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Iron and zinc isotope fractionation during uptake and translocation in rice (*Oryza sativa*) grown in oxic and anoxic soils☆

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ABSTRACT

Stable isotope fractionation is emerging quickly as a powerful novel technique to study metal uptake and translocation in plants. Fundamental to this development is a thorough understanding of the processes that lead to isotope fractionation under differing environmental conditions. In this study, we investigated Zn and Fe isotope fractionation in rice grown to maturity in anaerobic and aerobic soils under greenhouse conditions. The overall Zn isotope fractionation between the soil and above ground plant material was negligible in aerobic soil but significant in anaerobic soil with isotopically lighter Zn in the rice plant. The observed range of fractionation is in line with previously determined fractionations of Zn in rice grown in hydroponic solutions and submerged soils and emphasizes the effect of taking up different chemical forms of Zn, most likely free and organically complexed Zn. The Zn in the grain was isotopically lighter than in the rest of the above ground plant in rice grown in aerobic and anaerobic soils alike. This suggests that in the course of the grain loading and during the translocation within the plant important biochemical and/or biophysical processes occur. The isotope fractionation observed in the grains would be consistent with an unidirectional controlled transport from shoot to grain with a fractionation factor of $\alpha \approx 0.9994$. Iron isotopes showed an isotopic lighter signature in shoot and grain compared to the bulk soil or the leachate in aerobic and anaerobic soils alike. The negative direction of isotopic fractionation is consistent with possible changes in the redox state of Fe occurring during the uptake and translocation processes. The isotope fractionation pattern between shoots and grain material are different for Zn and Fe which finally suggests that different mechanisms operate during translocation and grain-loading in rice for these two key micronutrients.

1. Introduction

Soils deficient in zinc (Zn) and iron (Fe) constrain rice (*Oryza sativa*) production in large parts of the world (Dobermann and Fairhurst, 2000) and deficiencies of Zn and Fe in human populations with rice-based diets cause

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major health problems for millions of people (Graham, 2007). Identifying nutrient efficient rice lines, applying appropriate breeding techniques, and understanding the underlying mechanisms of Zn and Fe efficiency, are important for tackling this large societal challenge (Wissuwa et al., 2008).

Recent work demonstrated significant isotope fractionation during uptake and translocation of trace metals including Fe, Cu and Zn from soils to plants in a wide variety of plant species and this suggests that natural stable isotope compositions provide a powerful technique to study the chemical and biological processes that control micronutrient movement from soils into plants (Alvarez-Fernandez et al., 2014). Isotopic fractionations differ between plant species and between genotypes of the same species, as well as with soil and nutritional conditions (Arnold et al., 2010a; Aucour et al., 2011; Deng et al., 2014; Tang et al., 2012; Weiss et al., 2005; Weinstein et al., 2011).

Work on the isotopic fractionation during Zn uptake in hydroponic cultures showed that plant shoots are enriched with ^{64}Zn relative to ^{66}Zn in comparison to the source, consistent with an unidirectional process during transport of free Zn^{2+} across cell membranes (Aucour et al., 2011; Deng et al., 2014; Weiss et al., 2005). Plants grown in natural soils, however, show negligible or slight heavy isotope enrichment in the shoots (Arnold et al., 2010a; Tang et al., 2012; Viers et al., 2007). Recent work suggests that uptake of a Zn(II)-phytosiderophore (PS–Zn) complex could account for an observed heavy isotopic fractionation in Zn uptake by rice (Arnold et al., 2010a). Likewise Smolders and co-workers account heavy isotopic fractionation during Zn uptake by tomato plants in resin-buffered hydroponics by the uptake of a PS–Zn complex (Smolders et al., 2013). Investigations of Zn complexation by organic molecules using experiments in the laboratory (Jouvin et al., 2009) and ab initio calculations (Fujii and Albarède, 2012) support this model. Few studies determined the stable isotope fractionation of Zn during translocation in higher plants but enrichment with light isotopes in leaves with increasing distance from the root (Caldelas et al., 2011; Moynier et al., 2009; von Blankenburg et al., 2009) and during the translocation from root to shoot (Caldelas et al., 2011; Jouvin et al., 2012; Tang et al., 2012) have been reported.

To date, Fe isotope fractionation has not been studied in rice but a significant body of work exists for graminacae (Guelke and von Blankenburg, 2007; Kiczka et al., 2010b; von Blankenburg et al., 2009; Guelke et al., 2010). The observed range of isotope fractionation is around 2.25‰ per atomic mass unit (pamu). Initial pot studies found an enrichment with light Fe during plant uptake in strategy-I plants as opposed to an enrichment with heavy isotopes in strategy-II ones (Guelke and von Blankenburg, 2007). These observation are in line with known plant uptake and isotope fractionation mechanism, whereby a reduction reaction and subsequent uptake of ferrous iron is responsible for the enrichment with light Fe isotopes in strategy-I plants, and the complexation of Fe^{3+} by organic ligands and the subsequent uptake of the PS–Fe complex is responsible for the enrichment with heavy isotopes in strategy-II plants. Subsequent work found significant variation in isotopic fractionation among different graminaceous species, showing positive and negative isotope signatures suggesting that the controls are far more complex (Guelke-Stelling and von Blanckenburg, 2012; Kiczka et al., 2010b). One proposed explanation is that strategy-II plants possess transporters for ferrous Fe and thus can take up Fe^{2+} directly (Cheng et al., 2007; Kim and Guerinot, 2007). Kiczka et al., 2010b suggested two consecutive stages during Fe uptake to explain light Fe enrichment in graminacae, and in particular processes preceding active transport such as mineral dissolution which favours for trace metals the removal of light isotopes (Chapman et al., 2009; Kiczka et al., 2010a, 2011; Weiss et al., 2014; Wiederhold et al., 2006, 2007a, 2007b) and selective Fe uptake at the plasma membrane level. These more recent observations emphasize the possible effect of mixing of different chemical forms on the Fe and other trace metal isotope signature found in stems, grains and leaves during the transport in phloem and xylem (Guelke-Stelling and von Blanckenburg, 2012; Moynier et al., 2013). Although Fe speciation during root uptake is a major contributor to the final isotopic signature, possible mechanisms that influence isotope distribution within plants include successive oxidation and reduction steps during translocation, ligand exchange reactions, remobilisation from older plant tissues and mixing effects of short- (phloem) and long-distance (xylem) transport (Alvarez-Fernandez et al., 2014; Yoneyama et al., 2010).

The aim of the present study was to investigate the controls of stable isotope fractionation of Zn and Fe in rice (*Oryza sativa*) grown under aerobic and anaerobic soil conditions. We conducted experiments in a nutrient-sufficient soil with a widely grown rice genotype. We analysed soil, leachable fractions, shoots and grains, and we discuss the observed fractionation patterns in light of the possible mechanisms outlined above.

2. Materials and methods

2.1. Plant growth experiments

Rice (*Oryza sativa* L. cv. Oochikara) was grown in pots in a greenhouse at Rothamsted Research under anaerobic (flooded) and aerobic conditions as described elsewhere (Xu et al., 2008). The soil was taken from the plough layer (0–20 cm depth) of an arable field at Rothamsted. It is a moderately well-drained Aquic Paleudalf (USDA classification) or Luvisol (FAO classification) with silty clay loam texture (26% clay, 53% silt, 21% sand), 2% organic C, 0.2% total N, 5.93 g kg^{-1} amorphous Fe oxides (by ammonium oxalate extraction) and pH 6.4. The soil was air-dried, sieved to < 8 mm and homogenized. Two watering treatments (aerobic and flooded) with four pots per treatment were randomly arranged on a bench inside a greenhouse (day/night temperatures 28/25 °C, light period 16 h per day with natural sunlight supplemented with sodium vapour lamps to maintain light intensity of > 350 $\mu mol\ m^{-2}s^{-1}$). Five pre-germinated rice seeds were planted in each pot.