofuf soil showed neither a response to P nor to Zn fertilization (P>0.05) (Fig. 2.2 a). Plant uptake of P and Zn increased significantly due to P (P≤0.0001) and Zn (P≤0.0001) fertilization on the Anatolia soil (Fig. 2.2 b,c). Plants grown on the Hofuf soil responded to P (P=0.002), but not to Zn fertilization. Zinc uptake was significantly higher in the -P/-Zn treatment than in the +P/-Zn treatment from this soil (Fig. 2.2 b,c).
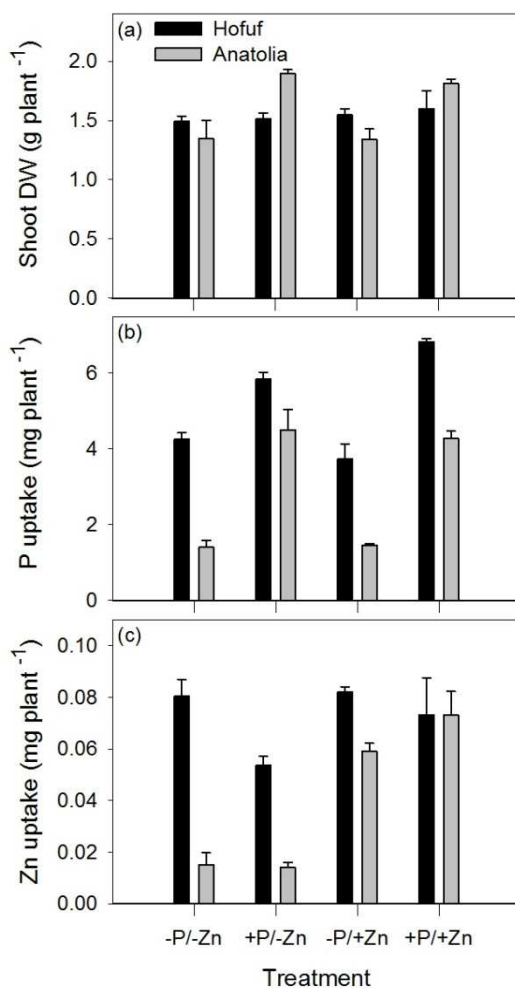
Fig. 2.2 Shoot dry weight (a), P plant uptake (b), and Zn plant uptake (c) of white lupin plants (27 days after germination) grown on two calcareous soils with (+) and without (-) fertilization with Zn or P. Error bars are standard errors (n=2).

Cluster root abundance in both soils was around 2.5 times higher in the -P compared to the +P treatments. The total number of cluster roots per plant in the -P treatment was about 20 and 8 in the Anatolia and the Hofuf soil, respectively. Phosphorous content in the shoot tissue of the Anatolia (r=-0.88, P=0.04, n=8) and Hofuf soil (r=-0.78, P=0.02, n=8) correlated negatively with the number of cluster roots per plant. The Zn content in the shoot tissue did not correlate with the number of cluster roots (P>0.05).

Citrate concentrations in the soil solution of the cluster rhizosphere peaked 3 days after emergence of the cluster roots (Fig. 2.3). Maximum citrate concentrations in the Anatolia and Hofuf soils were about 1.5 mM and 0.5 mM, respectively. At this point in time citrate

concentrations in the -P treatments were significantly higher than in the +P treatments. Zn fertilization did not affect the citrate concentrations in the cluster rhizosphere (Fig. 2.3).

At the same time Zn and P application had increased the respective Zn and P concentrations in the cluster rhizosphere solution of both soils compared with the bulk soil (-P/-Zn) (Table 2.2). However, except for the Zn concentrations in the Anatolia cluster rhizosphere soil solution, the Zn and Fe concentrations in the cluster rhizosphere of the -P treatments (-P/-Zn; -P/+Zn) were significantly higher than in the +P (+P/-Zn; +P/+Zn) treatments and the bulk soil (-P/-Zn), respectively (Table 2). The P concentrations in the cluster rhizosphere of the -P treatments (-P/-Zn; -P/+Zn) were also significantly higher than in the bulk soil (-P/-Zn).

The bulk soil pH was almost constant over time at ~7.8 and ~7.6 in the Anatolia and Hofuf soil, respectively. The pH values in the soil solution of the cluster rhizosphere were increasing ~3 days after the emergence of the cluster roots (Fig. 2.4). Nitrate concentrations in the soil solution of the cluster root rhizosphere were higher in the -P treatments compared to the +P treatments. In all treatments, a sharp decline of the NO_3 concentrations could be observed around 3 days after the emergence of the cluster roots (data not shown).
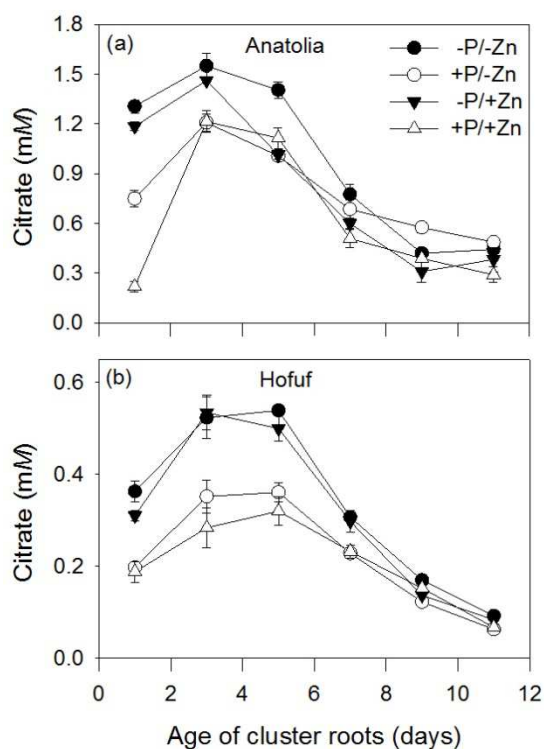
Fig. 2.3 Citrate concentrations in the cluster rhizosphere soil solution of the Anatolia (a) and Hofuf (b) soil depending on the age of the cluster roots. Error bars represent standard errors (n=2).

2.4.3 Extraction experiment

After shaking soil samples in a citrate solution, adsorbed citrate appeared to be linearly related to equilibrium citrate concentration in the extract. The highest added concentration of citric acid (7 mM) resulted in 1.3-1.7 mM citrate in solution after reaching equilibrium (24h) (Fig. 2.5 a). The pH values in the extractants of the two soils were decreasing with an increasing citrate concentration in solution. In the Anatolia soil, the pH decreased ±1 pH unit at the highest citrate concentrations in solution, whereas in Hofuf soil, the pH decreased ±1.3 pH units (Fig. 2.5 b).

Table 2.2 Metal micronutrients and phosphorus ($\mu g\ L^{-1}$) in the soil solution of the cluster rhizosphere and the bulk soil 3 days after cluster root emergence (19 days after germination). Values in brackets are standard errors (n=2).

Element	Rhizosphere				Bulk
	Anatolia soil				
	-P/-Zn	+P/-Zn	-P/+Zn	+P/+Zn	-P/-Zn
Zn	3.7 (0.1)	3.7 (0.4)	7.6 (0.3)	7.1 (0.2)	4.4 (0.2)
Fe	124.0 (9.0)	71.2 (7.0)	121.5 (13.5)	108.5 (12.5)	101.7 (12.0)
P	29.6 (5.3)	423.5 (34.5)	36.9 (3.4)	438.5 (17.5)	17.8 (3.5)
	Hofuf soil				
Zn	19.9 (0.6)	9.9 (0.3)	21.8 (0.6)	13.4 (0.5)	7.8 (0.8)
Fe	131.0 (5.2)	98.9 (4.7)	136.9 (11.3)	107.6 (16.5)	119.0 (5.6)
P	143.2 (27.4)	563.3 (9.6)	192.5 (9.1)	582.2 (20.3)	68.9 (3.6)

Extractable P increased linearly with the addition of higher citrate concentrations. Extracted P from the Hofuf soil was about 40 times higher compared to the Anatolia soil (Fig. 2.6 a). Extractable Zn increased with the addition of higher citrate concentrations in the Hofuf soil.
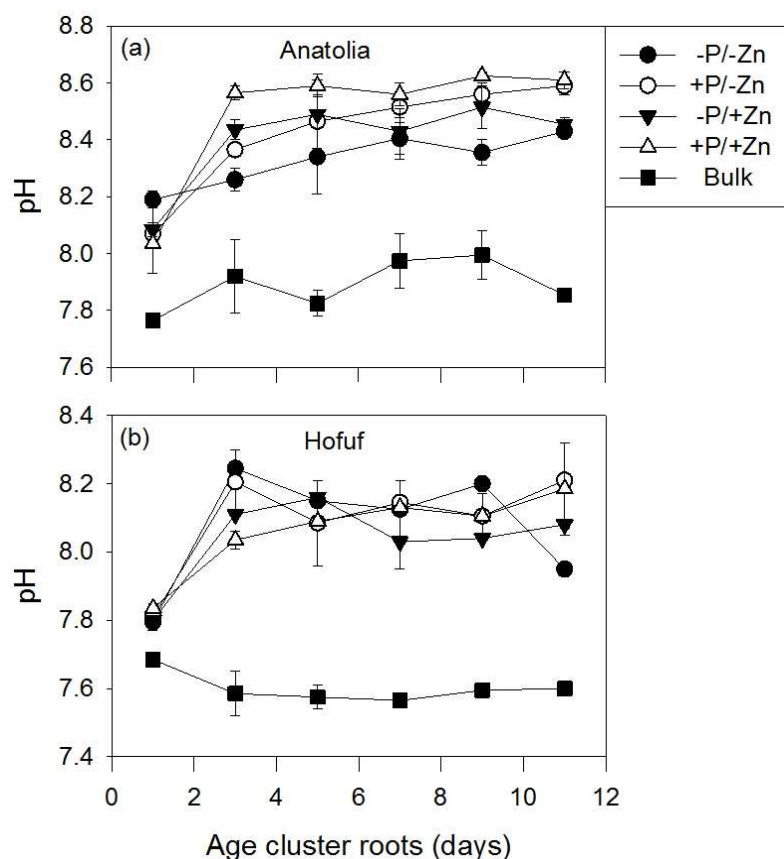
Fig. 2.4 The pH in the soil solution of cluster rhizosphere or bulk soil of the Anatolia (a) and Hofuf (b) soil depending on the age of the cluster roots. Error bars represent standard errors (n=2).

No Zn was extracted at all from the Anatolia soil regardless of the original citrate concentration in the extractant (Fig. 2.6 b).

2.5 Discussion

We showed that white lupin did not respond to Zn deficiency with cluster root formation and citrate exudation. Even plants grown in nutrient solution in the absence of any Zn neither formed cluster roots (Fig. 2.1) nor exuded any detectable citrate. Zn deficiency caused no increase in citrate concentration in the soil solution around cluster roots, even though Zn shoot tissue content was at a critical level (Reuter et al. 1997). Neumann et al. (2000) studied the physiological aspects of cluster root function and development of white lupine and suggested that cluster root induction may be stimulated by low Zn-supply. Our experiments

did not confirm this suggestion. Cluster root formation and citrate exudation were increased as a response to low P shoot tissue concentrations (Fig. 2.3) as shown before (Dinkelaker et al. 1995; Neumann et al. 2000). The citrate concentration in the soil solution peaked 3 days after the cluster root emergence, followed by a rapid decline which was also observed by Peñaloza et al. (2002) and Dessureault-Rompré et al. (2006). The measured citrate concentrations are in equilibrium with their environment since some of the exuded citrate is sorbed on Fe (hydr)oxides (Filius et al. 1997) and/or degraded by soil microorganisms (Ström et al. 2001).

To our knowledge we for the first time determined *in situ* citrate, metal and P concentrations in the rhizosphere of calcareous soils. The maximum citrate concentrations we detected can be considered as high compared with other studies in a non-calcareous soil (Dessureault-Rompré et al. 2007). The highest citrate concentration (~1.5 mM), which was detected in the cluster rhizosphere soil solution of the Anatolia soil (-P treatments) (Fig. 2.6), did mobilize P, but not Zn (Table 2). In the Hofuf soil the highest citrate concentration (~ 0.5 mM) (-P treatment) (Fig. 2.6) mobilized both P and Zn (Table 2).
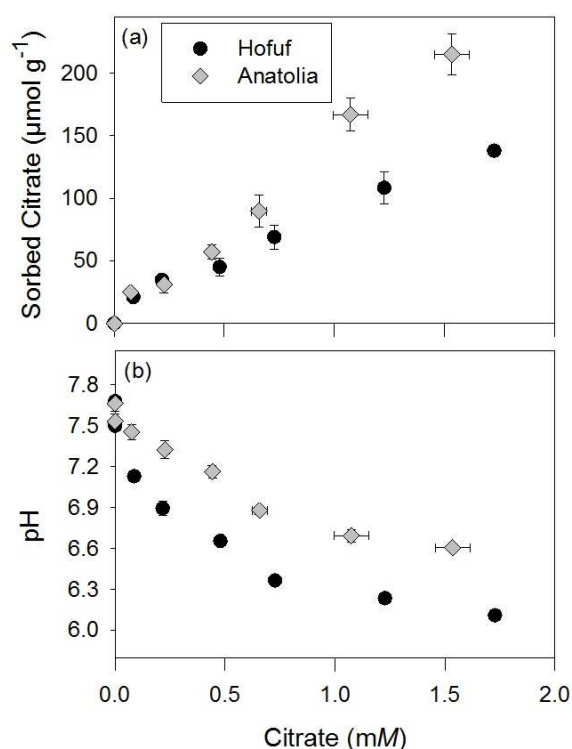
Fig. 2.5 Adsorption isotherms of citrate (a) on the Anatolia and Hofuf soil and the pH (b) after 24h shaking (20°C). Citrate concentrations in solution (x-axis) were determined at equilibrium with soil after shaking. Error bars represent standard errors (n=2).

The conclusion that P (and Zn) were mobilized is based on a comparison of P and Zn concentrations in the soil solution of the cluster rhizosphere of the -P treatments with the +P treatments and with the bulk soil (Table 2). The P concentrations in the cluster rhizosphere solution in the -P treatments were, due to mobilization, 5-7 times higher in the Hofuf soil than in the Anatolia soil (Table 2) and resulted in adequate shoot P tissue contents in all treatments (Reuter et al. 1997). Zinc mobilization by citrate in the -P treatments of the Hofuf soil (Table 2) was the reason that the Zn fertilization had no significant main effect due to the adequate Zn shoot tissue contents in the -P/-Zn treatment. This shows that the exuded citrate in the Hofuf soil was able to increase Zn mobilization and Zn plant uptake.
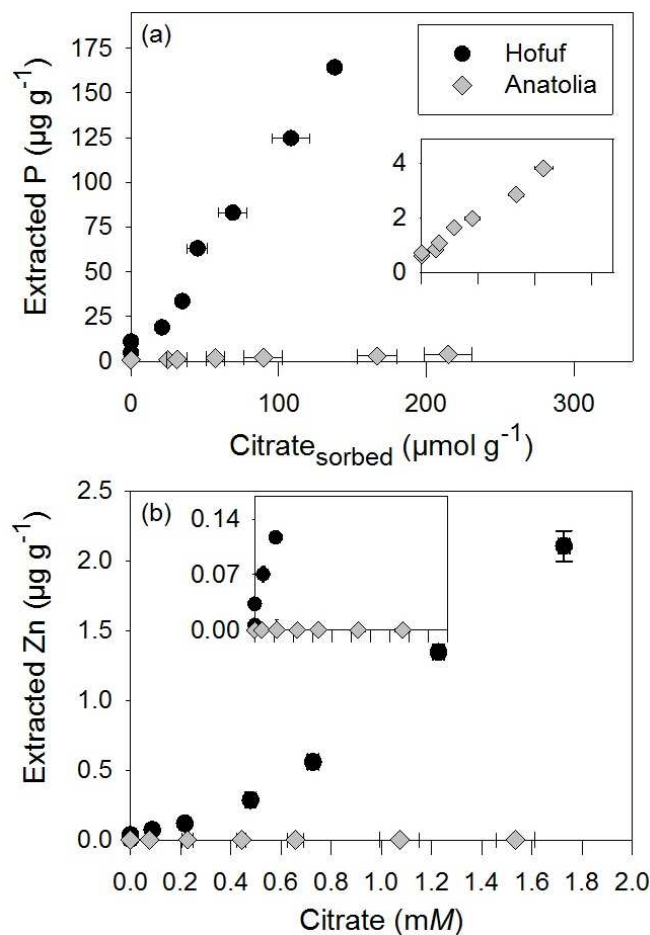
Fig. 2.6 Relationships between the extractable P in the extractant and sorbed citrate (a) and between the extractable Zn in solution and the citrate concentration in the extractant (b) after 24h shaking at 20°C of the Anatolia and Hofuf soil. The x-axis ticks and values of the inserted small graphs are the same as of the main graphs, respectively. Error bars represent standard errors (n=2).

Zinc was not mobilized by citrate in the Anatolia soil neither in the rhizobox (Table 2.2) nor in the extraction experiment (Fig. 2.6 b), even though the citrate concentrations in the cluster rhizosphere solution were ~3 times higher than in the Hofuf soil. This difference in Zn mobilization can be explained by Fe-Zn competition. Both soils had similar *aqua regia* extractable Zn concentrations. The DTPA-extractable Zn was about 5 times lower in the Anatolia soil, whereas the DTPA-extractable Fe was two times higher as in the Hofuf soil (Table 2.1). Considering the stability constants (log K, based on MINTEQA2 (Allison et al. 1991)) of citrate with Fe (13.1) and Zn (6.21), citrate probably mainly formed complexes with Fe. This is in line with the mobilized Fe concentrations in the soil solution of the cluster root rhizosphere (Table 2.2) when the citrate concentrations reached their maximum. Therefore more free Zn^{2+} remained in solution in the Anatolia soil. As a result no Zn^{2+} desorbed from soil surfaces and no Zn^{2+} dissolved from labile solid phases to replenish the Zn^{2+} in the soil solution. The Zn extraction efficiencies of citrate (% release as mol Zn per mol citrate in solution) in Hofuf and Anatolia soils were 0.19% and <0.00%, respectively. Therefore, metal competition in the soil solution should be considered when evaluating the mobilization capacity of citrate (or another ligand) for Zn (Kinniburgh et al. 1999).

Phosphorus, in contrast, was mobilized probably due to competitive adsorption of phosphate and citrate on metal (hydr)oxides (Geelhoed et al. 1998). In the extraction experiment, 40 times more P was extracted from the Hofuf soil (Fig. 2.6 a), which had about 8.5 times less citrate bicarbonate dithionite (CBD) extractable Fe (Table 2.1) than the Anatolia soil. Since the CBD-extractable Fe is considered to represent the total iron (hydr)oxide content (Hiemstra et al. 2010), there was a stronger competition between P and citrate in the Hofuf soil. In the Anatolia soil, more citrate was bound in total (Fig. 2.5 a), but the competition was less strong.

Other authors reported that, due to P deficiency, an increased exudation of citrate leads to a decrease of the soil pH (Neumann and Römheld 1999; Sas et al. 2001) and a correspondingly higher mobility of P and Zn. However, in our rhizobox experiment, citrate exudation caused no pH decrease since the high NO_3^- uptake of the plant and the corresponding excess of negative charges was counterbalanced by releasing equivalent amounts of OH^- or HCO_3^- into the rhizosphere (Imas et al. 1997). Therefore, a pH decrease was excluded to explain the mobilization of P and Zn. In the extraction experiment without plant-soil interactions, the pH value decreased (Fig. 2.5). This explains why the relative amount of mobilized P and Zn in the extraction experiment was higher than in the rhizobox experiment. The stronger pH decrease in the Hofuf soil was explained with the lower $CaCO_3$ content compared with the Anatolia soil.

We conclude that white lupin does not respond to low Zn supply with increased citrate exudation by cluster root formation, as was shown at low P supply, which is not supportive of

a multiple stress response. However, citrate exudation can, dependent on the type of soil, mobilize both Zn and P. To improve the understanding of the mechanisms which control Zn mobilization by root exudates in (calcareous) low Zn soils requires metal speciation techniques and mechanistic multicomponent modeling such as those used in studies with polluted soils (Koopmans et al. 2008).

3. Predicting zinc bioavailability to wheat improves by integrating pH dependent nonlinear root surface adsorption

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3.1 Abstract

Our aim was to improve the prediction of Zn bioavailability to wheat grown on low-Zn soils. The classical approach that directly relates Zn in a certain soil extract to Zn uptake has been shown to be inadequate in many cases. We tested a stepwise approach where the steps of the uptake process are characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot. Two pot experiments were done with wheat grown on nine low-Zn soils varying widely in pH, clay and organic matter content. Soluble Zn concentrations in two soil extracts (DTPA and $CaCl_2$) were measured. Free Zn ion concentrations in $CaCl_2$ soil extracts were determined with the Donnan Membrane Technique. These Zn concentrations were then related to plant Zn uptake following both the direct and the stepwise approach. In the direct approach, Zn in the DTPA extract was a better predictor for shoot Zn uptake than Zn in the $CaCl_2$ extract. In the stepwise approach, the relationship between Zn in $CaCl_2$ extracts and the root surface adsorbed Zn was pH-dependent and nonlinear. Root surface adsorbed Zn was linearly related to root Zn uptake, and the latter was linearly related to the shoot Zn uptake. The stepwise approach improved the Zn uptake prediction compared to the direct approach and was also validated for different wheat cultivars. The adsorption of Zn on the root surface is pH dependent and nonlinear with respect to the soil Zn concentration, and a useful proxy for bioavailable Zn over a wide range of soils.

3.2 Introduction

Zinc is an essential element for plants (Sommer and Lipman 1926), but it is toxic at high concentrations (Broadley et al. 2012) so prediction of its bioavailability is of great importance. Zinc toxicity in crops is far less widespread than Zn deficiency which limits crop production on ~30% of the world's agricultural soils (Sillanpää and Vlek 1985). Over the past decades, several soil extraction tests have been developed to predict Zn bioavailability in soils (Menzies et al. 2007). Mostly, bioavailability is predicted using the relationship between Zn concentrations in soil extracts and Zn concentrations in the aboveground parts of plants (Fig. 3.1 a) (McLaughlin et al. 2000). There is, however, no agreement on which soil extracted Zn concentration is the best predictor for plant Zn uptake, especially in low-Zn soils (Menzies et al. 2007; Degryse et al. 2009; Tandy et al. 2011).

Soil extraction with diethylenetriamine penta-acetic acid (DTPA) was developed to determine bioavailable Zn in near-neutral and calcareous soils (Lindsay and Norvell 1978) and is considered as one of the standard methods to determine bioavailable metals in non-polluted
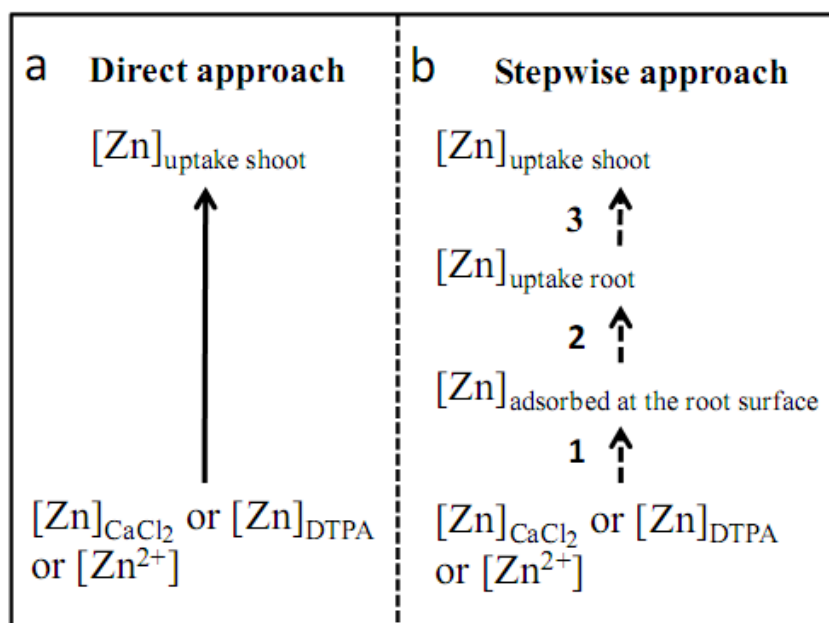
Fig. 3.1 The direct approach (a) and the stepwise approach (b) to relate Zn concentrations in soil extracts or Zn^{2+} to Zn bioavailability, expressed as Zn shoot uptake. The numbers 1-3 in (b) indicate the three steps of Zn uptake in the stepwise approach

soils (Tandy et al. 2011). The method is based on the formation of soluble complexes of DTPA with free metal ions, which reduces the activity of the free metal ions in solution. In response, metal ions desorb from soil surfaces or dissolve from labile solid phases to replenish the free metal ions (Lindsay and Norvell 1978). However, there are several studies where the DTPA method did not accurately predict the Zn content in the shoot (Feng et al. 2005; Tandy et al. 2011). This is particularly the case if the soil test is applied under conditions were the method were not developed for, like acid soils, where the buffering capacity (pH 7.3) of the DTPA solution may cause unrealistic concentrations or if the soil test is applied to soils with high metal loadings, which exceed the complexing capacity of the chelate (O'Connor 1988).

Extraction with a 0.01 *M* CaCl$_2$ solution utilizes a neutral salt extractant to determine the readily available nutrient concentrations in the solution of a wide range of soils (Houba et al. 1990). This procedure is based on equilibrating the soil with a surplus of cations (Ca^{2+}) which exchange a certain amount of the metal ions from the soil surfaces by competitive adsorption without influencing the soil pH. The CaCl$_2$ soil extraction has been successfully applied to determine Zn bioavailability in contaminated and non-contaminated soils (Pueyo et al. 2004;

Meers et al. 2007). In low-Zn soils, the $CaCl_2$ extraction is sometimes not applicable because the extracted Zn concentrations may not exceed the determination limit (10^{-7}-10^{-9} M) of the analytical equipment, like inductively coupled plasma mass spectrometry (ICP-MS) (Duffner et al. 2012).

The free metal ion concentration is often considered a key factor determining metal bioavailability (Parker and Pedler 1997; Weng et al. 2001b). Accurate measurement of the free Zn^{2+} ion is difficult, especially at low concentrations (µM range). Various techniques have been applied to experimentally determine the free Zn^{2+} ion concentration in soil solutions, such as the chelation method (Catlett et al. 2002), Permeation Liquid Membrane (PLM) (Gramlich et al. 2012), Diffusive Gradients in Thin-films (DGT) (Tandy et al. 2011), Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) and the Donnan Membrane Technique (DMT) (Chito et al. 2012). The advantage of the DMT is the possibility to measure several free metal ion concentrations simultaneously. The detection limit of this analysis can be decreased (up to 500 times) by making use of ligands for accumulation (Kalis et al. 2006b; Weng et al. 2011a). The relationship between bio-uptake and free metal ion concentration, however, was mainly established in aquatic environments (Van Leeuwen et al. 2005) and conceptualized, for example, with the Free Ion Activity Model (FIAM) (Brown and Markich 2000). It is disputable whether this approach applies to metal uptake by terrestrial plants, because metal complexing ligands in the rhizosphere and metal-root cell surface complexes may alter the chemical speciation (Parker and Pedler 1997; Duffner et al. 2012).

To improve the prediction of bioavailable Zn, an alternative to directly relating plant Zn uptake with soil-extracted Zn fractions is to account for the different steps that follow each other in the uptake process, noting that not all of these steps may be linear. Fried and Shapiro (1961) distinguished four steps in ion uptake by plants from a soil: (1) the release of the ion from the solid phase into the soil solution, (2) the transport of the ion from the soil solution to the rhizosphere ("vicinity of the root"), (3) uptake of ions from rhizosphere solution into the root and (4) the translocation of the ion from the root to the shoot. In line with this concept, Kalis et al. (2007a) successfully applied this four step approach to predict metal uptake by *Lolium perenne* in contaminated soils. They made the term "vicinity of the root" operational by defining it as the metal ions adsorbed to the root surface. The root surface adsorbed metals can be defined as extracellularly adsorbed metals, i.e. metals bound to one or more negatively charged biotic ligands in the apparent free space of the root cell wall (Santa María and Cogliatti 1988; Wu and Hendershot 2009; White 2012). Even though the adsorption of cations in apparent free space is not a prerequisite for ion uptake across the plasma membrane, the fixed negative charges in the apparent free space can, especially at low ionic activities, determine the cation concentration in the apoplasm (White and Broadley 2003). Zhang et al.

(1991) showed that the apparent free space can also serve as a Zn storage pool, which can be mobilized and translocated to the shoot.

The pH is an important factor controlling metal adsorption to the root surface. A decreasing pH increases the competition between the protons and the metal ions at the biotic surface, which can be described by a two-species Freundlich equation (Eq. 1) (Temminghoff et al. 1994; Weng et al. 2003):

$$[Q]_B = K[M^{2+}]^m [H^+]^a \tag{1}$$

where $[Q]_B$ is the quantity of a metal bound to the biotic surface, K is a constant related to the number of binding sites and their affinity for protons and metal ions, $[M^{2+}]$ represents the free metal ion concentration in the solution, $[H^+]$ is the proton activity, and *m* and *a* are empirical parameters. The K value, *m* and *a* are surface specific due to the differences of the density and type of binding sites (Plette et al. 1999). Due to the pH dependency of metal adsorption at the root surface the integration of root adsorption was crucial for the success of the stepwise approach of Kalis et al. (2007a). They tested this approach on ten soils with moderate and high Zn contents and with a pH range from 4.2-7.0.

The objectives of the present study were to improve our understanding of the Zn uptake process from soils that are low in Zn, using a stepwise approach in which the consecutive intermediate Zn pools were considered explicitly. In particular, we considered nonlinearity at the root surface with regard to Zn concentration in different soil extracts and pH.

3.3 Materials and Methods

3.3.1 Soils

Soil samples originated from nine sites located in Turkey (Anatolia), Spain (Xeraco), Italy (Bologna), Western Australia (Dalwallinu and Lancelin), Ethiopia (two sites in Wukro; Wukro-S (sand) and Wukro-C (clay)), India (Patancheru) and the Netherlands (Droevendaal) were used in this study. The soils were named after the location of origin. At each site samples were taken from the topsoil (0-20 cm) except for the Droevendaal soil where samples were taken from the subsoil (60-90 cm). The soils varied in clay content (4-50%), pH (4.2-7.6), soil organic carbon (SOC) (4-13.7 g kg^{-1}), dissolved organic carbon (DOC) (1.7-12.1 mg

L^{-1}) and $CaCO_3$ content (0-154 g kg^{-1}). The total Zn content of the soils ranged from 0.2-82.7 mg kg^{-1} (Table 3.1).

3.3.2 Soil extractions

Two soil extractions were conducted to determine different Zn pools in the soil samples. A 0.005 M diethylenetriamine penta-acetic acid (DTPA) extraction was done based on Lindsay and Norvell (1978). Samples of 10 g air-dried soil were placed in 50 mL plastic centrifuge tubes and 20 mL of the DTPA extracting solution adjusted to pH 7.3 was added. The tubes were shaken horizontally for 2 h at 20 °C and centrifuged at 3000 g for 10 min. The supernatant was filtered (0.45 μm) before determining the metal concentrations with ICP-MS (Elan 6000, Perkin Elmer). The extraction with 0.01 M $CaCl_2$ was done based on Houba et al. (1990). Samples of 3 g air-dried soil were placed in 50 mL plastic centrifuge tubes and 30 mL 0.01 M $CaCl_2$ was added. The tubes were shaken horizontally for 2 h at 20 °C and centrifuged at 3000 *g* for 10 min. The supernatant (10 ml) was filtered (0.45 μm), acidified (0.1 ml 0.43 *M* HNO_3) and stored in pre-cleaned ICP-MS tubes before determining the Zn concentration with ICP-MS. Both soil extractions were done in triplicate.

3.3.3 Free Zn ion concentration

The free Zn^{2+} concentration was determined in the $CaCl_2$ extracts by means of the Donnan Membrane Technique (DMT), which is described in detail by Temminghoff et al. (2000). Samples of 90 g soil were suspended in 1 L polypropylene centrifugation containers with 900 mL 0.01 M $CaCl_2$. Soil suspensions were shaken in a horizontal position on a reciprocal shaker for 2 h at 20 °C and centrifuged for 10 min at 3000 g. Extracts were separated from the soils by decanting supernatants in 1 L polyethylene containers.

Table 3.1 Soil characteristics

Origin/name	Country	Region	pH[a]	Clay	CaCO$_3$[b]	SOC[c]	DOC	WHC	Zn (*Aqua regia*[d])
				g kg^{-1}	g kg^{-1}	g kg^{-1}	mg L^{-1}	ml kg^{-1}	mg kg^{-1}
Anatolia	Turkey	Anatolia	7.5	220	93	10	6.3	482	33.1
Bologna	Italy	Bologna	7.4	232	144	9	12.1	445	66.6
Dalwallinu	Australia	Western Australia	4.2	72	0	6	1.7	223	2.0
Droevendaal	The Netherlands	Gelderland	6.2	48	0	4	2.5	255	8.0
Lancelin	Australia	Western Australia	5.2	38	0	13	5.1	201	0.2
Patancheru	India	Medak district	7.2	500	14	10	2.0	594	50.0
Wukro-C (Clay)	Ethiopia	Tigray	7.6	401	138	13	1.9	612	48.0
Wukro-S (Sand)	Ethiopia	Tigray	7.3	125	3	8	3.4	261	29.0
Xeraco	Spain	Valencia	7.5	357	154	14	9.4	435	82.7

[a] 0.01 M CaCl$_2$, ISO/DIS 10390; [b] ISO 10693, [c] Soil organic carbon, Walinga et al. (1992); [d] ISO 11466

All DMT cell parts were cleaned by successive washings in 0.1 M HNO_3 and ultra-pure water (UPW) prior to use. Teflon tubes were rinsed with 0.01 M HNO_3, UPW and the acceptor solution. The membranes were cleaned and saturated with Ca according to Kalis et al. (2006b). The supernatant was used as donor solution. The acceptor solution consisted of 0.01 M $CaCl_2$ (17 mL) prepared in UPW and held in 50 mL polypropylene tubes. Based on preliminary experiments, the free Zn^{2+} concentrations in the acceptor were expected to be below the determination limit (0.3 $\mu g\ L^{-1}$) of the analytical technique (ICP-MS). Therefore 10 μM nitrilotriacetic acid (NTA) was added as ligand to the acceptor solution for Zn accumulation (Weng et al. 2011a). Donor and acceptor solutions were circulated (7.5 ml min^{-1}) through the DMT cell by a peristaltic pump (Gilson Minipuls 3). The experiment was carried out at 20 °C, and samples were taken from the donor and the acceptor solution at t = 0, 24 and 48 h. In a preliminary experiment we found that the equilibrium was reached after 48 h (data not shown). Samples taken from the acceptor side of the DMT cell (6 mL) at 0 and 24 h were replaced by blank acceptor solution. Donor samples were filtered through a 0.45 μm filter. The pH in the donor and acceptor samples was measured with a combined glass-electrode. The Zn concentrations in the acceptor and donor samples were measured with ICP-MS and Ca concentrations in the acceptor and donor samples were measured with ICP-AES. Dissolved organic carbon (DOC) in the samples was measured at t = 0 and 48 h (Sievers 900, GE Analytical Instruments). The DMT experiment was done in triplicate.

The free Zn^{2+} concentrations were derived from Zn concentrations in the acceptor solution, which were calculated using the Equilibrium Calculation of Speciation and Transport (ECOSAT) program (Keizer and Van Riemsdijk 1995) by taking into account Zn complexation with NTA. Calcium was used as reference ion to correct for the ionic strength difference between the donor and acceptor. The inorganic ion complexes were calculated based on the reaction constants in the ECOSAT database. Zinc binding to dissolved organic matter was calculated by the Non-Ideal Competitive Adsorption (NICA)–Donnan model (Kinniburgh et al. 1999). We assumed that 30% of the DOC consisted of fulvic acid and the rest was inert (Weng et al. 2002). Total metal concentrations in $CaCl_2$ extracts were used as input variables.

3.3.4 Pot Experiments

Two pot experiments, a calibration and a validation experiment, were conducted to determine the Zn uptake by wheat (*Triticum aestivum* L.) and the Zn adsorption to the root surface. Both experiments were conducted in the greenhouse at Wageningen University (photoperiod of 15 h (6:00-21:00 h), light intensity 400 W m^{-2} (16 SON-T agro lamps), day/night temperature 21/19°C, rel. humidity 60%). Both pot experiments were done in triplicate.

3.3.5 Calibration experiment

Nine soils (Table 3.1) and one wheat cultivar (*Triticum aestivum* L. var. Minaret) were used to calibrate the direct and the stepwise approach (Fig. 3.1 b). One kg of air-dried and sieved (2 mm) soil was mixed with a nutrient solution and filled in 1 L plastic pots to a bulk density of 1.2 g cm^{-3}. The final nutrient concentration in the soil was: 150 mg N kg^{-1} (as $Ca(NO_3)_2 \cdot 4H_2O$), 80 mg P kg^{-1} (as KH_2PO_4), 101 mg K kg^{-1} (as KH_2PO_4) and 1.8 mg Fe kg^{-1} (as Fe-HBED, (Chaney 1988)). In four of the nine soils (Anatolia, Xeraco, Bologna and Droevendaal) Zn (10 mg Zn (as $ZnSO_4$) kg^{-1}) was also applied in an additional treatment to test the plant growth response to Zn application. The soil was adjusted to 60% of its water holding capacity every day with deionized water. The moist soils were allowed to equilibrate for 2 weeks in the greenhouse before sowing. In each pot twenty seeds were sown and were thinned to sixteen plants after germination. The plants were harvested 7 weeks after germination. Roots were washed thoroughly with distilled water to remove all soil particles. Afterwards, the roots were washed for 1 min with 100 mL of 0.01 M disodium ethylenediaminetetraacetic acid (Na_2H_2-EDTA) to remove Zn ions that were adsorbed to the root surface (Kalis et al. 2007a). The roots were then washed with UPW. The roots and shoots were dried at 70°C for 3 days to determine their dry weight and milled (Planetary mill with agate milling balls) and digested with nitric acid, hydrofluoric acid and hydrogen peroxide in a closed-system microwave according to the procedure described by Novozamsky et al. (1996). The Zn concentrations in digests and the EDTA solution from root washing were measured with ICP-MS.

3.3.6 Validation experiment

The Anatolia and Droevendaal soils and five different wheat cultivars (*T. aestivum* L. *var.* Eskischir, Italyan, Karachow, Kose and Sandomir) were used to validate the stepwise approach (Fig. 3.1 b). Different cultivars were used to test whether relationships within the approach were cultivar specific. In each pot fifteen seeds were sown and were thinned to ten plants after germination. After 8 weeks the plants were harvested. Except for the plant density, the experimental setup, plant treatment and analysis were the same as in the calibration experiment (see calibration experiment).

3.3.7 Statistical analysis

Statistical analysis of data was performed with SPSS analytical software (SPSS Inc., Chicago, IL, USA; version 19). Both linear and nonlinear stepwise regressions were used to relate the

Zn concentrations in the soil extracts with plant Zn uptake steps (Fig. 3.1). The variation among data was homogeneous (Levene's test).

3.4 Results

3.4.1 Soil extractions and free Zn ion concentration

The 0.01 M $CaCl_2$ extractable Zn was low in all soils and varied by one order of magnitude among the different soils (0.1-1.2 µmol kg^{-1}) (Table 3.2). The Zn concentrations in the DTPA extracts showed more variation. The DTPA extractable Zn concentrations of the Anatolia, Dalwallinu and Lancelin soil were in the range of the $CaCl_2$ extracted Zn concentrations, whereas those of the Xeraco and Wukro-S soil were around 100 times higher (Table 3.2).

The free Zn ion (Zn^{2+}) concentration in the $CaCl_2$ extracts ranged from $10^{-7.10}$ – $10^{-9.65}$ M (Table 3.2). The highest and lowest Zn^{2+} concentrations were found in the Dalwallinu and Patancheru soil, respectively. The highest and lowest proportion of Zn^{2+} on the total Zn in the $CaCl_2$ extract was found in the Dalwallinu (~70%) and the Wukro-C (~0.2%) soils (Table 3.2).

3.4.2 Calibration experiment

This pot experiment was used to calibrate the direct and the stepwise approaches (Fig. 3.1). Wheat plants grown in the Xeraco and the Wukro-C soil had the highest root and shoot dry matter production (Table 3.3). Biomass production in the two Australian soils (Dalwallinu and Lancelin) was about 50% less.

The highest root tissue Zn concentration (57 µg g^{-1} dw) was found in the Xeraco soil, whereas the root tissue Zn concentration of the Bologna and Wukro-C soil were about 3-4 times lower. The highest shoot tissue Zn concentration (56 µg g^{-1} dw) was also found in the Xeraco soil. The tissue Zn concentration in the shoots grown in the Anatolia, Lancelin and Wukro-C soil were about 3-4 times smaller as in the Xeraco soil (Table 3.3).

Four soils (Anatolia, Xeraco, Bologna and Droevendaal) received a Zn application (10 mg Zn kg^{-1}) to determine the corresponding plants' response. The tissue Zn concentrations in the shoot and the root increased on average by 68% and 56%, respectively (Fig. 3.2). The plants grown in the Anatolia soil showed the strongest response, whereas the plants grown in the

Xeraco soil showed the lowest response. The response of the shoot dry weight to the Zn application was positive and ranged from 3-20% increase (Fig. 3.2).

Table 3.2 Zn concentrations in the DTPA and the CaCl$_2$ soil extracts, Zn^{2+} concentration and the proportion of Zn^{2+} concentration to the total Zn concentration (means and standard error; n=3)

Soil	0.005 M DTPA [Zn] μmol kg^{-1}	0.01 M CaCl$_2$ [Zn] μmol kg^{-1}	$-\log[Zn^{2+}]$# M	[Zn^{2+}] /[Zn] ‡ %
Anatolia	1.7 (0.1)	0.21 (0.00)	9.3 (0.4)	2.4
Bologna	5.8 (0.2)	0.50 (0.02)	8.9 (0.4)	2.5
Dalwallinu	0.8 (0.0)	1.14 (0.03)	7.1 (0.3)	69.9
Droevendaal	7.0 (1.0)	1.18 (0.04)	7.8 (0.2)	13.4
Lancelin	1.0 (0.0)	0.64 (0.02)	8.0 (0.3)	15.7
Patancheru	8.3 (0.3)	0.14 (0.01)	9.7 (0.2)	1.4
Wukro-C	7.6 (1.2)	1.44 (0.05)	9.5 (0.5)	0.2
Wukro-S	34.0 (0.7)	0.85 (0.03)	8.6 (0.3)	3.0
Xeraco	99.6 (4.0)	0.17 (0.00)	9.0 (0.3)	5.7

Determined with the Donnan Membrane Technique

‡ Proportion of Zn^{2+} concentration to the total Zn concentration in the CaCl$_2$ extract

Table 3.3 Root and shoot dry weight of the harvested plants per pot (16 plants) and root and shoot tissue concentrations ($\mu g\ g^{-1}$) in the calibration experiment (means and standard error; n=3)

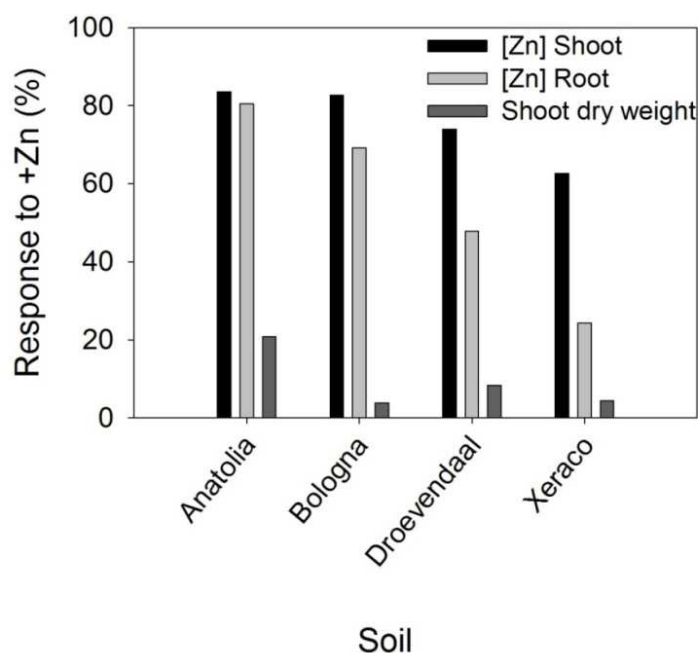| Soil	Dry weight (g pot^{-1})		Zn concentration ($\mu g\ g^{-1}$)	
	Roots	Shoot	Roots	Shoot
Anatolia	1.60 (0.12)	5.09 (0.26)	14 (0.0)	12 (0.9)
Bologna	1.31 (0.05)	5.76 (0.11)	17 (1.7)	19 (0.9)
Dalwallinu	0.99 (0.09)	2.50 (0.04)	24 (4.4)	36 (5.8)
Droevendaal	1.38 (0.07)	4.10 (0.21)	21 (0.9)	27 (0.3)
Lancelin	0.90 (0.08)	2.80 (0.17)	12 (0.3)	15 (0.9)
Patancheru	1.27 (0.04)	4.28 (0.13)	11 (0.7)	21 (1.2)
Wukro-C	1.59 (0.14)	6.86 (0.44)	11 (0.3)	17 (0.3)
Wukro-S	1.39 (0.09)	5.14 (0.59)	27 (1.9)	35 (1.7)
Xeraco	1.71 (0.07)	5.92 (0.06)	57 (1.0)	56 (5.7)

Fig. 3.2 Relative response to Zn application of the shoot tissue Zn concentration, root tissue Zn concentration and shoot dry weight grown in four selected soils.

3.4.3 Relating soil extracted Zn and free Zn^{2+} with Zn plant uptake

In line with the direct approach (Fig. 3.1 a) linear regression analyses between soil Zn (concentrations or fractions) and shoot Zn uptake were done. This resulted in the following equations:

$$[Zn]_{Shoot} = 0.072[Zn]_{DTPA} \qquad\qquad (r^2 = 0.79, P = 0.001) \quad (2)$$

$$[Zn]_{Shoot} = 1.29[Zn]_{CaCl_2} \qquad\qquad (r^2 = 0.42, P = 0.044) \quad (3)$$

$$[Zn]_{Shoot} = 1.50[Zn^{2+}]_{CaCl_2} \qquad\qquad (r^2 = 0.08, P = 0.42) \quad (4)$$

where $[Zn]_{Shoot}$ is the shoot Zn uptake expressed as nmol plant^{-1} and soil $[Zn]_{DTPA}$, $[Zn]_{CaCl_2}$ and $[Zn^{2+}]_{CaCl_2}$ as nM. Both $[Zn]_{DTPA}$ and $[Zn]_{CaCl2}$ related significantly to shoot Zn uptake, whereas there was no significant relationship between $[Zn^{2+}]_{CaCl2}$ and shoot Zn uptake.

In the first step of the stepwise approach (Fig. 3.1 b), Zn concentrations in the various soil extracts or free Zn^{2+} in $CaCl_2$ were related to root surface adsorbed Zn, by nonlinear regressions that account for pH dependency, assuming a two-species-Freundlich relationship, resulting in the following equations:

$$[Zn]_{RS} = 5.3[Zn]_{DTPA}^{0.22} [H^+]^{-0.19} \qquad\qquad (r^2 = 0.17, P = 0.31) \quad (5)$$

$$[Zn]_{RS} = 10.1[Zn]_{CaCl_2}^{0.37} [H^+]^{-0.18} \qquad\qquad (r^2 = 0.93, P = 0.001) \quad (6)$$

$$[Zn]_{RS} = 35.1[Zn^{2+}]_{CaCl_2}^{0.55} [H^+]^{-0.21} \qquad\qquad (r^2 = 0.29, P = 0.19) \quad (7)$$

where $[Zn]_{RS}$ is the Zn adsorbed at the root surface expressed as µmol kg^{-1} root dw, $[Zn]_{DTPA}$, $[Zn]_{CaCl_2}$ and $[Zn^{2+}]_{CaCl_2}$ as nM and $[H^+]$ as µM. Total Zn in the $CaCl_2$ extract ($[Zn]_{CaCl2}$) and the root surface adsorbed Zn were significantly related ($r^2 = 0.93$) (Eq. 6) though DTPA extractable Zn (Eq. 5) and $[Zn^{2+}]_{CaCl2}$ (Eq. 7) did not relate significantly to root surface adsorbed Zn.

The second step of the stepwise approach relates root surface adsorbed Zn and root Zn uptake (Fig. 3.1 b), and was both significant and strong:

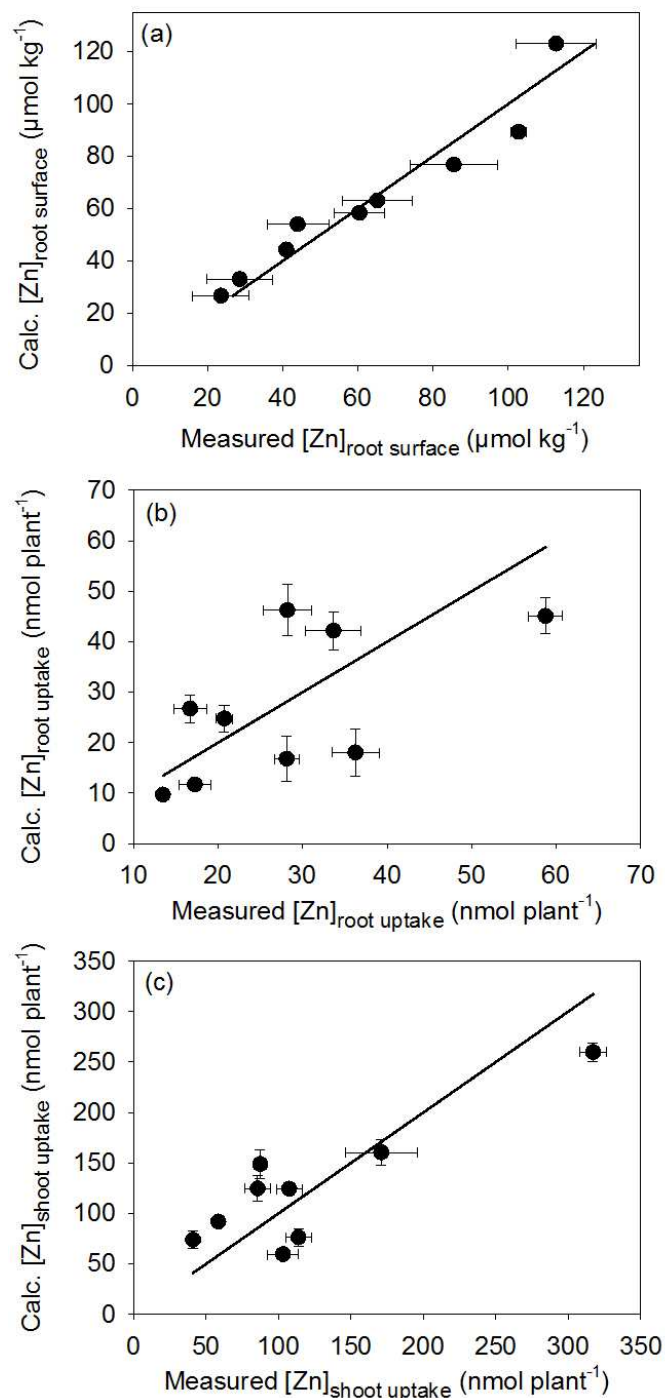$$[Zn]_{Root} = 0.41[Zn]_{RS} \qquad\qquad (r^2 = 0.90, P = <0.001) \quad (8)$$

Fig. 3.3 Measured and calculated root surface adsorbed Zn (a), root Zn uptake (b) and shoot Zn uptake (c) based on the equations (Eq. 6, 8 and 9) of the (non)linear regression analysis with the nine different soils (calibration experiment), respectively. The black solid line represents the 1:1 line. Error bars represent standard errors; n=3

where $[Zn]_{Root}$ and $[Zn]_{RS}$ is expressed as nmol plant^{-1} and µmol kg^{-1}, respectively.

In the third and last step, root Zn uptake was related to shoot Zn uptake by a linear regression, which showed a strong and significant relationship (Eq. 9):

$$[Zn]_{Shoot} = 4.42[Zn]_{Root} \qquad\qquad (r^2 = 0.92,\ P = <0.001) \quad (9)$$

where $[Zn]_{Shoot}$ and $[Zn]_{Root}$ are both expressed as nmol plant^{-1}.

The predicted results were in good agreement with the measured values as is shown by the 1:1 line in Fig. 3.3. The shoot Zn uptake prediction with the direct approach (Eq. 2) and the stepwise approach (combining Eq. 6, 8 and 9) were compared with each other. Predicting the shoot Zn uptake with the stepwise approach was done by calculating the root surface adsorbed Zn (Eq. 6), using $CaCl_2$ soil extracted Zn and soil pH. The calculated root surface adsorbed Zn was used to calculate root Zn uptake (Eq. 8) and calculated root Zn uptake (Eq. 8) was used to calculate shoot Zn uptake (Eq. 9). The direct approach underestimated the shoot Zn uptake, particularly in soils with low shoot Zn uptake (values below the 1:1 line), whereas the values of the stepwise approach were distributed around the 1:1 line (Fig. 3.4).
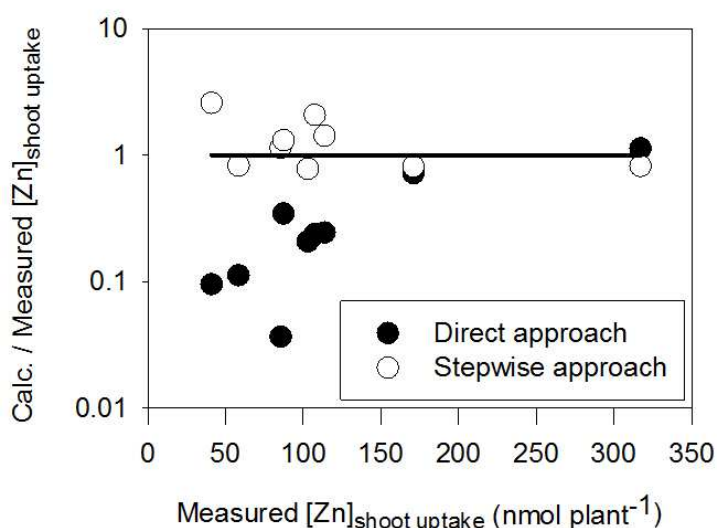
Fig. 3.4 Measured shoot Zn uptake of the calibration experiment plotted against the quotient (log scale) of the calculated and the measured shoot Zn uptake. The calculated shoot Zn uptake is based on the outcome of the direct approach (Eq. 2) and the stepwise approach (Eq. 6, 8 and 9), respectively. The calculated root surface adsorbed Zn, using $CaCl_2$ soil extracted Zn and soil pH, (Eq. 6) was used

as input to calculate the root Zn uptake (Eq. 8). The calculated root Zn uptake (Eq. 8) was used as input to calculate the shoot Zn uptake (Eq. 9). The black solid line represents the 1:1 line.

3.4.4 Validation experiment

This experiment was used to validate the equations developed for the stepwise approach. The calculations for Zn adsorbed to the root surface (Eq. 6) of the five different wheat cultivars grown in the Anatolia and the Droevendaal soils were in good agreement (i.e. within ± 2 x standard errors around the 1:1 line) with the measured values (Fig. 3.5). Measured Zn uptake by root and shoot was also in good agreement with the values calculated by equations 8 and 9 (Fig. 3.6).
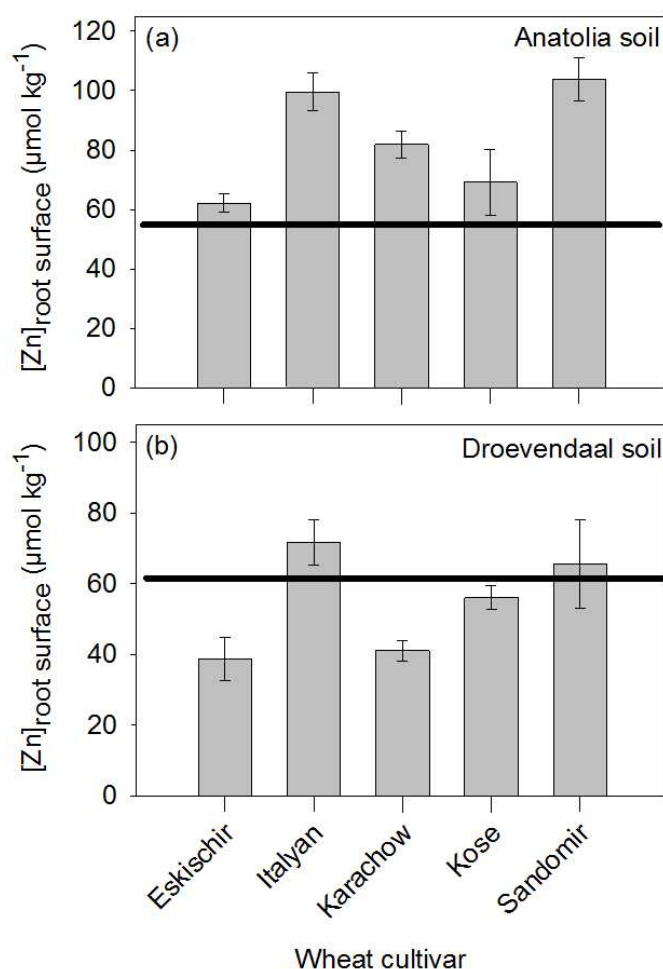
Fig. 3.5 Measured root surface adsorbed Zn of the five wheat cultivars grown in the Anatolia (a) and the Droevendaal (b) soil in the validation experiment. The black solid line represents the calculated values based on equation 6. Error bars represent standard errors; n=3
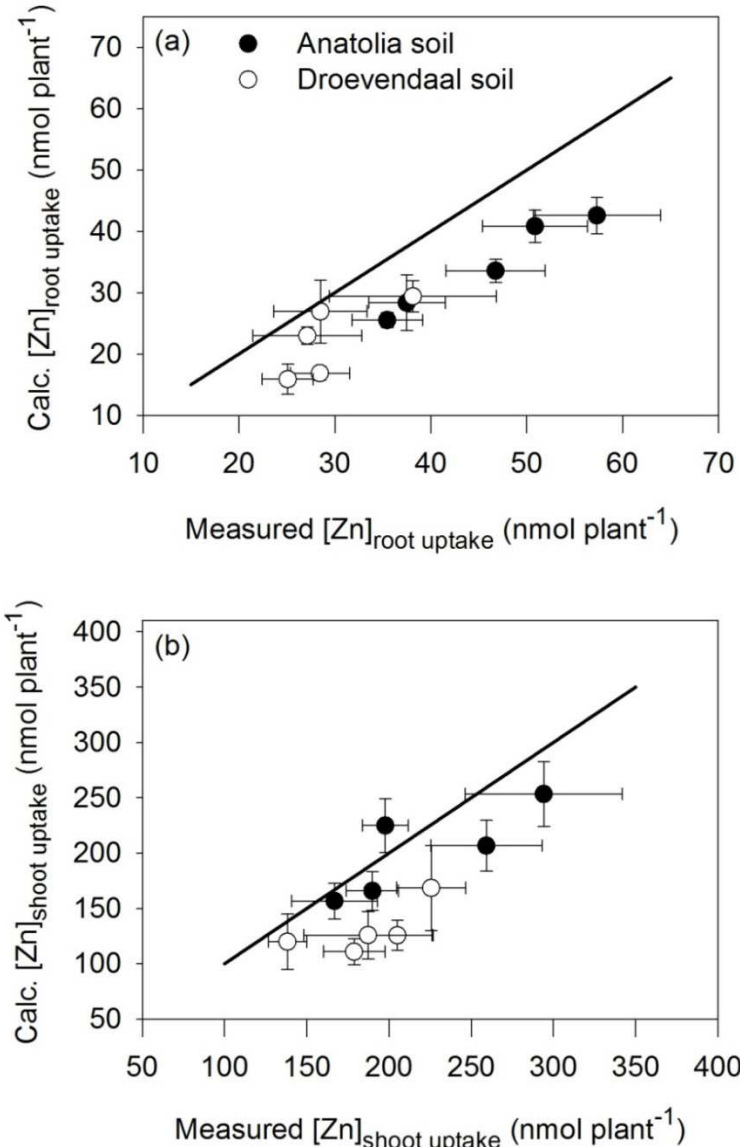
Fig. 3.6 Measured root Zn uptake (a) and shoot Zn uptake (b) of the five wheat cultivars grown in the Anatolia and the Droevendaal soil in the validation experiment plotted against the values calculated with equation 8 and 9, respectively. The black solid line is the 1:1 line. Error bars represent standard errors (n=3).

3.5 Discussion

Including the pH dependent, nonlinear Zn adsorption at the root surface and root Zn uptake as intermediate steps improved the prediction of the shoot Zn uptake significantly compared with directly relating the Zn concentrations in the $CaCl_2$ extracts with the shoot Zn uptake. This was also observed by Kalis et al. (2007a) in contaminated soils.

With increasing pH the Zn adsorption at the root surface increased (Eq. 6 and 7). This is contrasting to the study of Plette et al. (1999), where they showed that with increasing soil pH, less copper was bound to the maize root cell walls because at high pH soil ligands most probably outcompete the maize ligands. However, Von Wirén et al. (1996) showed that phytosiderophores are also able to form complexes with Zn (in addition to Fe) and this complexes can be adsorbed by maize roots. Such mechanisms were also observed for other graminaceous plant species (Arnold et al. 2010). Therefore exuded ligands from the wheat root probably outcompete the soil ligands when forming complexes with Zn.

That is also in line with our outcome that the free Zn ion concentration (Zn^{2+}) in the $CaCl_2$ extract was only weakly related to the root surface adsorbed Zn (Eq. 7). That indicates that depending on the pH most probably other Zn species like $Zn(OH)^+$, $ZnCl^+$ or phytosiderophore-chelated Zn were adsorbed to the root surface or diffused into the apparent free space of the root cell wall (Papanicolaou and Nobeli 1977; Chairidchai and Ritchie 1990; Sadiq 1991; Von Wirén et al. 1996; Broadley et al. 2012). Gramlich (2013) showed with plant experiments that Zn uptake by wheat was enhanced in the presence of citrate and histidine compared to treatments with solutions of the same free Zn concentrations buffered by EDTA. This shows that the Free Ion Activity Model (FIAM) is not valid in the presence of ligands. These results also indicate that the dissociation of these complexes or the adsorption of the whole complex can play an important role for Zn uptake.

Root surface adsorbed Zn seems to be a more accurate proxy variable for the bioavailable Zn than the Zn concentrations in the different soil extracts, which was shown by the significant linear relationship between root uptake and root surface adsorbed Zn (Eq. 8). This is in line with the conclusion of Degryse et al. (2009) that the Zn uptake obeys Michaelis–Menten kinetics also at low Zn activities at the root surface, because if the metal activity is lower than the Michaelis constant the Michaelis–Menten equation can be reduced to a linear relationship. Our results indicate that the root surface adsorbed Zn is taken up via the apoplastic pathway. This is in line with findings of Redjala et al. (2010) who showed the importance of Ni adsorption on root apoplast, which represented 81–95% of the total root uptake in maize plants grown in nutrient solutions. They concluded that this uptake process might act as a driving force to extract the metal from the soil. Similar observations were made for Cd uptake

(Redjala et al. 2009). This shows that the root surface adsorbed Zn acts as a proxy of the Zn that is available for plant uptake.

Root Zn uptake showed also a strong linear relationship with shoot Zn uptake (Eq. 9). That suggests that Zn was translocated from the root to the shoot without reaching a maximum, which was also observed by Kalis et al. (2007a) in contaminated soils and recently by Gramlich (2013) under low Zn conditions in nutrient solution experiments. That shows that the translocation from root to shoot is not related to the Zn concentration in the soil solution, but only to root Zn content regardless whether the soil is low in Zn or contaminated with Zn.

The strong pH dependency of Zn adsorption to the root surface most probably also explains the weak relationship between DTPA extractable Zn and root surface adsorbed Zn (Eq.5), because the DTPA extraction solution is adjusted and buffered at pH 7.3, which excludes the pH effect. The fact that the direct approach based on DTPA extractable Zn underestimated shoot Zn uptake in soils with low Zn uptake, whereas the predictions of the stepwise approach were around the 1:1 line (Fig. 3.4) means that the stepwise approach based on $CaCl_2$ extractable Zn is more universally applicable compared to the direct approach based on DTPA-extracted Zn, even though both approaches had similar coefficients of determination. The improved applicability is an important aspect considering the variety of soils with low Zn bioavailability. The nine soils in our work are characteristic for this variation which could be divided into two groups: the highly weathered Australian soils (Dalwallinu and Lancelin) with a small but labile total Zn pool and low pH and the other soils with a larger but relatively less soluble total Zn pool and higher pH (Table 3.1 and 3.2).

Our validation experiment confirmed that the stepwise approach was also valid for wheat cultivars that were not used for the calibration (Fig. 3.5, 3.6). The differences between the measured and the calculated root surface adsorbed Zn (Fig. 3.5) could possibly be explained by variation of the root surface area per plant among the cultivars (data not shown). Rengel and Wheal (1997) showed that Zn-efficient wheat cultivars had a greater proportion of fine roots than Zn inefficient wheat cultivars. Accounting for the root surface area as an additional parameter may therefore further improve the accuracy of the stepwise approach.

In conclusion, unraveling the uptake process in distinct steps demonstrated that the Zn bioavailability prediction is nonlinear due to pH dependent adsorption of Zn on the root surface. Zn uptake was shown to be strongly and linearly related to Zn adsorption at the root surface. Including these processes at the root surface improves the prediction of the Zn uptake based on Zn concentrations in soil extracts and pH. Integrating these root surface interactions most probably also improves the prediction of the bioavailability of other trace metals, since

this has already been shown for other biotic surfaces (e.g. bacteria cell wall (Plette et al. 1996)).

4. Multisurface modeling to predict free zinc ion concentrations in low zinc soils
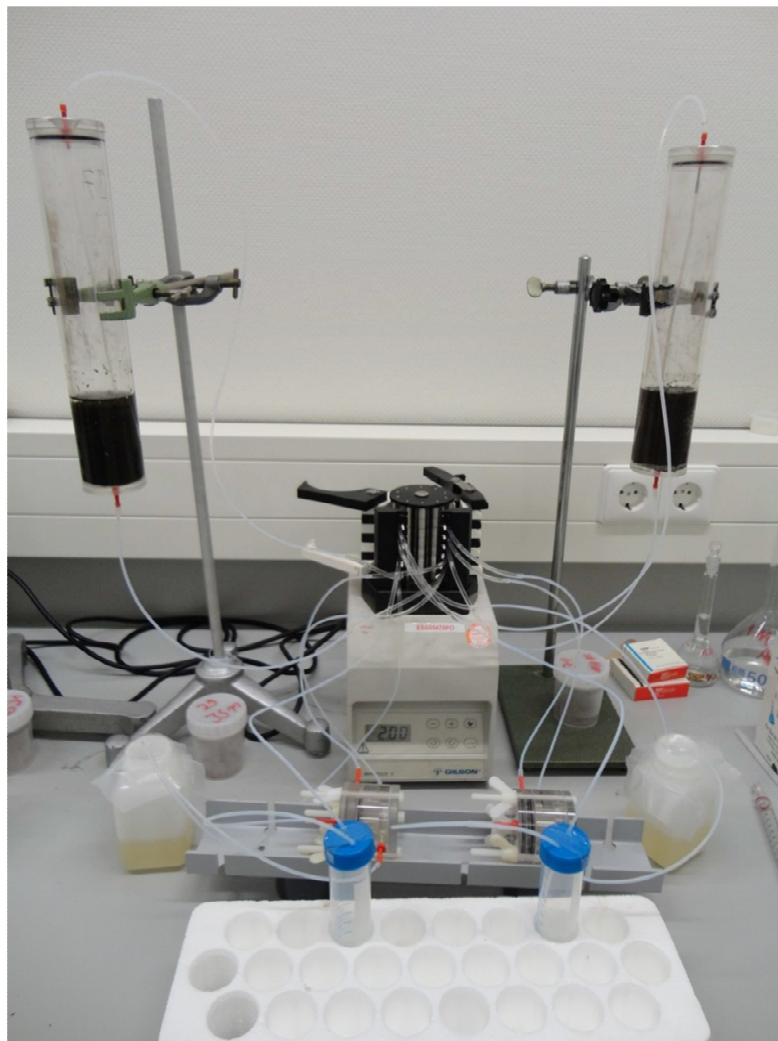
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4.1 Abstract

Multisurface models are widely used to assess the potential ecotoxicological risk in metal-contaminated soils. Their accuracy in predicting metal speciation in soils with low metal levels was not tested yet. Now highly sensitive analytical techniques are available to experimentally validate such models at low concentration levels. The objective of this study was to test the accuracy of a multisurface model to predict Zn^{2+} concentration and to improve our understanding of Zn bioavailability in low Zn soils. High Zn soils were included as controls. Model parameters were determined independently based on earlier peer reviewed publications. Model output was validated against free Zn^{2+} concentrations determined with the soil column Donnan Membrane Technique in a range of soils varying in potentially available Zn, organic matter, clay silicate and iron (hydr)oxides contents and pH. Deviations between predicted Zn^{2+} concentrations and experimentally determined values over the whole Zn concentration range were less or equal to the experimental standard error, except for one low Zn soil. The Zn^{2+} concentration was mainly controlled by adsorption, where organic matter was predicted to be the dominant soil sorbent. The predicted Zn^{2+} concentration depends more sensitively on changes of the reactive Zn pool (application of 0.6, 1.2, 2.4 and 3.6 mg Zn kg^{-1} soil) and organic matter content ($\pm$ 0.2 and 0.4 %) than on pH changes ($\pm$ 0.5 and 1 pH unit).

4.2 Introduction

Zinc is an essential element for plants (Sommer and Lipman 1926), but it is toxic at high concentrations (Broadley et al. 2012). Zinc deficiency is potentially limiting crop production in about 30% of the world's soils (Sillanpää and Vlek 1985; Alloway 2009). It is generally assumed that soils with DTPA-extractable Zn < 0.8 mg kg^{-1} are limiting crop production due to low Zn availability (Dobermann and Fairhurst 2000) and uptake. However, the relationship between soil Zn-DTPA levels and yield or Zn uptake is often very poor and statistically not significant (Duffner et al. 2014a). Accurate prediction of Zn bioavailability is important to reduce the risk of crop failure or low crop Zn content. Zinc availability to plants is poorly related to total soil Zn concentration, because it is largely determined by Zn speciation in the pore water and Zn replenishment from the solid phase (Nolan et al. 2003). Improving the understanding of Zn bioavailability in low Zn soils would allow for development of tools to evaluate management options for an increased Zn plant uptake. A quantitative understanding of the mechanisms controlling Zn speciation, such as adsorption or precipitation, could be improved by modeling with mechanistic multisurface models.

Multisurface models consider soil as a set of independent sorption surfaces (organic matter, iron (hydr)oxides (amorphous and crystalline), clay, $CaCO_3$). They are widely used to assess the ecotoxicological risk of Zn and other metal ions in contaminated soils (Weng et al. 2001a; Cancès et al. 2003; Dijkstra et al. 2004), but have not been used before to predict Zn bioavailability in low Zn soils. Now that analytical equipment allowing for accurate analyses of nanomolar range Zn concentrations is available, validation of multisurface models for low Zn soils has become feasible. In contaminated soils, soil organic matter was shown to be the dominant sorbent for most metal ions (Weng et al. 2001a; Dijkstra et al. 2009). The pH was also found to be an important determinant of bioavailable Zn through its controls on surface charge and metal speciation in the soil solution and because protons compete with metal ions for binding sites. However, the contribution of sorption surfaces and sites for metal binding may be different in low Zn soils with potentially different binding surface compositions, metal loading and higher pH. Compared with contaminated soils, in soils with low metal loadings, a larger fraction of Zn may be adsorbed to high affinity sites (Bonten et al. 2008). Model parameters used in multisurface models usually have been calibrated at a relatively high metal concentration using synthetic or purified materials. Therefore, it is uncertain if under low Zn conditions, Zn speciation can still be predicted accurately with thus calibrated multisurface models.

It is not known yet if Zn precipitates also play a significant role in controlling the Zn^{2+} concentration in (calcareous) low Zn soils. The role of Zn precipitates in controlling the Zn^{2+} concentration in Zn contaminated soils was discussed in several studies (Voegelin et al. 2002; Jacquat et al. 2008; Degryse et al. 2011). Jacquat et al. (2008) studied precipitation of Zn in contaminated calcareous soils and concluded that the precipitation of Zn-phyllosilicate, Zn-Layered Double Hydroxide (LDH), and hydrozincite allows accumulation of increasing amounts of Zn, that exceed the capacity of adsorption. Voegelin et al. (2002) used soils with lower Zn concentrations in the soil solution (0.1-0.2 mM) and showed that the formation of a Zn Al LDH precipitates played a significant role in slow Zn sorption. However, the Zn concentrations in the soil solution in their soils were still an order of magnitude higher than in soils where Zn deficiency was observed in crops. Hence, the role of Zn precipitates in these soils cannot be confirmed.

Here, we test if a multisurface model (implemented in ECOSAT (Keizer and Van Riemsdijk 1995)) that has been parameterized for relatively high metal levels, accurately predicts Zn^{2+} concentration too in (calcareous) low Zn soils on which Zn deficiency limits crop production. High Zn soils were included as a control. The predicted Zn^{2+} concentrations were validated against free Zn^{2+} concentrations determined with the soil column Donnan Membrane Technique (DMT) (Weng et al. 2001b). The effects of changing organic matter contents, pH

and the reactive Zn pool in the low Zn soils were simulated with the multisurface model to assess potential management options.

4.3 Materials and methods

4.3.1 Soil analysis

Samples of eight soils with low Zn levels (low Zn soils) (0.1-21 mg Zn kg^{-1} soil in 0.43 M HNO$_3$ extraction) originated from sites located in Hofuf (Saudi Arabia), Dalwallinu and Lancelin (Western Australia), Wukro (Ethiopia), Droevendaal (The Netherlands), Anatolia (Turkey), Bologna (Italy) and Xeraco (Spain). The soils are named after the location of origin (Table 4.1). These soil samples were obtained from fields on which crops showed Zn deficiency symptoms, except for the Droevendaal soil. Samples of seven other soils with high Zn levels (high Zn soils) (41-619 mg Zn kg^{-1} soil in 0.43 M HNO$_3$ extraction) originated from gardens in Veenendaal (The Netherlands), located on an old Zn contaminated waste dump. These soils were named V1-V7 (Table 4.1). At each site, subsamples were randomly taken from spatially different positions and mixed to form bulked samples. The samples were taken from the topsoil (0-20 cm) except for the Droevendaal soil, where samples were taken in the subsoil (60-90 cm).

Soil samples were air-dried and sieved (< 2 mm) before analysis. The soil organic C content was determined spectrophotometrically, using the absorbance of the green chromium(III) complex formed when organic matter is oxidized by potassium dichromate in acidic medium (Walinga et al. 1992). The pH was determined in 0.01 M CaCl$_2$ soil suspension (soil: solution ratio=1:10) (Houba et al. 1989). The clay content (< 2 µm) was measured by the sieve and pipet method in which particles were separated by their differences in speed of settlement and the size was calculated using Stokes' law (Gee and Bauder 1986). The CaCO$_3$ content was determined according to ISO 10693 (volumetric method). Ammonium-oxalate-extractable Fe was determined according to Schwertmann (1964) and the dithionite-citrate-bicarbonate extractable Fe was determined according to Hiemstra et al. (2010). The reactive metal pool was determined with a 0.43 M HNO$_3$ extraction (soil: solution ratio=1:10, 2 h shaking) (Houba et al. 1989). Additionally, Zn concentrations were also measured in soils extracted with 2 M HNO$_3$ (soil: solution ratio=1:10, 2 h shaking). Concentrations of Zn in the solutions were measured on High Resolution ICP-MS (Thermo Scientific Element 2) after being acidified to a final concentration of 0.14 M HNO$_3$.

4.3.2 Soil column-Donnan Membrane Technique experiment

A soil column Donnan Membrane Technique (DMT) experiment was conducted to determine the free Zn^{2+} concentration in the soil solution (Weng et al. 2001b). The DMT is based on a cation exchange membrane which separates the donor (substrate solution) and the acceptor solution (blank solution). At the Donnan membrane equilibrium, the charge corrected activity ratios of the cations in the donor and the acceptor are equal and the free metal concentration can be derived (Temminghoff et al. 2000).

One hundred g of air-dried soil was put into a Perspex column (length 25 cm, diameter 4 cm) to conduct the soil column DMT experiment. Two hundred mL of a 2 mM $CaCl_2$ solution was used in the donor side and 18 mL was used in the acceptor side. The donor solution was percolated (2 mL min^{-1}) through the soil in the column, which determined the composition of the solution based on the buffering capacity of the soil. The experiment was carried out at 20 °C, and samples were taken from the donor and the acceptor solution at t = 0, 24 and 48 h. Samples taken from the acceptor side of the DMT cell (6 mL) at 0 and 24 h were replaced by blank acceptor solution. Donor samples were filtered over a 0.45 µm nitrate-cellulose-membrane filter. The pH in all samples was measured with a combined glass-electrode. The concentrations of microelements were measured with High Resolution ICP-MS (Thermo Scientific Element 2) and macroelements were measured with ICP-OES (Vista pro, Varian). Dissolved organic carbon (DOC) was measured in the samples taken at t = 0 and 48 h (Sievers 900, GE Analytical Instruments). The experiment was done in triplicate for all soils. Free Zn^{2+} concentrations in the acceptor was calculated from the total Zn concentrations and pH in the acceptor using the Equilibrium Calculation Of Speciation And Transport (ECOSAT) software (Keizer and Van Riemsdijk 1995), taking into account the formation of soluble Zn complexes with Cl^-, OH^- and CO_3^{2-}. The Cl^- concentration was estimated from the total Cl concentration in the soil solution. The dissolved CO_3^{2-} concentration was calculated assuming equilibrium with CO_2 in the atmosphere. The inorganic ion complexes were calculated based on the reaction constants in the ECOSAT database. Calcium was used as reference ion to correct for the ionic strength difference between the donor and acceptor, to derive free Zn^{2+} concentration in the donor solution from that in the acceptor solution, while assuming that all calcium in both the sample solution and acceptor solution is free ion.

4.3.3 Multisurface model

Multisurface models consider ion adsorption to multiple soil constituents, assuming that ion adsorption to each type of surface is the same as in the single-surface systems and there is no impact of the interaction between constituents for ion adsorption (Weng et al. 2001a). Site

density and other adsorption parameters are generic to the type of adsorbate. This approach has been called the "component additivity approach" (Davis et al. 1998). Compared to the generalized composite approach, the multi-surface model approach has the advantage that no fitting is needed and the modeling outcome is pure prediction. We model ion adsorption to four types of surfaces: clay, soil organic matter, amorphous and crystalline iron (hydr)oxides (Fig. 4.1).

Zinc binding to clay minerals was modeled using a Donnan approach (Tipping 1994). Illite was considered as the representative clay silicate (Weng et al. 2001a). The clay silicate content in the soil samples was estimated from the measured clay content (<2 μm). The charge of illite is between 0.1-0.4 mol kg^{-1} (McBride 1994). For model calculations the average and the extreme values for the charge of illite (0.1, 0.25 and 0.4 mol kg^{-1}) were used in combination with the corresponding reactive fractions of soil organic matter (see below). This resulted in variation among predictions. The Donnan volume of illite was estimated as 1 L kg^{-1}. Electrostatic competition of Al^{3+}, Fe^{3+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, and H^+ with Zn^{2+} for binding to clay was considered in the model calculation.
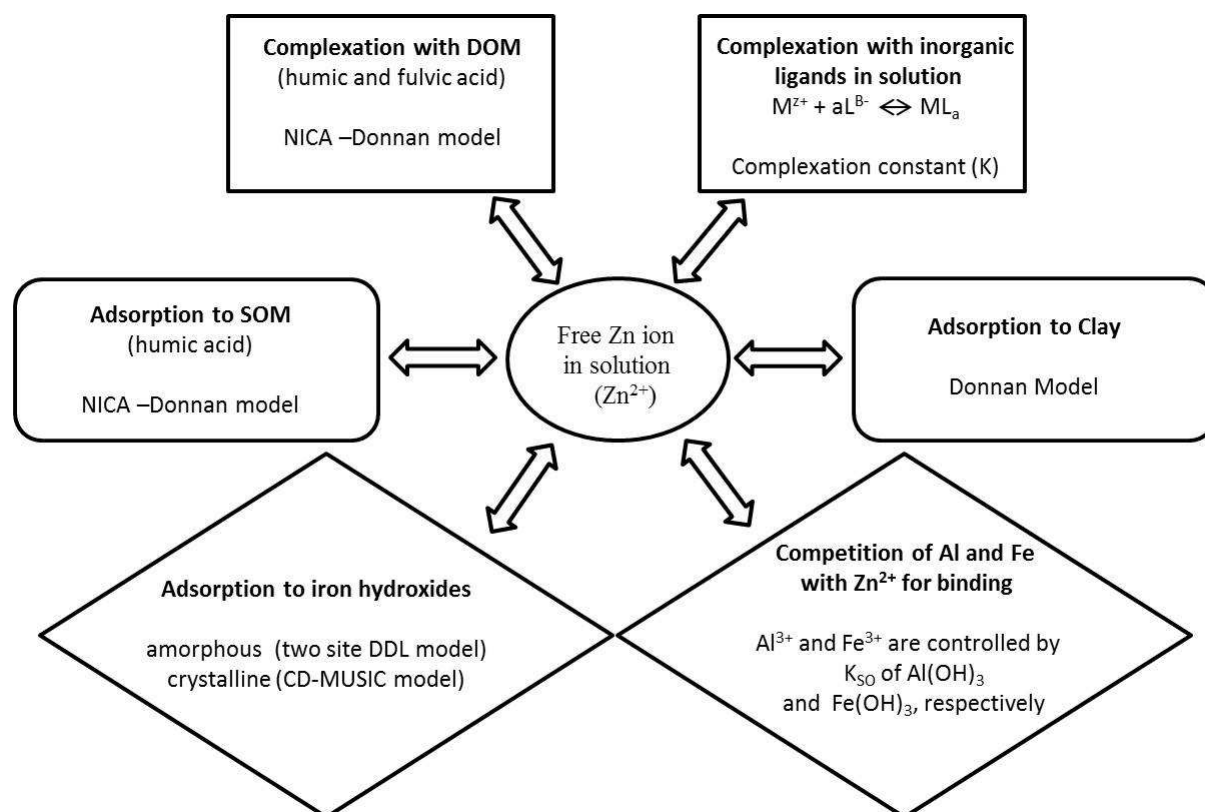

Fig. 4.1 Conceptual structure of the reactions and models used for multisurface modeling in ECOSAT. Modified after Weng et al. (2002)

Zinc binding to soil organic matter was modeled with the Non Ideal Competitive Adsorption Donnan model (NICA-Donnan) (Kinniburgh et al. 1999). Humic acid was considered as the model material of the organic matter fraction that reacts with metals. The generic model parameter set derived for humic acid (Milne 2000; Milne et al. 2001) was used (see Table S4.1 for supporting Information). As the site density of soil organic matter is smaller than that of purified humic acid, the soil organic matter content is corrected for this difference. This fraction was estimated from the charge of soil organic matter calculated from the difference of soil CEC and charge of clay, an approach successfully applied before (Weng et al. 2001a). The reactive fraction for Zn sorption was therefore different for each soil. Due to similar sorption properties of humic and fulvic acid, we used humic acid as representative of the reactive fraction. The hydrophilic small molecules, which can also be a part of the dissolved organic matter, were not considered, because the humic fraction is more metal-reactive than the hydrophilic fraction. Based on the measured soil CEC, clay content (Table 4.1) and the above mentioned range of charges of clay, the site density of soil organic matter was estimated for each soil, which was in the range of 15-45% (on average 30%) of purified humic acid. The total amount of soil organic matter (SOM) was calculated as two times of that of soil organic C (Table 4.1). Competition of Al^{3+}, Fe^{3+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, and H^+ with Zn^{2+} for binding to SOM was considered in the model calculation. Zinc binding to DOC was also predicted with the NICA-Donnan model, assuming Zn sorption to suspended humic acid.

Zinc binding to iron (hydr)oxides in the multisurface model was separated into binding to amorphous and crystalline iron (hydr)oxides. As model substances for amorphous and crystalline iron (hydr)oxides, hydrous ferric oxide (HFO) and goethite were chosen, respectively (Weng et al. 2001a). Zinc binding to goethite was simulated with the Charge Distribution Multi-Site Complexation (CD-MUSIC) model (see Table S4.3 for supporting Information) (Hiemstra and Van Riemsdijk 1996; 1999). Zinc binding on HFO was simulated by a two-site diffuse double layer (DDL) model (see Table S4.2 for supporting Information) (Dzombak and Morel 1990). The binding of phosphate on goethite and HFO was included in the calculation. Based on the measured total soluble P concentration in the soil solution (Table 4.1) and the pH, the PO_4^{3-} concentration was calculated, assuming that all soluble P is orthophosphate. This PO_4^{3-} concentration was used as model input. The model parameters used for the CD-MUSIC and the DDL model can be found in Weng et al. (2001a). For binding to both HFO and goethite, effects of H^+, Ca^{2+} and Cl^- on Zn^{2+} adsorption were taken into account in the model, in addition to the effects of PO_4^{3-}. Effects of the presence of adsorbed soil organic matter at the surface of iron (hydr)oxides on Zn adsorption to oxides were not taken into account due to the difficulty to calculate, in a numerically stable way, simultaneously OC sorption by oxides and Zn sorption by OC adsorbed to oxides. The second reason is that experimentally, adsorbed OC is difficult to assess. As we accounted for phosphate sorption, which competes with OC for the oxide surface, in a way the OC sorption

effect on Zn adsorption is mimicked. The amount of crystalline iron (hydr)oxides (goethite) was estimated from the difference between dithionite-citrate bicarbonate (DCB) and oxalate extractable Fe, using the molar mass of $Fe(OH)_3$. The amount of amorphous iron (hydr)oxides was estimated from the oxalate extractable Fe (Weng et al. 2001a). The surface area of goethite and HFO was assumed to be 100 m^2 g^{-1} and 600 m^2 g^{-1}, respectively (Dzombak and Morel 1990; Filius et al. 1997). This agrees with Hiemstra et al. (2010) who determined a specific surface area of 630 $\pm$ 470 m^2 g^{-1} of Fe- and Al-(hydr)oxides extractable with DCB based on phosphate adsorption capacity in the soils (Hiemstra and Van Riemsdijk 2009).

The calculations were done with the computer program ECOSAT (Keizer and Van Riemsdijk 1995). It combines the above described adsorption models, solution speciation and precipitation reactions (Fig. 4.1) (The aqueous reactions and their associated equilibrium constants are shown in Table S4.4 as supporting Information). Other software that combines the same chemical reactions and parameterization should yield the same results (Nowack et al. 2006). However, ECOSAT is one of the few computer programs with the ability to simultaneously implement a CD-MUSIC and a DDL model. The 0.43 M HNO_3 extractable metal (Zn, Cu and Ni) concentrations were used as input data for the model and were considered as reactive metal pool for solution complexation, adsorption and precipitation processes. The 0.43 M HNO_3 extractable Al and Fe content were used as model input for total Al and Fe. Free Al^{3+} and Fe^{3+} activities were controlled in the model calculation by the precipitation of $Al(OH)_3$ ($logK_{so}$ = -33.96) and $Fe(OH)_3$ ($logK_{so}$ = -38.46), respectively (Lindsay 1979). Specific conditions of the soil column DMT experiment, such as pH, ionic strength, temperature and soil : solution ratio were accounted for in the calculations.

To test if Zn concentration in these soils is oversaturated with regards to Zn-containing minerals, the free Zn^{2+} concentration as controlled by formation of Zn-phyllosilicate and hydrozincite was calculated for the pH range of the soils. The dissolution reactions are expressed as $logZn^{2+}$ = log K_{so} – 2pH, and the solubility products (log K_{so}) for Zn-phyllosilicate and hydrozincite are 6.7 and 8.7, respectively (Jacquat et al. 2008).

The sensitivity of the Zn^{2+} concentrations to changes of pH ($\pm$ 0.5 and 1 pH unit), organic matter ($\pm$ 0.2 and 0.4 %) and Zn application (2.5, 5, 10 and 15 kg Zn ha^{-1}) was tested within ranges that can be influenced by management options. Applying between 2.5 and 15 kg Zn ha^{-1} corresponds with common application rates in low Zn soils (Haileselassie et al. 2011; Phattarakul et al. 2012). For converting the application rate to mg Zn kg^{-1} soil, we assumed a soil depth of 0.3 m and a soil density of 1.4 kg m^{-3}.

Table 4.1 Soil characteristics

Soil name	pH	CEC	Clay (<2µm)	SOC	CaCO$_3$	Oxalate extractable	DCB extractable	HNO$_3$ extractable metals					Zn^{2+}	CaCl$_2$ extractable	DOC
								0.43 M				2 M		0.01 M	
						Fe	Fe	Zn	Fe	Al	Ca	Zn		P	
		mmol kg^{-1}			g kg^{-1}					mg kg^{-1}			(-log M)	mg kg^{-1}	mg L^{-1}
Hofuf	7.3	35	39	7	62.0	0.3	0.6	20.8	41	172	630	22.0	6.8	20.70	6.4
Dalwallinu	4.2	10	72	6	0.0	0.1	5.7	0.1	9	108	52	0.2	7.1	0.01	1.7
Lancelin	5.2	20	38	13	0.1	0.1	1.8	0.1	13	90	438	0.2	7.6	0.19	5.1
Wukro	7.3	100	125	8	2.7	0.6	6.6	5.8	200	437	470	6.7	7.4	6.97	3.4
Droevendaal	6.2	30	48	4	0.0	0.9	4.5	1.3	126	384	552	1.7	7.4	0.53	2.5
Anatolia	7.5	320	220	10	93.0	0.3	5.7	0.2	45	214	523	0.3	8.2	0.55	6.3
Bologna	7.4	172	232	9	144.0	1.6	7.1	2.8	406	508	647	3.0	7.8	0.13	12.1
Xeraco	7.5	303	357	14	154.0	0.9	13.2	13.4	114	401	721	14.0	8.0	0.79	9.4
Veenendaal, V1	6.7	150	23	32	1.0	2.6	3.8	513.0	1566	1480	7860	491.0	5.8	7.11	22.6
Veenendaal, V2	6.7	120	23	44	0.1	3.3	6.7	270.0	1714	1190	4110	225.0	5.9	2.66	44.0
Veenendaal, V3	5.7	80	17	35	0.0	1.5	2.5	95.8	973	812	1770	91.1	5.5	4.09	61.5
Veenendaal, V4	5.9	130	28	38	0.1	2.8	6.2	399.0	1538	1360	3470	383.0	5.1	9.17	45.7
Veenendaal, V5	5.0	60	15	26	0.0	1.9	2.2	40.5	1318	761	1280	42.0	5.4	2.42	38.7
Veenendaal, V6	5.0	100	26	48	0.0	1.8	2.1	162.0	914	1420	2070	136.0	4.8	3.24	74.5
Veenendaal, V7	6.4	180	25	43	0.5	5.6	10.9	619.0	3135	1450	2100	563.0	5.6	21.48	56.5

4.4 Results

4.4.1 Soil characteristics

The selected soils varied considerably in basic soil characteristics (Table 4.1). In the low Zn soils (total reactive Zn 0.1-21 mg Zn kg^{-1} soil) the pH ranged from 4.2-7.5 and in the high Zn soils (total reactive Zn 41-619 mg Zn kg^{-1} soil) from 5.0-6.7. The texture of the collected soils ranged from sand to loam. The soil organic matter content in the low Zn soils was considerably lower than in the high Zn soils. Ammonium-oxalate-extractable Fe in the low Zn soils was in average lower than in the high Zn soils, whereas the range of dithionite-citrate-bicarbonate extractable Fe was similar in both soil groups. Five low Zn soils contained CaCO$_3$, whereas in the other soils the CaCO$_3$ content was ≤ 1 g kg^{-1}. The Zn concentrations in the 0.43 M HNO$_3$ and 2 M HNO$_3$ extracts were in the same order of magnitude (Table 4.1).

4.4.2 Measured and predicted free Zn^{2+} concentration

The predicted free Zn^{2+} concentrations, and therefore the overall accuracy of the model, was validated against the Zn^{2+} concentrations determined with the soil column DMT. The Donnan membrane equilibrium of Zn in the soil column DMT experiment was reached after 48 h for all soil samples (see Fig. S4.1 for supporting Information). The free Zn^{2+} concentrations in the low Zn soils were on average two orders of magnitude lower than in the high Zn soils (Table 4.1).

The free Zn^{2+} concentrations predicted by the generic multisurface model were in good agreement (within ± 1 standard error around the 1:1 line) with the measured values, except for one low Zn soil (Dalwallinu; Fig. 4.2). At pH ≥ 7 the experimentally determined Zn^{2+} concentrations in the low and high Zn soils were in the range of the Zn^{2+} concentrations controlled by the solubility of Zn-phyllosilicate (Fig. 4.3). At lower pH, the Zn^{2+} concentrations is less than what is given by the solubility of the Zn-phyllosilicate and hydrozincite.
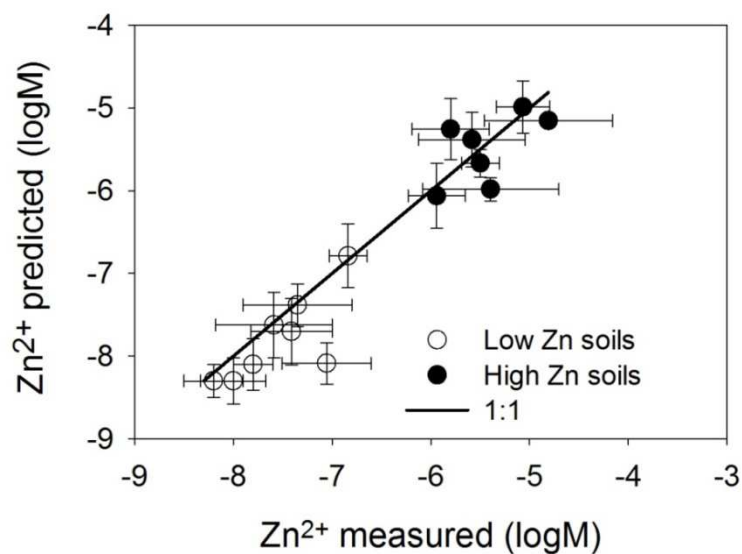
Fig. 4.2 Free Zn^{2+} concentrations measured with the soil column Donnan Membrane Technique plotted against the free Zn^{2+} concentrations in the soil solution predicted with the multisurface model. Vertical error bars show the standard errors around the mean of model predictions using three different clay charges and site densities of SOM (n=3). Horizontal error bars are the standard errors of measured Zn concentrations n = 3).
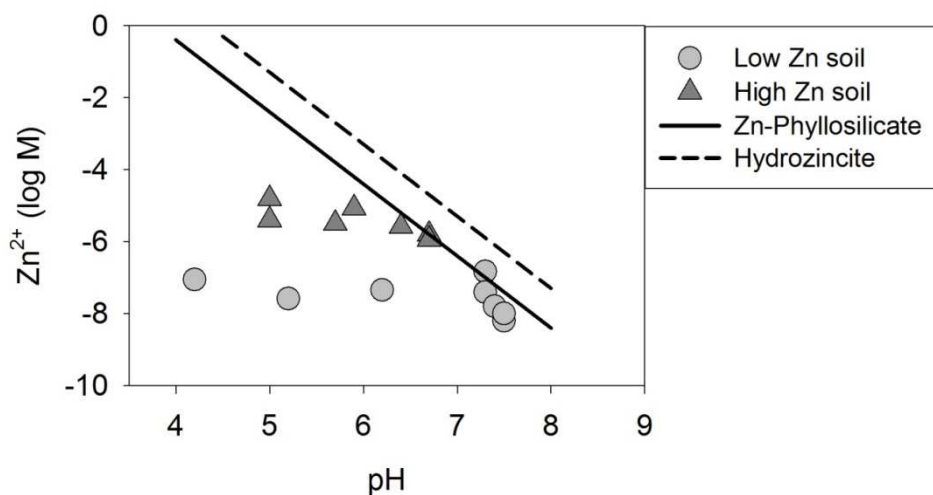
Fig. 4.3 Comparing measured Zn^{2+} concentrations in the low and high Zn soils with the Zn^{2+} concentration controlled by the solubility of Zn-phyllosilicate and hydrozincite.

4.4.3 Modeled Zn sorption to soil constituents

Model predictions revealed that soil organic matter was the dominant sorbent for Zn in the low and the high Zn soils (Fig. 4.4). Amorphous iron (hydr)oxides also contributed considerably (16-26%) to Zn sorption in the low Zn soils from Hofuf, Wukro and Droevendaal and in the high Zn soils V1 and V7. Contributions of clay and crystalline iron (hydr)oxides were predicted to be negligible for all soils.
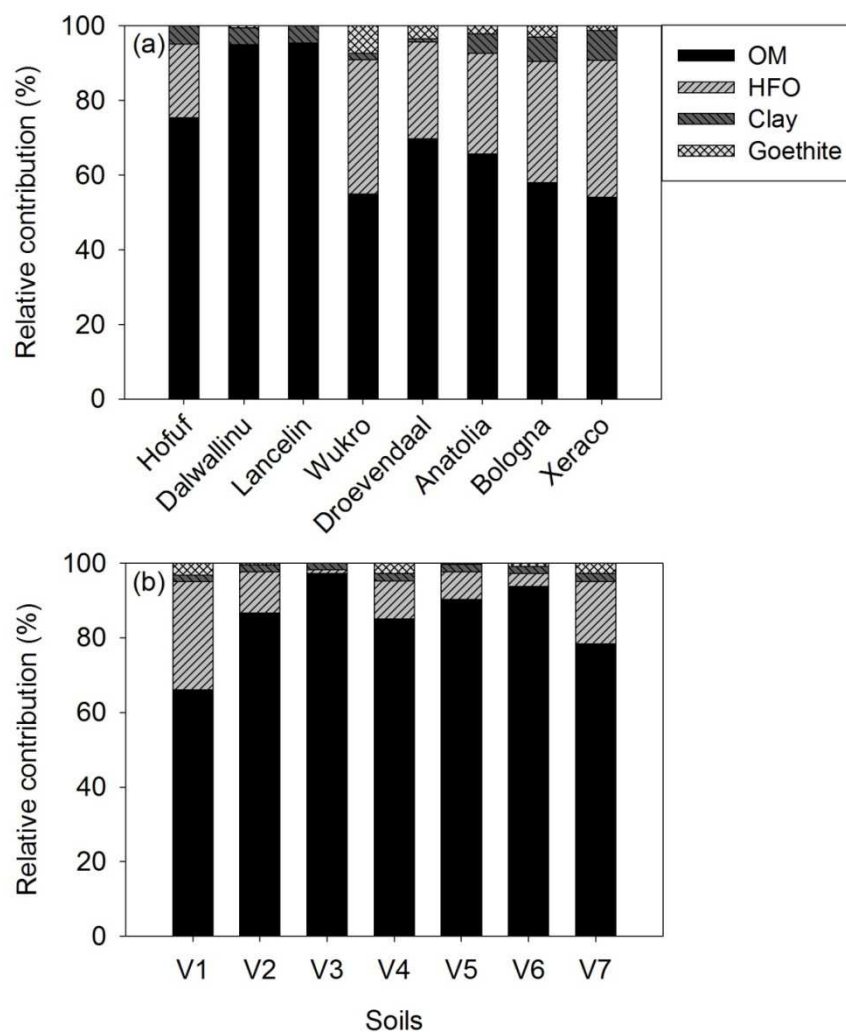
Fig. 4.4 Contribution of soil sorbents (OM = Organic Matter, HFO = Hydrous Ferric Oxides) to Zn adsorption in low Zn (a) and high Zn soils (b).

Due to the relatively small contribution of clay to Zn sorption, model results are not sensitive to clay type. Using kaolinite and montmorillonite as representative clay silicate did not change the minor role of clay (data not shown). The predicted relative contribution of both soil organic matter and amorphous iron (hydr)oxides to Zn sorption was pH dependent. With increasing pH, the contribution of amorphous iron (hydr)oxides increased, whereas the contribution of soil organic matter decreased (Fig. 4.5). Consideration of phosphate binding to iron (hydr)oxides improved the model accuracy, since it promotes Zn sorption to iron oxides due to synergistic electrostatic effects. The effect on the free Zn ion concentration was not significant, whereas it has an effect on the relative contribution of iron (hydr)oxides as sorption site for Zn (data not shown).
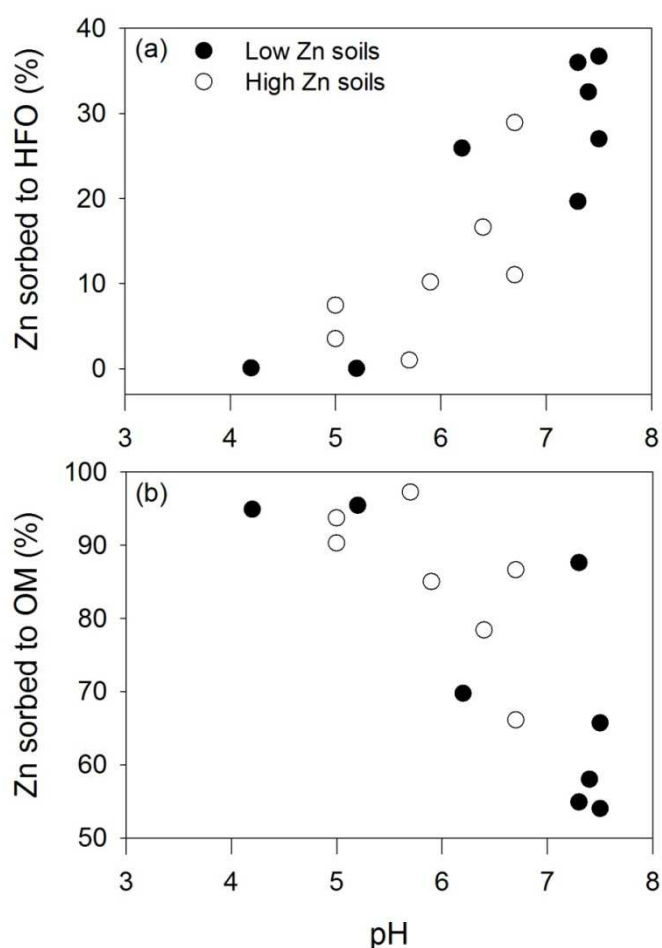
Fig. 4.5 Relationship between the pH of the low and high Zn soils and (a) Zn adsorbed to the hydrous ferric oxide (HFO) (b) and Zn adsorbed to organic matter (OM) as percentage of total amount of adsorbed Zn.
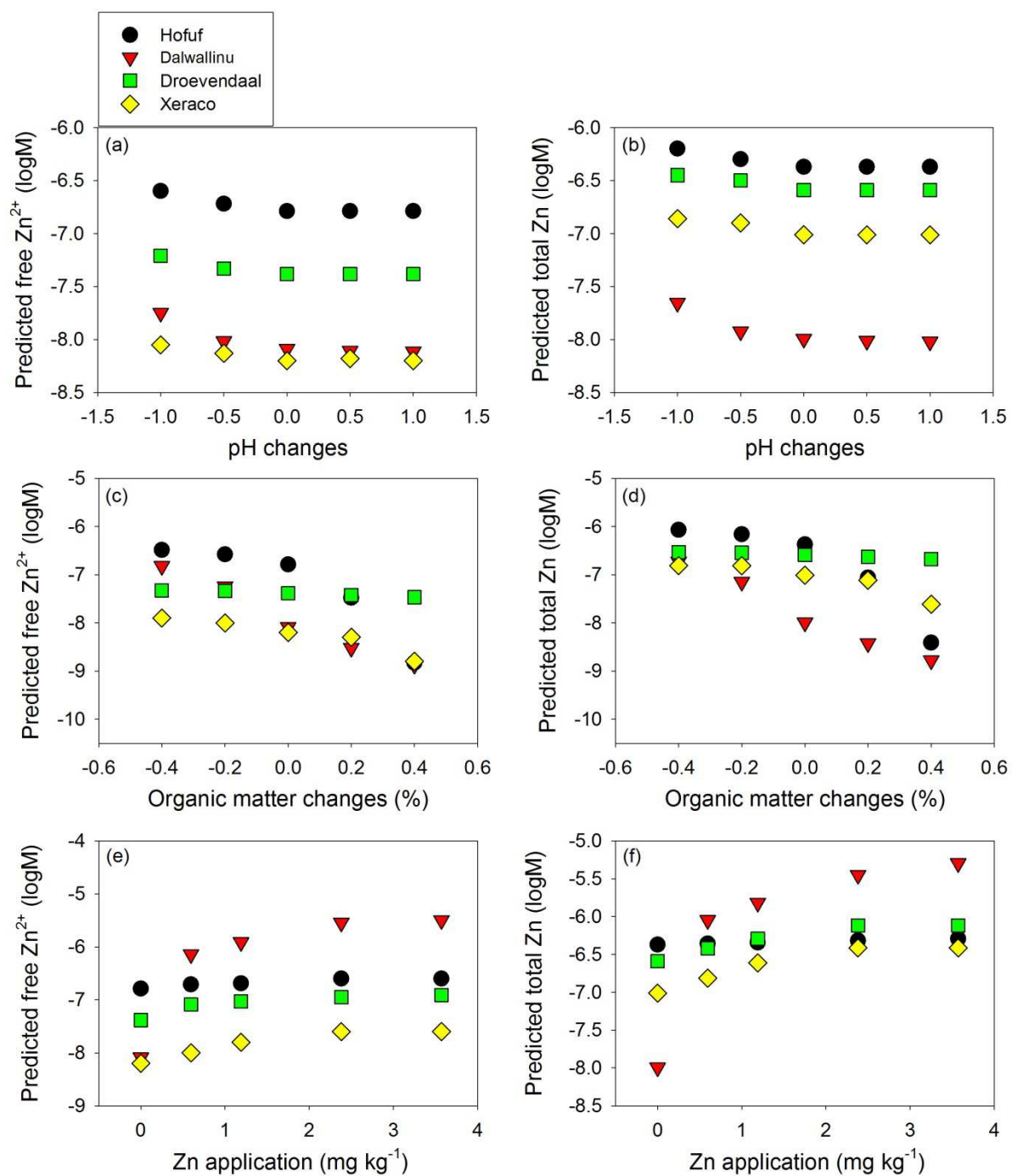
Fig. 4.6 Sensitivity of the predicted free Zn^{2+} concentrations and the total Zn concentration in the soil solution to changes in (a+b) pH ($\pm$ 0.5 and 1 pH unit), (c+d) organic matter ($\pm$ 0.2 and 0.4 %) and (e+f) Zn application (0.6, 1.2, 2.4 and 3.6 mg Zn kg^{-1} soil) for a representative selection of low Zn soils, respectively.

4.4.4 Evaluation of management options

The sensitivity test was carried out for the low Zn soils using the change-one-factor-at-a-time approach. Decreasing the pH by one unit caused a small increase of the free Zn^{2+} concentration in the low Zn soils (Fig. 4.6 a), whereas increasing by one pH unit had virtually no effect (Fig. 4.6 a). An increase or decrease in the organic matter content (up to 0.4 %) reduced or increased, respectively, the free Zn^{2+} concentration predicted for all low Zn soils, except of the Droevendaal soil (Fig 4.6 c). Increasing the reactive Zn pool in the low Zn soils by fertilization (2.5-15 kg Zn ha^{-1}, equivalent to 0.6-3.6 mg Zn kg^{-1} soil) increased the free Zn^{2+} concentration in the Dalwallinu and Lancelin soil by two orders of magnitude. The effect was smaller in the Hofuf and Wukro soil (Fig. 4.6 e). The sensitivity of the total Zn concentration in the soil solution to changing pH (Fig. 4.6 b), organic matter content (Fig. 6 d) and reactive Zn pool (Fig. 6 f) was similar to that of the free Zn^{2+} concentration.

4.5 Discussion

The accurate prediction of the Zn^{2+} concentration in low Zn soils with a multisurface model that was parameterized for high Zn levels indicates that our quantitative understanding of Zn speciation in soils is widely valid. The low Zn soils had total Zn concentrations close to the detection limit of current metal analytical techniques. The Zn^{2+} concentrations predicted by the model agreed well with observed Zn^{2+} concentrations over a wide range ($10^{-8} – 10^{-5}$ M; Fig. 4.2) without any parameter adjustment. Only in one low Zn soil, the Zn^{2+} concentration was significantly underestimated by the model (Dalwallinu soil, Fig. 4.2). In this soil, the ratio of charge density between soil organic matter and generic humic acid was lower than in the other low Zn soils at the same pH.

Our calculations indicate that Zn precipitates play only a minor role in controlling free Zn^{2+} concentrations in low Zn soils. At low pH (pH < 7), the measured free Zn^{2+} concentrations are below those controlled by solubility of Zn-phyllosilicate (Fig. 4.3). Only in soils with pH $\geq$ 7, the measured free Zn^{2+} concentrations are close to those controlled by solubility of Zn-phyllosilicate (Fig. 4.3). For all soils, except one, the model output agreed well (within one standard error difference between calculated and observed free Zn^{2+} concentrations) assuming no Zn precipitation (Fig. 4.2). Combination of these results shows that for low Zn soils, adsorption is the major process controlling free Zn^{2+} concentration over quite a wide pH range.

The contribution of $CaCO_3$ in controlling the Zn^{2+} concentration was small. The small difference between the 0.43 M HNO_3 and 2 M HNO_3 extractable Zn concentrations (Table

4.1) indicates that the proportion of Zn occluded in $CaCO_3$ is low. Zinc sorption to $CaCO_3$ can be considered as insignificant due to the small specific surface area of $CaCO_3$ (Inks and Hahn 1967). Low Zn sorption to $CaCO_3$ was also found by Trehan et al. (1977). They studied the effect of clay, organic matter, and $CaCO_3$ content on Zn adsorption in soils with pH and $CaCO_3$ contents similar to the soils we used. The importance of $CaCO_3$ in controlling the Zn^{2+} concentration could increase in soils with pH > 8 and $CaCO_3$ contents > 10% as proposed by Wenming et al. (2001). But such soils were not investigated in our study.

The multisurface model provides a new tool to evaluate effects of soil management options on the Zn^{2+} concentrations in low Zn soils. In soils with a low reactive Zn pool (Dalwallinu and Lancelin), for instance, the application of Zn fertilizer is expected to effectively increase Zn^{2+} concentration. The application of only 2.5 kg Zn ha^{-1} is predicted to increase the Zn^{2+} concentration by up to two orders of magnitude (Fig. 4.6 e). Increasing the application rate to 10 or 15 kg Zn ha^{-1} will still cause a significant further increase in Zn^{2+} concentration. In regions with expensive Zn fertilizers, application of only small amounts of Zn fertilizer in the planting hole or row could therefore effectively increase Zn^{2+} concentrations (Ebrahimian et al. 2010). In soils with a reactive Zn pool of > 5 mg kg^{-1} the application of Zn will cause only a minor increase in Zn^{2+} concentration according to our prediction. This was due to the formation of Zn-phyllosilicates (modeling results not shown), because the Zn^{2+} concentrations in the soils with the lowest response to Zn application were closest to the Zn^{2+} concentration which is controlled by the solubility of Zn-phyllosilicate (Fig. 4.3). Such insights by multisurface modeling help to prevent Zn application to soils where Zn^{2+} concentration and crop yield do not respond well to fertilization (Haileselassie et al. 2011; Zou et al. 2012).

Changing the organic matter content had a strong effect on the Zn^{2+} concentration in all low Zn soils, except in the Droevendaal soil (Fig. 4.6 c): Reducing the organic matter content increased the Zn^{2+} concentration and vice versa, because soil organic matter is a major Zn sorbent (Fig. 4.4 a). Even in soils with low soil organic matter contents (Table 4.1), organic matter is still the dominant sorbent (Fig. 4.4 a). If, however, applied organic matter would contain 80 mg Zn kg^{-1} (dry weight), which is a common Zn content in compost or cattle farmyard manure (Nicholson et al. 1999; Walker et al. 2003), the predicted free Zn^{2+} concentration in the studied low Zn soils would increase (data not shown) due to an increase of total Zn loading. Our multisurface model accounts for Zn complexation to dissolved organic carbon as well, and we consider an increase in soil DOC concentration with increasing soil organic matter content. As a result of increasing DOC upon organic matter application, DOC-complexed Zn increases. This could lead to an additional increase in Zn uptake (Van Leeuwen and Buffle 2009). The effect of organic matter addition on Zn bioavailability depends therefore mainly on the Zn concentration and the DOC content of the applied organic matter (Bolan et al. 2004; Schulin et al. 2009; Gao et al. 2012).

The predicted concentration of Zn^{2+} showed only a small response to pH changes of ± 0.5 and 1 pH units in the pH range of 4.2-7.3 (Fig. 4.6 a). Lowering the pH increases the contribution of SOM to Zn sorption, whereas increasing the pH leads to more contribution of iron (hydr)oxides (Fig. 4.5). However, soil organic matter remains the dominant sorbent in all cases (Fig. 4.4, Fig. S4.2 as supporting information). We did not focus on the occupation of single binding sites, rather on the contribution of each sorbate, because even in the high Zn soils saturation of the binding sites did not occur.

To evaluate potential management effects on Zn bioavailability, it may be relevant to predict effects on the total Zn concentration in the soil solution (Fig. 4.6 b, d, f), because this can be a good predictor for Zn uptake (Duffner et al. 2013). The simulated management effects on the total Zn concentration were similar to their effects on the Zn^{2+} concentration. A reliable prediction of free Zn^{2+} guarantees accurate prediction of other soluble inorganic Zn complexes (e.g. $ZnOH^{+}$), since the formation constants of common Zn inorganic complexes are reliable (Allison et al. 1991; McLaughlin et al. 2000). The prediction of other Zn species is relevant because besides Zn^{2+} also they can be taken up by plants (Broadley et al. 2012; Duffner et al. 2013). In our soils, $ZnOH^{+}$ concentrations between $10^{-6.8}$ and $10^{-9.1}$ M were predicted, which could contribute to uptake (Broadley et al. 2012). Correct prediction of free Zn^{2+} concentration is also the basis for accurate prediction of soluble organic Zn complexes. However, the formation constants of several organic Zn complexes (e.g. Zn-phytosiderophore) are not reliable enough yet to allow for accurate prediction. Further research should also include bioavailable organic Zn species (Zn-phytosiderophore (Von Wirén et al. 1996), Zn-citrate or Zn-histidine complexes (Gramlich et al. 2013)) when predicting bioavailable Zn with multisurface models.

Supporting information
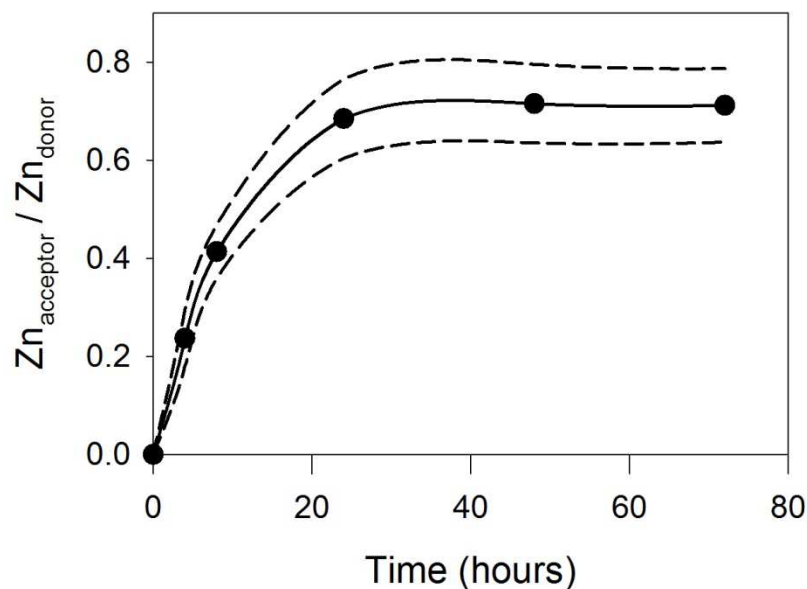
Fig. S4.1 Ratio of the acceptor (free Zn ion concentration) and donor (total Zn concentration) Zn concentrations in the soil column DMT experiment as a function of time. The solid line shows the average ratio of the acceptor and donor Zn concentractions in the soil column DMT experiment for all soils. The dashed lines represent the confidence interval of all soils (n=3). When the curves reach the maximum and the ratio does not change with time anymore, this indicates Donnan membrane equilibrium. Complexation of part of the Zn in the donor solution with dissolved organic matter was the reason that the ratio of Zn in the acceptor solution and Zn in the donor solution remained smaller than one, after reaching the Donnan membrane equilibrium.
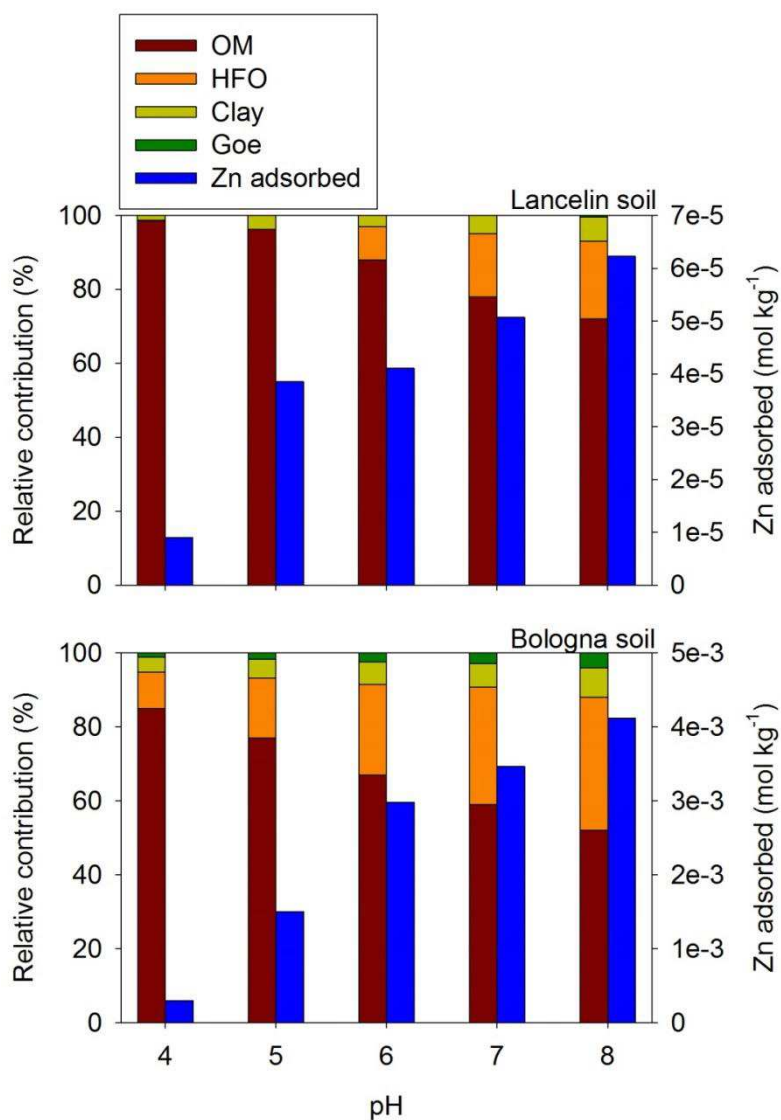
Fig. S4.2 Relative contribution of soil sorbents (OM = Organic Matter, HFO = Hydrous Ferric Oxides) to Zn adsorption and the total adsorbed Zn in the Lancelin and Bologna soil with varying pH, respectively. Lowering the pH increases the contribution of SOM to Zn sorption, whereas increasing the pH leads to more contribution of iron (hydr)oxides. However, soil organic matter remains the dominant sorbent in all cases

Table S4.1 NICA-Donnan Model parameters for generic humic acid. The K1 values are the constants for the low affinity or carboxylic type site and the K2 values are the constants for the high affinity or phenolic type site and were taken from Milne et al. (2001) and Milne (2000), where also further details regarding the constants can be found.

Electrostatic Donnan b value	-0.49
Eq. Log V_D = a+blogI	

	low affinity or carboxylic type site		high affinity or phenolic type site	
Site density Q max.H (mol kg^{-1})	3.15		2.55	
heterogeneity p	0.62		0.41	
Ion specific parameters	affinity $logK_1$	non-ideality n_1	affinity $logK_2$	non-ideality n_2
H	2.93	0.81	8.00	0.63
Ca	-1.37	0.78	-0.43	0.75
Al	-1.05	0.40	8.89	0.30
Fe$^{(III)}$	5.00	0.30	17.50	0.25
Zn	0.11	0.67	2.39	0.27

Table S4.2 DDL model parameters of hydrous ferric oxide (HFO). The constants for the two different sorption sites were taken from Dzombak and Morel (1990), where also further details regarding the constants can be found.

Surface area: 6×10^5 $m^2 kg^{-1}$

S1-OH site 0.056 nm^2

S2-OH site 2.255 nm^2

Surface species	$logK_0$	Surface species	$logK_0$
S_1-OH	0.00	S_2-OH	0.00
S_1-OH$_2^+$	7.29	S_2-OH$_2^+$	7.29
S_1-O	-8.93	S_2-O	-8.93
S_1-PO$_4^{2-}$	17.72	S_2-PO$_4^{2-}$	17.72
S_1-HPO$_4$-	25.39	S_2-HPO$_4$-	25.39
S_1-H2PO$_4$	31.29	S_2-H2PO$_4$	31.29
S_1-OHCa^{2+}	4.97	S_2-OHCa^{2+}	-5.85
S_1-OZn$^+$	0.99	S_2-OZn$^+$	-1.99

Table S4.3 CD-MUSIC model parameters of goethite. The constants were taken from Hiemstra and Riemsdijk (1996; 1999), where also further details regarding the constants can be found.

	110 face			021 face		
	Surface area:	4.5×10^4 m^{2}kg^{-1}		Surface area:	5×10^3 m^{2}kg^{-1}	
	FeOH$^{-0.5}$ site:	3.0 nm^{-2}		FeOH$^{-0.5}$ site:	3.75 nm^{-2}	
	Fe$_3$O$^{-0.5}$ site:	3.0 nm^{-2}		Fe$_3$O$^{-0.5}$ site:	3.75 nm^{-2}	

		Species parameters				
Surface species	logK$_0$	site 1	site 2	0-plane	1-plane $C_1 = 1.02$ F m^{-2}	2-plane $C_2 = 5$ F m^{-2}
FeOH$^{-0.5}$	0.00	1FeOH$^{-0.5}$		0	0	0
FeOH$_2^{+0.5}$	9.20	1FeOH$^{-0.5}$		1	0	0
FeOHK$^{+0.5}$	-1.00	1FeOH$^{-0.5}$		0	0	1
FeOH$_2$NO$_3^{-0.5}$	8.20	1FeOH$^{-0.5}$		1	0	-1
Fe$_3$O$^{-0.5}$	0.00	1Fe$_3$O$^{-0.5}$		0	0	0
Fe$_3$OH$^{+0.5}$	9.20	1Fe$_3$O$^{-0.5}$		1	0	0
Fe$_3$OK$^{+0.5}$	-1.00	1Fe$_3$O$^{-0.5}$		0	0	1
Fe$_3$OHNO$_3^{-0.5}$	8.20	1Fe$_3$O$^{-0.5}$		1	0	-1
Fe$_h$OH$^{-0.5}$	0.00	1Fe$_h$OH$^{-0.5}$		0	0	0
Fe$_h$OH2$^{+0.5}$	9.20	1Fe$_h$OH$^{-0.5}$		1	0	0
Fe$_h$OHK$^{+0.5}$	-1.00	1Fe$_h$OH$^{-0.5}$		0	0	1
Fe$_h$OH$_2$NO$_3^{-0.5}$	8.20	1Fe$_h$OH$^{-0.5}$		1	0	-1
FeOHCa$^{+1.5}$	3.55	1FeOH$^{-0.5}$		0.2	1.8	0
FeOPO$_3^{-2.5}$	20.80	1FeOH$^{-0.5}$		0.25	-2.25	0
(FeO)$_2$PO$_2$H^{-1}	35.70	2FeOH$^{-0.5}$		0.9	-0.9	0
(FeO)$_2$PO$_2^{-2}$	29.40	2FeOH$^{-0.5}$		0.39	-1.39	0
(Fe$_h$O)$_2$PO$_2$H^{-1}	35.70	2Fe$_h$OH$^{-0.5}$		0.9	-0.9	0
(Fe$_h$O)$_2$PO$_2^{-2}$	29.40	2Fe$_h$OH$^{-0.5}$		0.39	-1.39	0
(FeOHFe$_3$O)Zn^{+1}	8.01	1FeOH$^{-0.5}$	1Fe$_3$O$^{-0.5}$	0.83	1.17	0
(FeOHFe$_3$O)ZnOH^0	-1.00	1FeOH$^{-0.5}$	1Fe$_3$O$^{-0.5}$	0.83	0.17	0
(Fe$_h$OH)$_2$Zn^{+1}	6.63	2Fe$_h$OH$^{-0.5}$		0.83	1.17	0
(Fe$_h$OH)$_2$ZnOH^0	-2.38	2Fe$_h$OH$^{-0.5}$		0.83	0.17	0

Table S4.4. Aqueous reactions and their associated equilibrium constants. The constants were taken from Lindsay (1979), Morel and Hering (1993) and Allison et al. (1991), where also further details about the constants can be found. (Table continuous on the next two pages).

Species	log K (298.15 K)	delta H kJ mol^{-1}	Reference
$(H_2PO_4)_2$	38.75	0.0	Lindsay (1979)
$Al(OH)_2$	18.70	0.0	Lindsay (1979)
$Al(OH)_3$	27.01	0.0	Lindsay (1979)
$Al(OH)_4$	32.67	0.0	Lindsay (1979)
$Al(OH)_5$	35.67	0.0	Lindsay (1979)
$Al_2(OH)_2$	20.31	0.0	Lindsay (1979)
$AlOH$	8.98	0.0	Lindsay (1979)
$Ca(OH)_2$	0.01	0.0	Lindsay (1979)
$CaCO_3$	3.14	0.0	Lindsay (1979)
CaH_2PO_4	20.95	0.0	Lindsay (1979)
$CaHCO_3$	11.45	0.0	Lindsay (1979)
$CaHP_2O_7$	30.29	0.0	Lindsay (1979)
$CaHPO_4$	15.09	0.0	Lindsay (1979)
$CaOH$	1.30	0.0	Lindsay (1979)
$CaOHP_2O_7$	12.18	0.0	Lindsay (1979)
CaP_2O_7	24.08	0.0	Lindsay (1979)
$CaPO_4$	6.46	0.0	Lindsay (1979)
$Cu(CO_3)_2$	9.82	0.0	Lindsay (1979)
$Cu(OH)_2$	11.80	0.0	Morel and Hering (1993)
$Cu(OH)_3$	15.25	0.0	Lindsay (1979)
$Cu(OH)_4$	16.41	0.0	Lindsay (1979)
$Cu_2(OH)_2$	17.32	0.0	Lindsay (1979)
$Cu_2P_2O_7$	17.25	0.0	Lindsay (1979)
$CuCO_3$	6.72	0.0	Lindsay (1979)
$CuH_2P_2O_7$	35.95	0.0	Lindsay (1979)
CuH_2PO_4	21.14	0.0	Lindsay (1979)
$CuHCO_3$	12.42	0.0	Lindsay (1979)
$CuHP_2O_7$	32.06	0.0	Lindsay (1979)
$CuHPO_4$	15.55	0.0	Lindsay (1979)
$Fe(OH)_3$	28.91	0.0	Lindsay (1979)

Fe(OH)$_4$	34.41	0.0	Lindsay (1979)
Fe$_3$2(OH)$_2$	25.10	0.0	Lindsay (1979)
Fe$_3$H$_2$PO$_4$	24.98	0.0	Lindsay (1979)
Fe$_3$HPO$_4$	23.26	0.0	Lindsay (1979)
Fe$_3$OH	11.81	0.0	Lindsay (1979)
H$_2$CO$_3$	16.69	-22.6	Lindsay (1979)
H$_2$P$_2$O$_7$	33.39	0.0	Lindsay (1979)
H$_2$PO$_4$	19.55	-18.9	Lindsay (1979)
H$_3$P$_2$O$_7$	35.67	0.0	Lindsay (1979)
H$_3$PO$_4$	21.70	-10.9	Lindsay (1979)
H$_4$P$_2$O$_7$	36.47	0.0	Lindsay (1979)
HCO$_3$	10.33	-14.9	Lindsay (1979)
HP$_2$O$_7$	26.69	0.0	Lindsay (1979)
HPO$_4$	12.35	-14.7	Lindsay (1979)
P$_2$O$_7$	17.28	0.0	Lindsay (1979)
Zn(OH)$_2$	11.20	0.0	Lindsay (1979)
Zn(OH)$_3$	14.32	0.0	Lindsay (1979)
Zn(OH)$_4$	17.71	0.0	Lindsay (1979)
ZnH$_2$PO$_4$	21.15	0.0	Lindsay (1979)
ZnHPO$_4$	15.65	0.0	Lindsay (1979)
ZnOH	6.31	0.0	Lindsay (1979)
Fe$_3$(OH)$_4$	49.70	0.0	Allison et al. (1991)
Ni(CO$_3$)$_2$	10.11	0.0	Allison et al. (1991)
Ni(OH)$_2$	9.00	0.0	Allison et al. (1991)
Ni(OH)$_3$	12.00	0.0	Allison et al. (1991)
NiCO$_3$	6.87	0.0	Allison et al. (1991)
NiHCO$_3$	12.47	0.0	Allison et al. (1991)
NiOH	4.14	0.0	Allison et al. (1991)
Zn(CO$_3$)$_2$	9.63	0.0	Allison et al. (1991)
ZnCO$_3$	5.30	0.0	Allison et al. (1991)
ZnHCO$_3$	12.4	0.0	Allison et al. (1991)
CaCl	-1.00	0.0	Lindsay (1979)
CaCl$_2$	0.00	0.0	Lindsay (1979)
CuCl	0.40	0.0	Lindsay (1979)
CuCl$_2$	-0.12	0.0	Lindsay (1979)
CuCl$_3$	-1.57	0.0	Lindsay (1979)
Fe$_3$Cl	1.48	0.0	Lindsay (1979)

Fe$_3$Cl$_2$	2.13	0.0	Lindsay (1979)
ZnCl$_3$	0.50	0.0	Lindsay (1979)
ZnCl$_4$	0.20	0.0	Lindsay (1979)

5. Predicting plant available zinc in the rhizosphere with a coupled multisurface- and radial transport model

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5.1 Abstract

A combined multisurface and transport model approach was used to predict the effect of root exuded citrate on Zn speciation in the rhizosphere and on plant Zn uptake. Interaction of Zn, Fe and citrate with goethite and soil organic matter (SOM), degradation of citrate, and diffusion of Zn towards and citrate away from the root surface were considered. Assuming citrate exudation for 24 h into a low Zn soil with varying goethite (0.3-12.3 g kg^{-1}) and SOM (8-28 g kg^{-1}) contents increased the Zn concentration in the soil solution close to the root surface (1-2 mm) for a relatively short time (<1 d) after exudation had ceased. The effect of root exuded citrate on Zn speciation and plant Zn uptake was therefore not based on a specific minimum concentration of citrate in the rhizosphere, but on the time after exudation ceased. A major part of the citrate in the solution was adsorbed to goethite within 1 d after exudation stopped, but the Zn concentration in solution was mainly controlled by Zn sorption to soil organic matter. The remaining citrate was either degraded or complexed by Zn or Fe. Citrate exudation increased cumulative Zn uptake by 2-7 x 10^{-10} mol m^{-1} root surface during the first 2 d after citrate exudation. Zinc adsorbed to soil organic matter (SOM) was only marginally affected by root exuded citrate. This model approach is also suitable for future studies to predict the effect of other root exuded ligands on the speciation and plant uptake of other micronutrients.

5.2 Introduction

Zinc (Zn) is an essential micronutrient for all plants (Sommer and Lipman 1926). Zinc deficiency is limiting crop production in ±30 % of the world's soils (Sillanpää and Vlek 1985). The availability of Zn to plants depends on concentrations and distribution of Zn species in the soil solution rather than on the total Zn content of soils (Nolan et al. 2003). The concentration and distribution of Zn species in the soil solution vary significantly between bulk and rhizosphere soil due to differing dissolved organic matter concentrations, pH and microbial activity caused by root exudation of the plant and plant Zn uptake (Puschenreiter et al. 2005; Duffner et al. 2012). The mobilization of Zn, for instance, can be increased due to acidification caused by exudation of protons (Hinsinger et al. 2003) and by formation of soluble complexes of exuded citrate with Zn^{2+} in solution, which reduces the activity of Zn^{2+} in the soil solution. As a consequence Zn^{2+} desorbs from soil surfaces or dissolves from labile solid phases to replenish Zn^{2+} in solution (Lindsay and Norvell 1978).

There are several studies which experimentally determined concentrations of root exudates in the rhizosphere and their effect for Zn speciation and/or Zn plant uptake (Degryse et al. 2008;

Dessureault-Rompré et al. 2008; Gao et al. 2009; Duffner et al. 2012). It is, however, challenging to experimentally determine the period and distance to the root surface where root exudates have a significant effect (acidification, complexation and decrease of Zn sorption to the solid phase) on Zn bioavailability. Duffner et al. (2012) showed that the effect of root exuded citrate on the Zn concentration in the soil solution strongly varied between different soil samples. They hypothesized, based on stability constants, that in soils with high reactive Fe concentration in solution, citrate forms mainly complexes with Fe and not Zn. Therefore the root exuded citrate hardly leads to increased bioavailable Zn concentrations in those soils. The minimum concentration of root exudates in the rhizosphere required to increase bioavailable Zn is under debate: Rose et al. (2011) observed that citrate or malate concentrations in the soil solution have to be > 0.1 mM to cause significant Zn solubilization. But Gao et al. (2009) found that 0.5 mM of malate is not sufficient to solubilize significant amounts of Zn. Duffner et al. (2012) showed that citrate (~0.6 mM) is able to increase the Zn concentration in the soil solution of one soil, whereas in another soil even 1.5 mM had no effect.

Using sophisticated computational tools developed in hydro-geochemistry offer the possibility to determine the main controlling factors of metal bioavailability in the rhizosphere in a temporal and spatial context. Geelhoed et al. (1999) for instance included solution and surface speciation into rhizosphere models to simulate the effect of citrate exudation on phosphate uptake. Szegedi et al. (2008; 2010) developed a coupled rhizosphere transport and speciation model ("RhizoMath") to simulate the transport of competitive uptake of P and As. However, both approaches were applied to simplified substrates (goethite-quartz sand mixtures) without considering soil organic matter as sorption surface. Schnepf et al. (2012) used mathematical modeling to simulate the phosphorus uptake by a growing and exuding root system whereas the sorption was described by a Langmuir isotherm. This study showed that the effect of root exudation on P uptake is strongly dependent on the age distribution of the root system.

Hydro-geochemical modeling of transport and speciation of metals in the rhizosphere has been mainly applied to contaminated conditions (Puschenreiter et al. 2005; Zhao et al. 2007) or to macronutrients (Calba et al. 2004; Nowack et al. 2006). But there are, however, also a few modeling approaches dealing with low Zn levels. Duffner et al. (2014b) showed that a multisurface model approach (considering metal sorption to clay, soil organic matter and iron (hydr)oxide) can also accurately predict concentrations of metal micronutrients, such as Zn, at low concentration levels and that soil organic matter was the dominant sorption phase for Zn in a wide variety of soils. Ptashnyk et al. (2011) predicted the effect of root exuded phytosiderophores on Zn plant uptake over time, but without considering the role of the solid phase as sorbent.

In this study we simulated the solid-solution distribution, speciation and radial transport of Zn in the rhizosphere as a function of time and distance to the root surface to predict the effect of root exuded citrate on plant Zn uptake.

5.3 Model development

5.3.1 Modeling framework ORCHESTRA

The rhizosphere model is implemented in the modeling framework ORCHESTRA (Objects Representing CHEmical Speciation and TRAnsport) (Meeussen 2003). The object-oriented structure makes it possible to design a generic object framework, based on a set of fundamental object classes. These object classes act as named templates that contain generic calculation "recipes". In contrast to standard chemical equilibrium algorithms, such as MINTEQ2A (Allison et al. 1991), PHREEQC (Parkhurst and Appelo 1999) and ECOSAT (Keizer and Van Riemsdijk 1995) model equations are not hardcoded in the source code. This framework was successfully used before to couple multisurface- and transport models for other objectives (Van Beinum et al. 2006; Szegedi et al. 2010; Schotanus et al. 2014).

5.3.2 Speciation and sorption

The chemical part of the model includes the speciation of Zn, Fe and citrate and the sorption of these compounds to the solid phase (goethite and soil organic matter (SOM)). Goethite is considered as sorption surface for citrate (Geelhoed et al. 1999; Filius et al. 2003) and SOM as sorption surface for Zn and Fe (Duffner et al. 2014b). The assumed amounts of goethite and SOM were based on an average value of eight low Zn soils which were used in a former study (Duffner et al. 2014b) (Table 5.1). Due to low contribution of clay as sorbent for Zn and Fe at low concentrations levels (Duffner et al. 2014b) clay was not considered as sorption surface. The activity of Fe in solution is controlled by the solubility of amorphous $Fe(OH)_3$ (Weng et al. 2001a). Ionic strength is 0.1 mol kg^{-1}. Equilibrium constants for minerals and soluble species were taken from the MINTEQA2 database (Allison et al. 1991).

Humic acid was considered as the model material of the organic matter fraction that reacts with metals. It was assumed that 50% of SOM is humic acid (Weng et al. 2001a). Soil organic matter was calculated as 2 times that of soil organic C. The generic model parameter set derived for humic acid (Milne 2000; Milne et al. 2001) was used to model metal binding with the Non Ideal Competitive Adsorption Donnan model (NICA-Donnan) (Kinniburgh et al.

1999). The site density and the heterogeneity of the carboxylic type site is 3.15 mol kg^{-1} and 0.62 and for the phenolic type site 2.55 mol kg^{-1} and 0.41, respectively. The value for the Donnan volume b parameter is 0.49. Competition of Al^{3+}, Fe^{3+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, and H^+ with Zn^{2+} for binding to SOM was considered in the model calculation. Zinc binding to DOC was also predicted with the NICA-Donnan model, assuming Zn sorption to suspended humic acid.

Binding of citrate to goethite was simulated with the Charge Distribution Multi-Site Complexation (CD-MUSIC) model (Hiemstra and Van Riemsdijk 1996; Geelhoed et al. 1999; Hiemstra and Van Riemsdijk 1999). For the specific surface area of goethite, values are given from 20 to 200 m^2 g^{-1} (Cornell and Schwertmann 1996). We assumed a specific surface area of 100 m^2 g^{-1} for goethite (Filius et al. 1997). The binding of phosphate on goethite was included in the calculation.

The citrate exudation rate was assumed to be 3 x 10^{-6} µmol cm^{-2} s^{-1}, which is the range of the values given by Kirk (1999) and Hoffland (1992) and was used before in the rhizosphere model of Schnepf et al. (2012). We assumed that the root apices exuded citrate for 24 h (Ptashnyk et al. 2011) at one spot, based on the root growth velocity and the length of the exudation zone (root apices) (Schnepf et al. 2012). Our modeling approach predicted therefore the Zn speciation and transport within such a spot which was influenced by citrate exudation for 24 h. For citrate exudation, a concomitant exudation of protons (H^+) was assumed. For citrate degradation, we assumed a first-order rate constant (k_{Cit}) of 4.16 x 10^{-5} s^{-1}, i.e. a half-life of 4.6 h, which was determined by Kirk et al. (1999b). It was assumed that citrate adsorbed to the solid phase is protected from degradation. The aqueous reactions and their associated equilibrium constants and the parameters for the CD-MUSIC and NICA-Donnan model are given in Duffner et al. (2014b).

5.3.3 Radial transport

The equations of the radial transport model were based on the work of Nye, who applied the convection–diffusion equation to rhizosphere modeling (Nye and Marriott 1969; Tinker and Nye 2000). This theoretical background was comprehensively summarized in Szegedi et al. (2008).

Root uptake was defined as a boundary condition

$$J_{upt} = D \frac{\partial C}{\partial r} + r_0 v C \qquad (1)$$

where J_{upt} [mol L^{-2} T^{-1}] is the nutrient uptake flux, D [L^2 T^{-1}] is the diffusion dispersion coefficient, C [mol L^{-3}] is the concentration of a given species in the solution, r [L] is the distance from the root center, r_0 [L] is the root radius and v [L T^{-1}] is the water flux at the root surface. We assumed that Zn is taken up at the same rate as it is transported toward the root surface (zero-sink uptake) (De Willigen and van Noordwijk 1994) and the water flux at the root surface was assumed to be 0 to simplify the calculation (Nowack et al. 2006; Szegedi et al. 2008).

Table 5.1 Parameter of the multisurface- and the radial transport model

Parameter	Unit	Value	Reference
Goethite	g kg^{-1}	6.3 (0.3-12.3)	Duffner et al. (2014b)
Solid organic matter	g kg^{-1}	18 (8-28)	Duffner et al. (2014b)
Ionic strength (I)	mol kg^{-1}	0.1	
Initial pH	-	7	
Initial total Zn concentration (C_{Zn})	mol dm^{-3}	4 x 10^{-5}	
Initial total Fe concentration (C_{Fe})	mol dm^{-3}	4 x 10^{-5}	
Citrate exudation rate over 24 h	µmol cm^{-2} s^{-1}	3 x 10^{-6}	Kirk (1999), Hoffland (1992)
Degradation rate citrate (k_{Cit})	s^{-1}	4 x 10^{-5}	Kirk (1999)
Number cells	-	50	
Position outer boundary	cm	5	
Distance from center cell to boundary	cm	0.05	
Water flux to the root (v)	dm s^{-1}	0	
Porosity	-	0.25	
Impedance factor	-	0.3	Nowack et al. (2006)
Diffusion coefficient Zn (D_{Zn})	dm^2 s^{-1}	7 x 10^{-8}	Ptashnyk et al.(2011)
Diffusion coefficient Fe (D_{Fe})	dm^2 s^{-1}	7 x 10^{-8}	Ptashnyk et al.(2011)
Diffusion coefficient citrate (C_{Cit})	dm^2 s^{-1}	7 x 10^{-8}	Kirk (1999)
Volumetric water content (θ)		0.3	
Soil bulk density	g cm^{-3}	1.4	

The solid-solution distribution and the speciation of the relevant elements can be implemented by coupling the convective–diffusive transport equation,

$$\frac{\partial C_i}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD\frac{\partial C_i}{\partial r} + \frac{r_0 vC}{\theta}\right) \tag{2}$$

with a set of corresponding mass action equations:

$$K_i = \gamma_i C_i \prod_j \gamma_j C_j^{n_{i,j}} \tag{3}$$

where K_i is the equilibrium constant, i is the species index which concerns all species, θ (dimensionless) is the (constant) volumetric water content, γ_i is the activity coefficient, j goes across all reaction partners of species i, and $n_{i,j}$ is the stoichiometric coefficient of the components in the reaction equations (Parkhurst and Appelo 1999). The set of mass action equations (Eq. 3) consider the speciation and sorption processes of the previous section. The parameters of the model are given in Table 5.1.

5.4 Results

In a first step we focused on the effect of citrate on Zn and Fe speciation in the presence of different sorbents and considered therefore only the chemistry and not the transport part of the model. In the absence of sorbents, citrate in solution mainly formed complexes with Fe^{3+} and not with Zn^{2+} (Fig. 5.1). If we included goethite as the only sorbent, the formation of Fe[Citrate] and Zn[Citrate]$^-$ decreased significantly and Zn^{2+} became the dominant Zn species. At pH >8, however, the Zn[Citrate]$^-$ and ZnOH$^+$ concentrations were in equal magnitude of Zn^{2+}. Including humic acid (HA) as representative reactive surface of soil organic matter (SOM) decreased the Zn^{2+} concentrations, compared to the previous step, up to several orders of magnitudes with increasing pH (Fig. 5.1). In the presence of both goethite and soil organic matter as sorption sites, Zn[Citrate]$^-$ was the dominant species at pH >7. To test the influence of varying goethite and SOM contents on the metal speciation and the Zn concentration in solution, the values of these two properties were varied among the range of eight low Zn soil (0.3-12.3 g kg^{-1} for goethite and 8-28 g kg^{-1} for SOM), which were used before by Duffner et al. (2014b). These changes had, however, no influence on the general pattern shown in Fig. 5.1 and 5.2 and variation of the Zn concentration was $\pm 10\%$ (data not shown). Changing the Fe(OH)$_3$ contents, which were assumed to control the Fe^{3+} activity, influenced the Zn speciation only in the absence of sorbents (data not shown).
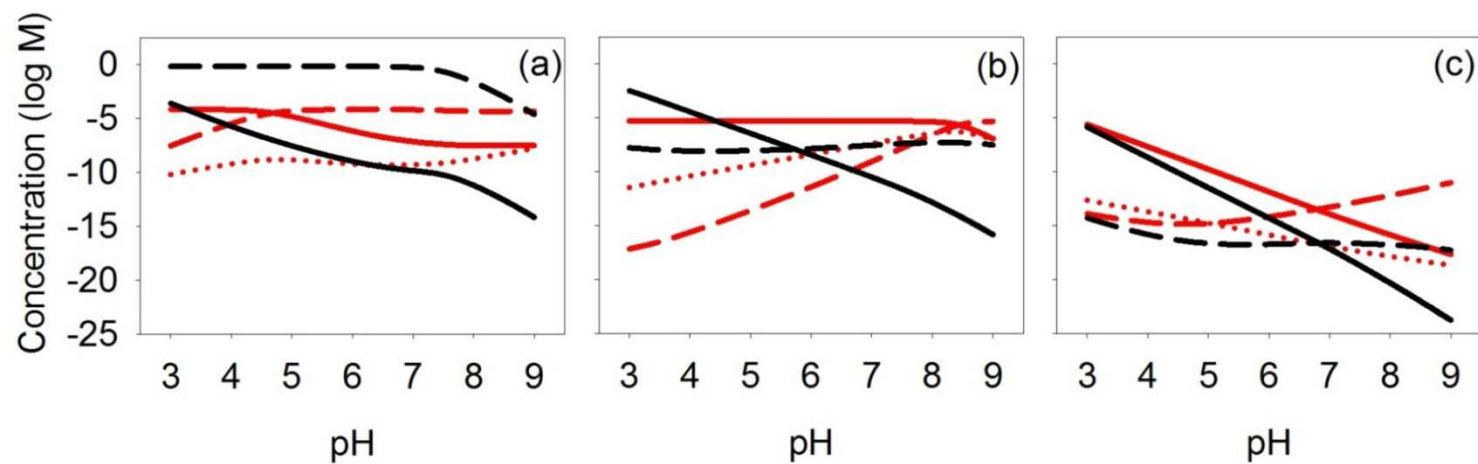
Fig. 5.1 Influence of pH (a) in absence of sorbents, (b) in the presence of goethite and (c) in the presence of goethite and soil organic matter on the concentration of Zn^{2+} (solid line, red), $ZnOH^+$ (dotted, red), $Zn[Citrate]^-$ (medium-dashed, red), Fe^{3+} (solid line, black) and $Fe[Citrate]$ (medium-dashed, black) in solution.
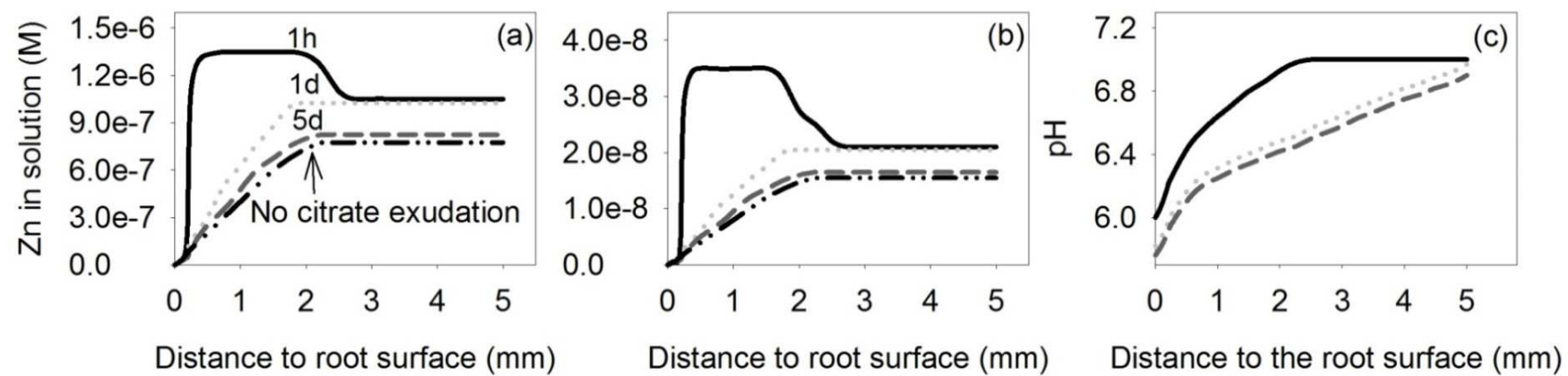
Fig. 5.2 Zinc concentration in solution in presence of (a) goethite and (b) goethite and soil organic matter 1 h, 1 d and 5 d after citrate exudation and (c) pH changes in the presence of goethite and soil organic matter 1 h after citrate exudation, respectively.

The combined speciation and transport model predicted that the effect of citrate on the Zn concentrations in the solution depended on the presence of sorbents (goethite and SOM), time after citrate exudation and the distance to the root surface (Fig. 5.2). Within the first hour after citrate exudation the Zn concentration increased within a distance to the root surface of 1.7 and 3 mm depending on the presence and absence of sorbents, respectively (Fig. 5.2 a, b). In the presence of the sorption surfaces the Zn concentrations were three orders of magnitude lower than in the absence of sorption surfaces. The effect of citrate on Zn concentrations disappeared after 1 d. The citrate exudation decreased the pH for ~1 pH unit close to the root surface (<0.5 mm) followed by a continuous increase towards the initial pH 7 (Fig. 5.2 c).

Citrate increased cumulative Zn uptake only within the first day after it was exuded (Fig. 5.3). However, considering SOM as sorption phase decreased the cumulative Zn uptake by two orders of magnitude compared with the absence of sorption sites. Including citrate degradation reduced the cumulative Zn uptake only marginally within 2 d after citrate exudation.
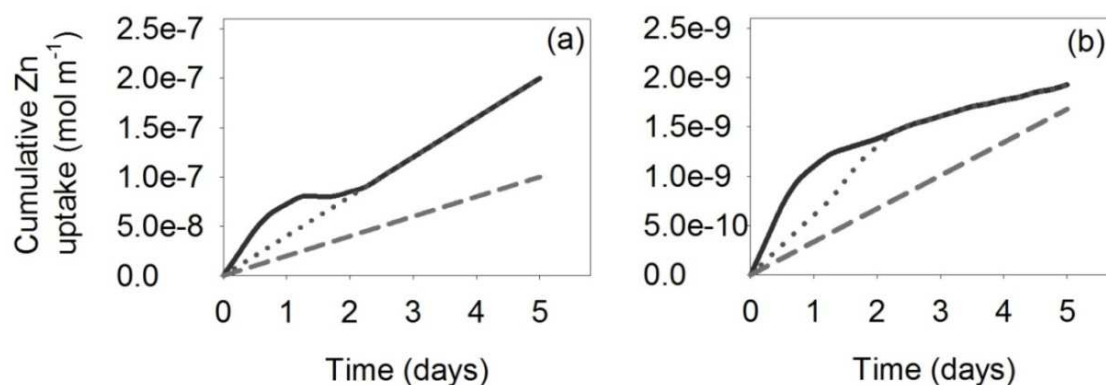

Fig. 5.3 The influence of citrate exudation with (dotted line) and without (solid line) citrate degradation on cumulative Zn uptake compared with no citrate exudation (dashed line) in the presence of (a) no sorption sites and (b) goethite and soil organic matter.
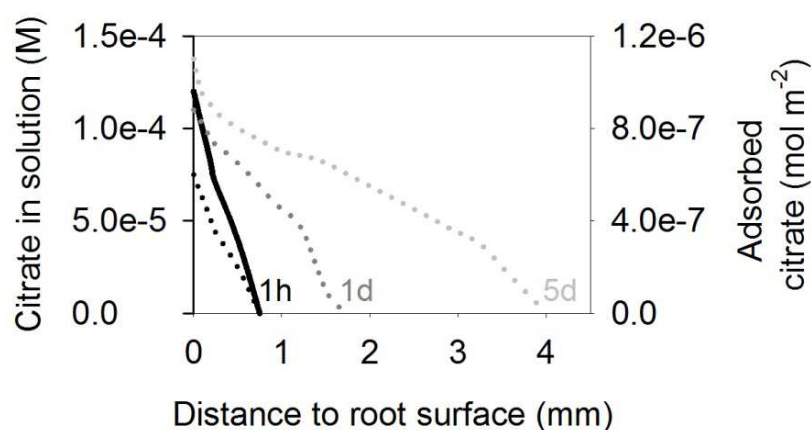
Fig. 5.4 Adsorbed citrate (dotted lines) 1 h, 1 d and 5 d and citrate in solution (solid line) 1 h after citrate exudation, respectively.
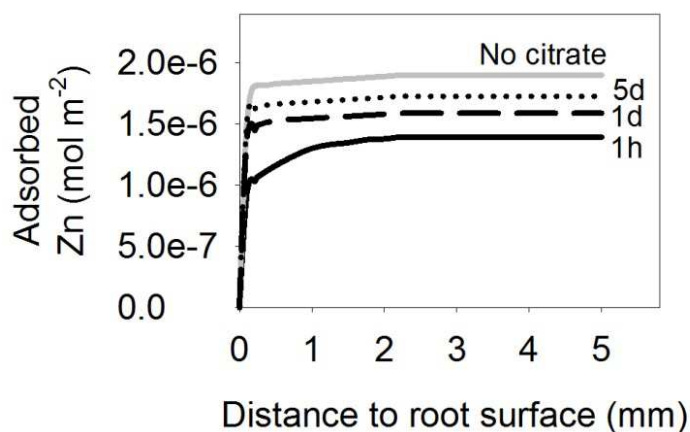
Fig. 5.5 Adsorbed Zn at different distances to the root surface 1h, 1d and 5d after citrate exudation and without citrate exudation.

A significant amount of the exuded citrate was already adsorbed to goethite close to the root surface and after a relatively short period (>1 d) (Fig. 5.4). One hour after citrate exudation the maximum distance of adsorbed citrate to the root surface was <1 mm, whereas 5 d after citrate exudation the distance increased to 4 mm. Citrate degradation had no influence on the amount of citrate which was adsorbed close to the root surface (data not shown). The citrate concentration close to the root surface (< 1mm) and ~1h after citrate exudation was about 0.1 mM (Fig. 5.4). After 1 d the predicted citrate concentrations in solution were negligible. Most

of the Zn in the system is adsorbed to SOM also close to the root surface (Fig. 5.5), whereas saturation of sorption sites did not play a role. Only within the first hours after citrate exudation and close to root surface (~1 mm) citrate decreases the Zn sorption by around 1 x 10^{-6} mol m^{-2}.

5.5 Discussion

The Zn concentration in solution was mainly controlled by Zn sorption to soil organic matter. Considering soil organic matter as sorbent reduced the Zn concentration by several orders of magnitude (Fig. 5.1-5.3), which was already shown in a former study (Duffner et al. 2014b) without considering transport and Zn uptake. Zinc sorption to SOM is only marginally affected by the root exuded citrate, because the affinity of Zn to adsorb to SOM is higher than for complexation with citrate (Fig. 5.5), even at low SOM levels (<10 g kg^{-1}, data not shown). This is also indicated by the pattern of the Zn concentrations in Fig. 5.2 b, where shortly after citrate exudation the maximum Zn concentration formed a plateau already close to the root surface (0.2 – 1.8 mm). The pH close to the root surface (<0.5 mm) was similar between 1 h - 5 d after citrate exudation, whereas the Zn concentrations between 1 h - 5 d after citrate exudation were significantly different. This indicates that the pH decrease of ~1 pH unit close to the root surface (<0.5 mm), due to concomitant H$^+$ exudation during citrate exudation, had a minor influence on increasing the Zn concentration in the soil solution.

The effect of exuded citrate for the cumulative Zn uptake was limited in time to ~1.5 days after citrate exudation. At the initially given pH 7 most of the Zn in solution was either present as Zn^{2+} or as Zn[Citrate]$^-$(Fig. 5.1). Zinc-citrate complexes could therefore have contributed to the Zn uptake for a short period after exudation by the supply of Zn^{2+} by diffusion to and dissociation of Zn-citrate complexes at the root surface, which was also suggested by Gramlich et al. (2013). They showed that un-dissociated complexes such as Zn-histidine can be taken up by roots, whereas for Zn-citrate complexes most probably complex dissociation was responsible for the increased Zn uptake. The relatively small difference between Zn uptake with and without citrate exudation indicates that citrate is not very efficient to increase Zn uptake. It is therefore arguable if citrate exudation is an effective response of plants which are tolerant to Zn deficiency like it is known at P deficiency (Zhu et al. 2005; Hoffland et al. 2006; Wang et al. 2010).

The effect of citrate on Zn speciation is limited, because citrate adsorption to goethite reduces the citrate concentration in solution already close to the root surface which was also indicated by Szegedi et al. (2010). The citrate concentrations in solution close to the root surface (~1 mM) were in the order of magnitude which were determined in experimental studies (Rose et

al. 2011; Duffner et al. 2012). A major part of the citrate (>55 %) in the solution is adsorbed to goethite within 1 d (Fig. 4). A saturation of citrate on goethite was not observed. That is in line with the experimental finding of Duffner et al. (2012) who showed that citrate sorption to goethite increased linearly with the citrate concentration in solution up to 2 mM. Only at low pH values (<4.5) the citrate sorption to the solid phase is low (Shen et al. 1996) but at that pH range Zn is anyway mainly present as free Zn ion (Zn^{2+}) (Fig. 5.1).

The presented combined multisurface- and transport modeling approach with parameters determined independently in earlier studies was useful to predict Zn speciation in the rhizosphere of low Zn soils and Zn plant uptake. Even though it was presented as case study for Zn, the parameters can also be set for other micronutrients such as Cu or Mn or other root exuded ligands. For further research this model setup may be combined with a root growth model which could consider the role of root growth velocity and the length of the exudation zone of the root in more detail.

6. General Discussion

6.1 Introduction

In 2010, an article entitled "An underground revolution" was published in *Nature* (Gewin 2010). There it was stated that *"Roots are the key for a second green revolution"*. In the previous chapters studies were presented which focused on the chemical and biological interactions at the root-soil interphase of low Zn soils to get a better insight about the relevance of rhizosphere processes for plant Zn uptake and therefore the Zn status of plants. This final chapter aims to discuss the outcome of this thesis in a more general context, based on the three objective groups presented in the introduction of this thesis, and gives implications of the results for practical management options and future research.

6.2 The role of root exuded citrate for increasing zinc bioavailability to plants

This thesis gave new insights about the effect of root exuded citrate on Zn bioavailability to plants grown on low Zn soils. In previous studies the critical citrate exudation rate or citrate concentration in the soil solution (Rose et al. 2011) which is able to increase plant Zn uptake was discussed. In this thesis it was shown that the effect of root exuded citrate for increasing plant available Zn is soil specific (Chapter 2 and Chapter 5). In addition it depends on the growth velocity of exuding root apices, time after citrate exudation and the distance to the root surface (Chapter 5) and not on a specific minimum citrate concentration in solution. In Chapter 2 it was shown that in one soil a citrate concentration of ~0.5 mM was able to increase the Zn concentration in solution, whereas in another soil a citrate concentration of ~1.5 mM did not increase the Zn uptake. Both soils had the same *aqua regia* extractable Zn concentration, whereas the ratio between *aqua regia* extractable Zn and 0.43 M HNO_3 extractable Zn was around 160 times higher in soil where citrate did not cause an increase in Zn uptake. In such soils the reactive Zn fraction (0.43 M HNO_3) is so small that even a high citrate concentration (> 10 mM), which can according to literature not be reached in the rhizosphere, is not able to increase plant available Zn. The non-reactive Zn fraction is not readily available because it is occluded in the matrix of soil constituents such as (hydr)oxides, clay minerals and SOM. The non-reactive part is assumed to be released from the soil matrix only by slow processes such as weathering (Groenenberg et al. 2010).

Observed citrate concentrations in the xylem sap (Hajiboland et al. 2005) and citrate exudation rates (Keller and Römer 2001; Ryan et al. 2001; Hoffland et al. 2006; Widodo et al. 2010; Jaitz et al. 2011) can be used as indication whether citrate exudation is a response of the plant to Zn deficiency, but cannot directly be linked to increased Zn bioavailability as it was shown in this thesis. The few available studies which measured *in situ* the Zn mobilization in

the soil solution of the rhizosphere in the presence of citrate (Dessureault-Rompré et al. 2007; Dessureault-Rompré et al. 2008) or malate (Gao et al. 2009) used only one soil in the experimental setup, respectively. Under such conditions it is not possible to assess the role of soil responsiveness to the root exuded LMWOA's. Using more soils at the same time in an experimental setup, like it was done in the thesis on hand, improved the understanding of soil-responsiveness to root exuded citrate and the effect on plant available Zn, such as the role of the ratio between *aqua regia* extractable Zn and 0.43 M HNO_3 extractable Zn (see previous paragraph).

To predict the effect of citrate on Zn bioavailability over time and space (distance to the root surface) multisurface modeling was useful (Fig 6.3). Despite the observations of this thesis, that root exuded citrate had only a limited effect on increasing Zn concentration in solution and Zn plant uptake, other exuded ligands could perhaps be more effective. Recently, Oburger et al. (2014) studied root exudation of phytosiderophores from soil-grown wheat. They sampled for the first time repeatedly and nondestructively phytosiderophore released of wheat grown in soil. They linked the phytosiderophores concentrations in the rhizosphere to Fe acquisition, but not to Zn acquisition. Further research could check if phytosiderophores or other root exuded ligands are more relevant for increasing Zn bioavailability than LMWOA`s such as citric acid. This step presumes of course that reliable parameters such as formation constants, diffusion coefficients and exudation rate of the corresponding ligand are available, which is only partly the case yet.

6.3 Opportunities of multisurface models to predict the zinc activity in low zinc soils

Multisurface models can be used to accurately predict the Zn activity also in a diverse set of soils with low Zn levels as it was shown in Chapter 4. The main advantage of using mechanistic multisurface models is the possibility to assess the relevance of processes at scales which are difficult to assess with experimental approaches. One of the main outcomes in our modeling studies (Chapter 4 and 5) was that soil organic matter was the dominant Zn sorbent and controlled the Zn activity also at low SOM levels (Fig. 6.3). This finding confirms and generalizes the work of Catlett et al. (2002) which hypothesized, based on observations made with the chelation method, that Zn activity in low Zn soils is mainly controlled by SOM.

The possibility to validate the predicted Zn activities experimentally in low Zn soils improved on the one hand the reliability of the prediction and gave new insights about the accuracy of the Donnan Membrane Technique on the other hand. Comparing the Zn^{2+} concentrations which were determined in low Zn soils with the Donnan Membrane Technique (Chapter 3)
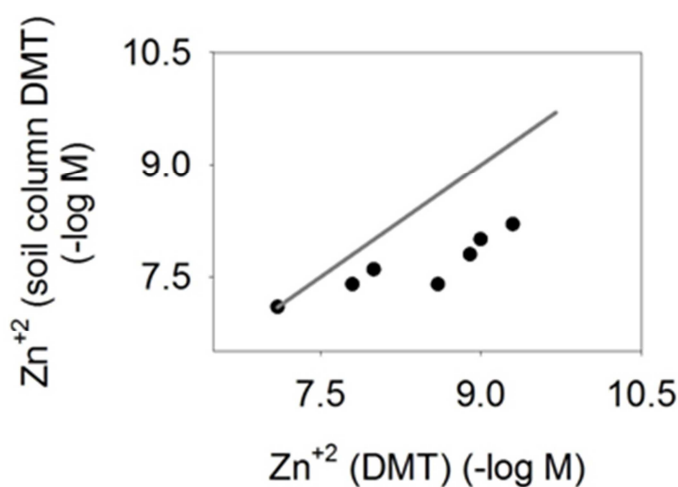
Fig. 6.1 Comparing Zn^{+2} concentrations determined with DMT and soil column DMT. The solid line represents the 1:1 line.

and the soil column Donnan Membrane Technique (Chapter 4), respectively, showed significant differences between these two approaches. The Zn^{2+} concentrations which were determined with soil column Donnan Membrane Technique were all higher (up to one order of magnitude) than with the other approach (Fig. 6.1). The reason here for is that in the soil column DMT approach the circulating solution is continuously in contact with the soil for 48 h. The Zn^{+2} concentration is therefore continuously buffered by the soil and is less influenced by the concentration and ionic strength of the extraction solution which is used as donor solution in the "normal" DMT approach. For the purposes presented in this work, the soil column DMT approach can therefore be considered as more accurate compared with the "normal" DMT approach.

The new insights which were gained with the multisurface modeling (Chapter 4 and 5) such as the dominant role of SOM as Zn sorbent or Zn precipitation can be used to assess management options. In Chapter 4 it was predicted that the application of organic matter with a common Zn content of 80 mg Zn kg^{-1} dry weight would increase bioavailable Zn. That is in line with few experiments with proper factorial designs (organic matter x applied Zn) which showed that the application of Zn fertilizer in combination with organic matter increases the agronomic efficiency of Zn fertilizer, whereas applying organic matter only decreases the agronomic efficiency of Zn due to the strong sorption of Zn to organic matter. Ahmad et al. (2012) for instance showed in a pot experiment that the recovery fraction of Zn from $ZnSO_4$ increased from 1 - 2 % till 2 - 5% when combined with compost. Similar but more modest
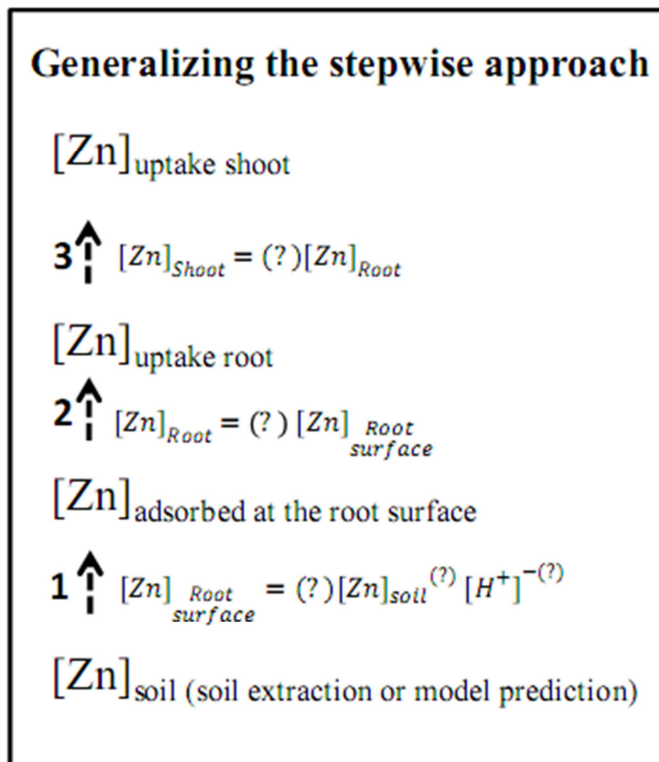
Generalizing the stepwise approach

$[Zn]_{\text{uptake shoot}}$

$3 \uparrow \quad [Zn]_{Shoot} = (?)[Zn]_{Root}$

$[Zn]_{\text{uptake root}}$

$2 \uparrow \quad [Zn]_{Root} = (?)\,[Zn]_{Root\ surface}$

$[Zn]_{\text{adsorbed at the root surface}}$

$1 \uparrow \quad [Zn]_{Root\ surface} = (?)[Zn]_{soil}^{(?)}\,[H^+]^{-(?)}$

$[Zn]_{\text{soil (soil extraction or model prediction)}}$

Fig. 6.2 Generalizing the stepwise approach. The question marks indicate the factors and exponents which need to be validated for a broader range of plants in order to generalize this approach.

results were obtained when $ZnSO_4$ was combined with cellulose in pots (Mandal et al. 2000) or biosludge in a field experiment (Srivastava et al. 2009). With the modeling approach used in this thesis it was, however, not possible to completely clarify if dissolved organic carbon (DOC) of the applied organic matter significantly increases the Zn bioavailability to crops by forming DOC-Zn complexes, because sorption of DOC to goethite was not considered. The effect of DOC varies of course also between the different crop residues, as different ligands are released in different proportions into the soil solution depending on their decomposition (Gramlich 2013).

Further developments of multisurface models should also consider the effect of more "extreme" soil properties in low Zn soils on accurate Zn activity prediction. One main objective could be soils with high $CaCO_3$ contents ($> 10\ \%$) and that $CaCO_3$ is considered as specific sorption surface. The current radial transport approach is also based on constant water content. Considering changing water contents could simulate alternate wetting and drying
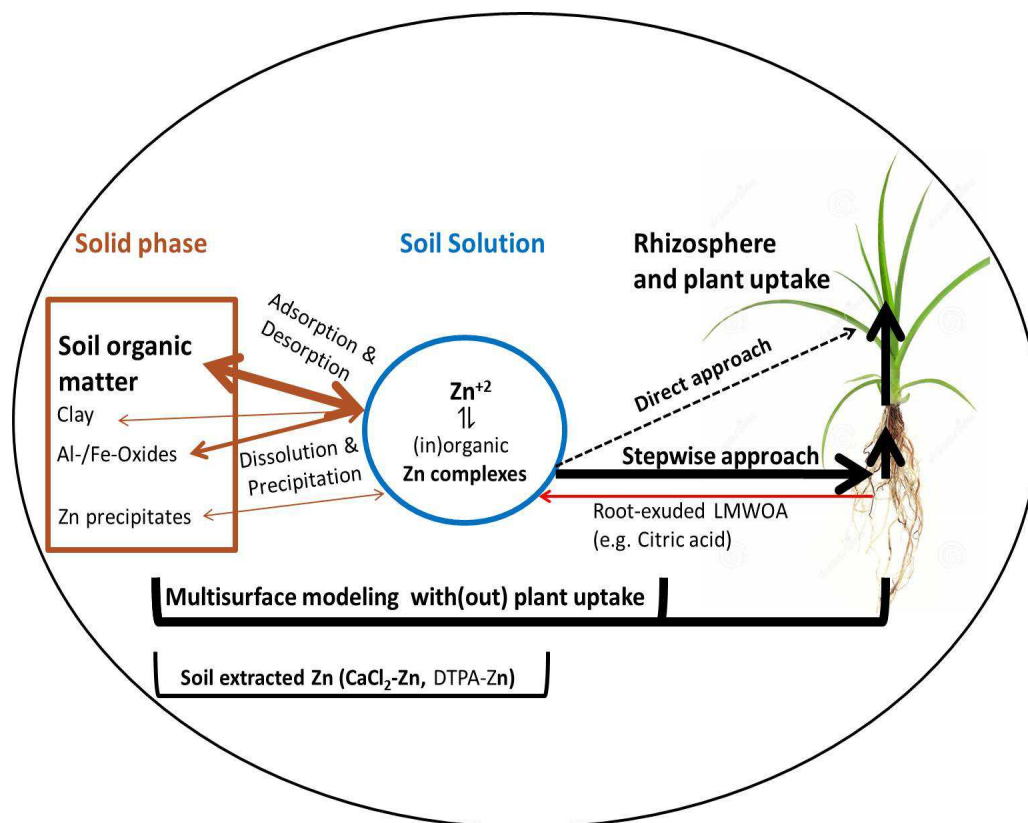
Fig. 6.3 Relevance of approaches (indicated by black arrows and brackets) and controlling processes (indicated by brown and red brackets) indicated by the size of the characters and arrows.

dynamics. The redox potential would then play an significant role in metal speciation due the formation of metal sulphide precipitates (MeS) and reduction of Fe-oxides which reduces the oxide surface available for adsorption of (in)organic anions such as citrate. Gao et al. (2010) considered redox reactions in a geochemical model (based on the work of Schröder et al. (2005) to predict the Zn bioavailability for rice, but without considering changing water contents and transport. This approach could be used as a basis to include more advanced features. In addition, soil pH will change during redox reactions, which also affects metal speciation. The challenge will be the parameterization and the link between soil organic matter mineralization and the inorganic chemistry.

6.4 Accuracy of estimating zinc plant uptake with soil extracted zinc fractions

This thesis showed, that using extracted Zn fractions to directly predict the Zn plant uptake at low Zn levels is not accurate, although it is still often used (Alvarez et al. 2006; Obrador et al. 2007; Johnson-Beebout et al. 2009; Takrattanasaran et al. 2010). One standard method which was actually developed to determine directly the bioavailable trace element fraction, such as Zn, is the extraction with DTPA. However, this method is restricted to a relatively small range of low Zn soils, when the limitations of this method are considered (O'Connor 1988).

For plant micronutrients at low concentration levels it is therefore required to apply a more general approach. Relating the soil extracted Zn concentrations to plant Zn uptake by a stepwise approach, which was initially developed by Kalis et al. (2007a) for polluted soils, can increase the accuracy of the prediction (Chapter 3) (Fig. 6.3). Besides the extracted Zn concentration ($CaCl_2$-Zn) only pH is required as additional information to predict the root surface adsorbed Zn, which is the key step in the stepwise approach. Using the predicted root surface adsorbed Zn as proxy for the bioavailable Zn has the advantage that potential effects of root exudates on the bioavailable Zn fraction are already considered. Even though root exuded citrate has a low effect on bioavailable Zn as it was discussed in the previous section (6.2), other ligands such as phytosiderophores could play a role as it was recently shown by Schenkeveld et al. (2014). They studied the metal (including Zn) mobilization from soils by phytosiderophores using experimental and modeling approaches. One of the soil they used (Xeraco) was also used in Chapter 3 and 4 of this thesis. Schenkeveld et al. (2014) showed that in the Xeraco soil complexes of Zn and deoxymugineic acid (DMA) represented between 10-30 % of the species in solution. The interaction between Zn and root exuded ligands at the root surface and the role of root surface adsorbed Zn for Zn plant uptake was, however, not yet considered in the model approaches presented in this thesis. The quantitative approach used by Plette et al. (1996) to describe competitive binding of H^+, Ca^{2+}, Cd^{2+} and Zn^{2+} to isolated cell walls of a gram-positive soil bacterium could be used as basis to implement this interactions in a rhizosphere model.

If the presented stepwise approach can be generalized and applied to a broader range of plants depends on whether the factors and exponents used in the equations (Fig. 6.2) are also accurate for broad range of plants. In Fig. 6.2, the critical factors and exponents are marked with a question mark. Applying the stepwise approach also to crops grown on low Zn soils in the field could be an opportunity to improve the accuracy of Zn bioavailability predictions under field conditions. Field studies such as from of Zou et al. (2012) and Phattarakul et al. (2012) could deliver a representative database for validating such an approach.

6.5 Implications for practical management options and future research

This thesis showed new insights, which can be implemented in management options to increase Zn plant uptake. Increased Zn concentrations in solution and plant Zn uptake can, however, only be related to increased plant biomass production, but not necessarily to increased Zn contents in the eatable parts as it was shown for wheat and rice, which are both relevant for human nutrition worldwide. Jiang et al. (2008) for instance showed that there is too little scope from a human nutrition perspective to enhance Zn mass concentration in the rice grain by simply increasing the Zn supply to crops, because Zn allocation to the rice grain is limited. Similar observations were made by Stomph et al. (2011) for wheat. They showed that at enhanced plant Zn uptake, grain Zn levels did not increase proportionally. Therefore a distinction is needed between the crop production and the related human nutrition when evaluating option to increase the Zn status of crops. This thesis focused on the chemical and biological rhizosphere interactions in low Zn soils that affect plant Zn uptake. The outcome of this thesis can therefore be used to increase the crop biomass production and therefore the production of carbohydrates and proteins for human nutrition, but cannot be directly related to human Zn intake. Options to increase human Zn intake via biofortification and food fortification of rice or the use of supplements was comprehensively reviewed by Duffner et al. (2014a).

The framework of experimental and modeling approaches which were developed and applied in this thesis are promising tools for future research to get further insights about the rhizosphere processes which control Zn bioavailability to plants and how they can be linked to plant Zn uptake. Especially the implementation of root growth models and the role of root surface adsorbed Zn in the presented multisurface- and transport modeling approach can be advised. The presented methodology is of course also appropriate to study the bioavailability of other micronutrients such as Fe, Cu or Mn. In addition also other root exudates such as phytosiderophores and specific uptake parameters of different plants could be included.

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Summary

Soil provides ecosystem services critical for life. The availability of micronutrients, such as zinc (Zn), in soils is a main factor for sustainable plant growth. Zinc is an essential micronutrient for the normal healthy growth and reproduction of plants, animals and humans. Zinc deficiency is, however, a global problem both in human populations and in crop production and it is estimated that about 50% of the cereal-cultivated soils worldwide are low in plant available Zn. It is therefore important to understand the factors and processes which control the Zn bioavailability in soils to plants, because there is a significant discrepancy between the total Zn content of soils and the bioavailable Zn fraction in soils.

The objectives of this thesis were (a) to identify the role of root exudates for increasing the Zn bioavailability in soils to plants and how this effect is changing over time and distance to the root surface, (b) to assess the accuracy of a multisurface- and radial transport model to predict the Zn activity in soils with low Zn levels and to experimentally validate such a model and (c) to improve the prediction of plant Zn uptake based on experimentally determined Zn fractions by considering interactions of Zn with the root surface.

In a first step, white lupin was used as model plant to clarify whether root exuded citrate could increase the bioavailability of Zn and phosphate (P) in calcareous soils (Chapter 2). White lupin was grown in nutrient solution and in two calcareous soils in a rhizobox. Soil solution was sampled *in situ* in the rhizosphere and the bulk soil to determine the corresponding citrate, metal and P concentrations. Based on the measured citrate concentrations, a soil extraction experiment with citrate as extractant was done to assess the mobilization capacity of citrate for Zn and P. The maximum citrate concentration ($\sim$1.5 mM) found in the rhizosphere soil solution of one soil mobilized P, but not Zn. In the other soil the highest citrate concentration ($\sim$0.5 mM) mobilized both elements. It was shown that the responsiveness of soils to root exuded ligands in terms of Zn mobilization is highly dependent on the soil characteristics.

In a next step the aim was to improve the prediction of Zn bioavailability to plants based on experimentally determined Zn fractions (Chapter 3). The classical approach that directly relates a specific Zn fraction or Zn concentration in a specific soil extract to Zn uptake has been shown to be inadequate in many cases. Therefore a stepwise approach was tested where the steps of the uptake process were characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot. Two pot experiments were done with wheat grown on nine low Zn soils varying widely in pH, clay and organic matter content. Soluble Zn concentrations in two soil extracts (DTPA

and CaCl$_2$) were measured. Free Zn ion concentrations in CaCl$_2$ soil extracts were determined with the Donnan Membrane Technique. These Zn concentrations were then related to plant Zn uptake following both the direct and the stepwise approach. In the direct approach, Zn in the DTPA extract was a better predictor for shoot Zn uptake than Zn in the CaCl$_2$ extract. In the stepwise approach, the relationship between Zn in CaCl$_2$ extracts and the root surface adsorbed Zn was pH-dependent and nonlinear. Root surface adsorbed Zn was linearly related to root Zn uptake, and the latter was linearly related to shoot Zn uptake. The stepwise approach improved the Zn uptake prediction compared to the direct approach and was also validated for different wheat cultivars. The root adsorbed Zn was shown to be a useful proxy for bioavailable Zn over a wide range of soils.

Based on the experimental insights the next aim was to test the accuracy of a multisurface model approach to predict the Zn^{2+} concentration and to improve our understanding of Zn bioavailability in low Zn soils (Chapter 4). Model parameters were determined independently based on earlier peer-reviewed publications. Model output was validated against free Zn^{2+} concentrations determined with the soil column Donnan Membrane Technique in a range of soils varying in potentially available Zn, organic matter, clay silicate and iron (hydr)oxides contents and pH. High Zn soils were included as controls. Deviations between predicted Zn^{2+} concentrations and experimentally determined values were less or equal to the experimental standard error, except for one low Zn soil. The Zn^{2+} concentration was mainly controlled by adsorption, where organic matter was predicted to be the dominant soil sorbent. Assessing management options with this modeling approach indicated that predicted Zn^{2+} concentration depends more sensitively upon changes of the reactive Zn pool (application of 0.6, 1.2, 2.4, and 3.6 mg of Zn kg^{-1} of soil) and organic matter content (± 0.2 and 0.4%) than pH changes (± 0.5 and 1 pH unit).

To assess the effect of root exuded citrate on Zn speciation in the rhizosphere over time and distance to the root surface the multisurface model approach of Chapter 4 was coupled to a radial transport model (Chapter 5). Interaction of Zn, Fe and citrate with the sorbents goethite and soil organic matter, degradation of citrate and diffusion of Zn towards and citrate away from the root surface, respectively, were considered. This comprehensive modeling approach showed that root exuded citrate increased the Zn concentration in the soil solution only close to the root surface and for a relatively short time after exudation had ceased. A major part of the citrate in the solution was adsorbed to goethite within 1 d after citrate exudation. The remaining citrate was either degraded or complexed with Zn or Fe. The effect of citrate for the cumulative Zn uptake was low and limited to the first 2 d after citrate exudation. Zinc adsorbed to soil organic matter is only marginally affected by the root exuded citrate and most of the Zn is sorbed to soil organic matter also close to the root surface.

In the last chapter (Chapter 6) the different studies are integrated and discussed in a broader context. The presented modeling and experimental approaches were useful to improve the determination of the Zn solid-solution distribution in the rhizosphere of soils with low Zn levels and the prediction of the plant available Zn fraction. The methodological framework can also be used in the future to study the availability of other micronutrients such as iron, copper and manganese to plants by including the effect of other root exuded ligands.

Samenvatting

Bodems leveren belangrijke ecosysteemdiensten. De beschikbaarheid van micronutriënten zoals zink (Zn) is een belangrijke factor voor een duurzame groei van gewassen. Zink is een essentieel micronutriënt voor een gezonde groei en voortplanting van planten, dieren en mensen. Een tekort aan zink is een wereldwijd probleem, zowel voor mensen en gewassen. Ongeveer 50 % van de met granen gecultiveerde bodems wereldwijd hebben een laag Zn gehalte. Het is daarom belangrijk om de factoren en processen te begrijpen die de biobeschikbaarheid van Zn in de bodem voor gewassen bepalen, omdat er een aanzienlijk verschil is tussen het totale Zn gehalte in de bodem en de biobeschikbare fractie Zn in de bodem.

De doelstellingen van dit proefschrift waren (a) het identificeren van de rol van wortelexudaten voor het verhogen van de Zn biobeschikbaarheid voor planten en te begrijpen hoe dit effect verandert over tijd en afstand naar de worteloppervlak, (b) het beoordelen van de nauwkeurigheid van een sorptie- en radiaal transport model voor het voorspellen van Zn concentraties in bodems met lage Zn gehalten en het experimenteel valideren van dit model en (c) het voorspellen van Zn plantopname, gebaseerd op experimenteel bepaalde Zn fracties, te verbeteren door rekening te houden met interacties van Zn en de worteloppervlak.

In een eerste stap werd witte lupine als modelplant gebruikt om te onderzoeken of citraat, afgescheiden door wortels, de biobeschikbaarheid van Zn en fosfaat (P) in kalkhoudende bodems verhoogt (hoofdstuk 2). Witte lupine werd gekweekt in een voedingsoplossing en in twee kalkhoudende bodems in een rhizobox. De bodemoplossing werd *in situ* bemonsterd in de rhizosfeer en de bulk grond om de concentraties van citraat , metalen en P te bepalen. Op basis van de gemeten citraatconcentraties werd een bodemextractie experiment met citraat als extractiemiddel uitgevoerd om de mobilisatiecapaciteit van citraat voor Zn en P te beoordelen. De maximale citraatconcentratie (~1,5 mM) die werd gemeten in de rhizosfeer van een bodem mobiliseerde wel P, maar niet Zn. In de andere bodem mobiliseerde hoogste citraat concentratie (-0,5 mM) beide elementen (P en Zn). Hiermee werd aangetoond dat de gevoeligheid van de bodem voor door wortel afgescheiden liganden qua Zn mobilisatie sterk afhankelijk is van de bodemeigenschappen.

In een volgende stap was het doel om de voorspelling van Zn biobeschikbaarheid voor planten te verbeteren op basis van experimenteel bepaalde Zn fracties (hoofdstuk 3). In veel gevallen is de klassieke aanpak om Zn gehalten in de bodem te relateren aan Zn opname in de plant niet nauwkeurig. Daarom werd een geleidelijk aanpak getest waarbij de stappen van het opnameproces werden gekarakteriseerd door: Zink distributie tussen vaste oplossing,

adsorptie van Zn aan het worteloppervlak, Zn wortel opname en Zn translocatie in de plant. Twee pot-experimenten werden gedaan, waarin tarwe werd geteeld op negen bodems met lage Zn gehaltes met een verschillende pH waarde, kleigehalte en organische stofgehalte. Zink concentraties in twee bodemextracten gemeten door middel van DTPA en $CaCl_2$. De vrije Zn ion concentraties in de bodem, via $CaCl_2$ extractie werden bepaald met de Donnan Membrane Techniek. Deze Zn concentraties werden vervolgens gerelateerd aan Zn opname van planten met zowel de directe als ook de geleidelijke aanpak. In de directe aanpak was de Zn concentratie in het DTPA extract een betere voorspeller voor Zn plant opname dan de Zn concentratie in het $CaCl_2$ extract. In de geleidelijk aanpak is de relatie tussen Zn in $CaCl_2$ extracten en het worteloppervlak geadsorbeerde Zn niet lineair en afhankelijk van pH. Het door de worteloppervlak geadsorbeerd Zn werd lineair gerelateerd aan Zn opname van de wortel, en de laatste werd lineair gerelateerd aan Zn opname van de plant. De geleidelijk aanpak verbeterde de voorspelling van de Zn opname vergeleken met de directe aanpak en werd ook gevalideerd voor verschillende tarwerassen. Door de wortel geadsorbeerde Zn bleek een bruikbare indicator voor biobeschikbare Zn over een breed scala van bodems te zijn.

Op basis van de experimentele inzichten was het volgende doel de nauwkeurigheid van een sorptie model voor het voorspellen van Zn^{2+} concentratie te testen en het begrip van Zn biobeschikbaarheid in bodems met lage Zn gehaltes te verbeteren (hoofdstuk 4). Modelparameters werden onafhankelijk bepaald op basis van eerdere peer-reviewed publicaties. Modelvoorspellingen werden gevalideerd tegen de vrije Zn^{2+} concentratie die met de bodem kolom Donnan Membraan Technique in verschillende bodems met variërend potentieel beschikbaar Zn, gehalte van organische stof, klei en ijzer(hydr)oxiden en pH bepaald werden. Bodems met hoge Zn gehaltes werden opgenomen als controle. Afwijkingen tussen de voorspelde Zn^{2+} concentraties en experimenteel bepaalde waarden waren kleiner of gelijk aan de experimentele standaardafwijking, met uitzondering van een bodem met een laag Zn gehalte. De Zn^{2+} concentratie werd hoofdzakelijk gecontroleerd door adsorptie, waarbij organisch materiaal de dominante sorbens was. Het beoordelen van management opties met deze model aanpak geeft aan dat de voorspelde Zn^{2+} concentraties mer gevoeliger zijn voor veranderingen van de reactieve Zn pool (applicatie van 0,6; 1,2; 2,4 en 3,6 mg Zn kg^{-1} bodem) en het gehalte aan organische stof (± 0,2 en 0,4 %) dan voor pH veranderingen (± 0,5 en 1 pH eenheid).

Om het effect van wortel uitgescheiden citraat op Zn speciatie in de rhizosfeer te beoordelen over tijd en afstand tot de wortel oppervlak werd een sorptie model gekoppeld aan een radiaal transport model (hoofdstuk 5). Interactie van Zn, ijzer (Fe) en citraat met de sorbenten goethiet en organische stof, afbraak van citraat en diffusie van Zn naar en citraat weg van het worteloppervlak werden daarin meegenomen. Deze uitgebreide modelleringsaanpak toonde aan dat het door wortel afgescheiden citraat de Zn concentratie in de bodemoplossing

verhoogde, maar alleen dicht bij het worteloppervlak en alleen voor een relatief korte tijd nadat de exudatie had opgehouden. Een groot deel van het citraat in de oplossing werd geadsorbeerd aan goethiet binnen 1 dag na de citraat exudatie. Het resterende citraat was of afgebroken of gecomplexeerd met Zn en Fe. Het effect van citraat voor de cumulatieve Zn opname was laag en beperkt tot de eerste 2 dagen na de citraat-exudatie. De door organische stof geadsorbeerde Zn was slechts marginaal beïnvloed door het citraat en het grootste deel van de Zn werd ook geadsorbeerd aan organische stof dicht bij de wortel oppervlak.

In het laatste hoofdstuk (hoofdstuk 6) zijn de verschillende studies geïntegreerd en bediscussieerd in een bredere context. De gepresenteerde experimenten en modellen zijn bruikbaar om de distributie tussen Zn in de vaste bodem fase en Zn in de oplossing van bodems te bepalen en de voorspelling van plant beschikbare Zn in bodems met een laag Zn gehalte te verbeteren. Dit methodologisch raamwerk kan ook in toekomstige studies gebruikt worden om de beschikbaarheid van andere micronutriënten zoals ijzer, koper en mangaan te bepalen inclusief het effect door andere van wortel afgescheiden liganden.

Zusammenfassung

Böden liefern vielfältige und wichtige Ökosystemfunktionen. Die Verfügbarkeit von Mikronährstoffen, wie zum Beispiel Zink, im Boden ist ein wichtiger Faktor für den Pflanzenwachstum. Zink ist ein essentieller Mikronährstoff für das Wachstum und die Reproduktion von Planzen, Tieren und Menschen. Zinkmangel ist ein weltweites Problem für die Pflanzenproduktion, sowie für die menschliche Ernährung. Schätzungsweise 50% der Böden, die für die weltweite Getreideproduktion benutzt werden, weisen geringe planzenverfügbare Zinkgehalte auf. Es ist darum wichtig die Faktoren und Prozesse zu verstehen, welche die Bioverfügbarkeit von Zink im Boden für den Pfanzenwachstum kontrollieren, weil in vielen Böden ein erheblicher Unterschied zwischen dem bioverfügbaren und dem totalen Zinkgehalt besteht.

Die Ziele dieser Arbeit waren (a) den Effekt von Wurzelexudaten auf die Zinkbioverfügbarkeit von Pflanzen zu bestimmen und wie sich dieser Effekt über die Zeit und Abstand zur Wurzeloberfläche verändert, (b) die Genauigkeit von Sorptions- und Transportmodellen zu bestimmen mit welcher diese die Zinkonzentration in Böden mit niedrigen Zinkgehalten vorhersagen können, sowie das Validieren dieser Modelle mit Experimenten und (c) die Vorhersage der Zink-Pflanzenaufnahme auf Basis von experimentell bestimmten Zinkfraktionen zu verbessern durch die Berücksichtigung von Interaktion zwischen Zink und der Wurzeloberfläche.

In einem ersten Schritt wurde Weiße Lupine als "Modell-Pflanze" verwendet um zu verdeutlichen ob von den Wurzeln ausgeschiedenes Citrat die Bioverfügbarkeit von Zink und Phosphat in kalkigen Böden erhöhen kann (Kapitel 2). Die Pflanzen wurde in Nährlösung und in "Rhizoboxen" mit zwei verschiedenen kalkhaltigen Böden kultiviert. Das Porenwasser in der Rhizosphäre und im Boden wurden *in situ* beprobt, um darin die Konzentration von Citrat, Metalen und Phosphat zu bestimmen. Auf Basis der gemessenen Citrat Konzentrationen wurde ein Bodenextraktions-Experiment durchgeführt um die Mobilisationkapazität von Citrat zu bestimmen. Die maximale Citratkonzentration ($\sim$1.5 mM), die in der Rhizosphäre in einem der Böden gemessen wurde mobilisierte nur Phosphat, aber kein Zink. In dem anderen Boden mobilisierte jedoch die maximale Citratkonzentration ($\sim$0.5 mM) beide Elemente. Es konnte daher gezeigt werden, dass die Reaktionsfreudigkeit von Böden gegenüber wurzelausgeschiedenen Liganden in Bezug auf die Mobilisierung von Zink sehr unterschiedlich und stark abhängig von den Bodeneigenschaften ist.

Das nächste Ziel war die Vorhersage der Zinkbioverfügbarkeit auf Basis von experimentell bestimmten Zinkfraktionen zu verbessern (Kapitel 3). Der klassische Ansatz bei dem eine

Zinkfraktion direkt mit der Zink-Pflanzenaufnahme in Verbindung gesetzt wird ist in vielen Fällen ungenau. Daher wurde ein schrittweiser Ansatz getestet, bei dem die Schritte aufgeteilt wurden in: Zinkverteilung zwischen fester Phase und der Lösungsphase, Adsorption von Zink an die Wurzeloberfläche, Zink-Aufnahme in die Wurzel und der weitere Zinktransport in den Spross. Zwei Topfexperimente wurden durchgeführt in denen Weizen auf neun verschieden Böden mit niedrigen Zinkkonzentration kultiviert wurden und die eine grosse Bandbreite an pH, Ton- und organischer Bodensubstanzgehalten aufwiesen. Die löslichen Zinkfraktionen wurden mit zwei verschiedenen Bodenextraktionsverfahren (DTPA und $CaCl_2$) bestimmt. Die freie Zink-Ionenkonzentration in $CaCl_2$ Extraken wurde mit der Donnan Membran Technik bestimmt. Diese Zinkkonzentrationen wurden dann mit dem direkten und dem schrittweisen Ansatz an die Zink-Pflanzenaufnahme relatiert. Im direkten Ansatz war DTPA-Zink genauer für die Vorhersage des Zink-Pflanzenaufnahme als mit dem $CaCl_2$-Zink. In dem schrittweisen Ansatz waren $CaCl_2$-Zink pH abhängig und nichtlinear an das von der Wurzeloberfläche adsorbierte Zink relatiert. Das von der Wurzeloberfläche adsorbierte Zink war dann linear an das an die Zink-Wurzelaufnahme relatiert, welche wiederum linear an die Zink-Aufnahme vom Spross relatiert war. Der schrittweise Ansatz verbesserte, im Vergleich zum direkten Ansatz, die Vorhesage der Zink-Pflanzenaufnahme und wurde auch für verschiedene Weizensorten validiert. Es konnte bei diversen Böden mit niedrigen Zinkgehalten gezeigt werden, dass das von der Wurzeloberfläche adsorbierte Zink ein guter Näherungswert für das bioverfügbare Zink ist.

Auf Basis der experimentellen Ergebnise wurde als nächstes die Genauigkeit von einem Sorptions-Modell zur Vorhersage der Zinkkonzentration bestimmt um das Verständnis der Zinkbioverfügbarkeit in Böden mit niedrigen Zinkgehalten zu verbessern (Kapitel 4). Die Modellparameter wurden vorhergehenden Puplikationen entnommen. Die Modellvorhersagen wurden validiert mit der freien Zink-Ionenkonzentrationen, die mit der Bodensäulen-Donnan Membran Technik in verschiedesten Böden mit niedrigen Zinkgehalten bestimmt wurden. Böden mit hohen Zinkgehaltem wurden als Kontrolle dazugenommen. Mit Ausnahme von einem Boden, waren die Abweichungen zwischen den vorhergesagten Zinkkonzentrationen und den experimentell bestimmten Zinkkonzentrationen alle innerhalb eines experimentellen Standardfehlers. Die freie Zink-Ionenkonzentration war hauptsächlich kontolliert durch Sorption, wobei die organische Bodensubstanz der dominante Sorbent war. Die Beurteilung von Bodenmanagementoptionen mit dem Modell zeigte, dass die freie Zink-Ionenkonzentration sensibler auf Änderungen des reaktiven Zinkpools (Zugabe von 0.6, 1.2, 2.4, and 3.6 mg Zn kg^{-1} Boden) und der organischen Bodensubstanz (±0.2 and 0.4%) reagierte als auf pH Veränderungen (±0.5 and 1 pH Einheit).

Um den Effekt von wurzelausgeschiedenem Citrat auf die Zinkspezierung in der Rhizosphäre in Abhängigkeit von Zeit und Abstand zur Wurzeloberfläche zu bestimmen wurde ein

Sorptionsmodell mit einem radialen Transportmodell kombiniert (Kapitel 5). Dabei wurden die Interaktionen von Zink, Eisen und Citrat mit den Sorbenten Goethit und organischer Bodensubstanz, der mikrobielle Abbau von Citrat und die Diffusion von Zink zur Wurzel hin und Citrat von der Wurzel weg berücksichtigt. Dieser umfangreiche Modellansatz zeigte, dass das von Wurzeln ausgeschiedene Citrat die Zinkkonzentration in der Bodenlösung nur sehr nahe der Wurzeloberfläche und für einen relativ kurzen Zeitraum erhöht. Ein Großteil des Citrats in der Bodenlösung war innerhalb eines Tages nach der Ausscheidung an Goethit adsorbiert. Das übrige Citrat wurde entweder mikrobiell abgebaut oder formte Komplexe mit Eisen und Zink. Der Effekt von Citrat für die kumulative Zinkaufnahme von Pflanzen war niedrig und limitiert auf die ersten zwei Tage nach der Citratausscheidung. Die Zinkadsorption an die organische Bodensubstanz war nur geringfügig beeinflusst durch das wurzelausgeschiedene Citrat und ein Grossteil des Zinks war daher auch schon nahe der Wurzeloberfläche an die organische Bodensubstanz gebunden.

Im letzten Kapitel (Kapitel 6) wurden die verschiedenen Versuche und Studien zusammengefügt und in eimem grösseren Zusammenhang diskutiert. Die vorgestellten Modellansätze und Experimente waren nützlich um die Spezierung von Zink in der Rhizospähre von Böden mit niedrigen Zinkgehalten zu bestimmen und die pflanzenverfügbare Zinkfraktion vorherzusagen. In zukünfigen Forschungsprojekten kann die hier angewandte Methodik auch benutzt werden, um die Pflanzenverfügbarkeit von anderen Metallen und Mikronährstoffen, wie zum Besipiel Eisen, Kupfer oder Mangan, unter der Berücksichtigung von anderen wurzelausgeschiedenen Liganden zu bestimmen.

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About the Author

Andreas Duffner was born on 18 December 1983 in Furtwangen im Schwarzwald, Germany. He obtained his Bachelor degree in Agricultural Sciences from Kassel University in 2008. He conducted his Bachelor thesis under supervision of Prof. Dr. Heinz Flessa at the Department of Soil Science of Temperate Ecosystems at Göttingen University about the effect of fertilization history on short-term emission of CO_2 and N_2O after the application of different nitrogen fertilizers. Afterwards he started his Master studies at Hohenheim University. During his Master studies he did an exchange semester (ERASMUS) at the Department of Environmental Sciences at Wageningen University. His master thesis was conducted at the Department of Biogeophysics at the Institute of Soil Science and Land Evaluation at Hohenheim University under supervision of Prof. Dr. Thilo Streck and Dr. Joachim Ingwersen. The topic was the identification of pesticide transport pathways from a sloped litchi orchard to an adjacent stream by hydrograph separation. In 2010, he graduated with the MSc. degree in Soil Science. Afterwards he started his PhD research at the Department of Soil Quality at the Wageningen University.

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o   Assisting practicals of the BSc course Soil-Plant Relations

Oral Presentations
o   *Predicting zinc bioavailability to wheat improves by considering adsorption to root surfaces.* 17th International Plant Nutrition Colloquium (IPNC), 19-22 August 2013, Istanbul, Turkey
o   *Bioavailability of trace metals in the rhizosphere of calcareous soils.* Workshop 'Mathematics and Rhizotechnologies: Rhizosphere processes on multiple scales', 23-25 March 2011, Vienna, Austria

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