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Fractionation of Metal Stable Isotopes by Higher Plants

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Higher plants induce chemical reactions in the rhizosphere, facilitating metal uptake by roots. Fractionation of the isotopes in nutrients such as calcium, iron, magnesium, and zinc produces a stable isotope composition in the plants that generally differs from that of the growth medium. Isotope fractionation also occurs during transport of the metals within most plants, but its extent depends on plant species and on the metal, in particular, on the metal's redox state and what ligand it is bound to. The metal stable isotope variations observed in plants create an isotope signature of life at the Earth's surface, contributing substantially to our understanding of metal cycling processes in the environment and in individual organisms.

KEYWORDS: higher plants, nutrition sciences, isotope fractionation

INTRODUCTION

Several metallic elements are essential nutrients for *higher plants* (words in italics are defined in TABLE 1), and the amounts required vary by several orders of magnitude, depending on the plant species. Of the major metals present in plant tissues, calcium (Ca) is used for stabilizing cell walls, and it acts as a counter-ion for inorganic and organic anions in the vacuoles, while magnesium (Mg) mainly supports photosynthesis. Potassium (K) activates enzymes and maintains cell pressure. The minor metals zinc (Zn), copper (Cu), and molybdenum (Mo), present at parts per million levels, are important cofactors of enzymes, and iron (Fe) is required for Fe–sulfur and heme proteins that participate in redox reactions (Marschner 1995).

The metabolic processes that control the behavior of metals in plants can be envisaged as a gigantic geochemical pump that continuously moves metals between reservoirs. By mobilizing metals from soils, plants accelerate chemical weathering by a factor of 2 to 5 (Berner et al. 2004), and by recycling metals, plants retard their release to drainage waters. The implication for global element cycling is immense. For example, globally, ~10^{15} g of K pass through vegetation each year, while only 2 to 3% of that amount is carried by rivers (Chaudhuri et al. 2007). Thus, K is recycled 50 to 100 times by plants following dissolution from primary minerals before it is released to rivers. In quantitative terms, Ca flux through plants is similar to that of K, while that of Mg is about an order of magnitude lower. Because of their higher mobility in soils, the annual river flux of Ca and Mg is roughly identical to the amounts cycled through plants. Fe and Zn flux through plants is ~3 to 4 times that of the annual river dissolved load.

As higher plants extract these bio-essential metals from the soil, chemical reactions occur that facilitate uptake of the metals by roots. The metals are then cycled through a variety of chemical species as they move from root to stem, from stem to leaf, and from leaf to seed. All of these processes can lead to isotope fractionation. Since the reactions rarely require complete transfer from one plant reservoir to another, metal fractions of different isotope composition are generated within the plant. Eventually, plants return their metals to the soil as leaf litter, but generally the *isotope composition* of the litter is different from that of the growth medium. Plants thereby generate a time-integrated metal isotope "fingerprint" of their activity.

During the past several years, metal stable isotope fractionation in higher plants has been demonstrated for Fe, Zn, Ca, and Mg (Weiss et al. 2005; Wiegand et al. 2005; Guelke and von Blanckenburg 2007; Viers et al. 2007; Black et al. 2008; Moynier et al. 2008; Page et al. 2008; Cenki-Tok et al. 2009). However, three major challenges must be overcome before these tools can serve as indicators of metal cycling: (1) Biochemical transformation processes associated with metal uptake by plant roots and metal *translocation* within plants must be characterized. (2) Isotope fractionation factors associated with these reactions must be quantified. (3) The relationship between mass balance and isotope fractionation in growth experiments under controlled conditions must be understood. Most progress has been made for Fe, Zn, Ca, and Si. We do not discuss Si because it is a metalloid, not a metal.

MOVING METALS FROM SOILS TO AND THROUGH PLANTS

On their way from soil into and through plants, metals repeatedly change the ligand they are bound to. This can lead to detectable isotope fractionation (FIG. 1): (1) in the *rhizosphere*, where some metals need dissolution from sparingly soluble solids in the soil, or in the cell *apoplast*, which may itself be isotopically heterogeneous (Marschner 1995); (2) during passage of metals across the plasma membrane of root cells, which is mediated by transport proteins and ion channels (Kim and Guerinot 2007); (3) in the *cytoplasm* of root cells, where slightly alkaline pH may require a change of binding form to maintain solubility or to protect sensitive cellular constituents from interaction with metals (Briat et al. 2007); (4) during export of metals from the cytoplasm into *xylem* vessels, which represent an acidic pipeline for the transport of metals from root to shoot (Kim and Guerinot 2007); (5) in the membrane passage from the xylem fluid into the cytoplasm of leaf cells; (6) during loading into the *phloem* vessel, which may involve membrane transporters (Briat et al. 2007) and which takes place during transfer of metals from old leaves to sink tissues (young shoots, seeds, fruits); and (7) during transfer from the phloem into the seed or fruit, which usually requires another passage across membranes and thus through an acidic apoplast because there is no symplastic (cell-to-cell) connection between maternal and filial tissue.

Transport mechanisms into and through plants are highly metal specific. For example, in waterlogged or acidic soils, Zn and Fe form aqueous complexes that are mobile, whereas in well-aerated and neutral to alkaline soils, Fe tends to precipitate as ferric iron [Fe(III)] in oxyhydroxides and phosphates, while Zn strongly adsorbs to the Fe hydroxides and clays. As a consequence, the free ion concentrations of Fe and Zn in the soil solution are too low to meet plant demand (Marschner 1995), so plants have developed specific uptake strategies. For Fe, roots of *dicotyledons* and nongraminaceous *monocotyledons* (“strategy I plants”, e.g. pea, bean) respond to Fe deficiency by releasing protons into the rhizosphere, thus promoting dissolution of Fe precipitates, and by inducing membrane-bound enzymes that reduce Fe(III) bound to chelates (FIG. 1). Reduced Fe [ferrous iron, Fe(II)] is then transported across the root plasma membrane by unspecific iron/metal transporters (Briat et al. 2007). The release of protons into the rhizosphere by strategy I plants also favors desorption of Zn from soils, so subsequent uptake by roots depends only on the induction of specific Zn transporters, which belong to the same protein family as the Fe transporters (Krämer et al. 2007). Graminaceous plant species (“strategy II plants”, e.g. maize, wheat) respond to Fe deficiency by releasing *phytosiderophores*, which form complexes with Fe(III) (Römheld and Marschner 1986; Kraemer et al. 2006).

These complexes are then carried across the plasma membrane by a specialized class of transport proteins (FIG. 1). For these plants, Zn acquisition benefits from the release of the phytosiderophores (Suzuki et al. 2006). Iron transport between plant cells is facilitated by complexation with organic acids or with nicotianamine (FIG. 1). Fe occurs in the xylem fluid mainly as the Fe(III)-citrate complex, and, in graminaceous plants, it is also complexed to phytosiderophores. These low-molecular-weight ligands are synthesized at elevated levels under Fe deficiency. Several steps of reduction and oxidation occur until Fe reaches the sink organ. Once there, transport through the apoplast and across the plasma membrane occurs, which, depending on the properties of the metal chelate, may lead to the dissociation of the metal from its ligand and another change of its binding form (von Wirén et al. 1999). In the case of Zn, which does not undergo a valence change, intracellular compartmentalization in the root, allocation to the shoot, and export and import required to cross plant membranes are thought to occur, in part as the Zn^{2+} cation and in part as a Zn(II)-complex. In the latter case, Zn(II) is complexed with low-molecular-weight organic ligands, such as organic acids, nicotianamine, and phytosiderophores (Curie et al. 2009).

The situation for Ca is markedly different. Calcium is stable in soil solutions mainly as the free metal ion or in hydrated form. After entering the root apoplast, >90% of Ca is adsorbed on cell walls, and only a small fraction enters the cytoplasm. The subsequent transport of Ca across the plasma membrane of a root cell is mediated by specific transport proteins, which are regulated according to the nutrient demand of the plant (Amtmann and Blatt 2009). Inside the cytoplasm, some Ca is bound to enzymes while the remainder is either

compartmentalized, in particular into the vacuole of cells, or exported into xylem vessels. These processes, and the reuptake from the xylem into leaf cells, are mediated by transport proteins in the membrane (Amtmann and Blatt 2009). Finally, Ca is immobile in the phloem and thus is not retranslocated from older to younger leaves or seeds, unlike other divalent cations such as Mg (Marschner 1995). Thus, Ca supply to younger shoot tissue, flowers, and fruit relies entirely on translocation via the xylem.

IMPLICATIONS FOR ISOTOPE FRACTIONATION

The metal conversion processes in plants are likely to lead to isotope fractionation. The extent and direction of fractionation depend on the physicochemical nature of the process (i.e. kinetic versus equilibrium control). For example, Fe redox reactions result in large isotope shifts. Under equilibrium experimental conditions (22°C), aqueous Fe(III) has a $^{56}Fe/_{54}Fe$ ratio that is ~3‰ greater than that of coexisting aqueous Fe(II) (Welch et al. 2003). Conversely, partial reduction of an Fe(III)-solid produces aqueous Fe(II) with an $^{56}Fe/^{54}Fe$ ratio approximately 1 to 1.5‰ less than that in the remaining Fe(III)-solid (Johnson et al. 2004). Likewise, Fe(III)-complexes released from an Fe(III)-solid have lower $^{56}Fe/^{54}Fe$ ratios than those in the remaining Fe(III)-solid, but only as a transient phenomenon; at equilibrium, unfractionated Fe is partitioned into the complexed aqueous phase (Wiederhold et al. 2006). These differences explain the distinct isotope composition difference between strategy I and II plants.

Zinc isotope fractionation factors for processes relevant to the soil–plant environment were determined for adsorption on organic (root) and inorganic (plaque) surfaces and for Zn uptake from nutrient solution (Pokrovsky et al. 2005; Juillot et al. 2008). During adsorption, the extent and direction of the fractionation depend largely on sorbent surface structure and solution composition (i.e. pH, ionic strength, chemical speciation). Observed fractionation of $^{66}Zn/^{64}Zn$ between solution and solid is between –0.6 and 0.5‰ (Pokrovsky et al. 2005; Juillot et al. 2008), suggesting that fractionation of Zn isotopes during adsorption might enrich either heavy or light isotopes.

For Ca, plant species- or tissue-specific fractionation factors have yet to be determined. However, virtually all studies of stable isotope fractionation accompanying Ca partitioning from aqueous solutions have shown that Ca associated with the solid product is isotopically similar to or lighter than coexisting aqueous Ca, by up to several per mil for $^{44}Ca/^{40}Ca$, as a result of both equilibrium and kinetic effects. This is true, for example, for both inorganic crystallization of calcite and aragonite and biomineralization (Eisenhauer et al. 2009 this issue), incorporation of Ca as a trace element in a mineral (Griffith et al. 2008), and bone formation (Skulan et al. 2007). Thus, wherever Ca is precipitated in plant tissue, light Ca isotopes are likely to partition into tissue, while heavy Ca remains dissolved in the translocation pathway.

ISOTOPE FRACTIONATION OBSERVED IN PLANTS

Several plant species have now been characterized for their Fe, Zn, and Ca stable isotope composition in either controlled laboratory *growth experiments* or natural environments.

Iron

Guelke and von Blanckenburg (2007) measured $^{56}Fe/^{54}Fe$ ratios (reported as $\delta^{56/54}Fe$ in per mil relative to IRMM-014) in vegetables and cereal crops grown in a variety of soils. Strategy I plants had significantly lower $\delta^{56/54}Fe$ values, while strategy II plants had slightly higher $\delta^{56/54}Fe$ values than those of both the operationally defined *plant-available extractable* soil Fe and the dissolved Fe in soil solutions (FIG. 2). Preferential uptake of light Fe by strategy I plants can be attributed to the reduction of Fe(III) in the rhizosphere prior to Fe(II) uptake. The small fractionation accompanying Fe(III) complexation by strategy II plants supports the absence of such a reduction step.

$\delta^{56/54}Fe$ values in strategy I plants measured by Guelke and von Blanckenburg (2007) decreased from soils to stems, from stems to leaves, and from leaves to seeds, with seeds having $\delta^{56/54}Fe$ values as much as 1.6‰ lower than that of soil Fe (FIG. 2). Time-series experiments in growth solutions showed that younger leaves of strategy I plants receive a substantial proportion of their Fe from older leaves. In contrast, all parts of strategy II plants had similar $\delta^{56/54}Fe$ values. Apparently, there are differences in the way Fe is translocated in strategy I and II plants. Within the plant, a large part of the Fe is scavenged by complexing agents, for example, by nicotianamine within the cells or citrate in the xylem. Nicotianamine has the ability to bind ferrous and ferric iron (von Wirén et al. 1999). Current thinking is that strategy I plants change the redox state of Fe during translocation, while in strategy II plants, Fe is assumed to remain in the ferric state while changing its ligands.

Zinc

Weiss et al. (2005) measured $^{66}Zn/^{64}Zn$ ratios (reported as $\delta^{66/64}Zn$ in per mil relative to JMC-Lyon Zn) in the nutrient solution, in the roots, and in the shoots of tomato and rice plants grown hydroponically (FIG. 3A). The $\delta^{66/64}Zn$ values of the shoots were 0.2 to 0.4‰ smaller than that of the growth solution, depending on plant species and the speciation of Zn in the growth medium. In contrast, the $\delta^{66/64}Zn$ values of the roots were 0.2‰ larger than that of the nutrient solution, probably reflecting the preferred adsorption of heavy Zn onto the roots and plaques, as predicted from isotope fractionation factors described above. A study conducted in a tropical watershed in Cameroon with soil-grown plants (trees and various herbaceous species) revealed lighter Zn in shoots relative to roots in some cases, but the patterns observed were not as systematic as those in the hydroponic study (FIG. 3B) (Viers et al. 2007).

Translocation within the plant favors transport of the lighter Zn isotopes. Moynier et al. (2008) investigated Zn isotope fractionation during transport from the primary seed to leaves of lentil, a dicotyledon, in the absence of any soil or nutrient solution (FIG. 3A). Zn in the leaves had $\delta^{66/64}Zn$ values that were 0.35‰ smaller than those in the seed. In field-grown bamboo, a graminaceous plant, the extent of fractionation increased with leaf position along the stem: leaves harvested the farthest along the stem, at 80 cm, had the lightest Zn, up to 1‰ lighter than soil Zn in terms of $\delta^{66/64}Zn$ (FIG. 3B). In the study by Viers et al. (2007), leaves of trees and herbaceous species all contained Zn that was isotopically lighter than Zn in their shoots (most pronounced in the palm tree *Raphia* sp., the tree *Musanga* sp., and the herbaceous species *Renealmaia* sp.; FIG. 3B). These observations are consistent with light Zn isotopes being transported preferentially during nutrient exchange along the cell walls of the xylem, and Zn being transferred across the cell membrane from xylem and phloem into the seed compartment.

Calcium

The first Ca stable isotope results obtained for higher plants were from trees in hardwood (Page et al. 2008) and tropical (Wiegand et al. 2005) forests. Results suggest systematic behavior of Ca isotopes in trees and associated soils (FIG. 4): (1) Tree tissues generally contain lighter Ca than either acid-extractable and/or exchangeable fractions of coexisting upper mineral soils or bulk regolith. (2) In the hardwood forest, organic soil horizons contain lighter extractable Ca than underlying mineral soils. (3) In the tropical forest, Ca in tree tissues and the extractable fraction of shallow mineral soils becomes heavier as soils age. (4) Roots and stemwood contain the lightest Ca, while leaves contain the heaviest Ca.

These ecosystem-based studies of Ca isotopes in trees highlight the way in which metal isotopes trace the time-integrated turnover of metals through the soils that host the trees. For example, observation 1 led Wiegand et al. (2005) to propose that trees preferentially take up light Ca from forest soils. They noted that Ca uptake by roots follows the electrochemical gradient and involves transport proteins (Amtmann and Blatt 2009), a process that is likely to result in kinetic isotope fractionation. In this regard, Ca behaves similarly to Fe in strategy I plants and to Zn, but differently from Mg, a neighboring alkaline earth metal to Ca. Indeed, Black et al. (2008) reported that wheat plants grown hydroponically preferentially took up heavy Mg from the growth solution. However, although there is evidence that trees preferentially absorb light Ca from rock substrates, the organic soils in the hardwood forest have a Ca isotope composition similar to an average tree composition (i.e. intermediate in composition between root/stemwood and leaf). This leads to the intriguing hypothesis that plants preferentially take up light Ca directly from primary minerals early in forest development on fresh rock, but shift toward recycling of tree litter–rich organic soil materials with isotopically light Ca (consistent with observation 2) as the ecosystem develops and the soil profile thickens (Bullen and Bailey 2005). The data from samples of the tropical forest support this hypothesis, in that plant tissues have lighter Ca than bulk soils at the 300-year-oldsite, but similar $\delta^{44/40}Ca$ at the 4.1 Ma site. Observation 3, increasingly heavy tree and soil Ca with increasing soil age, suggests progressive leaching of light, plant-processed Ca from the soils as the ecosystem ages. Although atmospheric input must be considered, the chemical imprint of this evolution is likely to be recorded as systematic shifts in $^{44}Ca/^{40}Ca$ and surrogate ratios (e.g. $^{87}Sr/^{86}Sr$, Ca/Sr) of ecosystem components across soil chronosequences (e.g. Wiegand et al. 2005).

Observation 4, increasingly heavy Ca along the transpiration stream, differs from the situation for Zn and Fe but is similar to that for Mg (Black et al. 2008). Cenki-Tok et al. (2009) likewise demonstrated increasingly heavy Ca in the order roots–stemwood–leaves for both spruce and beech at a watershed in northern France. For Ca, the results suggest a chromatographic effect during translocation, in which light Ca is preferentially partitioned out of solution into plant tissue, consistent with the results of the Ca isotope fractionation studies.

PERSPECTIVES

The picture that emerges is that biochemical processes taking place during metal uptake and translocation in plants produces a characteristic isotope fractionation that depends on the metal, its chemical speciation, and the plant species. It will be fascinating to see where the research into metal isotope fractionation by plants will lead next, toward plant physiology or toward Earth-surface geochemistry, or both. In plant physiology, isotope ratios may be used to define the predominant binding forms in which the elements have entered the plant. Further, considering that retranslocation should result in isotope fractionation, isotope ratios might help to elucidate the extent of remobilization of a metal within the plant. In addition, isotope ratios of different metals in plant samples may provide information on competitive interactions among metals having similar physicochemical properties. In Earth surface geochemistry, perhaps the most promising approach lies in the isotopic fingerprinting of the vast turnover of metals through higher plants as metals are moved from unweathered parent rock to river water. The variation of Ca isotope composition from trees across soil chronosequences indicates the potential of Ca isotopes as time-integrated turnover markers. Furthermore, these markers may be preserved in the geological record, for example, in the form of precipitated calcites or oxides in paleosols and lake sediments. Using these indicators, the impact of plants on isotope fractionation can be traced as vegetation changes with climate.

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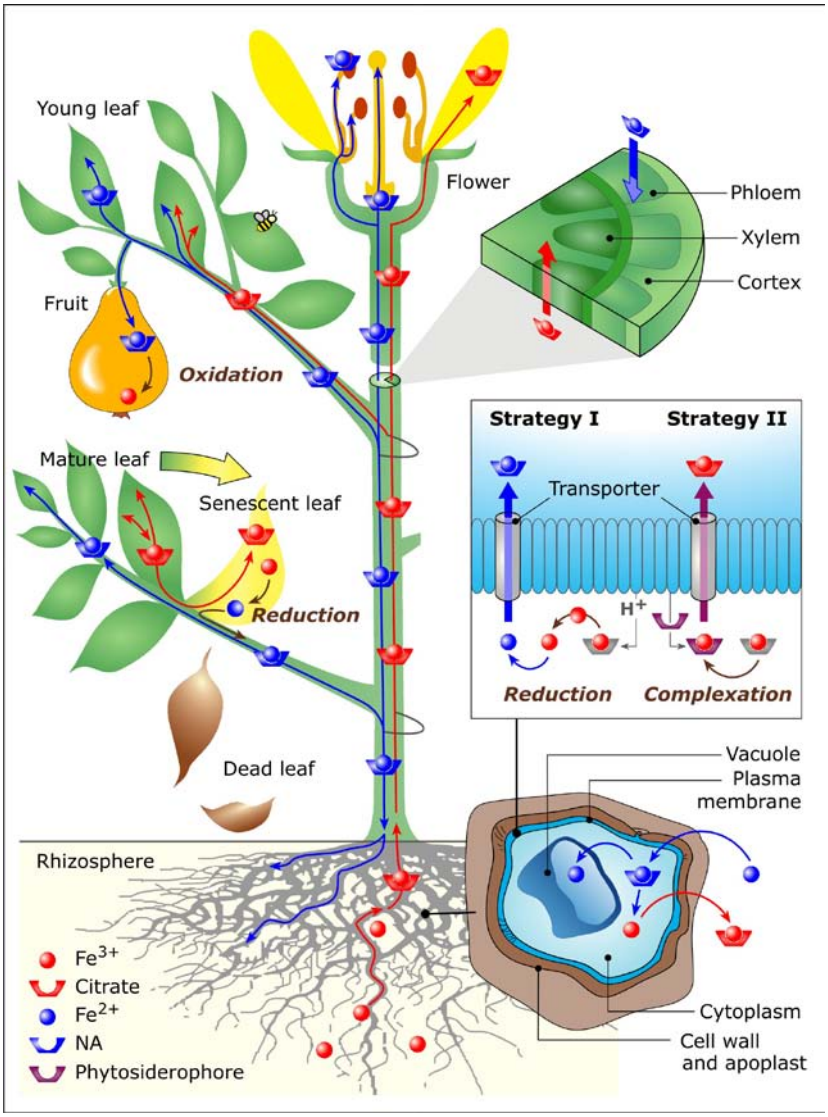

Figure 1: Metal uptake and translocation pathways in higher plants, emphasizing Fe reactions. Arrows represent the long-distance circulation of iron chelates within a flowering plant (after Briat et al. 2007). Uptake into the root is mediated by transporter proteins; in the case of Fe(III), this occurs either after reduction (strategy I) or after complexation (strategy II). Complex formation by organic acids or, in particular, nicotianamine (a secondary amino acid, "NA", blue arrows) moves Fe between cells. Transport in the xylem is as an Fe(III)-citrate complex, and in graminaceous plants, also an Fe(III)-phytosiderophore complex (red arrows). Transfer of Fe from the xylem into leaf cells requires Fe(III)-citrate reduction, followed by transport across the membrane as Fe^{2+} or as a ferric complex with nicotianamine or phytosiderophores (the latter only in strategy II plants). Within leaf and root cells, most of the physiologically active Fe is found as Fe(II) or Fe(III) in the protein fraction, as heme-bound Fe, or fixed in Fe–S clusters (Briat et al. 2007). When the plant enters the generative growth phase, root activity usually decreases, so elements become translocated to sink tissues (brown arrows). Fe can be reduced again before reaching the sink organs (Curie et al. 2009). Ca, Mg, and K are taken up by root cells via transport proteins, but not in a chelated form. Subsequent translocation in the xylem and phloem also occurs in ionic form.

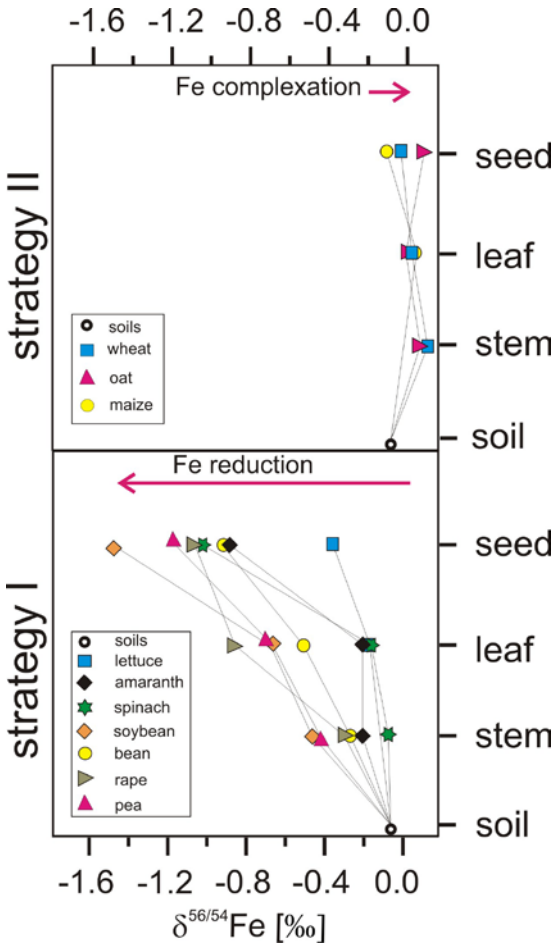

Figure 2: $\delta^{56/54}$Fe of extractable Fe in a loamy soil and of various parts of strategy I and strategy II plants after several months of growth (Guelke and von Blanckenburg 2007). Strategy I plants incorporate light Fe relative to Fe of the soil; the heavy isotopes are furthermore increasingly depleted from stem to leaf and leaf to seed. Strategy II plants have uniform composition throughout, similar to the operationally defined plant-available Fe in soils. The arrows show the direction and magnitude of apparent equilibrium fractionation compatible with reduction (lower diagram) and complexation to siderophores (upper diagram). The isotope fractionation observed in plants can be explained by these fractionation processes. Analytical error was ±0.05‰.

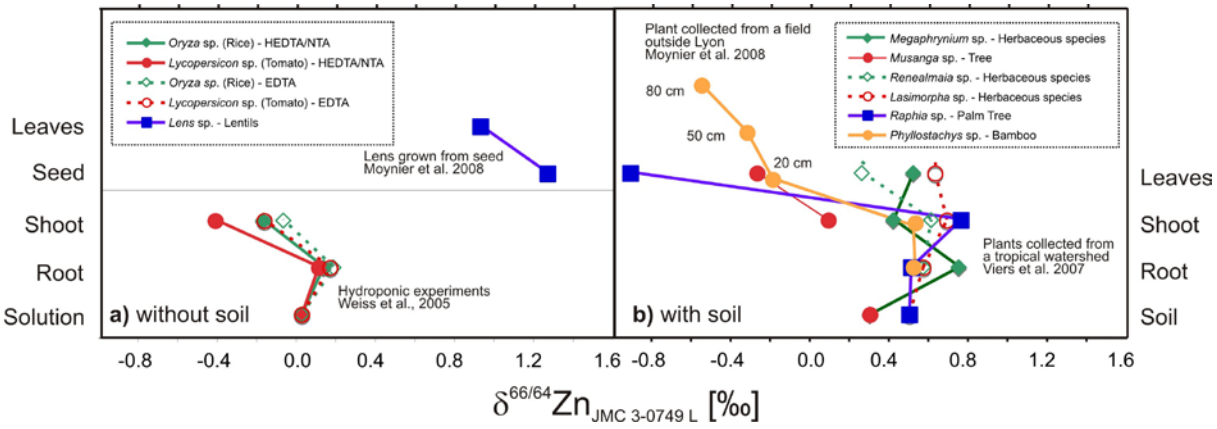

Figure 3: Zn isotope fractionation patterns in plants. (**A**) Data for plants grown without soil; tomato and rice were grown in nutrient solutions (Weiss et al. 2005), and lentils were grown from seed (Moynier et al. 2008). (**B**) Data from plants grown in soil; trees and various herbaceous species were collected from a watershed in Cameroon (Viers et al. 2007), and bamboo from a field outside Lyon, France (Moynier et al. 2008). The agreement between data from hydroponic experiments and from real ecosystems suggests that processes inside the plant (translocation) do not depend on the growth environment. In (A), HEDTA/ NTA and EDTA refer to the chelates used in the experiments. Analytical error was ±0.05 to 0.1‰.

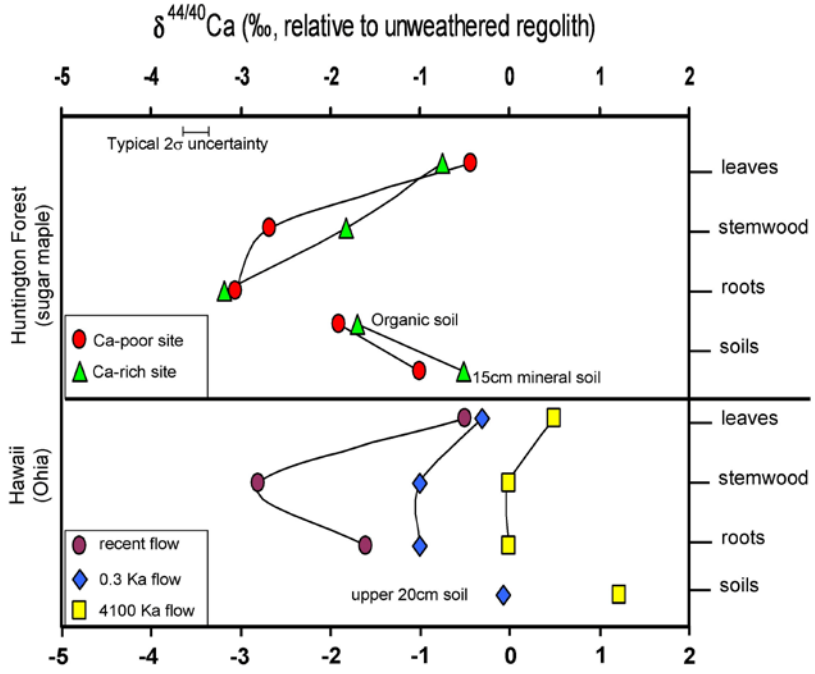

Figure 4: Ca isotope variations for tree tissues and soils collected at the granitic Archer Creek Watershed, Huntington Forest, New York, and from the basaltic Hawaiian islands of Hawaii and Kauai, plotted relative to $\delta^{44/40}$Ca for unweathered regolith at each site. Huntington Forest tree tissues are from sugar maples (*Acer saccharum*) sampled from neighboring stands with Ca-rich and Ca-poor regolith (Page et al. 2008). Hawaiian tree tissues are from mature Ohia (*Metrosideros polymorpha*) sampled from the youngest (300- year-old) and oldest (4.1 Ma) members of a soil chronosequence (Wiegand et al. 2005), and from an Ohia seedling sampled from a recent lava flow (T. Bullen unpublished data). Soil data represent combined exchangeable (ammonium acetate) and leachable (nitric acid) fractions for organic soils and the uppermost 15 cm of mineral soils from Huntington Forest, and the exchangeable (ammonium acetate) fraction for the uppermost 20 cm of mineral soils from Hawaii. Data represent the average of two or more total procedural replicates in all cases; the analytical error for replicate analyses was±0.15‰ or less.

	Tablee 1 GLOSSARY OF TERMS AND TOOLS
Apoplast	The extracytoplasmic compartment in the free space of the cell wall
Apoplastic metals	Within the root apoplast, the cell wall acts as a cation exchanger adsorbing metals and favoring the precipitation of Fe. Because these metals have not been taken up into the cells of roots, treatments using reducing chemicals are employed to remove them before accessing cell-hosted metals (Bienfait et al. 1985).
Cytoplasm	The cellular compartment inside the plasma membrane that hosts organelles and other intracellular compartments, such as the vacuole
Growth experiments	Plant metal isotope fractionation during uptake has been measured using (a) a growth solution containing an exactly known mix of nutrients (advantage: controlled conditions, mass balance possible); (b) growth experiments in controlled soil (advantage: close to real conditions, growth monitoring possible); (c) plants grown in a natural environment (advantage: real ecosystem condition, including the change of soil properties with time).
Higher plants	Plants with vascular tissues that serve to circulate water, nutrients, and photosynthetic products through the plant
Isotope composition reporting	Stable isotope ratios are commonly reported as a per mil (‰) difference relative to the standard (δ notation). Metals are reported as $^{26}Mg/^{24}Mg$, $^{44}Ca/^{40}Ca$, $^{56}Fe/^{54}Fe$, and $^{66}Zn/^{64}Zn$ ratios. Comparison of isotope ratio differences for different elements must be based on a common mass difference, because the magnitude of mass-dependent fractionation increases with mass difference.
Mass spectrometry	The Mg, Zn, and Fe isotope ratios reported here are from multicollector ICP mass spectrometry (MC–ICP–MS). Calcium ratios are from double-spike thermal ionization mass spectrometry (TIMS). A prerequisite of the measurements is the removal of organic and inorganic matrix by chemical-separation procedures.
Monocotyledons Dicotyledons	Flowering plants that appear with one leaf (monocotyledons) or two leaves (dicotyledons) after germination
Phloem	Vascular tissue responsible for the long-distance transport of assimilates and nutrients through the plant
Phytosiderophores	Hexadentate aminocarboxylate-type metal chelators that have a high binding affinity to ferric Fe and other metals
Plant-available (extractable) metal fraction from soils	Roots do not access the entire metal pool contained in soils; most of the metals are locked in crystalline, primary and secondary minerals. Available are metals in noncrystalline precipitates, bound to organic compounds, or dissolved in the aqueous form (in the soil solution). Between these fractions, the isotope composition of metals often differs. Various selective-extraction procedures are used; these extract some operationally defined plant-available metal fraction. It has to be ensured that isotopes are not fractionated during the extraction.
Rhizosphere	The soil volume surrounding plant roots that is subject to chemical reactions induced by the roots
Translocation	Long-distance transport of water and nutrients from one organ to another, for example, from roots to leaves via the xylem or from leaves to fruits via the phloem
Xylem	Conducting tissue responsible for the transport of water and soluble inorganic nutrients from the roots into above-ground plant tissues