# Root induced soil deformation influences Fe, S and P: rhizosphere chemistry investigated using synchrotron XRF and XAS

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## Abstract

Rhizosphere soil has distinct physical and chemical properties from bulk soil. However, besides root induced physical changes, chemical changes have not been extensively measured *in situ* on the pore scale.

In this study we couple structural information, previously obtained using synchrotron X-ray computed tomography (XCT), with synchrotron X-ray Fluorescence (SR-XRF) microscopy and X-ray Absorption Near-Edge Structure (XANES) to unravel chemical changes induced by plant roots.

Our results suggest that iron (Fe) and sulfur (S) increase notably in the direct vicinity of the root via solubilization and microbial activity. XANES further shows that Fe is slightly reduced, S is increasingly transformed into sulfate (SO42-) and that P is increasable adsorbed to humic substances in this enrichment zone. In addition, the ferrihydrite fraction decreases drastically suggesting the preferential dissolution and the formation of more stable Fe-oxides. Additionally, the increased transformation of organic S to sulfate indicates that the microbial activity in this zone is increased. These changes in soil chemistry correspond to the soil compaction zone as previously measured via X-ray CT.

The fact that these changes are co-located near the root and the compaction zone suggests that decreased permeability due to soil structural changes acts as a barrier creating a zone with increased rhizosphere chemical interactions via surface mediated processes, microbial activity and acidification.

Keywords: Fe, S, P, rhizosphere chemistry, synchrotron, X-ray Fluorescence, XANES, sulfate, phosphate

## Introduction

Soil resources are often inaccessible to plants even when they are abundant in soil because they are tightly bound to soil minerals and organic matter (Hinsinger, 2001). Roots have evolved strategies to ‘mine’ poorly accessible nutrients by adjusting their environment (Lambers *et al.*, 2008). As such, plants have the ability to “engineer” the direct soil environment the roots are exposed to; this zone is called the rhizosphere. The rhizosphere is a unique environment as it differs from the bulk soil (bio)chemically and physically (Hinsinger *et al.*, 2005; Koebernick *et al.*, 2018). Roots exude a wide variety of organic compounds, *e.g.* proteins, polysaccharides and organic acids (Watanabe *et al.*, 2008; Carminati *et al.*, 2010; van Veelen *et al.*, 2018), which make up the bulk of the rhizo-deposits (Jones *et al.*, 2004). It is thought that the role of these deposits is to improve contact between plant roots and mineral phases, nutrient mobilisation, immobilisation of toxic metals and they can act, crucially, as a carbon source for soil microorganisms (Hinsinger *et al.*, 2005; Bais *et al.*, 2006). In addition, plant roots alter the pore geometry of the adjacent soil by exerting combined hydraulic and mechanical stresses (Koebernick *et al.*, 2017; Koebernick *et al.*, 2018).

 Growing roots significantly augment the local soil structure in a manner that is beneficial to them and thus they can alter the soil bulk density, porosity and soil structure adjacent to them. Recent work has shown soil porosity variations with distance from the root surface, with an increased porosity directly adjacent to the root surface (Hinsinger *et al.*, 2005; Helliwell *et al.*, 2017; Koebernick *et al.*, 2018). Results from Koebernick *et al.* (2018) showed that there was an increase in smaller pores at the root surface accompanied by an increase in the total pore volume fraction. The resulting structural changes suggested an increased hydraulic conductivity in the rhizosphere, which would have profound implications for water and solute uptake by roots (Koebernick *et al.*, 2018). Neutron radiography conducted by Carminati *et al.* (2010) confirmed that water content in the rhizosphere soil was indeed higher than the bulk soil during a drying period due to active transpiration. However, after irrigation, this area remained strikingly drier than the bulk soil. These observed changes in wettability were explained by the presence of root exudates and mucilage in particular. Root mucilage is high molecular weight material which consists predominantly of polysaccharides, organic acids, amino acids, fatty acids and alcohols. As such, changes in the physical properties affecting soil structure reveal the major role of polysaccharides, which also changes the physical properties of soil solution. The sugar, amino acids and glycosidic linkages are known for high water-binding and gel-forming properties and achieve a water retention up to 589 times it’s weight (Fincher *et al.*, 1983; Capitani *et al.*, 2013). It has been estimated that approximately 20 – 25% of the total reduced carbon released by maize roots is in the form of high molecular weight root mucilage, which suggests that this is a substantial carbon source for soil microbes (Chaboud, 1983). The ability to utilize root mucilage and organic acids as a carbon source may be an important factor in the competitive colonization of roots by beneficial and pathogenic rhizosphere microbes (Knee *et al.*, 2001; Bertin *et al.*, 2003; Naveed *et al.*, 2017a; Naveed *et al.*, 2017b).

 Iron (Fe) is typically abundant in soil, but exists primarily in the insoluble ferric oxide form (Mendes *et al.*, 2013). Fe is an essential element for almost any living microorganism as it acts as a catalyst in enzymatic processes, oxygen metabolism, electron transfer and DNA and RNA syntheses (Ahmed & Holmström, 2014; Colombo *et al.*, 2014). However, its speciation in soil is still poorly understood. In order to regulate the poorly bioavailable Fe, plants exude phyto-siderophores and/or organic acids as to increase its solubility. Siderophores are low molecular weight molecules which are produced by plants and microbes to chelate ferric iron. For example, barley has been shown to increase the root exudation of organic acids and siderophores threefold during mild stress (Yang & Crowley, 2000). As a result, this caused possibly an enrichment of iron(hydr)oxide in the vicinity of the roots under oxic conditions. In addition, iron (hydr)oxides have the ability to facilitate the fixation and protection of microbes within microaggregates (Kögel-Knabner *et al.*, 2008). In a study by Vidal *et al.* (2018) most of the microbial cells were found within microaggregates rich in Fe-oxides. However, Keiluweit *et al.* (2015) proposed that increased exudation of oxalate by plants may facilitate the dissolution of iron (hydr)oxides, which in turn facilitates the release and loss of organo-mineral associated organic matter (OM) in the rhizosphere. This would also facilitate the release of the critical phosphorus, which is usually bound to Fe (and other cations such as Ca and Al) (Hinsinger, 2001). When looking at the Fe speciation, Prietzel, J. *et al.* (2007) detected several species of iron oxides depending on their location within aggregates. The iron oxides are identified as binding agent between the root-soil interface by forming bridges between primary soil particles (*e.g.* quartz) and as building blocks of microaggregates in the rhizosphere (Mueller *et al.*, 2017). Therefore, iron oxides are key components of the rhizosphere acting as binding agent as well as a potential source of iron for plants and microorganisms. As such, identifying these species is important in correlating rhizosphere microbial and physical functions with regards to nutrient bioavailability.

The increase in rhizosphere microbial biomass that results from C input by roots also plays an important role in the sulfur (S) cycle. Sulfur is an important element for the synthesis of proteins and a number of essential vitamins and cofactors, and plays a critical role in cells (Leustek & Saito, 1999). Although S is usually high in soils, directly available S (sulfate; SO4) is usually less than 5% as the major part of the S pool resides in soil organic matter and the microbial biomass (Kertesz & Mirleau, 2004). Importantly, the S pool in soils is extremely dynamic and complex, especially in the rhizosphere where microbes play a critical role in allowing plants to access soil organosulfur (Kertesz, 2000; Kertesz & Mirleau, 2004). As a result, S exists in a large variety of oxidation states ranging from -2 (inorganic sulfide) to +6 (inorganic sulfate) (Prietzel *et al.*, 2003). Remarkably, there is evidence that soil microbes actively compete with plants for the available sulfate in rhizosphere (Kertesz & Mirleau, 2004). Many bacteria and fungi are capable of mineralizing S from sulfate-esters (Klose *et al.*, 1999). The rates in which mineralization of organosulfur in soils takes place is dependent on the proportions of sulfate-esters and C-bonded sulfur in a given soil and by the type of crop. These effects are due to the differences in the quantities and chemical composition of root exudates, which as a result alters the microbial community (Bertin *et al.*, 2003). Sulfatases of the esterase class catalyze the sulfate-ester mineralization. Arylsulfatases enzymes act on aromatic sulfate-esters, while alkyl-sulfatase act on aliphatic sulfate-esters, both sulfate releasing enzymes/products are common in the rhizosphere. The largest pool of organic S is in the form of aliphatic or aromatic sulfonates (Zhao *et al.*, 2006). However, in order to mobilize sulfonate, an exclusive bacterial multicomponent enzyme known as mono-oxygenase complex is necessary (Kertesz & Mirleau, 2004). The limited abundance of plant available S makes it increasingly necessary to understand the pathways and interactions that mobilize sulfate-esters and sulfonates, which dominate the S-pool (Gahan & Schmalenberger, 2014). Therefore, the complex nature and the subtle differences in the rhizosphere makes it difficult to apply traditional wet-chemistry speciation, which is prone to artefacts and biases (Prietzel *et al.*, 2003; Prietzel, Jörg *et al.*, 2007). The use of X-ray absorption near-edge structure (XANES) in combination with synchrotron X-ray Fluorescence (SR-XRF) mapping have been proven to be a powerful non-destructive technique for direct speciation of various elements in biological and geological samples (Egerton *et al.*, 2015; Edwards *et al.*, 2016).

Spatial XRF investigations in combination with XANES spectroscopy into the composition, distribution and dynamics of plant nutrients is a new and exciting field of research with much potential. More recently, the focus has fallen upon nutrient speciation and distribution in bulk soils and the rhizosphere. This is an important development in understanding nutrient dynamics and bioavailability *in situ*. Singer *et al.* (2009) combines SR-XRF and XRD to study the distribution of U on mineral phases, and while this might be a more definitive analyses, it needs specific sample preparation, beamlines and energy ranges. Even though bulk soil chemical processes are well understood and investigated, the spatio-chemical relationships between plant and soil are poorly studied. Indeed, there are SR-XRF and XANES studies of Fe (Prietzel, J. *et al.*, 2007; Vidal *et al.*, 2018), S (Prietzel *et al.*, 2003) and P (Lombi *et al.*, 2006; Eriksson *et al.*, 2016) speciation in soils and aggregates, but these are generally limited to bulk samples, single aggregates or relatively small areas. In order to understand nutrient dynamics at the interface between roots and soil, it is necessary to identify the different species in the roots, rhizosphere and bulk soil. This is important as to improve the efficiency of fertilizer application and formulation of new fertilizer products. Therefore, in this study we will i) image rhizosphere elemental composition and its relative changes using SR-XRF, ii) identify the speciation of Fe, S and P within the rhizosphere and bulk (non-rhizosphere) soil using XANES, and iii) link this to soil physical parameters presented in Koebernick *et al.* (2018). In addition to the spectroscopy, traditional soil analytical experiments were conducted to support the spatial imaging results.

## Experimental

### Sample preparation

#### Chemical mapping

Chemical mapping was conducted on previously X-ray Computed Tomography (XCT) scanned samples from a study that focused on rhizosphere microstructural changes; see Koebernick *et al.* (2018). The soil analyzed in this study is an Ap horizon of a sandy loam textured Cambisol collected from the South Bullionfield at the James Hutton Institute, Dundee, UK. The soil was air dried and passed through a sieve with an aperture of 250 µm. A controlled water potential was maintained by using 25 cm long strings of carbon fiber wick (ø = 1mm) tied to a knot on one end and passed through a 1 mL syringe barrel (ø = 4.2 mm) such that the knotted end did not slip through the nozzle of the syringe. Approximately 1 mm of fine sand was layered onto the knot to ensure full contact. Next, the soil was poured into the syringe barrel and consolidated to a bulk density (ρd) of 1.31 ± 0.03 g cm-3. The individual syringe barrels were clustered in groups of seven and connected to a 3D printed seedling assembly as described in Koebernick *et al.* (2017).

Barley plants grew in the seed compartment of the seedling assembly that was filled with fine sand. Water was constantly resupplied into the syringe barrels by placing the wick ends into open water reservoirs at a distance of 20 cm resulting in a head of -2 kPa at the bottom of the syringes. From the original dataset, three contrasting treatments of barley were chosen: i) a root hair bearing wild-type (WT) of barley (*Hordeum vulgare* L. cv. Optic), ii) a mutant with reduced root hair proliferation (NRH) and iii) an unplanted control. Plant material was obtained from the barley mutant population at The James Hutton Institute (Caldwell *et al.*, 2004) and described previously by Brown *et al.* (2012). After the XCT analyses, the syringe barrels were snap frozen using liquid nitrogen and stored at -80 °C.

Prior to thin sectioning, five frozen syringe barrels were first dipped in liquid nitrogen and subsequently freeze dried for 24 hours. The syringe barrels were then placed in a custom-made syringe holder and perfused with Epo-Tek 301 epoxy resin and left to cure for 48 hours. To ensure full perfusion, the epoxy resin was 10% diluted with ethanol. After 48 hours, the cured barrels were cut at two heights: 20 mm and 40 mm below the top of the syringe barrel. To ensure a flat and clean surface, the cut samples were ground and polished with SiC and diamond paste respectively. Finally, the surfaces were wiped clean with ethanol.

#### Bulk samples

In order to study the effect of barley on the soil, two sets of bulk samples were prepared in 50 mL Falcon tubes for destructive analyses: one containing barley and one without plants. In this enlarged setup, soil parameters and growth conditions were kept similar to the above described imaging experiment, except that the growing time was 30 days as to ensure detectable changes. As a result, the compartments containing barley were full of roots creating a homogenous “rhizospheric-soil” in the tube. The compartment without plants was treated as bulk soil.

### Analytical chemistry

Prior to the analytical methods, the soil samples were sieved to remove barley roots and subsequently dried in an oven at 70 °C for 48 h (±2 h). The mineral fractions were determined via sieving and wet separation (see SI “Clay fraction separation from soil”). Amorphous Fe and Al were determined by extraction of 1 g soil in 0.2 M oxalate (H2C2O4) solution in a soil with solution ratio of 1:10 for two hours in the dark. Bioavailable/labile macronutrients Fe, Al, Ca and P were measured by extracting 1 g of soil in a 0.01 M EDTA + 1 M NH4OAc solution in a soil with a solution ratio of 1:10 for one hour. Effective cation exchange capacity (ECEC) of exchangeable base cations Na, Ca, K, Mn, Mg and Al were measured by extracting 1 g of soil in 1 M NH4Cl in a soil with a solution ratio of 1:5 for 4 hours. Finally, the pH and soil water extractable Fe, Al, Ca and P were analysed by suspending 2g of soil in MilliQ H2O with a soil:solution ratio of 1:2 for 12 hours. The pH was determined by a combined electrode pH meter calibrated against buffer solutions at pH 4, 7 and 10. All extractions took place at a roller bench and were conducted at room temperature. All extracts were filtered through 0.45 μm nylon syringe filters. Elemental concentrations in the extracts were measured using ICP-MS. Prior to the analyses, the samples were diluted in a 3% HNO3 solution containing internal standards of Be, In and Re.

### Synchrotron analyses

SR-XRF was conducted at beamline I18 at Diamond Light Source (DLS) Didcot, UK and at the PHOENIX beamline of the Swiss Light Source (SLS), Villigen, Switzerland. Elemental maps for light elements were collected at an incident beam energy of 2.7 keV (DLS) and 2.483 keV (SLS) respectively, just below the excitation of Cl present in the resin. In order to excite Ca and Fe, one WT and one NRH sample were mapped at 9 keV at DLS. Similarly, one WT sample was mapped at 7.2 keV at SLS. For the remaining maps, the higher harmonic contribution of the beam energy distribution was in purpose not rejected in order to allow high Z elements excitation (e.g. Ca and Fe).

 Soil thin sections were mounted on an x-y-z stage and rastered relative to the fixed incident beam. At DLS the maps were recorded under a He atmosphere, while at SLS the maps were recorded under vacuum (~10-6 mbar). Both beamlines use Kirkpatrick-Baez mirrors to produce a spot size of 10 µm and 5 µm (DLS) and 2.5 µm (SLS), respectively. The pixel size was equal to the selected spot size. In addition, a Si(111) monochromator was used to select the incident beam energy. X-rays were detected using a four element Vortex Si drift (DLS) and single element (SLS) Ketek Si drift detector. X-ray flux was estimated to be 1010-1011 photons sec-1. Finally, at both beamlines a full Energy Dispersive Spectrum (EDS) was recorded for each pixel of the XRF map. XRF spectra and maps obtained at DLS and SLS were fit using the PyMCA freeware (Solé *et al.*, 2007) from fundamental parameters of the experiment using a spessartine garnet and apatite mineral standards with known elemental concentrations for calibration (Table S1 and S2).

 XANES spectroscopy was performed for the phosphorus, sulfur and iron K-edge. The monochromator edge position was calibrated using apatite, a Zn-sulfate standard, and iron metal foil. A series of standards was collected in order to constrain the speciation of P, S and Fe in the soil samples (Table 1). Background subtraction, data normalization and linear combination fitting (LCF) were performed using Athena from the Demeter software package (Ravel & Newville, 2005).

### Image analyses

All image fitting, processing and analysis steps were performed in PyMCA, ImageJ (Schindelin *et al.*, 2012) and Matlab 2017b (The MathWorks Inc., Cambridge, UK). Since the chemistry of interest takes place in the organic/loamy/clay phases, the primary minerals (SiO2:quartz) were masked out as these minerals are unlikely to participate in important chemical reactions in the soil. For this, a global threshold was applied with Otsu’s method (Otsu, 1979). Roots, i.e., the root channels left behind by the dried-out root during resin fixing phase, were segmented out manually by carefully drawing the borders of the root channel using the fitted Si-maps. Then a 2D Euclidean distance transform was applied to the segmented root image to determine the distance of any soil pixel to the root surface. Pixel intensity (concentration) changes with distance from the root were recorded for each normalized elemental map within non-overlapping annuli of 10 µm thickness.

## Results

### Analytical

The mineral fractions of the soil in this study were determined to be 60% sand, 24% silt and 16% clay. Analytical results of the bulk and rhizosphere soil are presented in Table S1 and Figure 1. The results show that the rhizosphere soil chemical properties are different from the bulk soil. The results show both a decrease in the pH (~ 0.5 pH) and extractable amorphous Fe and Al fractions compared to the bulk soil (Table S1 and Figure 1). In contrast, there are no significant differences of EDTA bioavailable and water extractable Fe and Al fractions between rhizosphere and bulk soil. However, the bioavailability of Ca and P is higher in rhizosphere soil. Finally, the effective cation exchange capacity is increased in the rhizosphere compared to the bulk soil.

### Synchrotron analyses

#### Chemical mapping

SR-XRF imaging was performed on multiple root cross sections and at depths of 20 and 40 mm from the top of the syringe barrels (Figure 2A and B). Si maps confirmed that the primary minerals in the soil are quartz-like with mass fractions ranging from ~0.3 – 0.6. These observed variations are typical for quartz-bearing minerals.

 The chemical maps show that the distribution of Fe, S and P is constrained to the clay/loamy phase (unresolved structural features in XRF imaging). Variation in concentrations is large due to the different mineralogy in clay, loam and other soil particles. Maps show agglomerations of Fe, Al, P, Ca and S. For Fe, Al and P the stoichiometry show signs of crystalline phases, such as hematite and goethite clay minerals and apatite (see Figure 2A and B, Figure S2 – S5). The origin of the small apatite minerals is possibly either from past fertilization or bedrock, as this is a young soil. For the image analyses, these minerals were ignored. Fe-rich coatings around quartz particles were not detected. This is because the resolution was not high enough in order to accurately detect this.

Careful image analyses show gradients for Fe and S from the edge of the root. An enrichment zone of Fe and S is observed close to the root surface (Figure 3 and 4) and both elements show similar trends. Fe and S enrichment maxima compared to the bulk shows contrasting results at two depths: a 43.5 and 17.4 % for Fe and S at a depth of 40 mm and 24.3 and 36.7 % for Fe and S at a depth of 20 mm, respectively. The difference between the two sampling heights is translated to the rhizosphere age difference of maximum three days, with the 20 mm height being the older one. Between the two treatments NRH and WT no clear differences are observed. The phosphorus gradients (Figure 3, column 3) are much less pronounced and in general uniformly present in the clay/loamy phase. However, small enrichments are observed in maps containing WT and are corresponding with the enrichment of Fe and S. This is in line with P interacting with Fe-oxides and organic matter.

#### XANES analyses

Spectroscopic analysis was used to obtain further chemical details for the key elements Fe, S and P (Figure S5 – S7). XANES data at the Fe K-edge (Figure 5 and 6) shows different spectral features and edge-positions. The pre-edge analysis of the Fe spectra indicates a shift to lower energies in the rhizosphere compared to bulk soil (Figure 6), which is consistent with reduction of Fe. In addition, linear combination fits (LCF) of Fe XANES spectra indicates a zone decreased in ferrihydrite/ferric oxyhydroxide species. This zone extends to about 100 – 750 µm from the root surface. Furthermore, the results suggest that these FeOOH species are transformed to Fe-oxides, such as FeO and Fe2O3. These observations are seen in both NRH and WT and no clear differences are observed.

 Sulfur K-edge XANES data indicate that reduced organosulfur (cysteine, sulfoxide, sulfone and sulfonate) species are clearly present throughout the clay/loamy phase (Figure 5 and 7 left column). However, close to the root LCF results reveal a zone of a reduced organosulfur inventory compared to the bulk soil. In addition, this zone is characterised by a threefold increase of oxidised inorganic sulfate (SO42-). This zone extends from 100 – 500 µm from the root surface. While this technique is not sensitive to bacterial communities, the speciation can be directly linked to rhizosphere microbial activity.

 In accordance with Fe and S K-edge XANES, phosphorus also appears to show trends in speciation (Figure 5 and 7 right column). As such, in one of the NRH samples, where apatite was apparently more abundant, there are signs of apatite dissolution. Overall, the LCF results suggest predominant P interaction with humic substances in the rhizosphere. This affinity of organic substances correlates well to the substantial increase in microbial activity and root exudation in the rhizosphere (as shown in S-speciation). In addition, the interaction of P with ferrihydrite seems to decrease in the rhizosphere, suggesting that P released from mineral surfaces gets adsorbed to humic acid and dissolved organic matter. This zone extends from 50 – 1000 µm from the root surface. This is surprising as P is extremely fixating and normally it is expected to adsorb to Fe/Al-oxide mineral surfaces. As a result, an increase in Fe/Al-P type speciation would be expected.

## Discussion

This study is synergistic to the rhizosphere structural study of Koebernick *et al.* (2018) in which a compaction zone accompanied by changes pore size distribution were observed. Our chemical data in combination with XCT suggests that there are three fundamental processes dominating the alteration of the rhizosphere, see Figure 8. In our study we see an increase in Fe and S in a similar zone in the rhizosphere.

### Fe chemistry

The soil used in this study is a relatively young soil, so that weathering of phyllosilicates is still ongoing. However, in soil systems, and particularly in the rhizosphere, Fe reduction and complexation with ligands are the dominant mechanisms for mobilizing Fe (Schwertmann, 1991). Moreover, both plant roots, fungi and microbes produce siderophores to help to receive sufficient Fe (Marschner *et al.*, 1986). The reduction however, increases mobility of Fe so that it can move into the rhizosphere. There are likely to be three important scenarios in the enrichment and mobilisation of Fe when the general simplified reactions are considered (Schwertmann, 1991; Schwertmann *et al.*, 1999; Boukhalfa & Crumbliss, 2002):

 FeOOH + 3H+ + e- 🡪 Fe2+ + 2H2O reduction in soil solution [1]

 4FeOOH + CH2O + 8H+  🡪 4Fe2+ + 7H2O + CO2 reduction by biomass [2]

 FeOOH + H+ 🡪 Fe(OH)2+ OH- 🡪 Fe(OH)3 protonation [3]

Fe(OH)3 + HnSid- + 3H+ 🡪 Fe-Sid + (*n-3*)H+ +3H2O siderophores [4]

Observations of Fe enrichment at the root surface have been done by Kölbl *et al.* (2017) and Vidal *et al.* (2018). Their studies suggested oxidized Fe-oxides, most likely hematite dominate the root surface and rhizosphere. In line with their findings we also observe an oxidized region of Fe in the root-soil contact zone, followed by a zone of partly reduced Fe. This reduced area falls directly in the Fe enrichment zone. This suggest that reduction is the dominant mechanism in mobilizing Fe. XCT results of Koebernick *et al.* (2018) in addition showed an increased frequency of smaller pores, indicating coalescence and compression of aggregates. As a result, the inter aggregate pore structure is altered (Koebernick *et al.*, 2018). Consequently, the increase of a smaller pore size fraction will increase the mineral surface area, which will affect Fe speciation. Surface mediated processes allow for the oxidation and precipitation of dissolved Fe(II) first as the poorly crystalline ferrihydrite within the rhizosphere. Reduced Fe and ferrihydrite can readily be oxidized and transformed into the more stable goethite and/or hematite via H3O+ species or dissolved cations and anions (Cudennec & Lecerf, 2006). This is in line with the increased ECEC and decreased amorphous Fe of the rhizosphere soil (Table S1). Moreover, under conditions of pH ~7 α-hematite is the favoured endmember of the metastable ferrihydrite (Cudennec & Lecerf, 2006). Our XANES results confirms a decrease in ferrihydrite-like species and increase in goethite and hematite-like species which suggest ferrihydrite transformation. The formation of these species decreases the availability of Fe, since the stability of hematite (Ks=10-43) is higher than ferrihydrite (Ks=10-39) and goethite (Ks=10-41).

 Microbial degradation of organic matter will also have profound effects on the accumulation and speciation of Fe. Coalescence and compaction could temporarily cause reducing conditions within fully saturated micropores when the pore connectivity is poor. Under these conditions, microbes that oxidize organic matter can use Fe(III) as its terminal electron acceptor (Lovley, 1991; Nevin & Lovley, 2002). It has been shown that both anaerobic and aerobic bacteria preferably reduce amorphous Fe, which explains the lower concentrations of amorphous Fe in the rhizosphere (Wahid & Kamalam, 1993). Moreover, a study where amorphous Fe(III) was reduced by bacteria also shows that acetate can be used by bacteria as electron donor (Lovley & Phillips, 1988). As a result, the reduction will cause release of most of the adsorbed species from Fe-oxide surfaces thus speeding up other biochemical processes. The rate that ferrihydrite can be reduced also depends on the surface area and its crystallinity. Usually the poorly crystalline Fe-oxides are most susceptible to microbial reduction. However, these reactions are dynamic and re-solubilization is very likely.

 Finally, our XANES results show no unequivocal evidence of Fe complexation via organic siderophores (both plant and microbial derived). However, our sulfur (see next section) spectra suggest that there is evidence of increased microbial activity in the rhizosphere. It is known that plants, fungi and bacteria excrete multiple siderophores in order to access Fe (Hider & Kong, 2010; Johnstone & Nolan, 2015). In addition, modelling of siderophores in Zn deficient soil show an increased mobility and an accumulation front of Zn in the rhizosphere (Ptashnyk *et al.*, 2011). Even though Fe concentrations are increased, the bioavailability of Fe(III) still might be much lower due to slow dissolution processes. Without exception, siderophores have a higher affinity for trivalent iron and are much less competitive with other biologically important trivalent cations (Hider & Kong, 2010). However, although these processes almost certainly take place in the soil, our technique is not sensitive enough to detect these species. Moreover, other more important processes are likely to obscure the results of these organic complexes.

### Sulfur chemistry

The enrichment of sulfur in the rhizosphere follows a similar trend as that of Fe. As both elements are redox active, they are important in microbial and fungal processes. Linear combination fits indicate that the sulfate content is higher in the rhizosphere compared to bulk soil. Inorganic sulfate is the end product of desulfurization of organic sulfur compounds and are ascribed to bacteria and fungi (Gahan & Schmalenberger, 2014; Linder, 2018). The ability to transform sulfonate and sulfate esters in soil is also important for bacteria in order to access inorganic sulfate (Mirleau *et al.*, 2005). However, as plants also compete with bacteria for the produced inorganic sulfate, a depletion zone of sulfate is expected. In accordance with our XANES spectra, we see a steep decrease in sulfate fraction close to the plant roots, which suggest sulfate uptake. Although S is an essential element for bacteria, carbon is the limiting element for microbial activity. As 20% of rhizosphere carbon supply comes from plants (rhizo-deposits), a gradient of rhizo-deposits can be envisaged. This means that with increasing distance from the root carbon limitations increase dramatically (Mirleau *et al.*, 2005). In contrast, our results show a steep increase with maximum sulfate between 100 – 750 μm (Figure 3) instead of a gradual gradient. This suggests that increased microbial conversion coincides with the compaction zone as determined by XCT. We hypothesize that this compaction zone retards the transport of plant useable S. Thus, the net conversion of S is higher in this region, because of decreased competition and due to increased surface area and surface mediated processes (adsorption/desorption). Moreover, smaller pores also have important implications for the soil water holding capacity which will have an effect on nutrient movement and microbial activity. Moreover, the higher surface area will also create more sorption sites for SO4 onto hematite and goethite (Hinkle *et al.*, 2015). In contrast, the higher porosity close to the root surface has a profound effect on the S. In addition, increased assimilation by microbes and fungi will therefore also be higher and S mobilization is therefore be expected to increase (Gahan & Schmalenberger, 2014).

### Phosphorus chemistry

Although gradients in our study are not clearly visible, there are clear trends in Fe – P and humic matter interactions. In our study, the rhizosphere zone close to the root surface shows that humic like material is the dominant complexing material for P and to a lesser extent FeOOH-like surfaces. The interaction with humic substances seems to correlate to the exudation of organic substances from the root and from microbial derived organic matter. This, as corroborated by our analytical results, improves the bioavailability of P in the rhizosphere (Yang *et al.*, 2019). This effect is enhanced with the addition of root hairs, suggesting that these roots exude more organic substances. Moreover, our results show an increase in Fe in the rhizosphere linked to reductive processes, this and the formation of organo-mineral complexes directly or indirectly influences the adsorption of phosphate and bioavailability (Zhang *et al.*, 2014; Werner & Prietzel, 2015). However, a more recent study showed that in heavily manured soil the dominant P-speciation was P adsorbed to ferrihydrite and Al phases (Schmieder *et al.*, 2018). In contrast, our study shows that besides humic substances and FeOOH-like substances, illite is a steady and relative important P-adsorbent in the soil. This is in line with previous studies by Andersson *et al.* (2016) and Gérard (2016) which show that clay minerals, such as smectite and illite have higher adsorption capacities than Al and Fe-oxides for PO4 in soils. Moreover, the presence of apatite in our samples, either anthropogenic or naturally occurring, show signs of weathering in the rhizosphere. Similar changes of P speciation from apatite-P to organically bound and Fe bound P have been observed in deglaced topsoils (Prietzel *et al.*, 2013). The observed changes in speciation, are observed within 1 mm and correlate to both Fe and S speciation changes. Therefore, both redox and microbial activity have a direct impact on P speciation and availability.

### Relationship soil structure and chemistry

The goal of this study was to link soil structural changes observed with XCT with soil chemistry observed via SR-XRF and XAS spectroscopy. Spectroscopy of particularly Fe and S show remarkable changes at close distance to the barley root and correlates with soil porosity changes observed in Koebernick *et al.* (2018) (Figure S1). The heterogenous nature of soil makes it extremely complex to extract specific processes. Nevertheless, our data in combination with XCT suggests that there are three fundamental processes dominating the alteration of the rhizosphere, see Figure 8. We can identify three zones: 1) vicinal rhizosphere with increased porosity, 2) compaction zone, and 3) bulk soil. In the first two zones, the dominant processes are taking place, such as reduction, microbial conversion and surface mediated processes. Transformations and interactions of species is also much higher in this zone. Finally, the fact that we do not detect significant gradients and changes in speciation beyond the compaction zone suggests that the compaction zone acts as a barrier. Also, the age of plant root will clearly play a crucial role in the observed changes. For example, younger roots will exude more organic acids and siderophores than older roots. Therefore, we expect these changes to diminish over time.

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**Tables**

Table 1. List of selected standards for XANES analyses

|  |  |  |  |
| --- | --- | --- | --- |
|  | Formula | Oxidation state | XANES |
| Compound |   |   |  S  |  P  |  Fe  |
| *Fe-containing standards* |
| Wüstite\* | FeO | +2 |  |  | x |
| Vivianite | Fe2+3(PO4)2 · 8H2O | +2 |  | x | x |
| Spessartine | Mn2+3Al2(SiO4)3 | +2/+3 |  |  | x |
| Haematite | Fe2O3 | +3 |  |  | x |
| Ferrihydrite | (Fe3+)2O3 · 0.5H2O | +3 |  |  | x |
| Goethite | α-FeO(OH) | +3 |  |  | x |
| *Sulphur-containing standards* |  |  |  |
| Sulphur | S | 0 | x |  |  |
| L-Cysteine | C3H7NO2S | +0.5 | x |  |  |
| Methionine sulfoxide | C5H11NO3S | +2 | x |  |  |
| Methionine sulfone | C5H11NO4S | +4 | x |  |  |
| Sulfonate | C7H17NO3S | +5 | x |  |  |
| Zinc sulfate | ZnSO4 | +6 | x |  |  |
| *Phosphorus-containing standards* |  |
| Phosphonate | C3H9O3P | +3 |  | x |  |
| Apatite | Ca5(PO4)3(F,Cl,OH) | +5 |  | x |  |
| Phytic acid | C6H18O24P6 |  +5 |  x |  |  |  | x |  |
| Vivianite | Fe2+3(PO4)2 · 8H2O | +5 |  | x | x |
| FePO4 | Fe3+PO4 | +5 |   | x |  |
| PO4 adsorbed to FeOOH |  | +5 |  | x |  |
| PO4 adsorbed to Al2O3 |  | +5 |  | x |  |
| PO4 adsorbed to humic acid |  | +5 |  | x |  |
| PO4 adsorbed to illite |  | +5 |  | x |  |

\*van Veelen et al. (2014)

### Author contributions

AvV, NK and TR designed the study. AvV, NK, CSS, DMF, TH, CNB and JFWM collected the data. AvV and NK analysed the data. AvV wrote the manuscript and all other authors provided critical revision and approval before submission. AvV was PI on the Diamond Light Source experiments SP15971 and SP17888 and the Swiss Light Source experiments 20170623 and 20171809.

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**Figure captions**

Figure 1. Analytical results determined via ICP-MS for the different extraction methodologies. A—amorphous nutrients, specifically for Fe and Al; B – bioavailable nutrients extracted with EDTA; C – extractable soil fraction of amorphous and bioavailable nutrients; D – water extractable fraction of labile nutrients.

Figure 2A. Image analyses steps on (top left) XRF image of NRH cross section (20 mm depth) showing the distribution of elements. The composite map (top right) shows the complex nature of the chemical distribution within the soil. Root shows up in the composite map as a circular void. A Si mask (bottom left) was used to end up with a map of the clay/loamy/silt phase (bottom right) which has been used to extract gradients of Fe, S and P. Scalebar (red line) = 1 mm. Vertical scalebar (top left) = mass fraction of each element, where 1 = 1,000,000 ppm. Incident beam energy 2.7 keV.

Figure 2B. XRF image of WT cross section (depth 40 mm) showing the distribution of elements. Composite map shows the complex conglomeration of S and Fe. Root shows up in the composite map as a circular void with S enrichment in the dried root. Scalebar (red line) = 750 µm. Vertical scalebar (left panel) = mass fraction of each element, where 1 = 1,000,000 ppm. Incident beam energy 2.483 eV.

Figure 3. Extracted gradients from the analyzed cross sections of NRH and WT treatments. y – cross section depth at 40 mm; o – cross section depth at 20 mm.

Figure 4. Elemental distribution maps of NRH cross section (depth 20 mm). Note the increased concentration of S close to the root (showing in red). Maps show the heterogeneity, which is why Euclidian distance transforms were applied to these maps to extract the gradients from Figure 3. Scalebar (in red) = 1 mm. The elemental concentrations are expressed as mass fraction, where 1 = 1000,000 ppm.

Figure 5. XANES spectra of Fe, S and P K-edges. Standards of each elements are presented on the top of each column. Point analyses of cross sections NRH (Figures 2A and 3) and WT (Figure 2B) are presented on the bottom.

Figure 6. Linear combination fit results of the point analyses on all cross sections for Fe versus distance from the root surface. Error bars = standard deviation

Figure 7. Linear combination fit results of the point analyses on all cross sections for S and P versus distance from the root surface. Error bars = standard deviation

Figure 8. Postulated chemical interactions rhizosphere. Three dominant processes are responsible for the speciation changes observed in this study: reduction, microbial activity and complexation of organic materials e.g. siderophores and organic acids