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# Impact of Flooding–Drainage Alternation on Fe Uptake and Transport in Rice: Novel Insights from Iron Isotopes

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**ABSTRACT:** Iron (Fe) isotopes were utilized to provide insights into the temporal changes underlying Fe uptake and translocation during rice growth (tillering, jointing, flowering, and maturity stages) in soil–rice systems under typical flooding–drainage alternation. Fe isotopic composition ($\delta^{56}$Fe values) of the soil solution generally decreased at vegetative stages in flooding regimes but increased during grain-filling. Fe plaques were the prevalent source of Fe uptake, as indicated by the concurrent increase in the $\delta^{56}$Fe values of Fe plaques and rice plants during rice growth. The increasing fractionation magnitude from stem/nodes I to flag leaves can be attributed to the preferred phloem transport of light isotopes toward grains, particularly during grain-filling. This study demonstrates that rice plants take up heavy Fe isotopes from Fe plaque and soil solution via strategy II during flooding and the subsequent drainage period, respectively, thereby providing valuable insights into improving the nutritional quality during rice production.

**KEYWORDS:** *flooding–drainage alternation, Fe isotope fractionation, rice plant, uptake, transport, Oryza sativa*



## INTRODUCTION

Iron (Fe) is an essential mineral nutrient for plant growth and is highly abundant in the Earth's crust. Aerated soil predominantly contains Fe(III) hydroxides, which are highly insoluble and thus less available to rice plants.[1] Rice is a strategy-II plant that secretes iron-avid phytosiderophores of the mugineic acid family to chelate $Fe^{3+}$, and stable complexes are taken up.[2] Nongraminaceous plants and dicots absorb iron through a process involving ATPase-driven proton extrusion and the conversion of Fe(III) to Fe(II) by plasma membrane-bound surface reductase (FRO).[1] However, rice possesses an additional strategy-I-like IRT-dependent system for $Fe^{2+}$ uptake,[3] probably as an adaptation response to the excess $Fe^{2+}$ produced by dissimilatory Fe(III) reduction during the flooding season. Although rice has developed two elaborately regulated strategies that coexist to acquire iron from the rhizosphere[4,5] under flooding conditions, periodic flooding and drainage strongly affect the solubility and mobility of soil Fe, resulting in a sequential growth of rice that is characterized by either Fe excess or deficiency in the soil solution. Therefore, the mechanism underlying temporal changes in Fe uptake during rice growth in response to typical water regimes in paddy fields deserves further investigation to elucidate Fe bioaccumulation in rice grains.

Fe isotopes have been extensively used as fingerprints to track the sources and biogeochemical processes of Fe in soil–rice systems.[4,6−8] Strategy-I-related plants tend to uptake light Fe isotopes ($\Delta^{56}Fe_{plant-soil}$ = −1.6 to −0.15‰)[4−6,8] owing to the selective reduction of Fe(III) to Fe(II), and subsequent transmembrane transport favors the light Fe isotopes.[9] In contrast, strategy-II plants exhibit minimal or no heavy Fe isotope enrichment compared to soils ($\Delta^{56}Fe_{plant-soil}$ = 0.05–0.30‰),[6−8] similar in extent to the complexation of Fe from iron minerals in the soil onto phytosiderophores. The Fe isotopes in rice plants were moderately lighter than that found in the soil under drainage conditions[4,10] and unfractionated in comparison to the ones in the soil solution.[4] However, rice plants were found to be preferentially enriched in heavy Fe isotopes relative to the soil solution in a waterlogged paddy soil.[4,10] However, under sufficient Fe supply conditions ($\Delta^{56}Fe_{bulk\ plant-nutrient}$ = −1.36), rice plants prefer to take up light Fe isotopes from aqueous solutions.[8] The radial oxygen loss (ROL) from rice roots triggers the oxidation of ferrous ions that flow to the root surface, wherein heavy isotopes are preferentially partitioned into Fe plaques under flooding regimes ($\Delta^{56}Fe_{Fe\ plaque-soil\ solution}$ = 2.24 to 2.43‰).[4,5,10] It was recently reported that in contrast to the drainage conditions, the formation of Fe plaques under flooding regimes results in a much heavier isotope in rice plants than in the soil solution.[4] Consequently, Fe plaque formation is considered to be a key contributor to the enrichment of heavy iron isotopes in rice plants. However, the effect of flooding–drainage alternations on Fe uptake during rice growth remains unclear.

Fe isotope composition ($\delta^{56}$Fe) in cropland soil ranges from −0.05 to 0.15‰.[4,5,10] During flooding periods, Fe(II) is liberated from the soil into the soil solution and Fe plaques are

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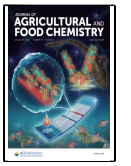





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subsequently formed following the kinetic fractionation effect. However, the formation of Fe plaques is probably triggered by a reversible isotopic equilibrium effect.[4] The introduction of $O_2$ upon drainage during grain-filling allows a rapid soil rhizosphere shift to aerobic conditions, which reduces soil Fe solubility and the formation of Fe plaques.[11] The redox-driven Fe transformation, precipitation, and dissolution of Fe (oxyhydr)oxides are generally accompanied by significant Fe isotope fractionation.[12] The Fe isotopes in rice plants at the maturation stage are isotopically heavier than those at the jointing stage.[5] Therefore, we hypothesized that the isotopic fractionation in the soil−soil solution-Fe plaque continuum may vary during typical flooding−drainage alternations, resulting in a temporal change in the uptake strategy and isotope compositions of rice plants during rice growth.

The incorporation of heavier Fe isotopes in rice plants typically results in the enrichment of rice grains with heavier Fe isotopes.[4,8] Iron homeostasis in rice plants is maintained through trafficking and storage of iron species such as iron chelators. Fe chelators such as citrate are responsible for Fe transport in the xylem, whereas nicotinamide (NA) and deoxymugineic acid (DMA) play an essential role in Fe translocation in the phloem.[4,5] The transport and accumulation of different Fe species in various rice plant organs cause isotope discrimination among rice compartments.[4] Density functional theory calculations indicated that Fe isotope fractionation between different Fe-chelator complexes can occur during Fe transport in higher plants.[13] Fe(III)-phytosiderophores can be 1.5‰ heavier than Fe(III)-citrate and up to approximately 3‰ heavier than Fe(II)-NA. A mixture of various Fe-chelator complexes may facilitate Fe transport since the fractionation associated with transport within rice is constrained by Fe excess and restriction. Mobility through the phloem during the vegetative period is crucial for grain Fe accumulation, particularly during stem-to-leaf transport. This process may also be affected by specific Fe chelators that are preferentially redistributed to the grains during the grain-filling stage. We hypothesized that flooding−drainage alternations may cause a change in the fractionation fingerprint during rice growth, which could help in deciphering the molecular Fe-transport processes in the shoots.

In this study, rice plants were subjected to flooding regimes during vegetative growth, followed by drainage during grain-filling stages. Samples of bulk soil, soil solution, Fe plaque, and specific rice compartments at the tillering, jointing, flowering, and mature stages of rice plants were collected for Fe isotope analysis. The objectives of this study were to reveal temporal changes in Fe uptake, transport, and isotope fractionation during rice growth in response to alternating redox conditions. Our results of Fe isotope fingerprinting provide insights into the regulation of Fe uptake and transport from soil to grains during regular rice growth under typical flooding−drainage alternation.

## MATERIALS AND METHODS

**Rice Growth Experiment.** Soil was collected from a depth of 0−20 cm in a paddy field located in Qujiang District, Shaoguan City, Guangdong Province, China (24.635485°N, 113.567534°E). The soil pH and Fe concentration values were 6.15 and 30.5 $g{\cdot}kg^{-1}$, respectively. After removing stones and other debris, soil samples were naturally air-dried, sieved to a size of <5 mm, and used for the pot experiment. For rice growth experiments, seedlings of the commonly grown rice (cv. *Oryza sativa* L. subsp. indica) variety Huanghuazhan were transplanted in pots, each containing 12 kg of paddy soil as the substrate. Throughout the vegetative stages, including tillering, jointing, and flowering, the soil was subjected to typical flooding regimes followed by drainage during grain-filling (from the flowering to maturity stages). Twelve pots were randomly distributed in a greenhouse; each experimental replicate consisted of three pots per growth stage, which were processed and harvested individually.

**Sample Preparation and Analysis.** The soil solution in the rhizosphere at each growth stage was collected using a Rhizon sampler (Rhizosphere Research Products, Netherlands), which was inserted 10 cm below the soil surface. Rice plants were harvested at the tillering, jointing, flowering, and maturity stages after 30, 50, 80, and 110 days of incubation, respectively. Subsequently, tillering- and jointing-stage rice plants were divided into roots, stems, total leaves, and flag leaves. At the flowering and maturity stages, roots, stems, total leaves, flag leaves, node I, husks, and grains (only at maturity stage) were collected from the rice plants. The rice samples were subsequently soaking and massaging in distilled deionized water, followed by dried at 55 °C for 48 h. One aliquot of the root sample was subjected to HCl extraction using ultrasonic cleaning to remove Fe plaques on the root surface, according to previous studies.[4,10]

Bulk soil and rice samples were digested with a 1:3 v/v mixture of $HNO_3$ and HF by using a high-performance microwave digestion system (Milestone; ETHOS UP, Italy). Inductively coupled plasma-optical emission spectrometry (ICP-OES-PerkinElmer Optima 8000) was used to measure the Fe concentration in subsamples from individual pots. Average values and standard deviations were derived from three experimental replicates. Three subsamples from individual pots were combined, digested, and purified for isotopic determination, and the assays were conducted in triplicate. The $\delta^{56}$Fe values of another biological replicate of soil solution, Fe plaque, and root at the jointing stage correspond to −0.91 ± 0.06, 0.15 ± 0.01, and −0.21 ± 0.06‰, which confirmed the reliability of the data of Fe isotope composition.

**Iron Isotope Analysis and Calculation.** Anion exchange chromatography using AG1-X8 resin (200−400 meshes, Bio-Rad) was used for the chemical separation of the samples following previously published procedures.[14] Briefly, the digested samples were dissolved in 6 M HCl and loaded onto the column, and the interfering elements and matrix fractions were removed by washing with 6 M HCl. Subsequently, purified Fe was obtained by using 0.4 M HCl. The separation procedure was performed twice to increase the Fe purity in each sample. The standard−sample−standard bracketing approach was used to correct the instrumental mass bias and time drifts for isotopic analysis using a *Neptune Plus* Multi-Collector inductively coupled plasma mass spectrometer (MC-ICP-MS, Thermo Fisher Scientific, Bremen, Germany). The in-house standards (GSB) were monitored multiple times to check the analysis accuracy with an external reproducibility of 0.03‰ (2sd) for $\delta^{56}$Fe. The repetitive measurements of U.S. Geological Survey (USGS) rock standards such as NOD-P-1 (−1.29 ± 0.04‰), BHVO-2 (0.08 ± 0.06‰), and AGV-2 (0.11 ± 0.04‰) were consistent with previously published results.[4,8,15] All acid solutions were cleaned in Teflon distills under sub-boiling conditions and prepared using high-purity water (18.2 MΩ cm, Milli-Q, Millipore). The procedural blank induced by digestion and purification processes was equivalent to 39.8 ng, and the recovery rate of Fe for the samples (~100 μg of Fe) of the chemical purification was higher than 98%. The samples were prepared on a Class 10 laminar flow bench in a Class 1000 clean room.

Fe isotopic compositions of the samples were expressed as delta per mil (‰) notation relative to standard IRMM-014 according to the following equation

$$\delta^{56}\mathrm{Fe} = \left[\frac{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{\mathrm{sample}}}{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{\mathrm{standard}}} - 1\right] \times 1000 \quad (1)$$

Isotopic discrimination between the two pools/compartments A and B was calculated as follows

$$\Delta^{56}\mathrm{Fe}_{\mathrm{A-B}} = (\delta^{56}\mathrm{Fe})_{\mathrm{A}} - (\delta^{56}\mathrm{Fe})_{\mathrm{B}} \quad (2)$$



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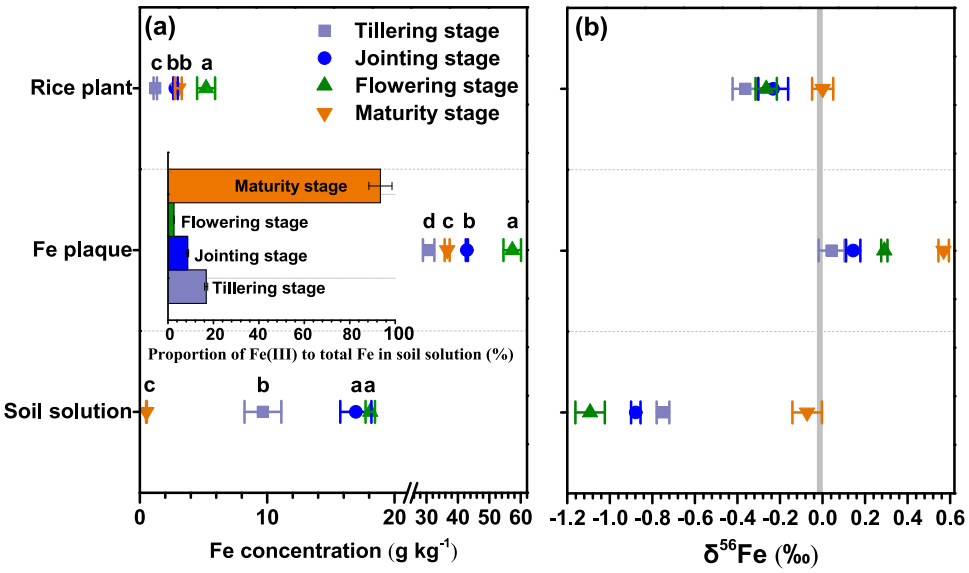


**Figure 1.** (a) Fe concentrations and (b) Fe isotope compositions in soil solution, Fe plaque, and rice plants at tillering, jointing, flowering, and maturity stages. The gray line represents the $\delta^{56}$Fe values of bulk soil. Three individual pot replicates were analyzed for Fe concentration with the error bars representing ±standard deviation (sd) values. The $\delta^{56}$Fe values are presented relative to the standard reference of IRMM-014 with the error bars denoting ±2 sd.

**Table 1. Dry Weight, Fe Concentration, Mass, and Isotope Compositions of Rice Compartments at the Tillering, Jointing, Flowering, and Maturity Stages**

| growth stage | rice compartment | dry weight (g) | Fe concentration (g·kg$^{-1}$) | Fe mass (g) | $\delta^{56}$Fe (‰) |
|---|---|---|---|---|---|
| tillering | root | 1.19 ± 0.16 | 6.83 ± 0.14 | 8.14 ± 1.11 | −0.35 ± 0.07 |
| | stem | 3.57 ± 0.15 | 0.41 ± 0.01 | 1.47 ± 0.07 | −0.30 ± 0.07 |
| | total leaf | 3.68 ± 0.11 | 0.17 ± 0.02 | 0.62 ± 0.08 | −0.66 ± 0.07 |
| | flag leaf | | 0.09 ± 0.01 | | −0.91 ± 0.07 |
| jointing | root | 1.91 ± 0.12 | 13.14 ± 0.19 | 25.12 ± 1.62 | −0.23 ± 0.03 |
| | stem | 4.37 ± 0.15 | 0.48 ± 0.01 | 2.11 ± 0.09 | −0.33 ± 0.03 |
| | total leaf | 3.81 ± 0.11 | 0.25 ± 0.03 | 0.95 ± 0.11 | −0.15 ± 0.03 |
| | flag leaf | | 0.12 ± 0.00 | | −0.58 ± 0.01 |
| flowering | root | 4.84 ± 0.6 | 15.68 ± 1.01 | 75.90 ± 10.60 | −0.28 ± 0.04 |
| | stem | 5.30 ± 0.43 | 1.12 ± 0.03 | 5.93 ± 0.50 | −0.47 ± 0.03 |
| | total leaf | 3.89 ± 0.48 | 0.86 ± 0.02 | 3.35 ± 0.42 | −0.01 ± 0.02 |
| | node I | | 0.40 ± 0.01 | | −0.40 ± 0.04 |
| | flag leaf | | 0.17 ± 0.01 | | −0.41 ± 0.07 |
| | husk | 2.37 ± 0.06 | 0.05 ± 0.00 | 0.12 ± 0.00 | −0.32 ± 0.05 |
| maturity | root | 5.58 ± 0.34 | 15.35 ± 0.87 | 85.67 ± 7.11 | 0.00 ± 0.05 |
| | stem | 7.21 ± 0.51 | 0.26 ± 0.02 | 1.88 ± 0.17 | −0.20 ± 0.06 |
| | total leaf | 4.22 ± 0.68 | 0.66 ± 0.04 | 2.76 ± 0.48 | 0.10 ± 0.05 |
| | node I | | 0.45 ± 0.01 | | −0.26 ± 0.06 |
| | flag leaf | | 0.20 ± 0.02 | | −0.07 ± 0.05 |
| | husk | 2.88 ± 0.11 | 0.15 ± 0.01 | 0.43 ± 0.02 | −0.05 ± 0.06 |
| | grain | 10.20 ± 0.69 | 0.02 ± 0.01 | 0.18 ± 0.01 | −0.81 ± 0.06 |

The $\delta^{56}$Fe values for whole rice and shoot were expressed on the basis of mass balance

$$\delta^{56}\mathrm{Fe}_{\text{whole rice/shoot}} = \frac{\sum_i m_i c_i \delta^{56}\mathrm{Fe}_i}{\sum m_i c_i} \quad (3)$$

where $m$ represents the dry weight (g), $c$ represents the Fe concentration ($\mu$g·g$^{-1}$), and $i$ refers to the plant tissues such as root, stem, total leaves, husk, and grain. All of the rice parts except roots were considered as shoots. The standard error of $\Delta^{56}\mathrm{Fe}_{A-B}$ was calculated by propagating the standard errors measured on the $\delta^{56}\mathrm{Fe}_A$ and $\delta^{56}\mathrm{Fe}_B$ values, and the standard errors on $\delta^{56}\mathrm{Fe}_{\text{whole rice}}$ and $\delta^{56}\mathrm{Fe}_{\text{shoot}}$ were calculated according to the combination of error propagation of addition, multiplication, and division according to a recent study.[4] The formulas for propagating error are provided in the Supporting Information section.

Under flooding periods, rice is expected to procure Fe(II) in the soil solution primarily due to its inherent strategy-I-like system in response to abundant Fe(II), while Fe(III) is acquired from the soil solution and Fe plaque upon grain-filling drainage and rice growth, respectively. This is because the redox state of Fe is sensitive to redox-alternation cycles and Fe plaque mainly consists of Fe(III) (oxyhydr)oxides.[4] Furthermore, the acquisition of Fe via strategy-I and -II systems may cause isotopic discrimination; however, this was not considered when calculating the relative contributions of the soil solution and Fe plaque to Fe uptake in this study. The above-mentioned relative contributions to Fe uptake of rice plants (that is, $f$


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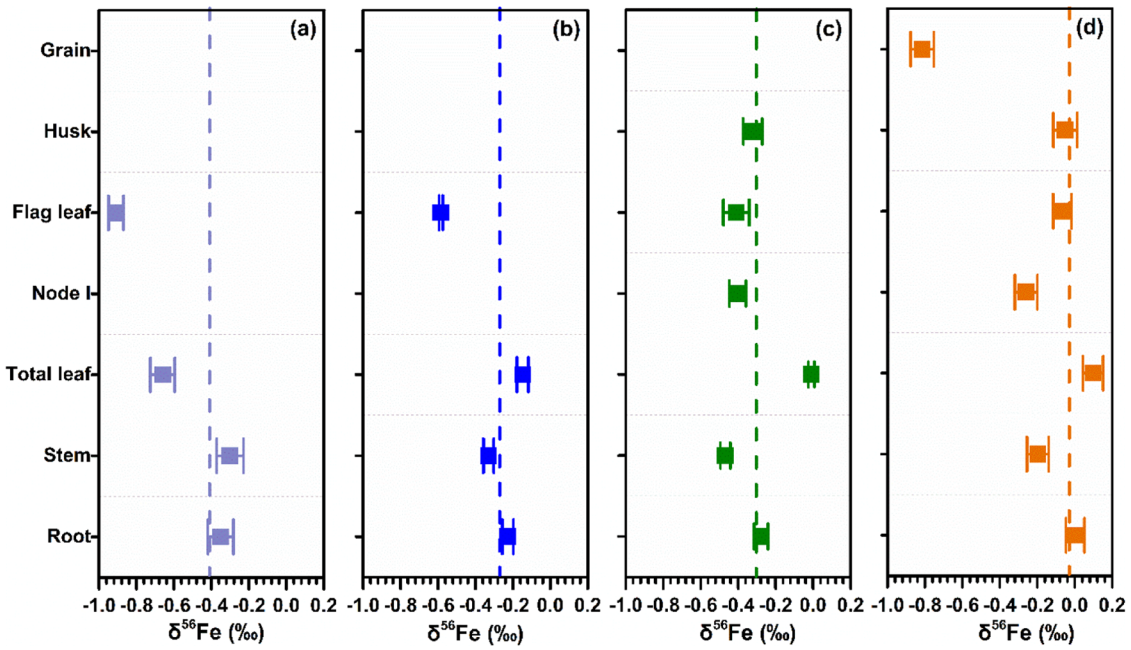


**Figure 2.** Fe isotope compositions of various rice organs including roots, stems, nodes I, total leaves, flag leaves, husks, and grains at (a) tillering, (b) jointing, (c) flowering, and (d) maturity stages. The dashed lines represent the $\delta^{56}$Fe values of shoots. The $\delta^{56}$Fe values are presented relative to the standard reference of IRMM-014 with the error bars denoting ±2 sd.

$_{\text{soil solution}}$ and $f_{\text{Fe plaque}}$) were calculated based on the mass balance as follows

$$\delta^{56}\text{Fe}_{\text{wholerice}} = f_{\text{soil solution}} \times \delta^{56}\text{Fe}_{\text{soil solution}} + f_{\text{Fe plaque}} \times \delta^{56}\text{Fe}_{\text{Fe plaque}} \quad (4)$$

$$f_{\text{soil solution}} + f_{\text{Fe plaque}} = 1 \quad (5)$$

**Statistical Analysis.** The normality of distribution and homogeneity of variance were tested by normality plot tests and Levene's test, respectively. An independent-sample *t*-test was performed to estimate significant differences in Fe concentration, dry weight, and Fe mass among the four rice growth stages at $P < 0.05$.

## RESULTS

**Fe Concentration in the Soil−Rice System.** Flooding of vegetative stages resulted in a gradual increase in Fe(II) concentration in the soil solution from the tillering stage (9.66 ± 1.44 mg·L$^{-1}$) to jointing (16.96 ± 1.22 mg·L$^{-1}$) and flowering stages (18.09 ± 0.37 mg·L$^{-1}$). This was followed by a significant decrease to 0.52 ± 0.02 mg·L$^{-1}$ upon grain-filling drainage (Figure 1a). A similar substantial increase followed by a marked decrease upon flooding followed by drainage was observed for Fe plaque formation. As a consequence, Fe concentrations in rice compartments (including the roots, stems, total leaves, and flag leaves) substantially increased during the vegetative stage. However, the opposite pattern was observed in roots, stems, and total leaves at the grain-filling stage, whereas flag leaves, nodes I, and husks increased marginally. The dry weight of rice tissues substantially increased during rice growth (Table 1). Accordingly, the Fe masses in whole rice plants, roots, and flag leaves increased during rice growth but decreased in Fe plaques, stems, and total leaves at the grain-filling stage.

**Fe Isotope Fractionation in the Soil−Rice System.** Fe in whole rice plants and soil solution was isotopically lighter than Fe in bulk soil ($\Delta^{56}\text{Fe}_{\text{whole rice−bulk soil}}$ = −0.39 to −0.03‰, $\Delta^{56}\text{Fe}_{\text{soil solution−bulk soil}}$ = −1.12 to −0.10‰, Figure 1b). In contrast, Fe plaques were enriched in heavy isotopes compared to the bulk soil ($\Delta^{56}\text{Fe}_{\text{Fe plaque−bulk soil}}$ = 0.01 to 0.54‰). $\Delta^{56}\text{Fe}_{\text{Fe plaque−soil solution}}$ values sequentially increased during vegetative stages (tillering stage: 0.79 ± 0.07‰, jointing stage: 1.02 ± 0.04‰, flowering stage: 1.38 ± 0.07‰), followed by a significant decrease to 0.64 ± 0.07‰ upon grain-filling. A similar isotopic fractionation pattern in terms of $\Delta^{56}\text{Fe}_{\text{rice plants−soil solution}}$ values was observed from the soil solution to Fe plaques during rice growth (tillering stage: 0.39 ± 0.07‰, jointing stage: 0.65 ± 0.04‰, flowering stage: 0.83 ± 0.03‰, maturity stage: 0.07 ± 0.04‰). Notably, the Fe isotope composition of whole rice was much lower than that of Fe plaques ($\Delta^{56}\text{Fe}_{\text{whole rice−Fe plaque}}$ = −0.57 to −0.38‰).

Fe isotopic compositions of rice shoots and roots were identical within the error margins, with no significant differences among rice growth stages ($\Delta^{56}\text{Fe}_{\text{shoots−roots}}$ = −0.06 to −0.03‰) (Figure 2a−d). Fe isotopes in the shoots and total leaves were moderately lighter than those in the stems at the tillering stage ($\Delta^{56}\text{Fe}_{\text{total leaves−stems}}$ = −0.36 ± 0.10‰). This result is generally opposed to that at the jointing, flowering, and maturity stages with an enrichment of heavy Fe isotopes in total leaves relative to Fe in the stems (0.18−0.46‰). The flag leaves were enriched in lighter Fe isotopes compared to total leaves ($\Delta^{56}\text{Fe}_{\text{flag leaves−total leaves}}$ = −0.44 to −0.16‰). Heavy Fe isotopes were gradually observed in shoots and flag leaves during rice growth, particularly in the grain-filling stage, with a strong enrichment of heavy Fe isotopes among the rice compartments. Accordingly, the extent of negative fractionation from shoots to flag leaves ($\Delta^{56}\text{Fe}_{\text{flag leaves−shoots}}$) sequentially decreased from the tillering stage (−0.50‰) to jointing (−0.31‰), flowering (−0.11‰), and maturity stages (−0.04‰). In addition, the Fe isotopic compositions of flag leaves and husks were similar to those of node I at the flowering stage but moderately heavier than those at the maturity stage. However, the grains were


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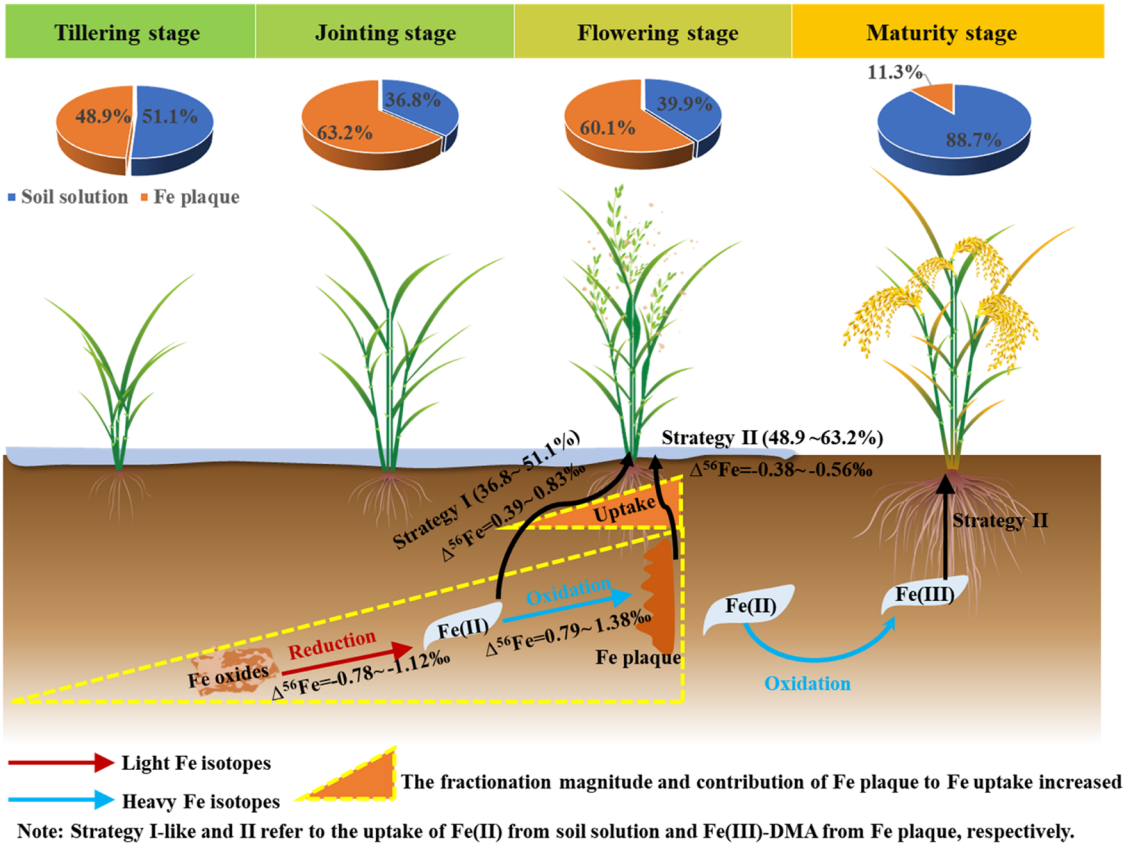


**Figure 3.** Conceptual model for Fe transformation and uptake at the soil−root interface under typical flooding−drainage alternation conditions based on the Fe isotopic fingerprint.

enriched in lighter Fe isotopes compared to node I, flag leaves, stems, and shoots.

## DISCUSSION

**Fe Isotope Fractionation in the Soil−Root Interface during Rice Growth.** The value of $\Delta^{56}Fe_{soil\ solution-soil}$ decreased from −1.12 to −0.78‰ upon flooding of vegetative stages (Figure 3a). The reductive dissolution with an enrichment factor for the $^{56}Fe/^{54}Fe$ ratio between reactive surface sites and solution can be up to −1.73‰.[16,17] The continuous reductive dissolution of iron (oxyhydr)oxides, probably controlled by the kinetic isotope effect, resulted in the enrichment of Fe(II) with light isotopes in the soil solution.[4,10] However, the soil solution was slightly enriched in heavier iron isotopes by approximately 0.11‰ compared to bulk soil upon grain-filling drainage. This indicated a depletion of light Fe isotopes by approximately 1.23‰ in the soil solution. Oxygen reintroduction into the soil upon drainage drastically decreased reducing conditions.[18] Consequently, the faster rates of Fe(II) oxidation and precipitation led to a dominant kinetic effect. Since the higher proportion of Fe(III) in soil solution contributes to its enrichment in heavier isotopes,[19] the marked increase in the $\delta^{56}Fe$ values of the soil solution upon drainage can be partly attributed to the high percentage of Fe(III). The $^{56}Fe/^{54}Fe$ ratios of $Fe(III)_{aq}$ were equal to or higher than those of iron oxyhydroxide precipitates ($Fe(III)_{ppt}$), and the isotopic fractionation was limited by the increasing precipitation rate as indicated by ferric iron precipitation experiments.[20] Additionally, the isotopically light Fe was preferentially enriched in the precipitates of iron oxyhydroxides or nodules[21] and Mn oxyhydroxides.[18] Therefore, soil-to-soil solution fractionation is linked to the redox-driven effect under redox-alternating conditions, and isotope depletion between soil and soil solution is erased when the reductive conditions do not prevail, owing to the oxidation and precipitation processes.

Fe plaque was enriched in heavier Fe isotopes compared to those in the soil solution, which is in accordance with the results of previous studies ($\Delta^{56}Fe_{Fe\ plaque-soil\ solution}$ = 2.24−2.43‰).[4,10] This could be due to the fact that radial oxygen loss (ROL) triggers Fe(II) oxidation to form iron oxyhydroxides on the surface of the roots with stronger bonds relative to the aqueous phases.[4] $\Delta^{56}Fe_{Fe\ plaque-soil\ solution}$ values increased during vegetative stages, all of which were lower than those observed in the same soil−rice system at the maturity stage with a continuous flooding regime (2.43‰).[4] The abiotic and biotic Fe(II) oxidation bring a significant Fe isotope fractionation from aqueous Fe(II) to ferrihydrite ranging from −1.5 to −0.9‰.[22,23] However, the rapid precipitation of iron oxyhydroxides from aqueous $Fe(II)_{aq}$ could be explained in terms of kinetic fractionation.[24] Additionally, the continuous release of isotopically lighter Fe from the soil to the soil solution driven by a kinetic effect and the subsequent flow to the root surface to form Fe plaques (Figure 1a) prevented the achievement of an equilibrium fractionation. An Fe plaque coating was continuously formed with an inner layer and an outer rim;[11] therefore, the sequentially positive $\delta^{56}Fe$ values of Fe plaques from tillering to jointing and flowering stages indicate the isotopic heterogeneity of the Fe plaque substrate. However, a much less pronounced fractionation was observed upon preharvest


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grain-filling drainage than during the vegetative stages. Soil drainage allows for a faster shift to aerobic conditions near the root surface Fe plaque than that in the bulk soil.[11] The Fe plaque surface sites can absorb Fe(II) before the grain-filling stage, after which it may be released into the soil solution or oxidate into Fe(III) at the fast acidic and oxic rhizosphere upon drainage; this result explains the production of high $\delta^{56}$Fe values in Fe plaques during grain-filling. Therefore, the fast rates of oxidation and precipitation upon soil oxygenation limit isotopic equilibrium.

The $\Delta^{56}Fe_{\text{rice plants−soil solution}}$ values gradually increased from the tillering stage (0.39‰) to the jointing (0.65‰) and flowering stages (0.83‰) in accordance with the observed fractionation pattern from soil solution to Fe plaques. In previous studies, rice plants in paddy fields incorporated heavy Fe isotopes from soil solutions.[5,10] It is plausible that $Fe^{2+}$ is taken up by rice since it is well adapted to submerged conditions where $Fe^{2+}$ is abundant.[1,3,25] The incorporation of $Fe^{2+}$ from the soil solution into rice plants could definitely account for slightly negative fractionation[4,8] but may not explain the observed fractionation solely. The highly positive $\Delta^{56}Fe_{\text{Fe plaque−rice plants}}$ value suggests that Fe plaques could offset the uptake of heavy Fe isotopes into rice plants. Thus, the sequential enrichment of heavy Fe isotopes in Fe plaques corresponds to increased $\delta^{56}$Fe values of rice plants at vegetative stages. Strategy-II plants exhibit a restricted $\delta^{56}$Fe variation between the plants and the pool of plant-available Fe.[8,26,27] The relative contributions of Fe plaques at the tillering, jointing, and flowering stages were 48.9, 63.2, and 60.1% (Figure 3), respectively, according to the two end-member mixing model. Therefore, Fe plaque formation is crucial for the Fe uptake by rice plants upon flooding during the vegetative stages. In any case, isotope fractionation implemented via strategy-I and -II systems may affect the contributions of Fe plaque and soil solution to Fe uptake by rice plants.

The Fe isotope composition of rice plants did not differ in terms of statistical significance with that of the soil solution at the maturity stage, with values ($\Delta^{56}Fe_{\text{rice plants−soil solution}}$ = 0.07‰) that were lower in magnitude than those observed in rice plants grown in soils with deficient Fe supply in a previous study (0.27‰).[8] This could be due to the fact that rice plants take up Fe(III) via strategy II. Accordingly, the greater enrichment of heavy isotopes in rice plants upon grain-filling drainage compared to that generated during the vegetative stages might be attributed to the Fe oxidation state in the soil rhizosphere (Figure 1b),[28] which is characterized by the preferential incorporation of heavy isotopes into rice plants. According to the two end-member mixing analysis, more than 88.7% of Fe in rice plants originates from the soil solution during grain-filling drainage. In this study, the Fe(III) incorporated via strategy II upon grain-filling drainage probably originates from the soil solution (Figure 1b), as indicated in a previous study reporting that the Fe uptake from soil solution is prevalent during drainage seasons.[4]

**Fe Isotope Fractionation within Rice during Rice Growth.** Fe isotopes in shoots were identical to those in roots within error irrespective of the growth stages ($\Delta^{56}Fe_{\text{shoots−roots}}$ = −0.05 to −0.03‰, Figure 1b), which minimally varied with the proportion of Fe in roots. This implies that root-to-shoot fractionation is more likely controlled by membrane transport. The maintenance of iron homeostasis involves Fe storage in the roots and upward translocation to the shoots of the plant. Fe(III)-DMA is the major form sequestered into the vacuoles of root cells, while the oxidation capacity of roots to limit excess $Fe^{2+}$ accumulation probably contributes to the deposition of Fe(III)-phosphate or Fe(III)-hydroxides in the apoplast.[7,29] The higher stability constant of the Fe(III)-phytosiderophore compared to that of Fe(III)-phosphate or Fe(III)-hydroxides indicates that the Fe(III)-phytosiderophore complex is highly enriched in heavy isotopes.[5] The enrichment of lighter Fe isotopes in the stems relative to roots can be attributed to Fe translocation in the form of Fe(III)-citrate from roots to shoots via the xylem[30] and as Fe(II)-NA and Fe(III)-DMA for intercellular transportation in the phloem.[4,5] Fe(III)-phytosiderophores were heavier than Fe(III)-citrate and Fe(II)-NA by approximately 1.5 and 3‰, respectively.[13] The presence of a mixture of Fe species (including Fe(III)-citrate, Fe(II)-NA, and Fe(III)-phytosiderophores) may explain the absence of fractionation from the roots to the shoots.

Fe in total leaves was isotopically lighter than that in stems at the tillering stage (−0.36‰), in contrast to the jointing, flowering, and maturity stages (0.18−0.46‰), which are similar to a previous result (0.52‰).[5] This is likely due to the lower stem-to-leaf transfer at the tillering stage (Table 1) and the favorable transport of Fe(III)-citrate or Fe(II)-NA through the xylem or phloem sap, respectively. However, the transport of the DMA-Fe(III) complex was favored after the tillering stage. This is likely due to rice iron homeostasis mechanisms alleviating excess Fe accumulation.[31] The extent of negative fractionation from the stem to flag leaves gradually decreased at the vegetative stage, and the slightly positive fractionation upon grain-filling drainage ($\Delta^{56}Fe_{\text{flag leaves−stems}}$, tillering stage: −0.61 ± 0.08‰, jointing stage: −0.25 ± 0.03‰, flowering stage: 0.06 ± 0.07‰, maturity stages: 0.13 ± 0.07‰) indicated that Fe(III)-DMA is the predominant form (over Fe(III)-citrate or Fe(II)-NA) during the transport of the complexes from stems toward flag leaves. This result is supported by the similar variation observed in isotopic fractionation from node I to flag leaves (from 0.01 to 0.19‰) upon grain-filling drainage. This confirms a preferable heavy Fe remobilization in the phloem sap. However, $\Delta^{56}Fe_{\text{flag leaves−total leaves}}$ values decreased during the vegetative stage and increased during grain-filling (−0.25, −0.44, −0.40, and −0.16‰). Thus, the transfer of light counterparts from total leaves to flag leaves is favored via phloem sap.

Iron is highly localized in parenchymal cells and is proposed to be strongly associated with phosphorus in insoluble forms.[32] Citrate can solubilize Fe deposited in the apoplastic part of the nodes, thus redistributing Fe(III)-citrate to the husks.[33] Fe transport from node I to husks mainly follows the xylem flow, which may favor the transport of Fe(III)-citrate with heavy isotopes to husks (0.09−0.21‰).[33] However, the absence of citrate solubilization barely influenced the Fe accumulation in the grains.[33] Grains were enriched in lighter Fe isotopes relative to nodes I (−0.21‰), in a similar extent to that of a previous study.[4] A higher level of DMA at the expense of NA facilitates Fe uptake but minimally impacts Fe accumulation in grains.[34] Furthermore, DMA-increased solubilization of Fe deposited at the node may also facilitate Fe remobilization,[35,36] while DMA-chelated Fe(III) may contribute less to Fe accumulation in the grains since the complex is heavier than Fe(III)-phosphate or Fe(III)-hydroxides.[7,13] It is suggested that Fe in the phloem sap of rice primarily chelates to NA, and a rice line with a high NA:DMA ratio promotes Fe loading into


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the grains.[34] Notably, mutants lacking NA exhibit a loss of iron-NA transport and failed long-distance iron transport, with decreasing iron levels in the leaves.[37] The preferential transport of Fe(II)-NA with lighter isotopes in comparison to DMA-Fe(III) could account for the observed fractionation. Therefore, Fe(II)-NA (which is characterized as a light isotope from phloem sap) should be preferentially loaded into the grains.

**Implications.** Paddy soils constitute the largest anthropogenic wetlands worldwide.[6] The redox-alternating conditions during rice planting are the main factors controlling soil Fe loss, which is strongly linked to biogeochemical cycling on the Earth's surface.[38−40] The uptake strategies and sources of Fe plaque and soil solutions in paddy rice were identified using Fe isotopes.[4,10] Rice undergoes Fe excess and restriction in the soil solution during typical flooding−drainage alternation during rice growth; however, the uptake mechanisms underlying the redox-alternating conditions remain largely unknown. This study indicated that Fe(III) uptake from Fe plaques predominantly occurs via strategy II during the flooding periods, and its contribution generally increases during the vegetative stages. The uptake of Fe(III) in the soil solution via strategy II was favored upon drainage of the grain-filling. These findings provide a more systematic and comprehensive insight into the temporal changes in Fe uptake by rice in response to typical water regimes in paddy fields. The combined strategies of distinct sources for Fe uptake have significant implications for the Fe biogeochemical cycle and may also apply to other strategy-II graminaceous plant species.

Soil iron availability generally far exceeds the demand for rice growth during flooding seasons.[1,31] However, iron transport from roots to grains is limited due to rice plant iron homeostasis.[4,31] The decreased magnitude of negative fractionation at the vegetative stage and fractionation toward heavy isotopes from stem/node I to flag leaves during grain-filling and the favored transport of light isotopes from leaves to grains caused a strong enrichment of heavy Fe isotopes in leaves. This strongly indicates that citrate- and DMA-chelated Fe(III) are mainly preferred during stem/node I-to-flag leaf transfer. Our findings and those of previous studies suggest that NA-chelated Fe(II) is critical for Fe transport from leaves to grains via the phloem during grain-filling.[34] The limited Fe accumulation in grains may also be attributed to the distinct preference for Fe-chelated species between stem-to-leaf and leaf-to-grain transfer. Therefore, the molecular function of NA in the proteins controlling and mediating iron homeostasis deserves further investigation, which will set the stage for a holistic understanding of the "iron efficiency" of rice cultivars. Identification of rice cultivars or transgenic rice lines with a high Fe transport capability in the phloem is also a promising regulatory strategy to improve the nutritional quality of crops and combat iron-deficiency-induced anemia in humans.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c07640.

Methods about calculation of error propagation (PDF)

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### Notes

The authors declare no competing financial interest.

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