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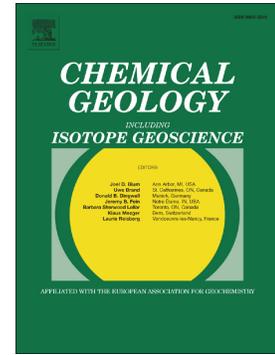
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Effect of rare earth elements on rice plant growth

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Abstract

The goals of this study were, (1) to quantify the effects of rare earth elements (REE) on rice plant growth and (2) to determine whether the presence of iron (III) oxides on the plant root surface (i.e. the iron oxide plaques) played a role in impeding any toxic effects caused by the presence of the REE. Hydroponic experiments were designed to grow rice plants in a greenhouse under controlled conditions, exposed to all rare earth elements simultaneously, and to iron (II) sulfate or iron (II) chloride. The results showed a significant decrease in root and plant height and biomass at rare earth element concentrations of 0.5 mg/L and 1 mg/L. Negative growth effects were observed for plant roots and shoots upon addition of 100 $\mu\text{mol/L}$ Fe(II) chloride or Fe(II) sulfate. Even when the root biomass was enhanced upon addition of Fe (II) chloride at a 1 mg/L rare earth concentration, however, statistically significant decreases in root length and plant height were recorded. In the presence of Fe(II) sulfate, a negative growth effect was present for all REE concentrations, being more pronounced at the highest REE levels. For the Fe(II) chloride experiments, speciation modeling showed that the rare earth elements would remain “free” as hydrated ions (Ln^{3+}) or would be complexed by Fe(III) oxyhydroxides. With Fe(II) chloride, the light rare earths (La, Ce, Pr, Nd; LREE) remained mostly soluble, whereas the middle (Sm, Eu, Gd; MREE) and heavy (Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu; HREE) elements were for the most part bound by Fe(III) solids. As negative growth effects were observed with Fe(II) chloride, the most soluble LREE could be concluded to play a role in rice plant growth inhibition. Furthermore, upon addition of Fe(II) sulfate, the MREE and HREE were significantly associated with SO_4^{2-} and a regained toxic effect for rice plants was observed at the highest REE concentration, suggesting also an effect of the dissolved MREE-SO_4^+ and HREE-SO_4^+ species on rice growth. This observation, coupled to the knowledge that SO_4^{2-} is an essential nutrient for plants, suggests absorption of the REE-SO_4^+ species by the rice plant. The recorded negative

growth effects for both the Fe(II) chloride or Fe(II) sulfate conditions, strongly suggest that all REE are detrimental to the development of rice. For both the Fe(II) chloride and Fe(II) sulfate conditions the negative growth effects may have been attenuated as a consequence of REE sorption to Fe(III) oxyhydroxides (i.e. iron oxide plaques) identified on the root surface, as suggested by surface complexation modeling of the REE to iron (III) oxides, at the pH and ionic strength conditions in this study.

1. Introduction

Despite the growing concern on the environmental impact of an increasing use of the rare earth elements (REE) in agriculture, little information is available concerning the effects of REE on plant growth (d'Aquino et al., 2009). The uptake of REE by plant roots is the controlling step for the subsequent distribution, enrichment and fractionation of these elements in the aerial and edible plant parts, such as rice grains (Tagami and Uchida, 2006; Thomas et al., 2014; Pagano et al., 2015; Zhuang et al., 2017). In wheat roots, REE fractionation was shown to result from phosphate induced precipitation of the REE (Ding et al., 2006). Accumulation of REE in roots has led to inhibition of primary root elongation and decrease plant dry biomass, as well as a marked diminution in mineral nutrient content (Hu et al., 2002). In the aerial parts of rice plants, positive Eu and Tb anomalies were observed, as well as a positive Eu anomaly in rice roots (Tao et al., 2008). Ning and Xiao (1989) reported that REE at concentrations of less than 5 mg/L could enhance the growth of new rice roots, however, at higher REE contents growth inhibition in rice and other main crops has been demonstrated (Pang et al., 2002). The mechanisms controlling the transfer of REE to plant roots, however, are not yet properly identified and quantified.

The transfer of the REE in soil-plant systems thus has been proposed to be intimately coupled with the Fe cycle (Brioschi et al., 2013). Iron has been shown to be essential for plant growth, suggesting the development of strategies by plants destined to maximize Fe absorption (Berner et al., 2003; Reichman and Parker, 2005; Robin et al., 2008). The proposed Fe/REE interaction in soils, however, would imply that the same processes that may mobilize iron in the rhizosphere (e.g. exudation of siderophores) may also target the REE (Wiche et al., 2016; Wiche and Heilmeyer, 2016). The roots of wetland plants, such as those of rice, have been demonstrated to become covered largely by iron (III) oxide minerals, known as iron plaques (Fu et al., 2011; Yamaguchi et al., 2014). The mechanism of formation of these iron (III) minerals has not yet been fully quantified. However, iron plaques have been proposed to play a central role in the control of trace metal uptake by rice plants (Hansel et al., 2001; Batty et al., 2002; Hansel et al., 2002; Liu et al., 2005; Møller et al., 2008; Fu et al., 2011; Yamaguchi et al., 2014). However, the fate of the REE during the formation of plant root iron (III) minerals, as well as their form of interaction with the surface of these reactive solids, have not yet been fully quantified.

Physical and chemical weathering of rocks and their forming minerals are the source of the REE in the soil solution and in aquatic systems (Dia et al., 2000; Gaillardet et al., 2003; Pédrot et al., 2015; da Silva et al., 2017; Zhuang et al., 2017). The chemical reactions in these processes, mainly hydrolysis, release the REE as “free” ions (i.e. Ln^{3+}). The Ln^{3+} species are prompt to complexation by ligands in solution, such as carbonate, sulphate, soluble organic matter, colloids and solid surfaces such as Fe or Mn oxides (Guthrie et al., 2003; Tang and Johannesson, 2003; Wang et al., 2005; Liu et al., 2017). The partitioning of trace elements, including the REE, among available ligands is controlled by complexation stability constants, reaction kinetics, element concentrations and physicochemical parameters (e.g. pH,

temperature) (Guthrie et al., 2003; Wang et al., 2005; da Silva et al., 2017; Zhuang et al., 2017). Quantifying the above parameters for REE organic and inorganic complexes is important to determine the concentration of the REE forms as a function of the reactive nature of dissolved ligands and solid phases. This is essential to understand the fate and interactions of these elements in aqueous environments, such as the rice root rhizosphere to quantify and identify the nature of the REE species which may detrimental to plant growth (Davranche et al., 2015; 2017).

In this study, rice plant growth was quantified in the presence of REE. Rice plants of the local *Nep cai hoa vang* Vietnamese variety were grown for a period of 14 days in hydroponic laboratory experiments. The plants were grown in: (1) the absence of REE (control conditions), (2) exposed to all REE from a standard solution at concentrations of 0.5 mg/L and 1 mg/L, and (3) in the presence of 100 $\mu\text{mol/L}$ Fe(II) chloride or Fe(II) sulfate and REE. The formation of REE sulfate complexes (LnSO_4^+) and amorphous iron (III) oxyhydroxide precipitates on the plant root, as determined by scanning electron microscopy and X-ray diffraction methods, played a key role in the solubility and absorption of the REE by the rice plant.

2. Material and methods

All chemicals used were of analytical grade. Ultrapure Mili-Q® water (18 M Ω) was used for the preparation of rice growth medium and all solutions for chemical analyses. Unless stated otherwise.

Whole grains of the local *Nep cai hoa vang* rice variety grown in the largest open-pit coal mining region in NE-Vietnam (Martinez et al., 2013), were acquired from the Quang Ninh Seedlings Joint Stock Company in Cam Pha and germinated under sterile conditions in a solution containing 0.049 g/L of Murashige and Skoog medium basal salt mixture, 0.050 g/L of MES (2-(N-morpholino) ethanesulfonic acid) biological buffer, and 6 g/L of phyto-agar. All three components were obtained from Duchefa Biochemicals (Haarlem, The Netherlands; product numbers: M0221.0025, M1503.0250, and P1003.1000, respectively). The pH of the resulting rice growth medium was adjusted to 5.7, with a sterile solution of 0.1 mol/L NaOH, prior to the addition of phyto-agar and after addition of Fe(II) chloride or Fe(II) sulfate to the corresponding experimental set-ups. The pH was adjusted for optimal rice plant growth as suggested previously (Yoshida et al. 1976; Hoai et al. 2003; Martinez et al. 2013). The medium was subsequently sterilized by autoclaving at 121 °C for 20 min.

To test the effects of the REE on the growth of the *Nep cai hoa vang* variety, in the presence of Fe(II) sulfate and Fe(II) chloride, rice grains (n = 533) were planted in sterile 15 mL Falcon® tubes, containing 12 mL of sterile rice growth medium, prepared as described previously (Martinez et al., 2013). The plants were grown in a greenhouse under controlled conditions with a humidity ranging from 80 to 90%, at a temperature of 28 to 32°C and under a day/night cycle, with a 55000 to 60000 lux illumination for a period of 14 hours. The rice plants were harvested after a period of 14 days, and any excess agar was removed from the plant roots. After measurement of the root length, whole plants were dried to a constant weight in an oven at 60 °C. This procedure was applied for control conditions, and for rice grains germinated in growth medium with a final concentration of 0.5 mg/L or 1 mg/L of all REE, exposed to 100 µmol/L of Fe(II) chloride or Fe(II) sulfate. Although iron has been shown to cause toxicity to rice plants, the final concentration of Fe in these experiments (i.e.

600 $\mu\text{mol/L}$, of which 500 $\mu\text{mol/L}$ are contained in the optimal growth medium), would at most exert non-significant toxic effect, when compared to those of toxic trace elements (Sahrawat, 2005). The final amounts of all the REE were obtained by dilution of a multi-component standard solution containing an initial concentration of 50 mg/L in 2% HNO_3 of all the REE (CRM TraceCERT® Sigma-Aldrich Rare earth 16 element mix for ICP). The extent of the rice plant growth under these different experimental conditions was quantified by measuring the dry weight and the length of the wet roots and shoots, as suggested previously (Gardea-Torresdey et al., 2004; Bashan and de-Bashan, 2005; Martinez et al., 2013). The wet rice root length was manually measured using a digital caliper (Mitutoyo® Digimatic 150 mm), as young rice plants after 14 days of growth, the root system was a single unbranched root whose length was quantifiable by a manual method as suggested previously (Himmelbauer et al., 2004; Muehe et al. 2014).

2.1 Analytical setups

To determine metal concentration in shoots and roots, 0.5 g (accurately weighed) of samples were digested using a mixture of 8 mL HNO_3 and 2 mL HCl and a microwave system at the University of Freiburg (MLS GmbH Microwave Laboratory System, Germany) according to the procedure reported by Avula et al. (2010). Rare earth element concentrations in the digest samples were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific XSERIES2) at UniLaSalle, Beauvais, France. Quantification was carried out by external calibration (REE multi elemental standard solution from Accu Trace Reference, USA) and using indium (2.5 $\mu\text{g/L}$) as an internal standard in order to correct for instrumental drift and matrix effects. The instrumental accuracy was assessed by analyzing the SPS-SW2 certified reference material (CRM) for measurement of elements in surface water (SpectraPur standards, Oslo, Norway). The analyses of real samples were carried out

provided that the bias of measured concentration was <5% compared to the certified values. The method for plants analysis was validated by the analysis of CRM 1573a - Tomato Leaves (NIST, Gaithersburg, USA). In addition, statistical agreement with the “determined but not certified data” for La, Ce, Sm and Gd was obtained.

2.2 Mineralogical investigations.

A qualitative mineralogical analysis of the root samples was carried out by using a Bruker AXS D8 Advance X-ray powder diffractometer, equipped with a Cu-K α radiation source, a diffracted-beam graphite monochromator, and a scintillation detector. The X-ray diffraction (XRD) patterns were collected from 2.0 to 60.0° 2 θ , with a step size of 0.02° 2 θ , and a dwell time of 2 s at each step. The patterns were evaluated with the DIFFRACplus 5.0 software. Iron (III) oxyhydroxides on rice plant roots were by using an environmental Scanning Electron Microscope (ESEM) (ElectroScan 2020) and a Quanta 250 FEG (FEI).

2.3 Statistical analysis.

Descriptive statistics were performed on shoot and root samples and normality of data and homogeneity of variances were verified. One-way ANOVA (Analysis of Variance) tested differences in metal concentrations in roots and shoots among rice plants exposed to REE, and Fe (II) chloride or Fe (II) sulfate. Post-hoc multiple comparisons (Tukey HSD) were applied after ANOVA when there was a significant difference (as expressed by the different letter labels, a, b and c, in Fig. 1 and Fig. 2)

2.4 Speciation modeling.

The speciation calculations were performed using the computer program PHREEQC version 3.1.5 (Parkhurst and Appelo, 2013) and the NAGRA/PSI data base (Hummel et al., 2002)

modified to include well-accepted infinite-dilution (25 °C) stability constants for REE inorganic complexes (i.e., in our study, chlorides (Luo and Byrne, 2001) and sulfates (Schijf and Byrne, 2004). Surface complexation of the REE with hydrous ferric oxides (HFO) was modeled as described by Liu et al. (2017).

3. Results and discussion

3.1 Effects of the REE on rice plant growth

Fig. 1 and Fig. 2 show the results of rice plants grown exposed to 0.5 mg/L or 1 mg/L REE, and 100 μ mol/L Fe(II) chloride or Fe(II) sulfate. In the absence of added Fe(II) salts, the REE concentrations of 0.5 mg/L and 1 mg/L result in a significant decrease in root length and plant height and respective biomass (Fig. 1 and Fig. 2). The root length decreased from an average of 85 ± 10 mm for control conditions to a mean of 45 ± 10 mm for a REE concentration of 1 mg/L (Fig. 1). Similarly, the average root biomass diminished from 35 ± 10 mg for the control to 20 ± 10 mg at the highest REE content (Fig. 2). Detrimental growth effects for plant shoots are shown in Fig. 1 and Fig. 2. The plant height was reduced from 200 ± 50 mm under control conditions to 150 ± 50 mm at 1 mg/L of REE (Fig. 1) and the shoot biomass diminished to 60 ± 20 mg from a control value of 85 ± 15 mg (Fig. 2). These results indicate a negative effect of the REE on rice growth at concentrations lower than those previously reported, where the negative plant growth effects of the REE were apparent at levels in excess of 5 mg/L (Pang et al., 2002). This suggests that the toxic effects of these elements are controlled by their speciation in solution and partitioning among specific organic and inorganic ligands, and reactive solids. These processes may facilitate root absorption of the REE, and further imply that the form of the REE species rather than their total nominal

dissolved concentrations in the aquatic environment, would account for detrimental plant growth (Ichihashi et al., 1992; Tang et al., 2003).

3.2 Speciation of the REE with Fe(II) chloride or Fe(II) sulfate and effects on rice growth

In aquatic systems, such as the plant rhizosphere, solution and interface chemistry is the controlling factor determining the dominant dissolved REE species and their concentrations (Sholkovitz, 1995; Davranche et al., 2015; 2017). Speciation calculations for the REE in the presence of Fe(II) chloride are shown in Fig. 3a. As reported previously, in the presence of chloride, LnCl_2^{2+} and LnCl_2^+ are the dominant species below a pH of 5, however at the pH of the experiments herein, the concentrations of these species is negligible (i.e. <0.1%) (Brookins, 1989). With the addition of 100 $\mu\text{mol/L}$ Fe(II) chloride, 90 to 70% of the light rare earth elements (LREE: La, Ce, Pr, Nd) are present as “free” hydrated ions, whereas 70% of the middle (MREE: Sm, Eu, Gd) and 70% to 80% of the heavy elements (HREE: Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) are bound by hydrous ferric oxides (HFO) (Fig. 3a). In the presence of Fe(II) chloride, the mean root length remained constant for all REE concentrations, however it showed a statistically significant decrease with respect to control condition in the absence of Fe(II). (Fig. 1). No significant difference in the rice root biomass was observed, with Fe(II) chloride, when comparing the results of control (no REE added) and low REE concentration (0.5 mg/L) experiments (Fig. 2). For the 1 mg/L REE condition, however, a significant increase in root biomass was recorded, suggesting that the addition of chloride enhanced root growth (Fig. 2). This result is consistent with those of previous studies, which reported beneficial effects of the application of chloride on root development (Kimura et al., 2004; Chen et al., 2016; Jia et al., 2017). In addition, this result is in good agreement with previous works, which suggest that chloride (Cl^-) stimulates root growth through two different mechanisms: (1) an osmoregulatory role of Cl^- in the plant, and (2) the indirect effect of Cl^-

through control of the water potential, which may influence the root length of rice plants (Kimura et al., 2004; Chen et al., 2016; Jia et al., 2017).

A further interpretation of the speciation results in Fig. 3a for rice plants exposed to REE and 100 $\mu\text{mol/L}$ Fe(II) chloride, suggests that the most mobile REE (i.e. the LREE) would be the cause of the mild, but nonetheless detrimental effects on rice root length or biomass observed in Fig. 1 and Fig. 2. The LREE remain, for the most part, mobile in the presence of Fe(II) chloride and HFO, and could be considered responsible for the negative effects on rice plant growth (Fig. 1 and Fig. 2), as in the presence of Fe(II) chloride, a marked portion of the MREE and the HREE was removed from solution by complexation to HFO (Fig. 3a). This implies that the LREE played a role in the negative effects observed on the root length and biomass, also recorded for experiments carried out in the absence of Fe(II) salts (Fig. 1 and Fig. 2).

Fig. 3b shows the speciation of the REE in the presence of Fe (II) sulfate. For the LREE, 5% to 10% of these elements are bound to HFO, whereas 30% to 35% are present as “free” Ln^{3+} ions, and the remaining 55% to 60% occur as the dissolved LnSO_4^+ species. Fig. 3b shows, that a significant portion of the MREE and HREE are complexed by sulfate to form the soluble LnSO_4^+ species. As such, 40% to 45% of the MREE and 25% to 40% HREE remain in solution and are therefore available for absorption by the rice root. At the highest REE concentration of 1 mg/L, a renewed negative effect on rice root length and biomass is observed with Fe(II) sulfate (Fig. 1 and Fig. 2). The magnitudes of the decrease in these two parameters are in good agreement with those of experiments conducted in the absence of Fe (II) salts (Fig. 1 and Fig. 2). The increase in MREE and HREE dissolved species

concentration with sulfate are also indicated by the lower fractions of MREE and HREE bound to HFO, as compared to the Fe (II) chloride condition (Fig. 3a and Fig. 3b).

Upon addition of Fe (II) sulfate, a decrease in root length and biomass was recorded for REE concentrations of 1 mg/L. At a pH of 5.7, the pH of the rice growth medium, LnSO_4^+ should be present as the main form of the REE interacting with the rice root. This is consistent with the results of Zhu et al. (2016), who showed that LnSO_4^+ is the dominant species within the pH range of 3.4 and 6.0 under similar solution conditions. The results of speciation calculations in Fig. 3b, indicate that SO_4^{2-} more strongly complexes the MREE and HREE ($\log_{10} K_{\text{average}} = 3.16 \pm 0.24$; Schijf and Byrne, 2004) rather than the LREE ($\log_{10} K_{\text{average}} = 2.14 \pm 0.32$; Schijf and Byrne, 2004). The stronger association of the MREE and HREE with SO_4^{2-} would lead to a partial remobilization of MREE and HREE as soluble LnSO_4^+ species. The fractions of the REE bound to HFO upon the initial addition of Fe (II) chloride decrease from ranges of 55 to 60% and 70 to 85% for MREE and HREE respectively, to 30 to 37% and 55 to 75% in the case of added Fe (II) sulfate (Fig. 3a and Fig. 3b). This suggests that the MREE and HREE, present in the form LnSO_4^+ , would be available as dissolved species to be the cause of the regained decrease in shoot and root growth at the highest concentration of 1 mg/L REE (Fig. 1 and Fig. 2). This negative effect may be induced by the requirement of the rice plant to maintain a cation/anion balance (Marschner, 2011). Upon exposure to most stable metal sulfate complexes (e.g. MREE-SO_4^+ and HREE-SO_4^+), the rice plant root can uptake SO_4^{2-} for plant nutrition and metabolism, inducing simultaneous cation (e.g. MREE and HREE) absorption to maintain osmotic balance (McLaughlin et al., 1998). This mechanism would then constitute a plausible explanation for the regained toxic effects to the rice plant observed for highest REE concentrations in the presence of Fe (II) sulfate (Fig. 1 and Fig. 2).

As mentioned earlier, the results in Fig. 1 and Fig. 2 involving Fe(II) chloride suggest that the LREE are toxic to rice plants, as well as the remobilization of the MREE and HREE in the presence of Fe(II) sulfate (Fig. 3b), which cause a detriment of plant growth at highest REE concentrations of 1 mg/L (Fig. 1 and Fig. 2). These mechanisms may serve to interpret the results of previous works involving field studies, which have shown that the REE patterns in plant roots are consistent with those of local soil particles, mainly composed of Fe-oxyhydroxides and possibly clay minerals (Brioschi et al., 2013). These soil particles near roots (i.e. in the rhizospheric zone) are the main source of REE to plants. Their enhanced chemical weathering in the rhizosphere, for example by acid mine drainage, leads to soil acidification resulting in the solubilization of HREE from stable inorganic ligands (Mn- and Fe-oxides) which can then be taken up by plant roots (Hopkins and Hüner, 2009; Brioschi et al., 2013). This is consistent with the REE speciation modeling results in this study, which in the presence of SO_4^{2-} suggest the formation of MREE-SO_4^+ and HREE-SO_4^+ species, and a lower fraction of the REE sorbing to HFO (Fig. 3b). The presence of more soluble REE species with SO_4^{2-} may explain the regained toxic effects on rice plant growth, through root absorption of REE sulfate complexes as suggested by McLaughlin et al. (1998) and by the results in Fig. 1 and Fig. 2.

3.3 The role of Fe(III) oxyhydroxides on the REE effects on rice plant growth

The oxidation of the Fe(II) in these experiments may play a key role in attenuating the effects of REE absorption and toxicity to the rice plant. The added Fe (II) would be readily oxidized to Fe (III) oxyhydroxide phases. Figure 4 shows the presence of Fe (III) oxyhydroxides (e.g. ferrihydrite), heterogeneously covering the rice root surface. These

Fe(III) phases have been suggested to act as filters of trace elements (e.g. MREE and HREE) (Liu et al., 2005; Fu et al., 2011; Yamaguchi et al., 2014). Mechanisms such as surface complexation or co-precipitation in the presence of these Fe(III) minerals would be expected to prevent metal cations, such as “free” Ln^{3+} , from being absorbed by rice plant roots (Liu et al., 2005; Fu et al., 2011; Yamaguchi et al., 2014). In this study, the formation of iron (III) minerals on the rice root was observed both in the presence of Fe(II) chloride or Fe(II) sulfate. Sorption of the REE to root iron (III) oxyhydroxides may be supported by \log_{10} values of metal sorption constants (K), of the form $K = [\text{REE-L}^{(n-m)}]/[\text{REE}^{n+}][\text{L}^{m-}]$, which show that for REE complexation to iron (III) oxyhydroxides, $\log_{10}K$ values increase as the ionic radii of the REE decreases. For example, La presents a $\log_{10}K$ of 1.68, whereas Lu shows a value of 3.47 (Liu et al., 2017). This suggests that as the radii of the REE decreases the stronger the binding to HFO as shown in Fig. 3a and Fig. 3b. This result further implies a weaker binding of the LREE to HFO (Fig. 3a and 3b). The higher solubility of the LREE, together with the negative growth effects observed upon addition of Fe (II) chloride, confirm that the LREE are toxic to the rice plant.

Previous works have demonstrated that solution complexation of the REE with organic and inorganic ligands plays a significant role for the absorption of these elements by plant roots (Ding et al., 2006; Liang et al., 2008). In this study, speciation calculation results may serve to better understand the effects of Cl^- , SO_4^{2-} and, Fe (III) dissolved species or mineral precipitates on the complexation and partitioning of the REE and their interaction and absorption by the rice root. The oxidation of Fe (II) leads to the development of amorphous Fe(III) oxyhydroxides, such as the iron precipitates observed on the root surface in Fig. 4. Precursor Fe(III) aqueous species, such as $\text{Fe}(\text{OH})^{2+}$ or $\text{Fe}(\text{OH})_2^+$ show $\log_{10}K$ values of 11.3 and 21.8 respectively (Baes and Mesmer, 1981), whereas, Fe(III) sulfate species, including

$\text{Fe}(\text{SO}_4)_2^-$ and $\text{FeH}(\text{SO}_4)_2^0$ have corresponding $\log_{10}K$ values in the ranges of 5.4 to 7.6 and 8.1 to 10.0 (Langmuir, 1997; Casas et al., 2005). The magnitude of $\log_{10}K$ for hydroxide and sulfate complexation by Fe (III) aqueous species, suggests a competition between SO_4^{2-} and OH^- anions for Fe (III). This could imply that a lower fraction of the total Fe(III) initially added may be available for the formation of Fe (III) oxyhydroxides, leading to the diminished fractions of the MREE and HREE scavenged by HFO, as shown in Fig. 3b.

Figure 5 shows the concentration of REE in mg/kg in shoot (Fig. 5a and 5b) or root (Fig. 5c and 5d) biomass for experiments in the presence of 0.5 mg/L and 1 mg/L REE, and with further addition of 100 $\mu\text{mol/L}$ Fe(II) sulfate or Fe(II) chloride, as mentioned previously. For all conditions in Fig. 5, a lower concentration of the REE can be observed in roots and shoots, in the presence of Cl^- , compared to SO_4^{2-} . This result may complement the modeling results in Fig. 3 and support an enhanced absorption of LnSO_4^+ species by the plant root, however this effect would have to be the subject of a subsequent study focusing on investigating Ln^{3+} speciation with iron (III) oxides on the rice root surface. The results shown in Fig. 5c and 5d for the quantification of REE in concentrations in roots, show a decrease in MREE and HREE concentrations, both in the presence of Fe(II) sulfate and Fe(II) chloride and at 0.5 mg/L and 1 mg/L REE. This result suggests the presence of Fe (III) plaques on the rice root, as $\log_{10}K$ values propose a stronger binding of the MREE and HREE to hydrous ferric oxide strong sites (Liu et al. 2017). In addition, the “flat” patterns observed for the REE series in Fig. 5, and the absence of a Ce anomaly for all experimental conditions, could suggest a first step binding of the REE to organic matter, and subsequently to mineral phases such as hydrous ferric oxides as reported in previous studies (Davranche et al., 2004; 2015), however further quantification of the REE sorption and speciation mechanisms in the rice root rhizosphere are required to provide conclusive evidence. In addition, normalization of the patterns shown previously in Fig. 5 to the control experiments, further evidences a lower concentration of REE in roots and

shoots, in the presence of Cl^- , as with SO_4^{2-} (Fig. 6). This better illustrates REE fractionation. Indeed, in the shoots, a LREE to MREE enrichment is observed, whereas MREE enriched patterns with a small Tetrad effect are observed for the roots. This latter results highlight the role of the iron oxides even if the slope of the REE patterns is smaller. These results suggest that plants may preferentially absorb the LREE. This can further indicate that they are able to discriminate between cations differing by diameter and charge. Calcium and LREE to MREE have almost identical ionic radii (Shannon, 1976), suggesting that LREE and HREE are therefore able to competitively replace Ca ions in biological systems.

As shown by Brioschi et al. (2013) the transfer of the REE in soil-plant systems thus has been proposed to be intimately coupled with the Fe cycle. Iron has been shown to be essential for plant growth, suggesting the development of strategies by plants destined to maximize Fe absorption (e.g., Robin et al. 2008). Iron/REE interaction in soils would imply that the same processes that may mobilize iron in the rhizosphere (e.g., exudation of siderophores; Kraemer et al., 2017; competition with bacteria; Martinez et al., 2014) may also target the REE (e.g., Wiche et al. 2016). Even if the mechanism of formation of these iron (III) minerals has not yet been fully quantified, iron plaques have been proposed to play a central role in the control of trace metal uptake by rice plants in combination with other competitors (i.e., siderophores, bacteria, simple organic acids, organic matter). REE patterns of iron plaques (Fig. 7a), are different relative to those typical of inorganic HFO (Fig. 7b). They show a decrease in the Ce anomaly and a preferential uptake of HREE relative to LREE. This can be interpreted as a competition of REE sorption with organic matter (humic materials or siderophores) that may reduce Ce anomaly, or as the enhanced sorption of the HREE by organic reactive solids, such as bacteria. Eventually, small organic acids (like oxalate and acetate, e.g. Fig. 7b) may play a complementary role and favor

MREE>LREE>HREE extraction from solution (e.g., Josso et al., 2018). Overall, the absence of cerium anomalies in these experiments, suggests that Ce occurred as Ce (III) during the metabolic processes of the *Nep cai hoa vang* rice variety or that the organic complexes, which usually depress amplitudes of Ce anomaly, drive the REE speciation in plant fluids (Davranche et al., 2008).

The shoot and root REE patterns of control experiments shown in Fig. 5 display HREE enrichment with La/Sm ratios ranging between 1.34 and 1.64 and Gd/Yb ratios oscillating within values of 0.73 and 0.89. At 0.5 mg/L REE and 100 $\mu\text{mol/L}$ Fe (II) sulfate or Fe (II) chloride, shoot and root REE patterns become rather flat with a La/Sm ratio between 1.17 and 1.39 and a Gd/Yb ratio in the range of 1.05 to 1.17. Eventually these patterns become LREE enriched when considering the 1 mg/L REE concentration in the presence of 100 $\mu\text{mol/L}$ Fe (II) sulfate or Fe (II) chloride with a La/Sm ratio between 1.23 and 1.47 and a Gd/Yb ratio of 1.09 to 1.22. These results can be interpreted to be a consequence of REE-FeOx-OM interactions as previously shown by Davranche et al. (2004). At higher iron concentration, the HREE enrichment, which is consistent with an increasing REE mobility with increasing atomic number, is in favor of a speciation driven by solution complexation (i.e. by sulfate or free ionic species) as already highlighted for *Vitis vinifera* by Censi et al. (2014).

3.4 Protective effects of iron plaques on rare earth element toxicity

Rice plants adapt to waterlogged environments by releasing oxygen from their roots to produce an oxidizing medium in their rhizosphere and minimize the risk of toxic effects, especially of ferrous iron and other reduced elements (Chen et al., 1980). Under these conditions, insoluble ferric iron precipitates form on the root surface, and although this

mechanism may be protective against toxic elements, it can also impede to a significant degree the uptake of essential nutrients (Chen et al., 1980; Fu et al., 2011). Both the generation of an oxidizing environment and the coating of the root surface by iron (III) mineral plaques control the speciation and partitioning of the REE in the rhizosphere of rice and in consequence, the uptake of these elements by the plant roots. The generation of iron (III) oxyhydroxide precipitates on the root surface, coupled to the oxidizing soil conditions near the rice roots developed, in part, from the release of O₂ from the root surface (Liu et al., 2005; Fu et al., 2011; Yamaguchi et al., 2014) would then constitute a mechanism of defense against REE absorption by the rice plant. However, the effects of iron (III) oxyhydroxides on REE mobility in soils may be influenced by the presence of other reactive solids such as rhizospheric bacteria. The REE have been shown to sorb to bacteria cell surfaces which could also affect REE mobility. The log₁₀ of REE sorption constants in the presence of *Bacillus subtilis* ranged from 1.08 ± 0.04 to 1.40 ± 0.04 for the light REE (LREE: La to Eu), and from 1.36 ± 0.03 to 2.18 ± 0.14 for the heavy REE (HREE: Gd to Lu) at biomass concentrations of 1.3 g/L (Martinez et al., 2014). Furthermore, as indicated by the experiments in this study, the presence of Cl⁻ and SO₄²⁻ influence the extent of REE sorption to HFO, and may play a significant role in the form and concentration of the REE species taken up by the plant root heterogeneously covered by iron (III) precipitates.

3.5 The role of organic and inorganic ligands on REE speciation

As suggested by the results of hydroponic studies in the present study, the REE negatively affect rice plant growth. As shown herein, the presence of inorganic ligands (i.e. SO₄²⁻ and Cl⁻), and iron (III) oxyhydroxide phases associated with the rice root played a key role in controlling the toxic effects of the REE. In the natural environment, however, the toxic

effects of these and other trace elements are dependent on their form in the aquatic soil solution, which is composed of a significant number of competing inorganic and organic ligands able to complex the REE (Tang and Johannesson, 2003; Johannesson et al., 2004; Davranche et al., 2015; Liu et al., 2017). The REE have been shown to interact with iron and manganese oxide surfaces, as well as to complex inorganic ligands. These elements are suggested to partition and fractionate in the presence of oxide reactive solids and clay minerals (Tyler, 2004; Liu et al., 2017), natural organic matter and mineral organic matter composites (Johannesson et al., 2004; Davranche et al., 2015). As indicated previously by Liu et al. (2017) and references therein, further control on the speciation of these elements in natural aquatic environments is exerted by inorganic ligand (e.g. OH^- , HCO_3^- , CO_3^{2-} , NO_3^- , Cl^- , PO_4^{3-}) complexation as well as, by the interaction of the REE with soil bacteria species (Martinez et al., 2014). Due to the complex nature of REE speciation in soils, an exhaustive quantification and understanding of the toxic effects of these elements, as “free” cations (i.e. Ln^{3+}) and their forms, to plants in contaminated soils, must result from the interpretation of complementary simpler hydroponic laboratory based experiments and future studies of the REE behavior in natural soils.

4. Conclusion

In this study, a significant decrease in rice plant growth was observed upon addition of increasing concentrations of REE, of 0.5 mg/L and 1 mg/L. Rice plants were exposed to all REE, and also to the presence of Fe(II) chloride or Fe(II) sulfate. During the experiments, iron (III) oxide mineral deposits or iron plaques were developed on the rice plant root surface. In the presence of iron (II) chloride, changes in plant growth were observed within the error in biomass or measured length of shoots and roots. For the case of iron (II) sulfate addition, a

toxic effect was recorded for the highest REE concentration of 1 mg/L. As a first account of this observation, speciation modeling showed that with iron (II) chloride, the REE were complexed for the most part to hydrous ferric oxides. These results showed that the LREE remained soluble as free ions with iron (II) chloride, whereas a large fraction of the MREE and the HREE were bound to the iron (III) oxides. Mild toxic effects were recorded from the REE for the iron (II) chloride experiments, suggesting that the LREE part take in exerting negative growth effects to the rice plant. These detrimental consequences for the rice plant are further indicated by the results from the speciation modeling in the presence of iron (II) sulfate, which showed that a significant portion of the MREE and HREE (previously bound to iron (III) oxide phase with iron (II) chloride) were present as soluble LnSO_4^+ species. This coincided with the regained negative effects on rice plant growth at 1 mg/L REE for these experiments. Therefore, this indicates a renewed decrease in growth in the presence of the dissolved and available LnSO_4^+ species. The effects of REE on plant growth are diverse, however their interaction with iron (III) reactive solids is one of the key mechanisms leading to the determination of the REE forms fractionated and enriched in plant parts, including roots and aerial plant portions. The presence of iron (II) sulfate or chloride and their effects on REE speciation reveal a complex system of reactions around the formation of iron (III) oxides on the root surface. Further studies are needed, however, to quantify the modes in which REE species interact with the surface of these iron (III) oxide. These further studies will allow obtaining precise results for REE binding constants, sorption mechanisms and sorption kinetics, able to form part of surface complexation and kinetic models, needed to predict the fate of the REE in the plant rhizosphere. The results in this study from laboratory hydroponic experiments will further serve to constrain the speciation modeling of the REE in complex natural systems, therefore contributing to the quantification of the forms of the REE and their interactions with reactive solids in the natural environment.

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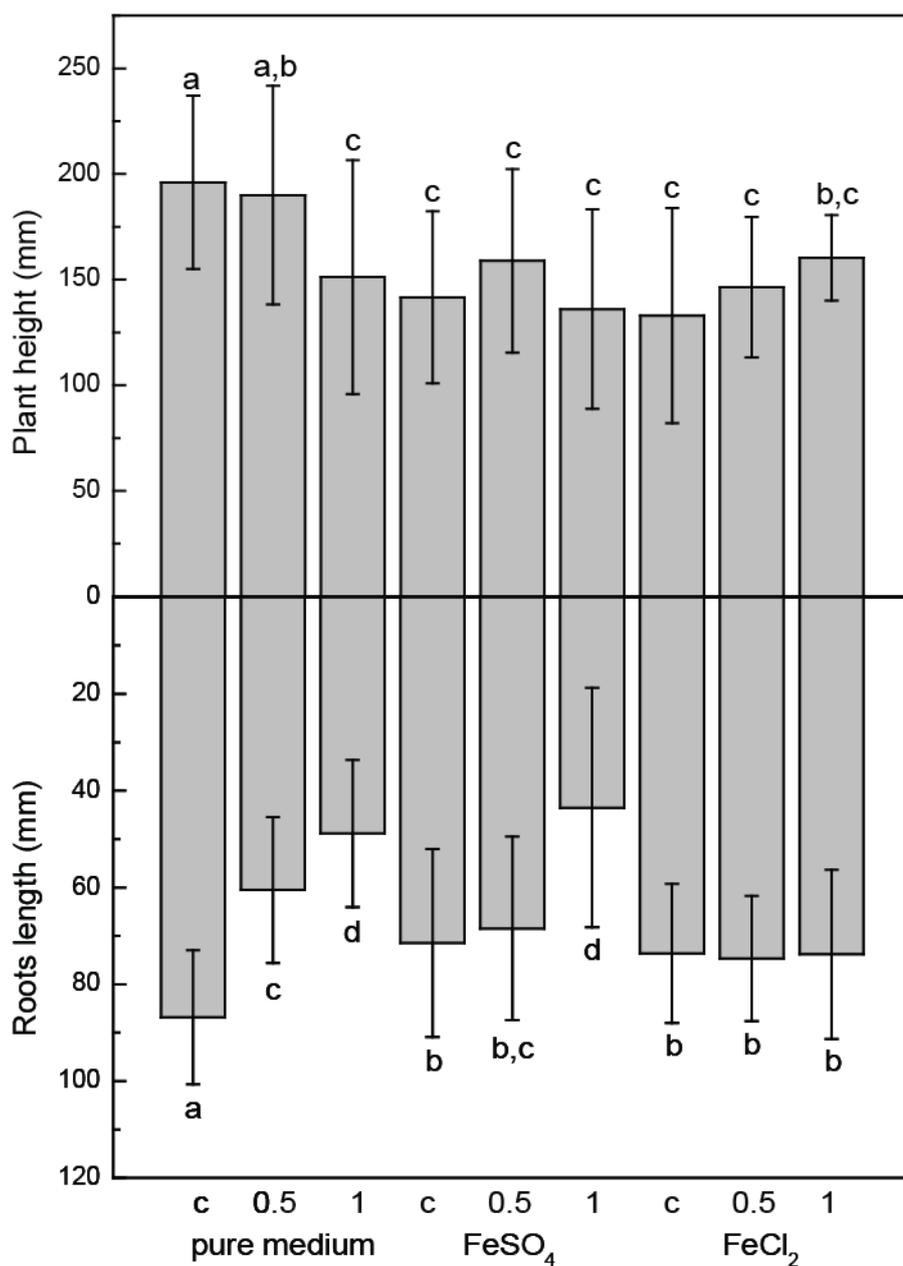


Fig. 1 Bar graphs showing the effects of REE on rice shoot and root length. The labels: “pure medium”, “FeSO₄” and “FeCl₂” correspond to experiments conducted in the presence of REE at 0.5 mg/L and 1 mg/L, REE + 100 μmol/L Fe(II) sulfate, and REE + 100 μmol/L Fe (II) chloride respectively. For each of these conditions, labels: “c”, “0.5” and “1” correspond to REE concentrations of 0 mg/L (control), 0.5 mg/L and 1 mg/L respectively. Error bars correspond to the standard deviation of measurements as indicated in the text. There is no statistically significant difference in length between conditions when the same letter is shown.

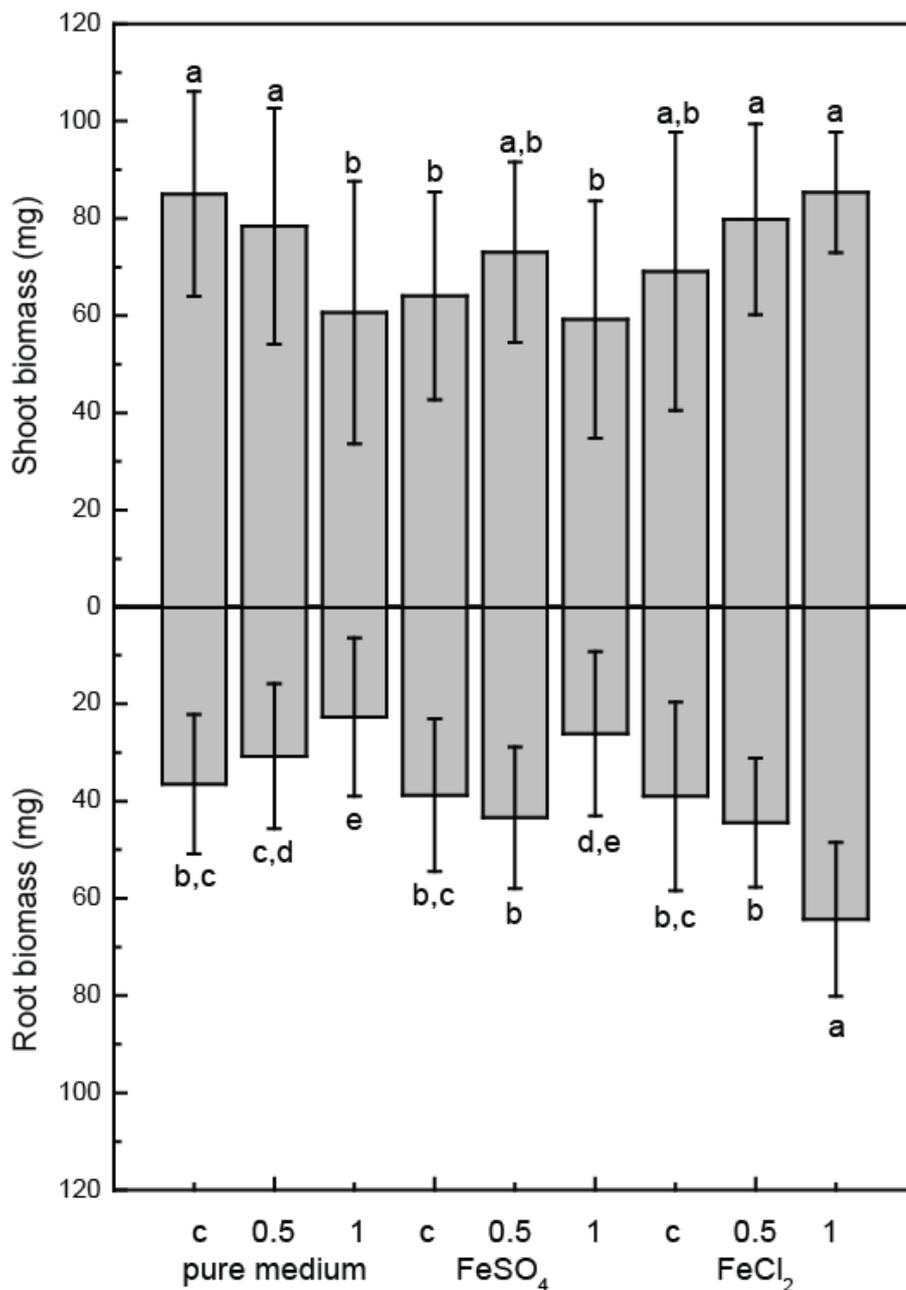


Fig. 2 Bar graphs showing the effects of REE on rice shoot and root biomass. The labels: “pure medium”, “FeSO₄” and “FeCl₂” correspond to experiments conducted in the presence of REE at 0.5 mg/L and 1 mg/L, REE + 100 μmol/L Fe(II) sulfate, and REE + 100 μmol/L Fe (II) chloride respectively. For each of these conditions, labels: “c”, “0.5” and “1” correspond to REE concentrations of 0 mg/L (control), 0.5 mg/L and 1 mg/L respectively. Error bars correspond to the standard deviation of measurements as indicated in the text. There is no statistically significant difference in biomass between conditions when the same letter is shown.

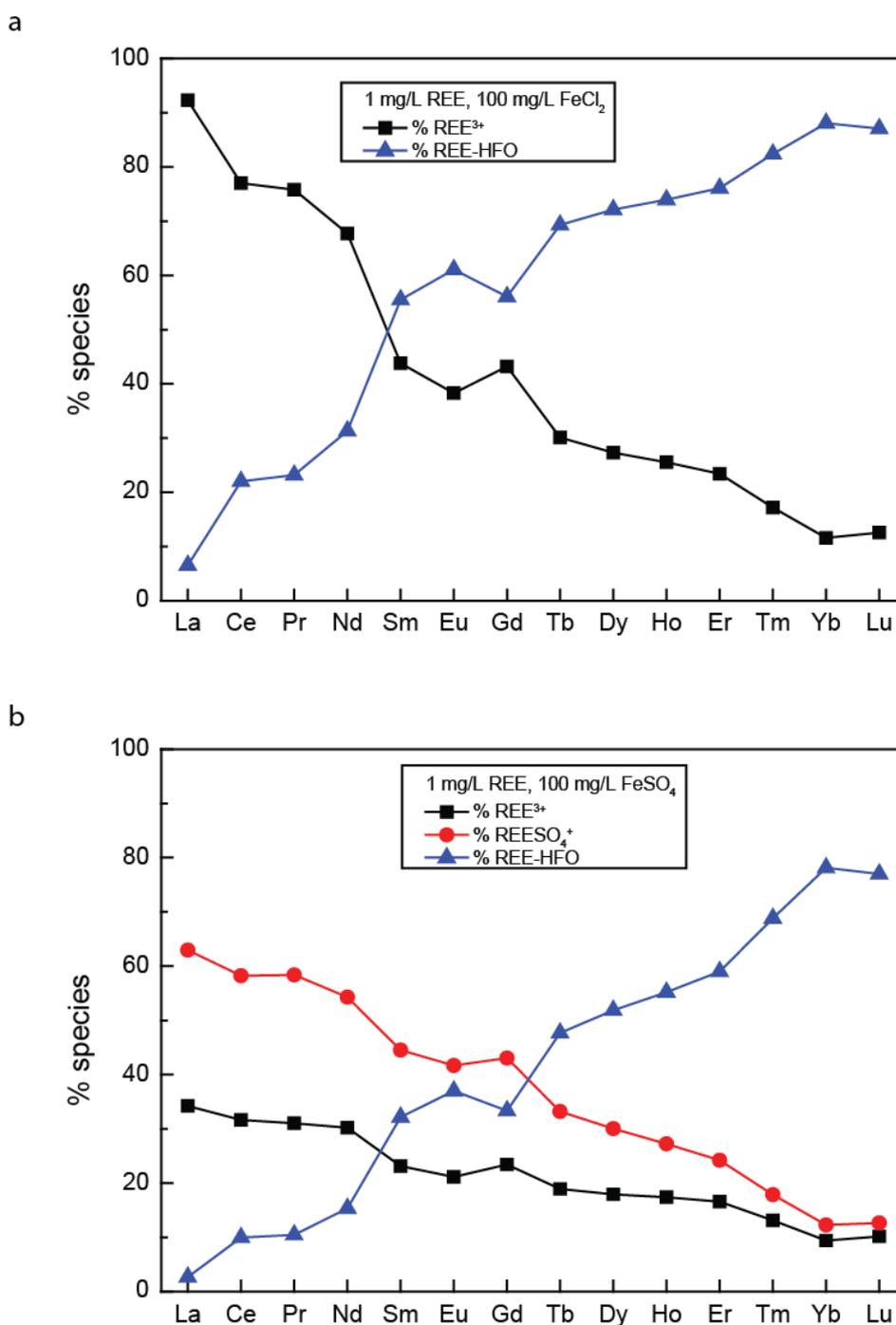


Fig. 3 Results of PHREEQC modeling of REE speciation for the conditions in this study, (a) in the presence of Fe(II) chloride and (b) with Fe(II) sulfate, for 1 mg/L REE and 100 μ mol/L Fe(II) salt. The black square, blue triangle and red circle markers, correspond to the fraction of REE as “free” cations (i.e. Ln³⁺ or REE³⁺), REE iron (III) oxyhydroxide complexes, and REE sulfate complexes, respectively.

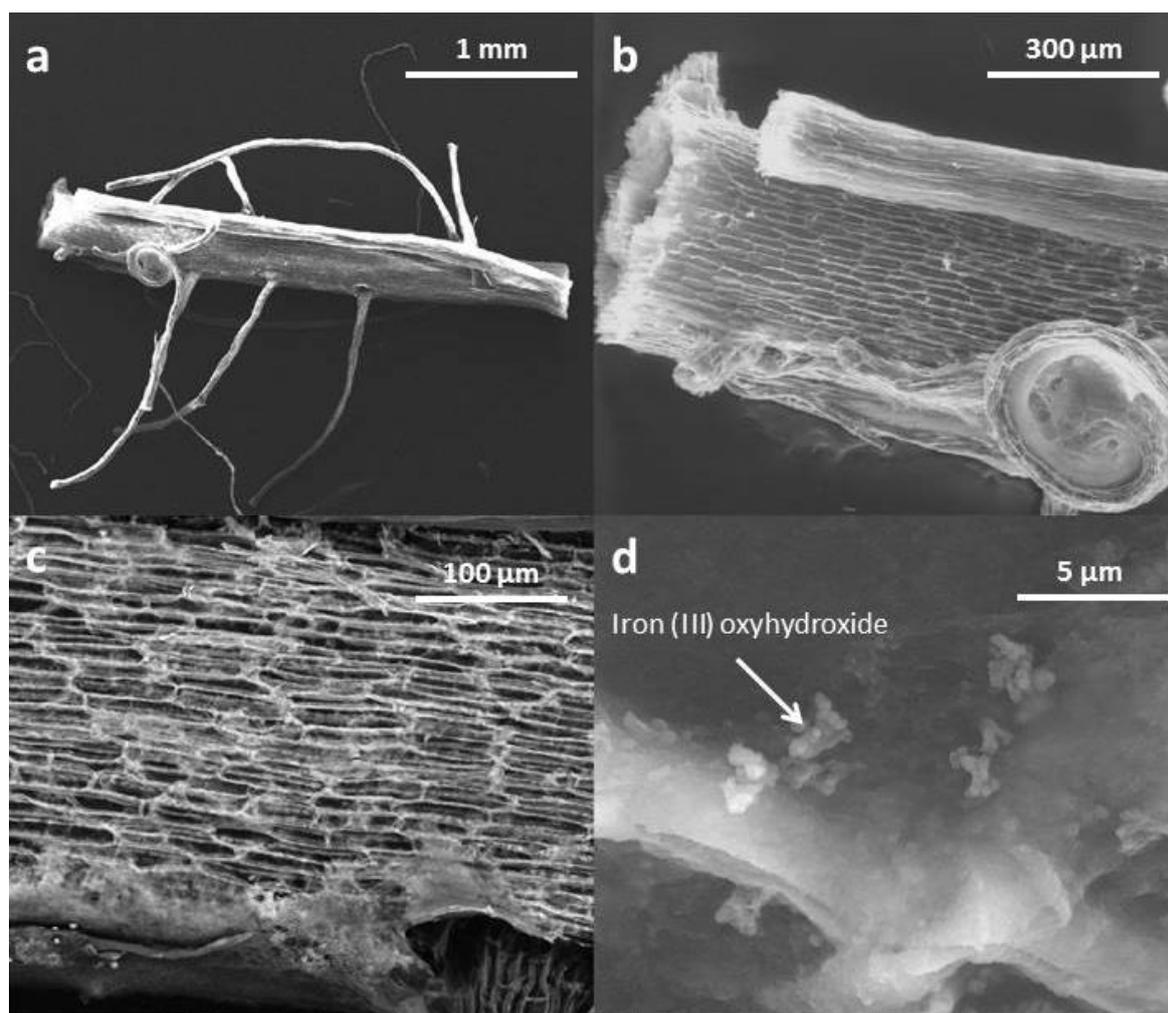


Fig. 4. SEM images of the rice root surface after 14 days of exposure to the 100 $\mu\text{mol/L}$ concentration of Fe (II) sulfate or Fe (II) chloride. (a), (b), (c) and (d) show the iron (III) oxyhydroxide solid phases present on the rice root surface, at increasing resolution.

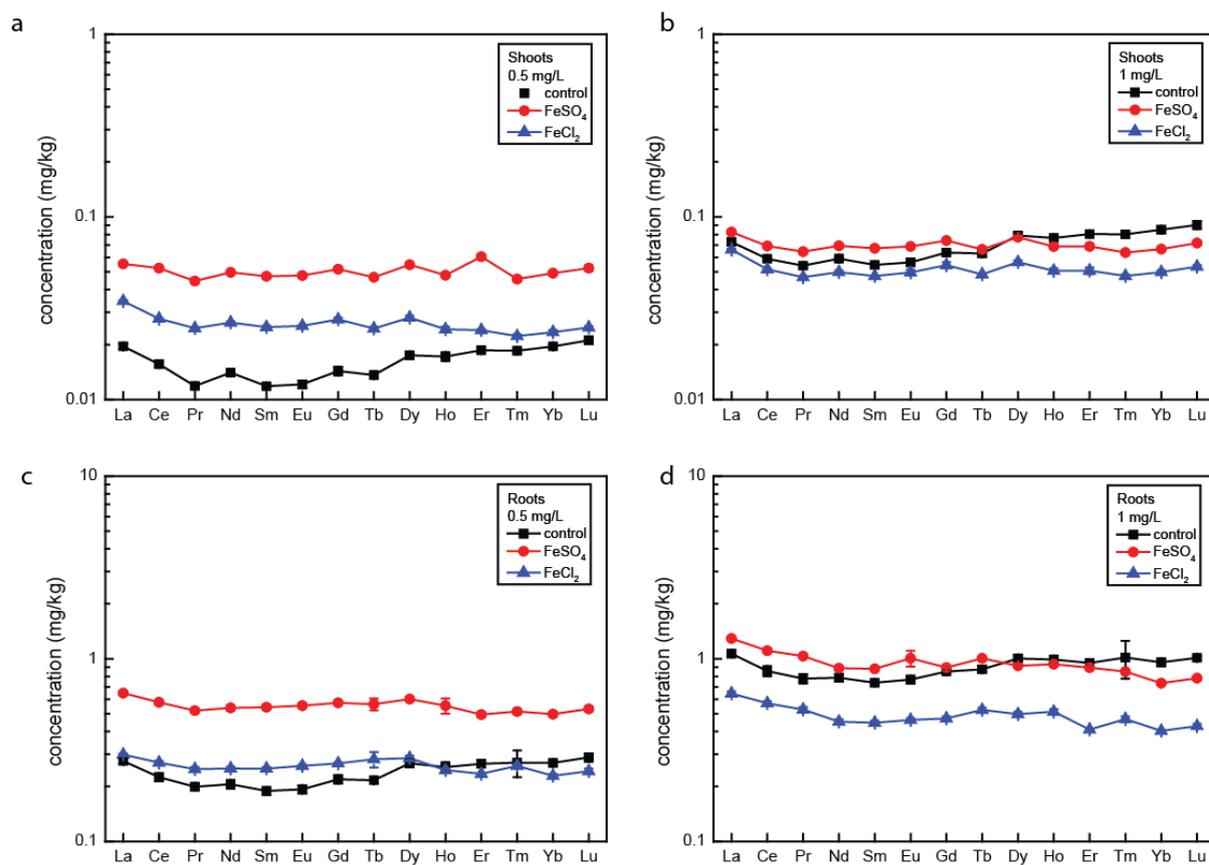


Fig. 5 Rare earth element patterns of shoots (a,b) and roots (c,d) at REE concentrations of 0.5 mg/L and 1 mg/L. Black square, red circle and blue triangle markers correspond to the control condition (no Fe(II) salts added), the Fe(II) sulfate and Fe(II) chloride experimental set-ups, respectively.

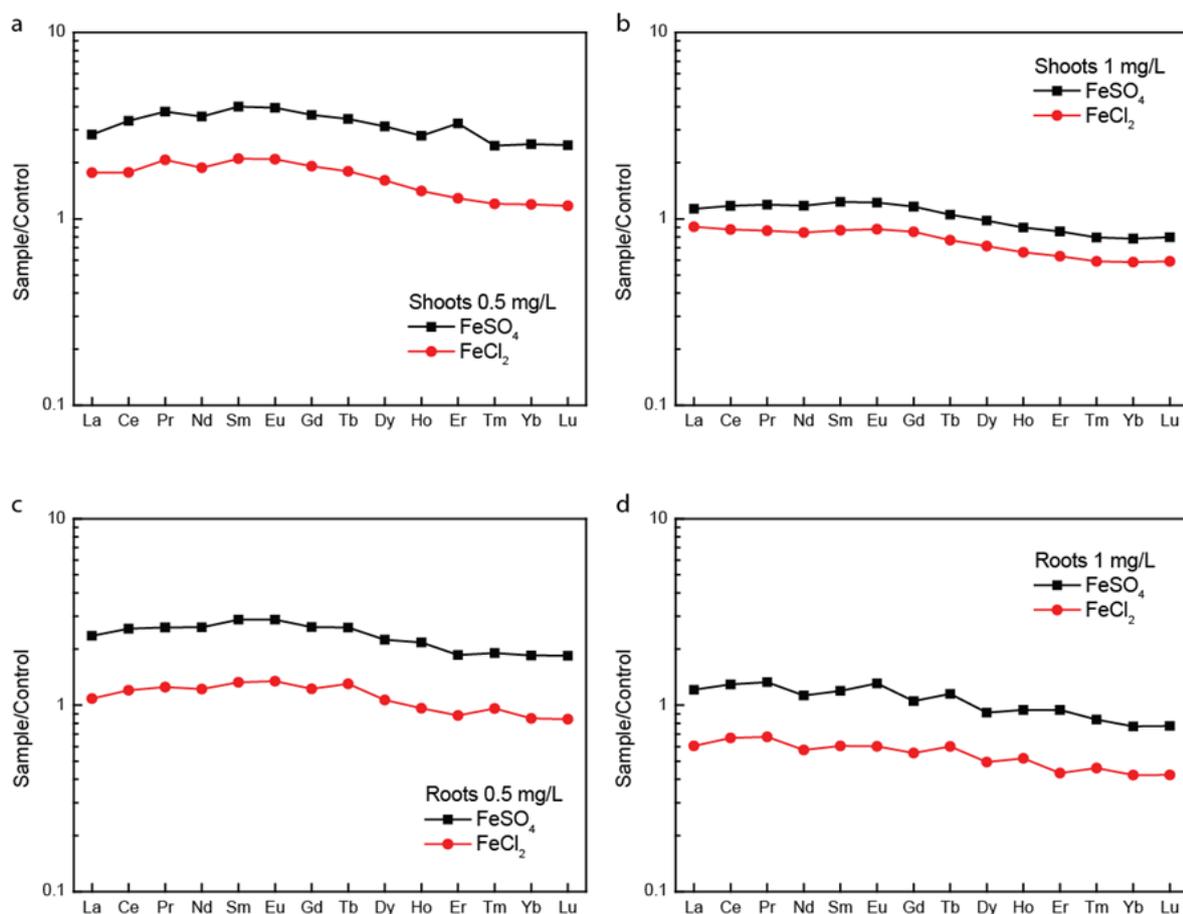


Fig. 6 Rare earth element patterns normalized to control experiment of shoots (a,b) and roots (c,d) at REE concentrations of 0.5 mg/L and 1 mg/L. Black square and red circle markers correspond to the Fe(II) sulfate and Fe(II) chloride experimental set-ups, respectively.

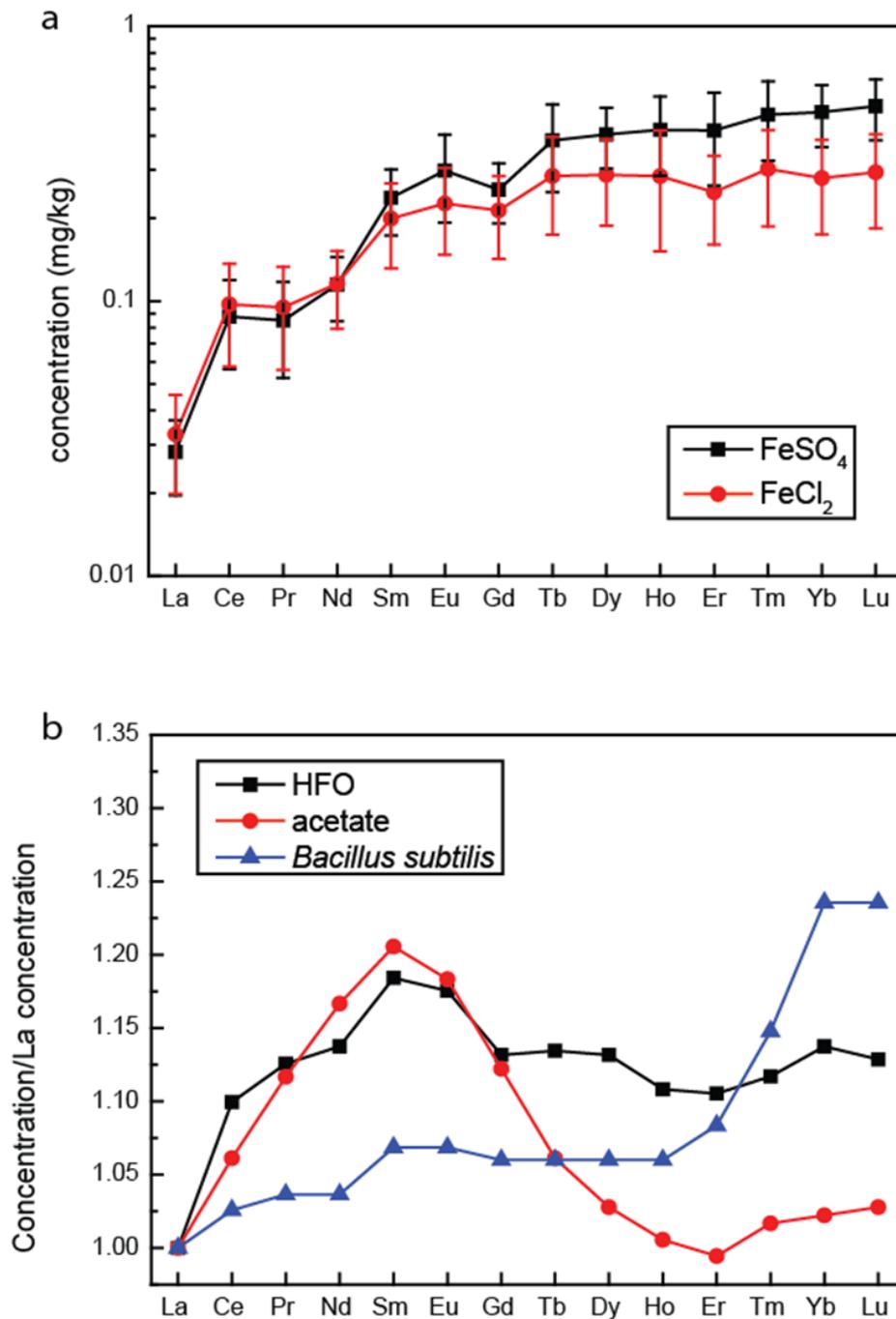


Fig. 7 Rare earth elements patterns of (a) iron plaques (Error bars are shown in the figure, n=2) and (b) HFO, acetate and *Bacillus subtilis* from literature (Davranche et al., 2004; Byrne and Li, 1995; Martinez et al., 2014). Concentrations are normalized to La for comparison purpose.

Effect of rare earth elements on rice plant growth

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Manuscript Highlights

1. Rare earth element affect the growth of rice plant roots and shoots.
2. The presence of sulfate increases the availability rare earth elements to the rice plant.
3. Iron (III) oxides, Cl⁻, and SO₄²⁻ play a role in the REE fractionation and plant growth.