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Magnesium-Isotope Fractionation During Plant Growth

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Magnesium is an essential nutrient, which activates more enzymes than any other mineral element and, thus, plays an important role in biogeochemical cycles. With three stable isotopes naturally abundant (^{24}Mg, 78.992%; ^{25}Mg, 10.003%; ^{26}Mg, 11.005%), magnesium stable isotope fractionation may provide insights into these cycles. Here, we detail for the first time the magnesium stable-isotope distribution in a higher plant, wheat (*Triticum aestivum* L.), during its growth cycle. Wheat plants were grown in a limiting nutrient supply hydroponically, some being left to mature through senescence and others detopped at maturity for collection of exudates. Measurements of the magnesium isotopic composition of chlorophylls, seeds, shoots, roots, leaves, exudates, and the limiting nutrient solution over time show that the plant appears to establish an isotopic equilibrium with the nutrient available to it and that the plant (in particular, the seeds and exudates) becomes enriched in the heavy isotopes of magnesium in a mass-dependent relationship as the plant reaches maturity. The preference of the plants for heavy magnesium isotopes suggests that a difference might exist in the bioavailable magnesium of agricultural and natural soils due to the periodic removal of heavy magnesium isotopes by harvest.

INTRODUCTION

Geochemists are now using nontraditional stable isotopes to characterize the composition of reservoirs and fluxes of materials in the Earth (*1*), including magnesium (*2*−*5*). These new isotope systems have great potential for fields such as palaeoclimatology, palaeooceanography, hydrology, and inorganic plant nutrition, but often, the measured fractionations are poorly linked to individual chemical reactions and biological processes. Deciphering causes of isotopic fractionation in nature is difficult because, in addition to the abiotic fractionations, the isotopic signals are complicated by enzymatic and transport fractionations in organisms. This is a particularly important point in trying to understand how magnesium isotopes are partitioned in biological systems, since magnesium is an essential nutrient which activates more enzymes than any other mineral element (*6*).

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The isotopic fractionation of magnesium in the soil−plant system involves both abiotic and biological mechanisms. Potential fractionation steps include the speciation of magnesium in the pore fluids of soil; the transport of magnesium across root cell membranes into the plant, making it part of the biosphere; and the transport of magnesium within the plant to its final destination and functions. Magnesium has three abundant stable isotopes and only one redox state (Mg(II)) in nature, which makes it particularly useful for identifying biogeochemical processes that fractionate isotopes because there will be no fractionation processes associated with changes in redox state. Isotope spiking has been used extensively to study the uptake and distribution of various elements, including magnesium, in trees (e.g., Scots pine (*7, 8*), spruce (*9, 10*), oak (*11*)). These studies have focused on nutrient cycling, but not fractionation of the stable isotopes within the plant, as we do here. Only a few studies have looked comprehensively at the distribution of nontraditional stable isotopes in plants, including Zn (*12, 13*), Si (*14*), and Fe (*15*). These studies suggest that isotope discrimination within higher plants is specific to the mode of transport. The variability in δ^{66}Zn values reflects different modes of transport across cell membranes and within a plant (*13*). Silicon shows a Rayleigh-like kinetic fractionation upward in the plant (*14*) and is possibly influenced by the kinetics and specificity of the transport system (*16*). The isotopic fractionation of iron isotopes depends on the pathways, for iron uptake-δ^{56}Fe is lighter in plants in which uptake of iron takes place after the reduction of Fe(III) to Fe(II) by up to −1.6‰, but slightly heavier (≈0.2‰) in plants where uptake is facilitated by the chelation of Fe(III) by siderophores (*15*), relative to the available Fe fraction in the soil.

Here, we examine magnesium isotope fractionation in wheat (*Triticum aestivum* L.) during its growth history and we compare the plant constituents with the nutrient solution. We specifically investigated if uptake and transport mechanisms produce a measurable fractionation of magnesium isotopes. Previous studies showed mass-dependent fractionation of the stable isotopes of magnesium in chlorophyll extracted from cyanobacteria (*2*) and English ivy (*3*), although whether the isotopic fractionation occurred during magnesium transport was not determined.

EXPERIMENTAL METHODS

Plant Growth. Wheat plants were grown from seed using methods previously described (*17*−*19*). Figure 1 shows the progression of growth of the wheat plants from seedlings to maturity. Samples of seeds used to grow the wheat plants and 2-week-old seedlings were taken for magnesium analysis. The chemical composition of the limiting nutrient supply is given in Table 1. The water level in the nutrient solutions was regularly topped up with deionized water to a level of 18 L. Samples (10 mL aliquots) were taken periodically for magnesium analysis. One plant was followed over the course of 31 days (plant 1) before running xylem exudate experiments

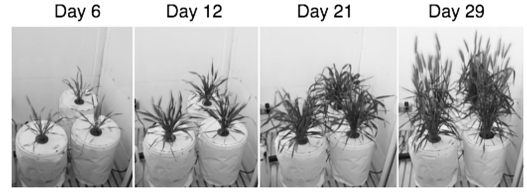

FIGURE 1. Sequence showing growth cycle of wheat plants in environmentally controlled chambers from seedling to maturity.

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TABLE 1. Initial Nutrient Solution Composition

compound	concentration (mM)
KNO_3	2.5
$Ca(NO_3)_2$	2.0
NH_4NO_3	0.5
KH_2PO_4–K_2HPO_4, pH 8.5	1.0
H_4SiO_4[a]	0.02
$MgSO_4$	0.5
KCl	25×10^{-3}
H_3BO_3	12.5×10^{-3}
$MnSO_4 \cdot H_2O$	1×10^{-3}
$CuSO_4 \cdot 5H_2O$	0.25×10^{-3}
$ZnSO_4 \cdot 7H_2O$	1×10^{-3}
H_2MoO_4	0.25×10^{-3}
$NiSO_4 \cdot 6H_2O$	0.1×10^{-3}
Fe–EDTA	100×10^{-3}

[a] Silicate was added as a basic Na_2SiO_3 solution. All nutrient solutions were then acidified to pH = 5.8 with 1.0 M HCl, accounting for an extra ~0.04 mM NaCl.

(see below), at which point the roots, shoots, leaves, and seeds were harvested for analysis. The nutrient solutions of another plant (plant 2) were followed through to senescence (52 days).

Exudate Experiments. The details of the xylem exudate experiments have been previously published (*19*). Figure 2 illustrates the design of the wheat stem support at the top of the nutrient solution container. The plastic containers were lined with a black sheath to prevent the buildup of bacteria and algae in the nutrient solutions by shielding it from light. Plants where algae were found growing around the base of the stem support (Figure 2, labeled part iv) were not used for exudate experiments or isotopic analysis. A pretreatment solution (*19*) was not used. Plant 1 was detopped in its growth solution (Figure 2c) so that the isotopic composition of the exudates could be compared with that of the nutrient solution composition. Three xylem exudate samples (~0.5 mL volumes of increasing [Mg] from 0.5 to 1 mM) were collected from plant 1 after 50 (initial), 120, and 150 (final) minutes from detopping of the plant.

Sample Preparation. Samples of nutrient solution were tested for their magnesium content using atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS). Six and eight samples of the nutrient solutions of plant 1 and plant 2, respectively, were chosen for isotopic analysis (Table 2). Aliquots containing ~0.4 μmol of magnesium were evaporated to dryness, digested in concentrated ultrapure nitric acid, evaporated to dryness, and then loaded on cation-exchange columns in 1 M HNO_3 for purification of the magnesium, as in previous studies (*2*).

Chlorophylls were extracted from a blend of flag leaf pulp using methanol. The chlorophyll fraction (Chl*a* + Chl*b*) was then purified on a column of anion exchange resin (*20*). Yields of Chl*a* and Chl*b* in the purified extract were determined using the UV–vis spectrum and the known extinction

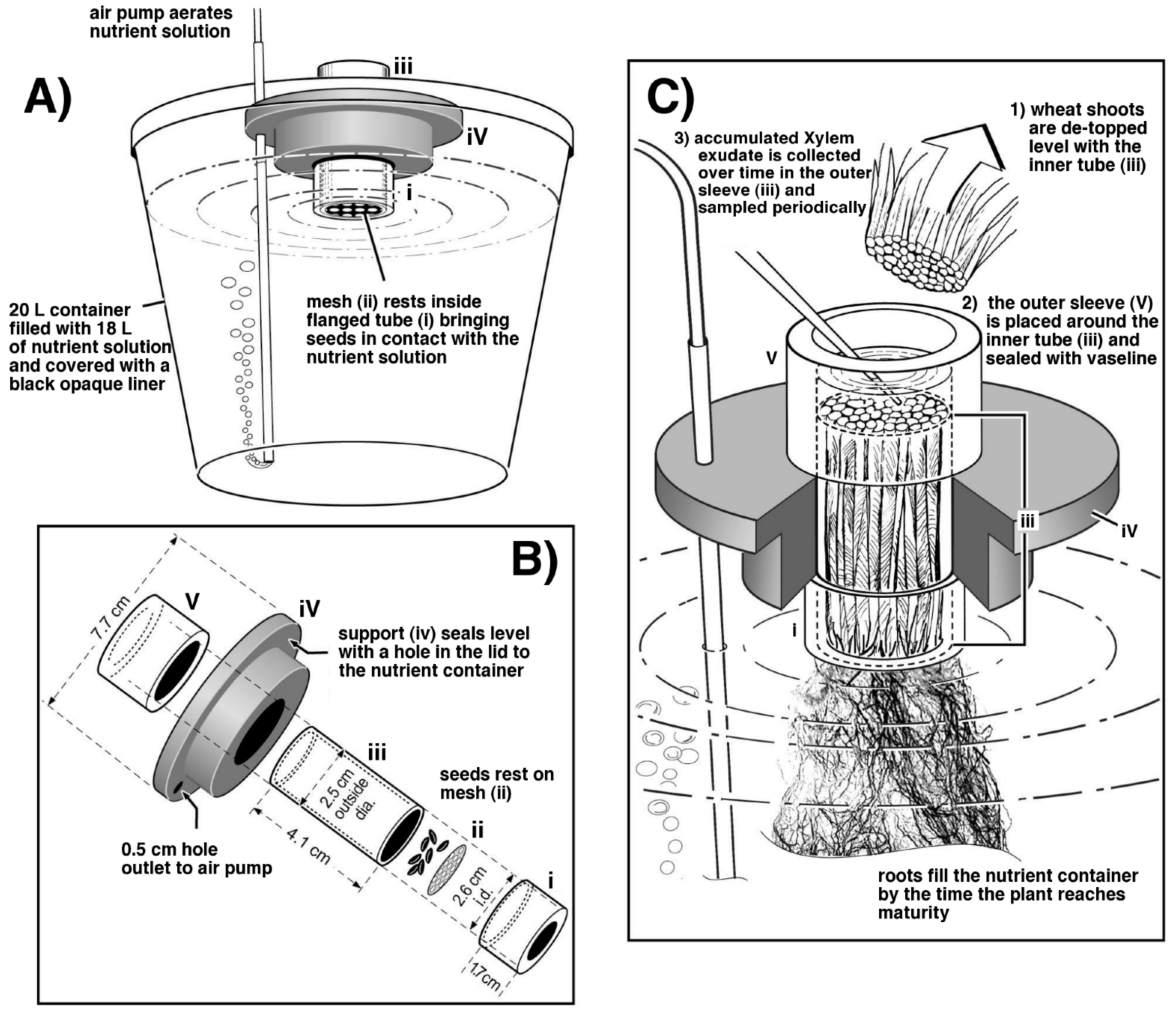

FIGURE 2. Schematics showing (A) 18 L nutrient container lined with a black sheath; (B) design of plastic rigging that supports the wheat stems at the interface between the nutrient solution and plant; (C) detopping of the plant for collection of exudates in the outer sleeve, V. The plants stems are sealed to the inner tube, iii, by the time the wheat plant has reached maturity.

TABLE 2. Magnesium-isotopic composition of samples

sample	δ^{26}Mg[a] (‰) ± 2σ	δ^{25}Mg[a] (‰) ± 2σ	N[b]	Mg yield (%)[c]
DSM3	0	0	28	
Cambridge 1	−2.59 ± 0.15	−1.34 ± 0.08	17	
Cambridge 1 (*22*)	−2.60 ± 0.14	−1.34± 0.07	35	
		Plant 1 Samples		
root	−0.15	−0.08	2	100.1[d]
harvested seed	0.61	0.30	1	99.2[d]
leaf	0.11	0.05	2	97.3[d]
shoot	−0.07	−0.03	2	95.9[d]
exudates				
initial	0.04	0.02	1	95.5[d]
final	0.43	0.22	2	94.0[d]
chlorophyll				
extract 1	−0.34	−0.18	2	102.4[e]
extract 2	−0.58	−0.27	2	102.7[e]
nutrient solution				
day 0	0.08	0.02	2	100.0[f]
day 9	0.19	0.09	2	99.2[f]
day 17	0.03	0.00	3	102.0[f]
day 22	0.01	0.01	2	98.6[f]
day 26	−0.06	−0.05	2	101.5[f]
day 31	−0.11	−0.06	2	102.5[f]
		Plant 2 Samples		
nutrient solution				
day 0	0.09	0.05	1	105.6[f]
day 9	0.15	0.09	1	101.1[f]
day 16	0.035	0.00	1	100.7[f]
day 22	−0.05	−0.03	1	99.3[f]
day 26	−0.10	−0.07	1	99.4[f]
day 31	−0.28	−0.14	1	98.3[f]
day 38	−0.32	−0.17	1	101.1[f]
day 52	−0.47	−0.22	1	96.9[f]

[a] The magnesium isotopic composition of the samples is expressed as a per-mil deviation from the DSM3 standard (*22*) using the formula: $\delta^x\text{Mg} = \{(^x\text{Mg}/^{24}\text{Mg})_{\text{sample}}/(^x\text{Mg}/^{24}\text{Mg})_{\text{DSM3}} - 1\} \times 10^3$. [b] N = number of separate standard-bracketed replicates. [c] Final yield of magnesium in purified sample (measured by AAS) compared to the initial concentration (measured by AAS. [d] UV−vis. [e] Or ICP-MS. [f] The precision of sample and standard measurements is represented by the reproducibility of the Cambridge 1 standard. Relative to DSM3, our repeatedly measured value for the Cambridge 1 standard is comparable to other measurements in the literature (*22*).

coefficients in methanol at 665 and 652 nm (*21*). The chlorophyll samples were then digested in concentrated nitric acid to liberate the magnesium, and the magnesium was purified as above.

Samples of plant material (roots, shoots, and seeds) from plant 1 were dried in an oven at 80 °C for a week. To liberate the magnesium, organic digests of plant 1 material were conducted by refluxing ~0.5 g of wet (leaf) or dry (root, shoot, and seed) plant material in 5 mL of concentrated ultrapure nitric acid in round-bottomed flasks with condensers attached for 1 h at 30 °C; 1 h at 80 °C; and finally, 1 h or longer (as required) at 170 °C until only a clear to yellowish solution was left. The solutions were cooled and diluted with 5 mL of concentrated ultrapure hydrochloric acid and 15 mL of deionized water. A 5 mL aliquot of each sample was taken for magnesium analysis using AAS; another aliquot equivalent to ~0.4 μmol of magnesium was taken for magnesium purification on cation exchange columns, as discussed above. Final yields of magnesium were calculated using AAS (Table 2) and averaged 99.7 ± 5.4%.

Magnesium Isotope Analysis. The purified magnesium fractions (obtained after cation exchange column chemistry, see above) from samples of nutrient solution, chlorophyll, and plant material (Table 2) were dissolved in 0.1 M ultrapure HCl to a final magnesium concentration of ~400 ppb. Measurements were conducted on an MC-ICP-MS (Nu Instruments) using a standard-sample bracketing technique in which every sample measurement was bracketed by a measurement of the international standard DSM3 (*22*). An estimate of the instrumental uncertainty was determined by measuring a second international standard, Cambridge 1, to monitor the established isotopic difference (*22*). The isotopic ratio of the DSM3 standard was extrapolated to the time of sample measurement to standardize the data. Details of this method are reported elsewhere (*2*). Two blank samples, run through the entire sample preparation procedure, had ~0.3% relative to the total magnesium in the samples.

Total Isotopic Composition of the Plant. Estimates for the δ^xMg (see Table 2 footnotes) in the plants (plotted in Figure 5) are calculated using two different equations. One based on the change in nutrient solution composition is

$$\delta^x\text{Mg}_{\text{plant}}^{\text{day}} = \left(\frac{\delta^x\text{Mg}_{\text{nutrient}}^{0} - \delta^x\text{Mg}_{\text{nutrient}}^{\text{day}} X_{\text{nutrient}}^{\text{day}}}{X_{\text{plant}}^{\text{day}}} \right) \quad (1)$$

where x = 25 or 26; day = given day nutrient was sampled; X_y = mol fraction of magnesium in compartment y; assuming it is a closed-system, i.e. $X_{\text{nutrient}}^{0} = X_{\text{nutrient}}^{\text{day}} + X_{\text{plant}}^{\text{day}}$.

The other equation is based on preliminary measurements of the plants magnesium composition at harvest (Table 3):

$$\delta^x\text{Mg}_{\text{plant}} = \delta^x\text{Mg}_{\text{roots}} X_{\text{roots}} + \delta^x\text{Mg}_{\text{shoots}} X_{\text{shoots}} + \delta^x\text{Mg}_{\text{leaves}} X_{\text{leaves}} + \delta^x\text{Mg}_{\text{seeds}} X_{\text{seeds}} \quad (2)$$

RESULTS AND DISCUSSION

The results are summarized in Tables 2 and 3 and Figures 3−6. The magnesium concentration over time in the nutrient solution is shown in Figure 3a for two wheat plants (plants 1 and 2), which were chosen for magnesium isotope analysis. Plant 2 was grown to senescence, at which point it had used 83% (±5%) of the available magnesium in the nutrient solution. Plant 1 was excised at day 31 of growth (having used 52% (±5%) of the available magnesium in the nutrient solution), and xylem exudates were collected for isotopic analysis. The content of magnesium in a sample of the seeds used to grow the wheat and a sample two-week-old seedling was <0.1% of the total magnesium in plant 1 at the point of detopping. Therefore, the magnesium content of the seeds used to grow the plants and two-week-old seedlings transferred to the final growth solutions were considered negligible, and their isotopic composition was not measured.

We observe systematic changes in the magnesium isotope composition of the plant and nutrient solutions during the growth cycle (Table 2). The evolution of the magnesium isotope composition of the nutrient solutions over time is shown in Figure 3b. There is a uniform decrease in the δ^{26}Mg (Figure 3b) and δ^{25}Mg of the nutrient solutions (Table 2). A three-isotope plot (Figure 4) shows that the fractionation of magnesium isotopes in the nutrient solution over time and sampled plant tissues follows a mass-dependent fractionation law. These trends do not follow a Rayleigh-style kinetic fractionation (Figure 5), as has been observed for the sequential fractionation of silicon isotopes in rice (*14*). It appears instead that magnesium uptake from the nutrient solution to the plant follows a closed-system equilibrium that is established across the plant's root membranes. The trends observed in plant 2 (Figure 5) support this hypothesis,

TABLE 3. Plant 1 Mass Balance

samples	total fresh wt (g)[a]	fresh wt, sample digested (g)[b]	dry wt, sample digested (g)[c]	Mg/dry weight (g/g)[d]	mg Mg total[e]
nutrient solution					
day 0					212
day 31					108
totals: estimate 1[f]					104[f]
roots	161.62	161.62	6.76	2.31×10^{-3}	16
seeds	81.34	1.63	0.40	1.16×10^{-3}	23
shoots + leaves	207.24				63[g]
shoots	~177.24[g]	4.62	1.16	7.08×10^{-4}	31.5[g]
leaf sample[h]	~30[g]	0.205		1.05×10^{-3}[h]	31.5[g]
totals: estimate 2[f]	450.20				102[f]

[a] The total weight of plant material harvested. [b] The weight of fresh plant material weighed out for digestion. [c] Dry weight of previous column. [d] Grams of magnesium per gram of dry weight of the given plant material. [e] Total magnesium in the plant (±5 to 10%). [f] Estimate 1 based on change in nutrient composition; estimate 2 based on composition of plant tissues. [g] These weights represent an estimate of the relative contribution of the leaves and shoots to the total "shoots + leaves" fresh weight (±20%). [h] An entire flag leaf sample was digested wet; therefore, the g/g weight reported in column 5 is g/g Mg/fresh weight for the leaf.

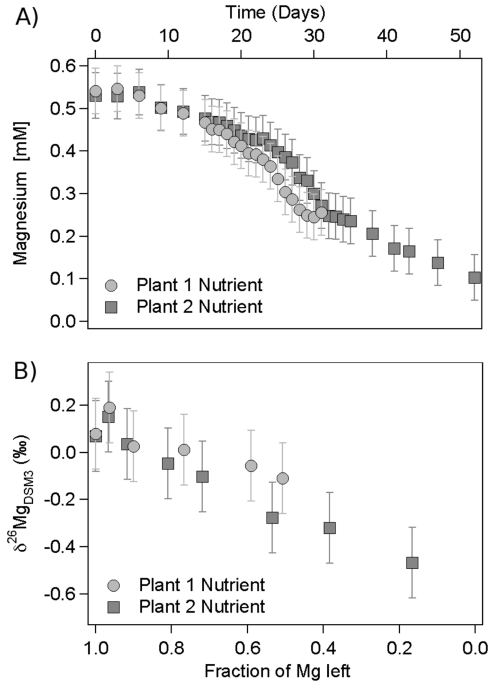

FIGURE 3. Evolution of magnesium reservoir in nutrient solutions showing (A) concentration of magnesium (in units of millimole per liter) over time in nutrient solution measured by AAS; (B) isotopic composition of the nutrient solution as a function of the fraction of magnesium left. Error bars for $\pm 2\sigma$.

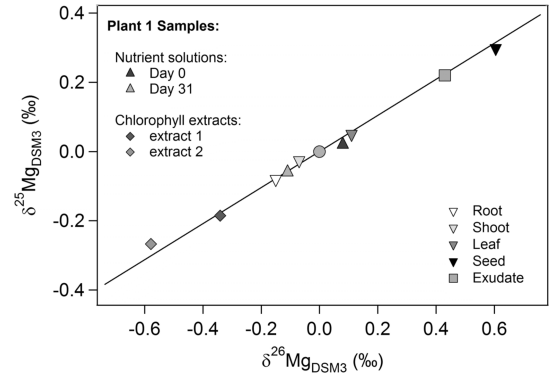

FIGURE 4. Magnesium isotope ratios relative to the DSM3 standard (gray circle). The average results of sample and standard measurements on a three-isotope plot. The error on Cambridge 1 (Table 2) is representative of the error on all sample averages. The solid line represents the equilibrium mass-dependent fractionation line.

the composition of the nutrient solution exhibiting a linear decrease in the δ^{26}Mg with an average fractionation factor of $\alpha_{plant2\text{-}nutrient} = 1.0007$ ($\varepsilon = 0.7$‰). Similarly, a fractionation factor of $\alpha_{plant1\text{-}nutrient} = 1.0004$ ($\varepsilon = 0.4$‰) can be fitted to the trend of the isotopic composition of the nutrient for plant 1 (Figure 5). However, it is more difficult to distinguish the nature of the fractionation in the case of plant 1 because the plant was detopped after using ~50% of the magnesium available in the nutrient solution. The closed-system equilibrium model fit to the data ($\alpha_{plant2\text{-}nutrient} = 1.00068 \pm 0.00008$), presented in Figure 5, is an average of the plant 1 and plant 2 isotope systematics weighted using the 2σ error.

The distribution of magnesium isotopes within plant 1 varies (Figure 6, Table 2). The roots and shoots have approximately the same isotopic composition as the final nutrient solution (day 31), δ^{26}Mg ≈ −0.15, −0.07, and −0.11‰, respectively (well within the uncertainty of replicate analysis of Cambridge 1 standard). Seeds are enriched in the heavy isotopes relative to the final nutrient solution composition (day 0), $\Delta^{26}Mg_{seed\text{-}nutrient} \approx 0.72$‰. The isotopic composition of a leaf relative to the final nutrient solution composition is slightly heavier, $\Delta^{26}Mg_{leaf\text{-}nutrient} \approx 0.22$‰. Two separate chlorophyll extractions from flag leaves are depleted in the heavy isotopes of magnesium relative to the final nutrient solution composition, $\Delta^{26}Mg_{Chl\text{-}nutrient} \approx -0.47$‰ and ≈ −0.23‰. There is a striking difference between wheat and English ivy, in that the magnesium in wheat chlorophyll is isotopically lighter than magnesium in the leaf as a whole, which is opposite from our previous work on English ivy (*3*). In interpreting these differences, it is important to remember that the propagated error of the Δ^{26}Mg is on the order of ≈ 0.21‰, as indicated by repeated analysis of the international standard, Cambridge 1 (Table 2).

The overall isotopic composition of plant 1 at the time of detopping is estimated to be $\delta^{26}Mg = 0.28$‰ and $\delta^{25}Mg = 0.10$‰ using the measured isotopic composition of the nutrient solution (Table 2) and the magnesium concentration of the nutrient solution at day 0 (0.48 mM) and day 31 (0.25 mM) (isotopic estimate 1). One can also estimate the overall magnesium isotopic composition of plant 1 by using the magnesium concentration and isotopic composition measured for each component of the plant (seeds, leaves, shoots, and roots, isotopic estimate 2). Given the isotopic composition of the root, shoots, seeds, and leaves of plant 1 (Table 2) and their contribution to the total magnesium in the plant

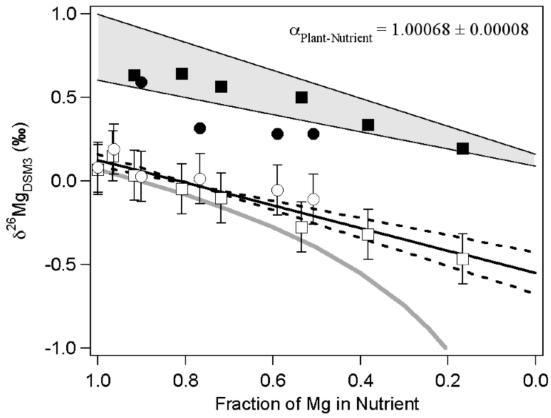

FIGURE 5. Fractionation models for nutrients and plants. Lines of best fit for a closed-system equilibrium given the fractionation factor presented in the top right-hand corner are plotted for the nutrient solution (thick, black, solid and dashed (2σ) lines) and the corresponding range of δ^{26}Mg for the plant (gray shaded region bound by black lines). The Rayleigh fractionation (thick solid gray line) corresponding to the fractionation factor in the top right-hand corner is plotted for the nutrient solution. Measured δ^{26}Mg is plotted for the nutrient solutions (hollow circles = plant 1; squares = plant 2; $\pm 2\sigma$ error bars) and the corresponding composition of δ^{26}Mg calculated in the plants is shown (solid circles = plant 1; squares = plant 2). Estimates of the plants composition at values of *x*-axis ~0.95 are not shown because they are very light relative to the nutrient.

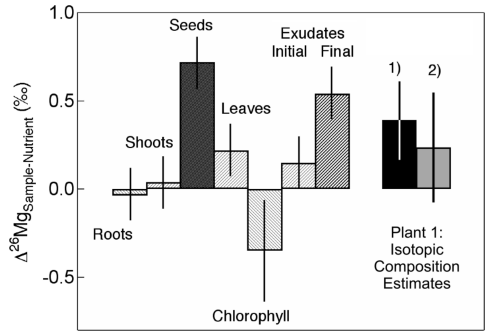

FIGURE 6. Magnesium stable isotope composition of various digested tissues from plant 1, extracted chlorophyll-bound Mg and sample exudates from the detopped plant. Isotopic composition estimates for plant 1 are based on (1) change in [Mg] and $\delta^{x}Mg_{DSM3}$ in nutrient solution by the time of detopping and (2) total weights and isotopic composition of various plant tissues sampled (Table 3). All values are referenced to the final nutrient solution composition on the day of detopping (day 31).

(Table 3), the overall magnesium isotope composition of the plant is calculated to be $\delta^{26}Mg = 0.13‰$ and $\delta^{25}Mg = 0.06‰$. The two independent estimates, one based upon changes in the nutrient compositions and the other from the sum of the plant compositions, are consistent with one another within the error of the isotopic measurements ($2\sigma = \delta^{26}Mg \pm 0.15‰$ and $\delta^{25}Mg \pm 0.08‰$, Cambridge 1, Table 2) and propagated error (plotted in Figure 6). Estimates of the total magnesium in plant 1 (Table 3, totals, estimates 1 and 2) using the two independent methods are also consistent with one another at ~103 mg.

The xylem exudates from plant 1 become enriched steadily in heavy isotopes relative to the final nutrient solution composition (day 31 sample for plant 1 in Table 2), $\Delta^{26}Mg_{exudate\text{-}nutrient} = \delta^{26}Mg_{exudate} - \delta^{26}Mg_{nutrient}$: in initial exudate, ≈0.15‰ (50 min after detopping of the shoots) and final exudate, ≈0.54‰ (150 min after detopping, Table 2). Associated with this enrichment is a rise in the concentration of dissolved magnesium in the exudate solution: the initial exudate contained ~0.79 mM magnesium, and the final exudate, ~1.88 mM magnesium. This increase clearly shows that uptake and transport mechanisms that are responsible for the depletion of heavy isotopes from the nutrient solution are leading to an accumulation of heavy isotopes in the plant.

It is appropriate to discuss briefly the transport of magnesium in plants. We note first, however, that this process has been studied much less than has been the case for the other macronutrient ions: potassium, calcium, and nitrogen. That adds relevance to the present research. Magnesium is initially absorbed by the roots of plants from the nutrient solution in which the predominant aqueous magnesium complex is the $[Mg(H_2O)_6]^{2+}_{(aq)}$ species. It enters the cell wall spaces of epidermal and cortical cells, and from there, it is transported across the outer cell membrane into the cell interior, much as is the case for potassium (*7, 23*). It is then moved into the vessels of the xylem and moved upward in the transpiration stream, which delivers it to the shoots, mainly the interior of leaf cells, that again being a multistep process similar to that in roots. Unlike calcium, magnesium is readily retranslocated from older to young, actively growing leaves and other sinks, including seeds. This translocation takes place through the phloem, the same pathway whereby sugars are transported from the leaves to the roots and other organs (*6*). This very brief survey shows that magnesium absorption and subsequent transport in plants involves a number of separate steps, each of which may be instrumental in affecting magnesium isotope fractionation. With little known about the speciation of magnesium in these transport streams, the stable isotope composition of magnesium will be an invaluable tool for future studies. The data from this preliminary study suggests that a model for a closed-system equilibrium can be used to model the data (Figure 5), with exudate experiments demonstrating that transport up the xylem led to a concentration of the heavy magnesium isotopes. The composition of the removed exudates was not taken into account in the second isotopic estimate presented (Figure 6), and this may in part account for why the measured isotopic composition of the roots of plant 1 was slightly lighter than the final nutrient composition (Figure 6). Further studies are needed to determine the nature of uptake across the nutrient−root interface and associated fractionation.

To summarize the experimental results, wheat produces a mass-dependent fractionation of magnesium isotopes during uptake and transport from the roots to the shoots, leaves, and seeds. Wheat appears to establish an isotopic equilibrium with the nutrient supply with an equilibrium fractionation factor (assuming a closed system equilibrium) between the plant and nutrient of 1.00068 ± 0.00008 (ε = 0.68 ± 0.08‰). The net result is a plant with isotopically heavy magnesium relative to magnesium in the nutrient solution; seeds are particularly heavy relative to the final nutrient composition ($\Delta^{26}Mg_{seed\text{-}nutrient} \approx 0.72‰$). The magnesium in chlorophyll is isotopically lighter ($\Delta^{26}Mg_{Chl\text{-}leaf}$ up to −0.69‰) than that of the whole plant. The seeds contain around 27% of the magnesium in the plant, whereas chlorophyll contains <6% of the total magnesium in the plant. Although wheat is the first higher plant that has been characterized in detail, we speculate that other higher plants will also select for isotopically heavy magnesium.

Environmental Implications. Several broader biogeochemical ideas arise from this work. First, this is one of the first reported cases of a higher plant using the heavy isotopes of an essential mineral nutrient. Two separate estimates of the overall isotopic composition of a wheat plant, one based on changes in the nutrient solution composition and one based upon measurements of plant tissues, show that the plant is isotopically heavier than the final composition of its nutrient supply by $\delta^{26}Mg \sim 0.2‰$. The exudate experiments

conducted suggest that the mechanism of heavy isotope concentration is due in part to processes taking place during the uptake of magnesium from roots to shoots. Second, repeated harvesting of plants, particularly if only the seeds are taken, will result in an isotopic distinction between natural and agricultural soils, particularly if the isotopic composition of bioavailable magnesium is not buffered by mineral dissolution. There is a growing body of work showing that the influence of the biosphere on heavy isotope fractionation is profound (*12*−*15*). These relatively unexplored stable isotope systems may lead to useful new tools for investigating environmental stress and our impact on the environment. It is therefore important that we understand the complex abiotic and biotic controls on observed fractionations.

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