



## Isotopic fingerprints indicate distinct strategies of Fe uptake in rice

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### ABSTRACT

Plants typically take up Fe through either strategy I or strategy II, whereas rice uses both. Stable Fe isotopes potentially reveal pathways of Fe uptake in rice plants. In this study we measured Fe isotopic compositions of rice grown under different conditions, i.e. paddy soil with deficient Fe supply and Fe<sup>3+</sup>-EDTA aqueous solution with sufficient Fe supply, to investigate whether Fe isotope fractionation is distinct under Fe-deficient and Fe-sufficient conditions as well as their possible controlling mechanisms. Our results show that rice grown in the Fe<sup>3+</sup>-EDTA aqueous solutions with sufficient Fe supply preferentially takes up light Fe isotopes ( $\Delta^{56}\text{Fe}_{\text{bulk plant-nutrient}} = -1.36\%$ ) and accumulates Fe in different parts of plant (roots, stems, leaves, husks, and grains) with large Fe isotope fractionation. Under such a growing condition, rice takes up Fe through strategy I and the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> results in plants enriching isotopically light Fe. Within the plant, the transportation of Fe is accompanied with changes in redox state, which thus causes significant Fe isotope fractionation among different plant tissues. In contrast, rice plants grown in soils with deficient Fe supply are slightly enriched in heavy Fe isotopes ( $\Delta^{56}\text{Fe}_{\text{bulk plant-soil solution}} = 0.27\%$ ) and accumulate Fe in different plant tissues with little isotope fractionation. Under this growing condition, the rice plant takes up Fe through strategy II. Plants take up Fe from soils as Fe<sup>3+</sup>-phytosiderophores (Fe<sup>3+</sup>-PS) complex and transport Fe as Fe<sup>3+</sup>-nicotianamine (Fe<sup>3+</sup>-NA) complex throughout the plant, which does not involve changes in redox state and thus results in very limited isotope fractionation. The observed difference in Fe isotope fractionation indicates two distinct Fe uptake and translocation strategies for rice grown under the two different conditions: strategy I under Fe-sufficient conditions, and strategy II under Fe-deficient conditions. Our results demonstrate that Fe isotope ratios may be used to distinguish Fe-sufficient versus Fe-deficient conditions and to elucidate Fe biogeochemical processes during rice growth.

### 1. Introduction

Iron is an essential micronutrient for plants, which is used in DNA synthesis, respiration, and photosynthesis (e.g. Chereskin and Castelfranco, 1982; Connolly and Guerinet, 2002). Although Fe is abundant in soils, Fe acquisition is challenging as it is mainly present in insoluble Fe<sup>3+</sup> forms that are not readily available to plants (Guerinet and Yi, 1994). Insufficient Fe uptake results in Fe-deficiency symptoms of plants and reduction of crop yields (Kim and Guerinet, 2007).

Two distinct strategies (known as ‘strategy I’ and ‘strategy II’) control plant uptake of Fe from the rhizosphere (Marschner et al., 1986). Strategy I plants, including dicots and non-grass monocots, excrete H<sup>+</sup> to enhance the solubility of Fe<sup>3+</sup> in the rhizosphere where Fe<sup>3+</sup> is reduced by the inducible Fe<sup>3+</sup>-chelate reductase to Fe<sup>2+</sup>, which is then transported into plant roots via an Fe<sup>2+</sup>-transporter (Robinson et al., 1999; Vert et al., 2002). Strategy II plants, which are represented by graminaceous plant species, can exude phytosiderophores (PS) to form Fe<sup>3+</sup>-PS complexes that are transported into plant roots via a specific

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membrane transport system (Mori, 1999). Strategy II is usually induced by Fe-deficiency conditions (Chen et al., 2015).

Iron isotope studies have demonstrated that strategy I and strategy II plants exhibit very different Fe isotopic compositions as strategy I plants enrich light Fe isotopes and strategy II plants show no or slight enrichment of heavy Fe isotopes relative to the pool of plant-available Fe (e.g. Guelke and Von Blanckenburg, 2007; Von Blanckenburg et al., 2009; Kiczka et al., 2010; Caldelas and Weiss, 2017; Dauphas et al., 2017; Wu et al., 2018). Similar results were found for Cu isotopes by Ryan et al. (2013), with isotopically light Cu being preferentially incorporated into strategy I plants whereas strategy II plants showed minimal isotope fractionation. It was hypothesized that the different isotope behaviors of Fe for these two groups of plants may be consistent with the different mechanisms of Fe uptake (Guelke and Von Blanckenburg, 2007, 2012; Caldelas and Weiss, 2017). According to these authors, the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> in strategy I plants results in greater Fe isotope fractionation than the non-reductive pathway of strategy II. Strategy II plants do not require reduction of Fe<sup>3+</sup>, and the direct uptake of Fe<sup>3+</sup>-PS complexes may slightly prefer uptake of heavy Fe isotopes, manifesting little or no Fe isotope fractionation in the plants. However, Guelke and Von Blanckenburg (2012) reported that oat, a strategy II plant, exhibited markedly Fe isotope fractionation ( $\Delta^{56}\text{Fe}_{\text{oat-nutrient}} \sim -0.5\%$ ) when it grew hydroponically with sufficient Fe<sup>3+</sup>-EDTA as the Fe source. They attributed the enrichment of light Fe isotopes in the strategy II plant to its complex Fe uptake processes, including reduction of Fe<sup>3+</sup> when Fe is abundant under hydroponic growing condition. This suggests that some plants, classified as either strategy I or II plants, may adapt their uptake mechanisms according to the availability of Fe in their rhizospheres during growth. As suggested by Guelke and Von Blanckenburg (2007), such adaptability of plants to environmental constraints (i.e., Fe sufficient versus Fe insufficient conditions) could be characterized using an Fe isotope approach.

This study was designed to use an Fe isotope approach to evaluate Fe uptake mechanisms for rice grown under Fe sufficient versus Fe deficient conditions. We chose rice as the target plant since it is the most important crop in Southeast Asia including China. Rice is generally considered as a strategy II plant (Robinson et al., 1999), but recent studies showed that rice plants could directly take up Fe<sup>2+</sup> via an Fe<sup>2+</sup> transporter (Ishimaru et al., 2006; Kim and Guerinot, 2007). It is therefore likely that Fe uptake mechanisms in rice depend on the bioavailability of Fe in paddy soils. Two groups of rice plants, one grown hydroponically in nutrient solutions with Fe<sup>3+</sup>-EDTA (sufficient Fe supply), and another grown naturally in a paddy field (deficient Fe supply), were used to study the Fe isotope ratios of soil/nutrient solutions and plant tissues. The Fe uptake and translocation strategies were assessed according to Fe isotope fractionation among soil/nutrient solutions and different tissues of the rice plant.

## 2. Materials and methods

### 2.1. Rice culture and pretreatment

Rice plants (*Youyou 128*) were hydroponically cultivated in an environmentally controlled greenhouse (day 25 °C/night 20 °C; RH 70–95%) using full strength Kimura B nutrient solution containing 370 μM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 550 μM MgSO<sub>4</sub>·7H<sub>2</sub>O, 90 μM K<sub>2</sub>SO<sub>4</sub>, 180 μM KNO<sub>3</sub>, 370 μM Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 180 μM KH<sub>2</sub>PO<sub>4</sub>, 1 μM CuSO<sub>4</sub>·5H<sub>2</sub>O, 5 μM MnSO<sub>4</sub>·H<sub>2</sub>O, 10 μM H<sub>3</sub>BO<sub>3</sub>, 0.5 μM Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 100 μM NaCl, 0.2 μM CoSO<sub>4</sub>·7H<sub>2</sub>O, and 1 μM ZnSO<sub>4</sub>·7H<sub>2</sub>O at pH 5.6 (Guo et al., 2007). In particular, the 50 μM Fe<sup>3+</sup>-EDTA solution was part of the nutrient solution but was added separately. The Fe<sup>3+</sup>-EDTA solution was prepared by mixing solid EDTA (Sigma-Aldrich, purified grade ≥ 98.5%) and FeCl<sub>3</sub> salt (Sigma-Aldrich, purified grade ≥ 99%) with ultrapure water (18.2 MΩ·cm). The pH of the solution was adjusted to 5.6 with sodium bicarbonate. Rice seeds were sterilized with 10% H<sub>2</sub>O<sub>2</sub> (v/v) for 10 min followed by rinsing with ultrapure water (18.2 MΩ·cm) for

**Table 1**

Key physicochemical properties and element concentrations of paddy soil.

pH	Density	TOC	Illite	Quartz	Kaolinite	Calcite
(H <sub>2</sub> O)	(g/cm <sup>3</sup> )	(g/kg)	(%)	(%)	(%)	(%)
7.54	0.83	28.5	5.1	61.3	29.1	4.4

SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MnO	TiO <sub>2</sub>	MgO	CaO	Na <sub>2</sub> O	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	LOI
(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
59.15	14.69	6.32	0.07	0.97	1.33	2.95	0.31	1.74	0.21	12.26

10 min. After being soaked in sterile deionized water for 1 day at 37 °C, they were germinated at 25 °C for 3 days on moist filter paper placed in Petri dishes. After germination, seedlings were grown in deionized water for 7 days, and subsequently cultivated in 1/2 Kimura B solution until the fifth leaf appeared. The plants were then divided and three plants were transferred into PVC pots (2 L-volume) filled with nutrient solution which was replaced every 3 days. The plants were matured and harvested after cultivation for 120 days.

The same kind of rice plant as the hydroponically cultivated rice in the mature stage was collected from the field of Guangxi Province, southwestern China (107° 56′ 26″ E, 23° 02′ 55″ N). Paddy soils are alkaline (pH > 7) and Fe is less active (showing an Fe-deficient condition) due to the dominant karst. Bulk plants and soil samples were collected, and then immediately sealed in airtight plastic bags and refrigerated in the field. The samples were transported to the laboratory within 24 h. An aliquot of soil sample was air-dried for 72 h. Soil solutions were obtained in the lab by centrifuging another aliquot of wet soil, followed by filtering the supernatant through 0.22-μm MCE syringe filters (Fisher Scientific, USA). Key physicochemical properties and element concentrations of paddy soil were listed in Table 1 and several of them were reported in Gao et al. (2018).

Both hydroponically and field grown rice was rinsed with ultrapure water (18.2 MΩ·cm) to remove soil particles. Roots, stems, leaves, husks, and grains of three hydroponic plants and two field plants were separated with ceramic scissors. Iron plaque on the root surface was removed using 0.5 M HCl. The plant tissues were dried in an oven at 105 °C for 1.5 h and then at 75 °C for 48 h before being weighed. All soil and plant tissue samples were crushed using an agate mortar before sample digestion.

### 2.2. Sample digestion

All reagents used during sample treatments were ultrapure grade and prepared with ultrapure water. The BV-III grade HNO<sub>3</sub>, HCl, and HF (Beijing Institute of Chemical Reagents, China) were further distilled by sub-boiling distillation (DST-1000, Savillex, USA). All preparation procedures were conducted in a clean lab in class 1000. Approximately 200 mg of plant samples and 20 mg of soil samples were digested using a microwave digestion system (Milestone, Italy) in the mixture of concentrated HNO<sub>3</sub> and HF, evaporated on a hotplate in Teflon beakers (Savillex, USA), and treated with a mixture of 30% H<sub>2</sub>O<sub>2</sub> (Fisher Scientific, USA) and concentrated HNO<sub>3</sub>. Solution samples were directly treated with 30% H<sub>2</sub>O<sub>2</sub> and concentrated HNO<sub>3</sub>, and then heated on a hot plate with cap at 70 °C for 1 h to oxidise organic compounds. Subsequently, each sample solution was split into two aliquots which were prepared in 3% HNO<sub>3</sub> and 6 M HCl for Fe concentration analysis and column purification, respectively.

### 2.3. Iron concentration and Fe isotope analyses

Iron concentrations were determined by ICP-OES (Perkin Elmer Sciex, USA) at the Guangdong Institute of Eco-environmental Sciences

& Technology, Guangdong, China. Iron isotope analysis was conducted on a Neptune Plus MC-ICP-MS (Thermo Scientific, USA) at the University of Science and Technology of China, Hefei, following the procedures recently described in Gong et al. (2017). Briefly, Fe was purified using AG1-X8 anion resin (100–200 mesh, Bio-Rad, USA) with HCl media. Matrix elements (e.g. Na, Al, Ca, and Mg) were removed by washing with 4 mL of 6 M HCl. Iron was consequently eluted using 4 mL of 0.4 M HCl, 1 mL of 8 M HCl, and 0.5 mL of H<sub>2</sub>O. The purified samples were analyzed for the content of Fe, and then diluted to 2 µg/mL in 2% HNO<sub>3</sub>. Iron isotopic compositions were determined on a Neptune Plus MC-ICPMS operated in wet plasma and high-resolution mode, with the sample-standard bracketing method. The Fe isotopic compositions were reported as δ<sup>56</sup>Fe and δ<sup>57</sup>Fe relative to the IRMM-014 standard:

$$\delta^x\text{Fe}_{\text{sample}} = [({}^x\text{Fe}/{}^{54}\text{Fe})_{\text{sample}}/({}^x\text{Fe}/{}^{54}\text{Fe})_{\text{IRMM-014}} - 1] \times 1000 \quad (1)$$

where x refers to mass 56 or 57. Long term external reproducibility was 0.05‰ (2SD) for δ<sup>56</sup>Fe based on replicate runs of an in-house standard. Repetitive analyses of USGS geological reference materials BHVO-2 (basalt) and AGV-2 (andesite) during the course of this study yielded average δ<sup>56</sup>Fe values of 0.09 ± 0.04‰ (2SD) and 0.12 ± 0.06‰, respectively (Table 2), which are in agreement with previously published values within uncertainty (He et al., 2015; An et al., 2017). The Fe isotopic compositions of all samples fall on a single mass-dependent fractionation line with a slope of 1.481 on the three-isotope diagram (not shown), indicative of mass-dependent fractionation of Fe isotopes.

### 3. Results

Iron concentrations and isotopic compositions of soils, soil solutions, nutrient solutions, roots, stems, leaves, husks, and grains are shown in Table 2. The average Fe isotopic composition of bulk plant (δ<sup>56</sup>Fe<sub>bulk plant</sub>) was calculated using the following equation:

$$\delta^{56}\text{Fe}_{\text{bulk plant}} = \frac{\sum (\delta^{56}\text{Fe}_n \times m_n \times c_n)}{\sum (m_n \times c_n)} \quad (2)$$

where n is the plant tissue (roots, stems, leaves, husks, and grains), m is the dry mass, and c is the Fe concentration.

#### 3.1. Iron in field rice

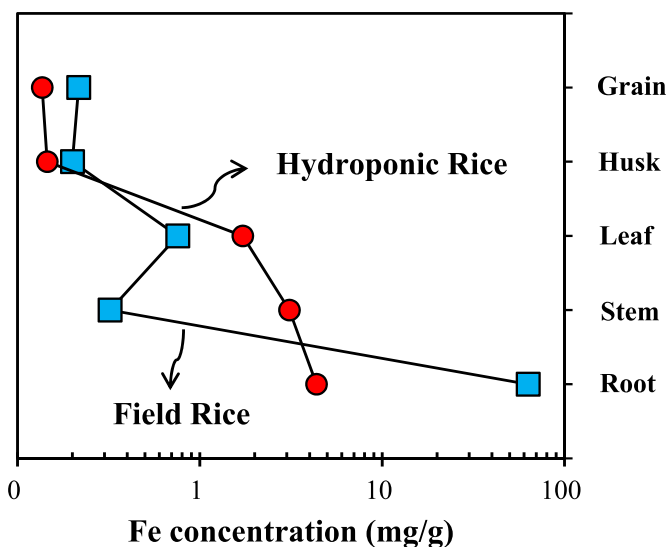
Field rice shows that the Fe concentration decreases from roots to stems, increases from stems to leaves, decreases from leaves to husks, and slightly increases from husks to grains (Fig. 1). The δ<sup>56</sup>Fe value of

**Table 2**

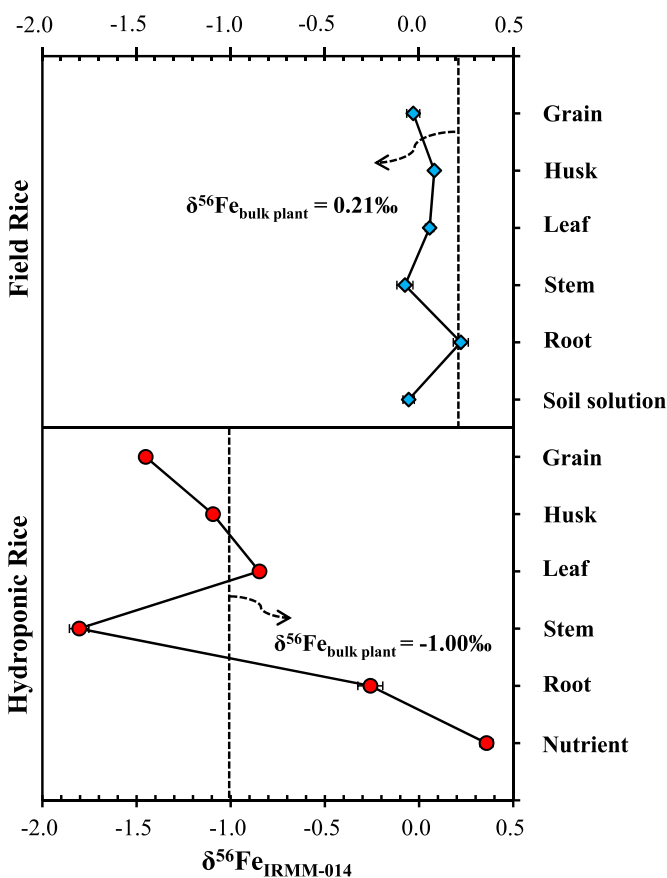
Iron concentrations and isotopic compositions of soils, soil solutions, nutrients, roots, stems, leaves, husks, grains, and geological reference materials.

Type	Sample	Fe conc. (µg/g)	δ <sup>56</sup> Fe	2SD	δ <sup>57</sup> Fe	2SD	N	
Hydroponic rice	Nutrient	2.8	0.36	0.03	0.55	0.14	3	
	Root	4360	-0.26	0.07	-0.36	0.14	6	
	Stem	3100	-1.80	0.05	-2.64	0.02	3	
	Leaf	1720	-0.85	0.01	-1.23	0.08	3	
	Husk	145	-1.10	0.02	-1.63	0.03	3	
Field rice	Grain	137	-1.45	0.02	-2.14	0.03	3	
	Soil	44,240	0.05	0.06	0.10	0.07	3	
	Soil solution	1.68	-0.06	0.03	-0.14	0.12	2	
	Root	63,150	0.22	0.04	0.35	0.08	3	
	Stem	321	-0.08	0.04	-0.11	0.08	3	
	Leaf	756	0.06	0.02	0.08	0.03	3	
	Husk	200	0.08	0.00	0.09	0.02	3	
	Grain	216	-0.03	0.03	-0.08	0.01	3	
	Geological reference materials	BHVO-2	/	0.09	0.04	0.11	0.02	3
		AGV-2	/	0.12	0.06	0.20	0.11	3

Notes: / represents no measurement.



**Fig. 1.** Iron concentrations of roots, stems, leaves, husks, and grains in the field and hydroponic rice.



**Fig. 2.** Iron isotopic compositions of soil/nutrient solutions, roots, stems, leaves, husks, and grains in the field and hydroponic rice. The dotted lines refer to the calculated Fe isotopic compositions of bulk plant. Error bars represent 2SD uncertainties.

rice tissues varies from -0.08‰ to 0.22‰ (Fig. 2). The δ<sup>56</sup>Fe value of the bulk plant is 0.21‰, which is slightly heavier by about 0.27‰ than that of the soil solution (-0.06‰). Within the plant, except for roots other tissues (stems, leaves, husks, and grains) have indistinguishable δ<sup>56</sup>Fe values (-0.08 to 0.08‰). However, roots (0.22‰) have slightly heavier δ<sup>56</sup>Fe values compared with other tissues. The δ<sup>56</sup>Fe value of

the soil solution is  $-0.06 \pm 0.03\text{‰}$ , slightly lighter than that of the bulk soil ( $0.05 \pm 0.06\text{‰}$ ).

### 3.2. Iron in hydroponic rice

Hydroponic rice shows that the Fe concentration successively decreases from roots to grains (Fig. 1). The  $\delta^{56}\text{Fe}$  values of plant tissues range from  $-1.80\text{‰}$  to  $-0.26\text{‰}$  (Fig. 2). Except for the stem with the lightest Fe isotopic composition ( $-1.80\text{‰}$ ), Fe in different tissues becomes increasingly lighter from the root to grain ( $-0.26$  to  $-1.45\text{‰}$ ). The  $\delta^{56}\text{Fe}$  value of the bulk plant is  $-1\text{‰}$ , which is  $-1.36\text{‰}$  lighter compared to the nutrient solution ( $0.36\text{‰}$ ).

## 4. Discussion

### 4.1. Uptake of Fe in the rice plant with sufficient and deficient Fe supplies

Mass balance (Eq. (2)) shows that the bulk plant being hydroponically cultured has a lighter  $\delta^{56}\text{Fe}$  value than the nutrient solution (Fig. 2), indicating a preferential uptake of light Fe isotopes by the plant. This finding is consistent with the preferential incorporation of isotopically light Fe by the rice root, in which the  $\delta^{56}\text{Fe}$  value is  $0.62\text{‰}$  lighter than the nutrient solution (Fig. 2). Our results for hydroponic rice are incompatible with most previous reports, which showed that strategy II plants exhibited no or slightly positive Fe isotope fractionation during uptake of Fe (Guelke and Von Blanckenburg, 2007; Guelke et al., 2010). However, our results agree with the observation that the strategy I plant significantly enriches isotopically light Fe (Guelke and Von Blanckenburg, 2012). It was suggested that the distinct behaviors of Fe isotope for these two groups of plants result from the different uptake mechanisms (Guelke and Von Blanckenburg, 2007, 2012; Von Blanckenburg et al., 2009; Caldelas and Weiss, 2017; Dauphas et al., 2017; Wu et al., 2018). The reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  in strategy I plants results in a greater Fe isotope fractionation, whereas the non-reductive pathway of strategy II plants fractionates Fe isotopes limitedly (Guelke and Von Blanckenburg, 2007, 2012; Guelke et al., 2010). The hydroponic rice in this study was grown on the  $\text{Fe}^{3+}$ -EDTA solution being replaced every 3 days and thus with sufficient Fe supply. The speciation of the nutrient solution was modeled with the MINTEQ 3.0 (Gustafsson, 2011). Modeling results show that about 99.2% of the EDTA is present as  $\text{Fe}^{3+}$ -EDTA, suggesting that Fe is highly available to plants. Under such a Fe-rich condition, plants prefer to take up  $\text{Fe}^{2+}$  and the stimulation of their plant-specific Fe mobilization strategies is not required, because the  $\text{Fe}^{2+}$  acquisition strategy is especially advantageous for rice compared to the strategy II (Kim and Guerinet, 2007). This is consistent with the observation that the exudation of PS is suppressed in Fe-sufficient conditions (Marschner, 2011). In this case, the hydroponically cultured rice can behave like a strategy I plant and reduces  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  before being incorporated (Fig. 3). It is known that the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  is associated with a mass-dependent isotope fractionation and prefers light isotopes (Johnson et al., 2002; Welch et al., 2003), thereby resulting in enriched isotopically light Fe in the hydroponic rice. A similar enrichment result of light Fe isotopes has also been found in oat, which was even classified as a strategy II plant (Guelke and Von Blanckenburg, 2012). They found that oat was markedly enriched in isotopically light Fe ( $\Delta^{56}\text{Fe}_{\text{oat-nutrient}} \sim -0.5\text{‰}$ ) when it grew hydroponically with sufficient  $\text{Fe}^{3+}$ -EDTA as the Fe source. These authors attributed the enrichment of light Fe isotopes to the reduction of  $\text{Fe}^{3+}$  when Fe is abundant under hydroponic growing conditions. It thus can be concluded that the plants, classified as either strategy I or II plants, can use strategy I to take up Fe when Fe is abundant and highly available to plants.

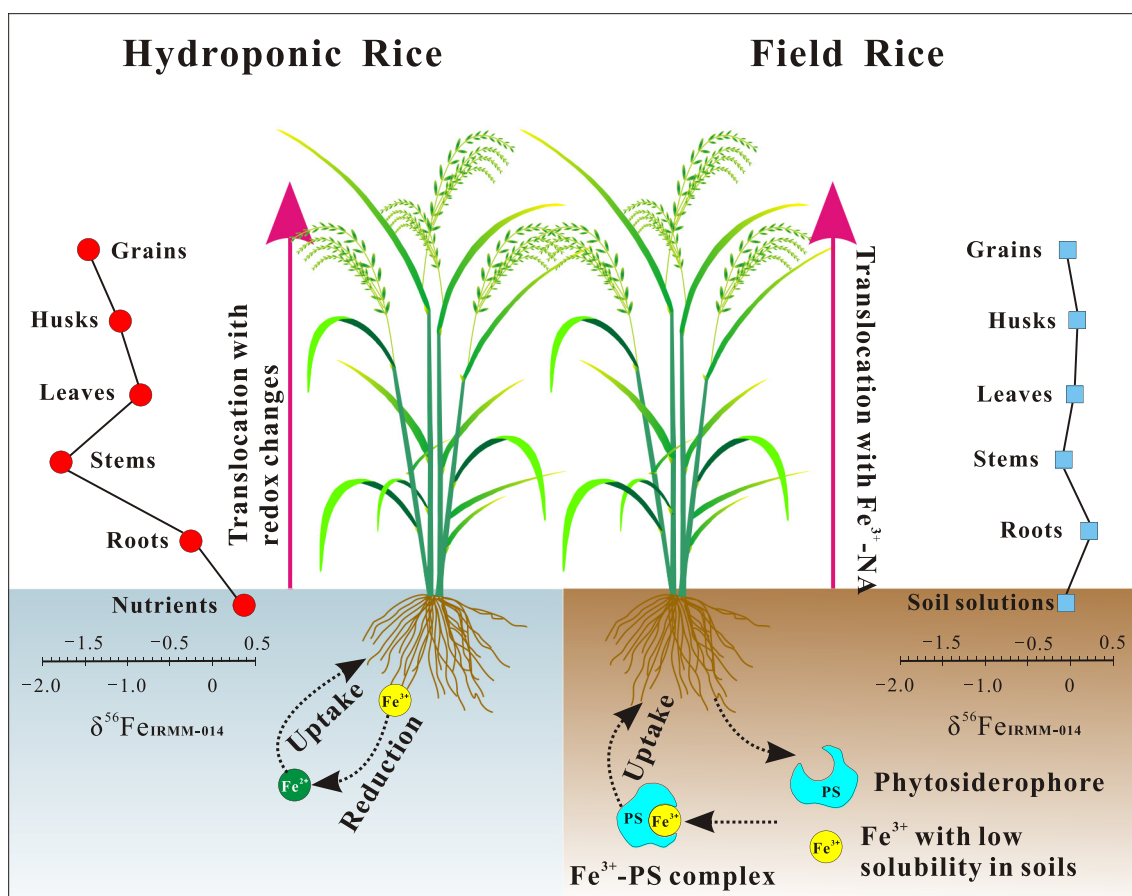
Unlike the hydroponic rice plants, the bulk rice grown in soils has Fe isotope ratios slightly heavier than the soil soluble Fe (Fig. 2). This is consistent with the observation that the root slightly accumulates heavier Fe isotopes compared with the soil soluble Fe ( $\Delta^{56}\text{Fe}_{\text{root-soil}}$

$\sim 0.28\text{‰}$ ). These findings agree with previously reported Fe isotopic compositions of strategy II plants grown in soil substrates, which are similar to, or slightly heavier than that of the plant-available Fe pool (Guelke and Von Blanckenburg, 2007; Guelke et al., 2010). Field rice was grown on latosolic red soils where karst is widely developed. The carbonate is the dominant rock, which contributes to the high soil pH ( $> 7$ ) in this area (Table 1). Because Fe(III) oxides remain stable and not soluble in alkaline environments, the concentration of free Fe in the soil solution is quite low, even less than that required for the optimal growth of plants ( $10^{-9}$ – $10^{-4}$  M) (Guerinet and Yi, 1994; Marschner, 2011). For example, Kim and Guerinet (2007) concluded that the concentration of free  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  in well-aerated soils at physiological pH is  $< 10^{-15}$  M, which is far below that required for optimal growth. As such, rice grown in soils is forced to excrete PS to satisfy the demand of Fe and behaves like a strategy II plant for Fe uptake (Fig. 3) (Curie et al., 2001; Kim and Guerinet, 2007; Chen et al., 2015). However, the  $\text{Fe}^{3+}$ -PS membrane transport process will unlikely result in significant Fe isotope fractionation, because 1) the relative mass difference between the  $\text{Fe}^{3+}$ -PS and  $\text{Fe}^{3+}$ -EDTA complex is too small (both the species as a whole are too big), and 2) no redox changes occur.

In summary, the rice plant may adapt their uptake mechanisms according to the availability of Fe in their rhizospheres during growth. Rice grown in the  $\text{Fe}^{3+}$ -EDTA aqueous solution with high Fe content behaves like a strategy I plant, with significantly light Fe isotopes enriched in plants. In contrast, rice grown in soils with deficient Fe supply behaves like a strategy II plant, with little isotope fractionation occurred during uptake of Fe.

### 4.2. Translocation of Fe in the rice plant with sufficient and deficient Fe supplies

Except for the increased  $\delta^{56}\text{Fe}$  value from stems to leaves,  $\delta^{56}\text{Fe}$  value decreases from roots to stems, from leaves to husks, and from husks to grains in hydroponic rice plants (Fig. 2). This is in agreement with the mechanism previously proposed for Fe translocation in strategy I plants (Guelke and Von Blanckenburg, 2007, 2012; Kiczka et al., 2010), within which different redox transitions may occur during Fe translocation (Fig. 4). Once Fe enters the root symplasm of strategy I plants, Fe is commonly bound by chelating compounds, such as nictotianamine (NA) and citrate, and then transports throughout the plants (Hell and Stephan, 2003; Kim and Guerinet, 2007). It was suggested that, within the root symplasm, Fe is chelated by NA as  $\text{Fe}^{2+}$ -NA (step 1, Fig. 4) (Hell and Stephan, 2003). The release of Fe from the root into the xylem requires oxidation of  $\text{Fe}^{2+}$  and then transports as  $\text{Fe}^{3+}$ -citrate complex to the xylem (step 2) (Tiffin, 1966; Hell and Stephan, 2003; Kim and Guerinet, 2007). Although oxidation reaction occurs at this step, limited Fe isotope fractionation is expected because of the quantitative export of Fe from the root to upper parts. When Fe transfers from the xylem to the leaf cytoplasm (step 3),  $\text{Fe}^{3+}$  should be reduced to  $\text{Fe}^{2+}$  and then transports as  $\text{Fe}^{2+}$ -NA complex (Briat et al., 2007). Isotopically light Fe would therefore accumulate in the leaf cytoplasm, given that the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  prefers light isotopes (Johnson et al., 2002; Welch et al., 2003). After transportation from the xylem to the leaf,  $\text{Fe}^{2+}$  is again oxidized and then stored as  $\text{Fe}^{3+}$ -ferritin in the leaf cytoplasm (Marschner, 2011). Remobilization of Fe from the leaf to the stem phloem involves another reduction reaction, with  $\text{Fe}^{2+}$ -NA complex stored in the phloem (step 4) (Briat et al., 2007), which thus further favors light Fe isotopes. Multiple reduction reactions result in the phloem Fe with lighter isotopic compositions than the xylem Fe. The Fe isotopic composition in the stem is controlled by the relative proportion of phloem Fe and xylem Fe. When the phloem Fe dominates in the stem, the rice stem exhibits the lower  $\delta^{56}\text{Fe}$  value than that in the root and leaf. In this case,  $\delta^{56}\text{Fe}$  value in rice tissues would follow the order of stem  $<$  leaf  $<$  root as observed in the hydroponic rice (Fig. 2). When remobilized through importing Fe from the leaf into



**Fig. 3.** Conceptual model of Fe isotope fractionation processes in the field and hydroponic rice. For hydroponic rice, plants directly take up  $\text{Fe}^{2+}$ , after the reduction of  $\text{Fe}^{3+}$ , via an  $\text{Fe}^{2+}$ -transporter as strategy I plants. For field rice, plants exude PS to form  $\text{Fe}^{3+}$ -PS complexes that are then transported into plant roots via a specific membrane transport system.

the seed, Fe might be transported as  $\text{Fe}^{2+}$ -NA complex (step 5) (Chasteen, 1998; Hell and Stephan, 2003), in which isotopically light Fe is thus expected to be enriched in the seed. The detailed isotope fractionation process when Fe enters the husk is not clear due to the lack of detailed knowledge about how Fe transports into the husk, in what form, and if redox changes occur. According to the visible variation in Fe isotopic composition among the leaf, husk, and seed, it is expected that redox changes may have occurred when Fe enters the husk.

Unlike the hydroponic rice plant, field rice plants grown in soils have more uniform Fe isotopic compositions among different tissues (Fig. 2). This agrees with the finding that the translocation of Fe within strategy II plants produces limited Fe isotope fractionation (Guelke and Von Blanckenburg, 2012). The detailed mechanism corresponding to the Fe transfer as  $\text{Fe}^{3+}$ -PS complex in strategy II plants remains unclear and controversial (Briat et al., 2007). Hell and Stephan (2003) suggested that NA chelates  $\text{Fe}^{2+}$  but citrate chelates  $\text{Fe}^{3+}$  in the plant (Hell and Stephan, 2003; Kim and Guerinot, 2007). The changes in chelating compounds are thus coupled with redox changes of Fe. However, von Wirén et al. (1999) demonstrated that  $\text{Fe}^{3+}$  can be chelated by NA rather than  $\text{Fe}^{2+}$ . In this case, Fe taken up by strategy II plants as  $\text{Fe}^{3+}$ -PS complexes would not need to be reduced and NA can directly chelate  $\text{Fe}^{3+}$  to form the  $\text{Fe}^{3+}$ -NA complex for subsequent internal transport to other parts of the plant. If this process was shown to proceed in the rice grown in soils, we can speculate that the Fe accumulated in rice plants may translocate mainly in the form of  $\text{Fe}^{3+}$ -NA without redox changes, which thus lead to limited Fe isotope fractionation throughout the plant (Fig. 3).

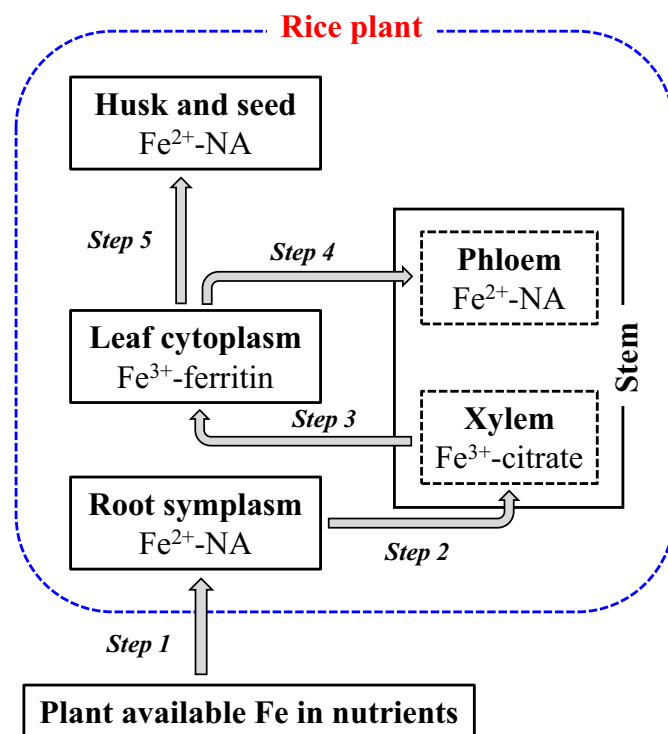
In summary, the rice plant grown in the  $\text{Fe}^{3+}$ -EDTA aqueous solution transports Fe is accompanied by changes in redox state, which results in significant Fe isotope fractionation throughout the plant. In contrast, Fe in the rice plant grown in soils is transported as  $\text{Fe}^{3+}$ -NA without redox changes, with limited isotope fractionation occurred among plant tissues.

## 5. Conclusions and implications

The results of this study revealed distinct patterns of Fe isotope fractionation in rice plants grown in Fe-rich nutrient media hydroponically and in paddy soils. The hydroponic rice plants showed much greater Fe isotope fractionation than the rice plants grown in the paddy field. This difference indicates two distinct Fe uptake strategies under Fe-sufficient and Fe-deficient conditions. Under Fe-sufficient hydroponic conditions, rice plants take up and translocate Fe mainly following strategy I with redox changes, which can produce large Fe isotope fractionation. By contrast, under Fe-deficient conditions in field, rice plants take up Fe via the  $\text{Fe}^{3+}$ -PS complex (strategy II) and translocate Fe via  $\text{Fe}^{3+}$ -NA complex, during which no redox changes occur and Fe isotope fractionation is limited. The present results indicate that Fe isotopic compositions may be used to fingerprint different strategies of Fe uptake and translocation in related species of plants.

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**Fig. 4.** Scheme of Fe pools in the rice system grown in Fe<sup>3+</sup>-EDTA nutrient solutions. Arrows indicate the dominant Fe pathways and transfers between these pools, with numbers indicating the potential fractionation steps that are explained in the text.

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