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Journal

ACS Earth and Space Chemistry, 2(7)

ISSN

2472-3452

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Publication Date

2018-07-19

DOI

10.1021/acsearthspacechem.8b00035

Peer reviewed

Potassium and Calcium Isotopic Fractionation by Plants (Soybean [*Glycine max*], Rice [*Oryza sativa*], and Wheat [*Triticum aestivum*])

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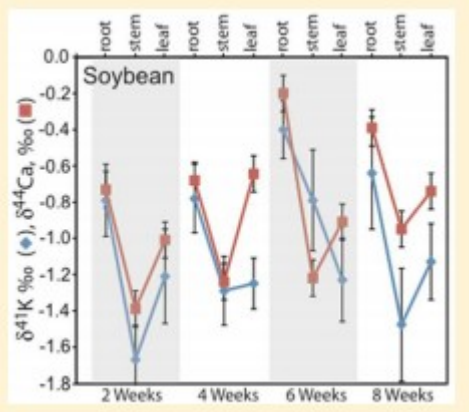
Abstract

We conducted hydroponic experiments growing soybean (*Glycine max*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) under K and Ca replete conditions to establish the degree of K isotopic fractionation by plants, and compare the isotopic fractionation of Ca and K. Each of the test plants displays fractionation relative to the growth solution favoring the light isotopes of K and Ca. The average $\delta^{41}K$ values of the roots from the three plant species were similar, and have an overall average of −0.55 ± 0.24‰ 2s, while the overall average $\delta^{44}Ca$ for roots is −0.67 ± 0.44. For leaves, the overall average of $\delta^{41}K$ is −0.97 ± 0.4‰, compared to an overall average leaf $\delta^{44}Ca$ of −0.83 ± 0.09‰. In the case of the soybean plants, the lightest K and Ca occurs in the stems with average $\delta^{41}K$ of −1.31 ± 0.40‰ 2s and average $\delta^{44}Ca$ of −1.20 ± 0.19 ‰ 2s. We present a simple box model involving the relative fluxes of K and its isotopic fractionation that reproduces our K isotopic observations and suggests a fractionation of ~0.8‰ with K uptake from solution by roots. Directly comparing the per amu fractionation of K and Ca reveals an average factor of 2.05 ± 0.50 2s greater fractionation of K isotopes which may reflect their different roles and behaviors in plants.



Introduction

Potassium (K) and calcium (Ca) are key elements for plant physiology, as evidenced by being the highest concentration cations in plants and, together with N and P, among the most crucial elements for plant growth and productivity.(1−3) K is centrally involved in a wide range of crucial biophysical and biochemical processes in plants, including cell osmotic pressure regulation, growth, regulation of photosynthesis, ion homeostasis, control of cell membrane polarization, enzyme conformation/activation, water conservation and salt resilience.(4,5) Ca has its own crucial roles including as a significant component in cellular walls, the maintenance of membrane integrity against the passive transfer of H^+, K^+, Na^+, and other monovalent cations, and biochemical signaling.(2,6,7) Due to these various essential roles, much research has focused on the mechanisms of K and Ca uptake by and transport within plants.(8−15) For Ca and Mg, another key nutrient element, isotopic fractionation by plants has been investigated both experimentally in the lab to elucidate processes of acquisition and transport, (16−19) but also in the field (e.g., refs (20−25)) as monitors of the local and global biogeochemical cycling (weathering) of these elements. The isotopic fractionation by plants of micronutrients such as Fe, Cu, Zn, and Mo have also been measured.(26−32) In the case of K, it has been suggested that terrestrial plants represent a significant reservoir for K, with as much as 40–70% or more of the dissolved K in the world’s rivers coming from the decay of plant matter,(33) resulting from its crucial role as a plant nutrient. Therefore, potassium isotopic systematics potentially provides a new tool for tracking and quantifying nutrient cycling in ecological systems (e.g., boreal and tropical forests); a proxy for global geochemical cycling; and a research avenue for understanding the optimal fertilization for efficient agricultural food production. To fulfill that potential, the degree of K isotopic fractionation by plants needs to be established.

Two published studies do suggest that plants may fractionate K isotopes. Deviations in the $^{41}K/^{39}K$ ratio of ~1‰ from an in-house reference material favoring the light isotope have been measured by Li et al.(34) in several plant materials (rice grains, chili peppers, tea leaves and Wolfberry fruit), but

with no measurement of the K sources. Likewise, in their broad survey of natural geologic and organic materials Morgan et al.(35) found that a banana and a potato purchased in a Scottish grocery had $^{41}K/^{39}K \sim 0.4‰$ lighter and ~0.1 ‰ heavier respectively than seawater K, but again with no measurements of the K sources. Here we use hydroponically grown plants, with isotopically characterized K and Ca nutrient sources, to quantify and compare the isotopic fractionation of K and Ca by three species of vascular plants: soybean (*Glycine max*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*). These three plants were chosen to cover a selection of important food crops; and each are C3 plants. We choose to compare K and Ca isotopic fractionation for several reasons. First, the atomic weights of K and Ca are relatively close so that mass fractionations can be easily compared. Second, K and Ca have contrasting roles in plant physiology as described above, and have contrasting valencies. Third, there is a body of experimental data on Ca isotopic fractionation by plants that we can compare our Ca isotopic results to (e.g., 18).

Methods

Soybean, rice, and wheat were grown from seeds and cultivated in a large hydroponic system at the U.C. Berkeley Oxford Facility greenhouse. The hydroponic solution, a modified Hoagland's solution replete with Ca nitrate (1.25 mM) and K nitrate (2.5 mM, somewhat higher than the typical range of 0.1–1 mM in soil porewaters,(36) was replenished periodically to provide an isotopically constant source of Ca and K during plant growth. Plants were harvested before full maturity for analysis after growth periods of 2, 4, 6, and 8 weeks. Harvested plants were divided broadly for soybeans into root, stem, and leaf samples, and for rice and wheat plants into samples of roots and leaves. The plant samples were rinsed, dried, and weighed, and then ashed before complete dissolution in high-purity nitric acid. Aliquots were taken of the sample solutions for K and Ca isotopic analyses. K was separated from the sample aliquots using AG50×8 cation resin and 1 M HNO_3.(37) The K isotopic analyses were conducted on an IsoProbe MC-ICPMS at Lawrence Berkeley National Lab (LBNL) using a sample-standard bracketing technique where Ar-based mass interferences were removed (and peak tailing avoided) by the introduction of Ne + H_2 gas to the hexapole collision cell.(38) Results are reported as per mil deviations of the $^{41}K/^{39}K$ ratio ($\delta^{41}K$) relative to an in-house K standard using a spectroscopic concentration standard (ULTRA Scientific). We estimate that our in-house standard on the Bulk Silicate Earth (BSE) scale of Wang and Jacobsen(39) has an approximate $\delta^{41}K_{BSE}$ of +0.5‰. For Ca isotopic analysis, the sample aliquots were spiked with a $^{42}Ca–^{48}Ca$ double spike before chemical separation using Eichrom DGA resin eluted with 3 N HNO_3 and separated Ca collected with DI H_2O.(40,41) The spiked Ca separates were then analyzed for isotopic composition using thermal ionization mass spectrometry with a Triton multicollector instrument using a multidynamic Faraday cup routine. The Ca isotopic results are reported as per mil deviations of the $^{44}Ca/^{40}Ca$ ratio ($\delta^{44}Ca$) from the Bulk Silicate Earth

(BSE) $^{44}Ca/^{40}Ca$ of 0.0212035.(42) On this scale our long-term average $\delta^{44}Ca$ of SRM915A is −1.0 ± 0.1 2s. Further details of the K and Ca separation and isotopic analyses are presented in the Supporting Information.

Results

The K isotopic composition of the K-nitrate used in the hydroponic solutions is indistinguishable from the in-house standard (Table 1). Likewise, the Ca isotopic composition of the Ca nitrate is indistinguishable from BSE (Table 1). For both K and Ca isotopic compositions, each of the test plants show fractionation favoring the light isotope (Figure 1). No resolvable trends with growth time in K or Ca isotopic composition are observed, which indicates that replenishment of the hydroponic solution provided an effectively infinite K and Ca source reservoir during plant growth, and that the size of the plants had no discernible effect on the magnitude of the isotopic fractionation for each species. The greatest degree of K and Ca isotopic fractionation is seen in the soybean plants, followed by wheat and then rice. The $\delta^{41}K$ analyses of plant roots represents K acquisition, and indicates a fractionation of −0.65 ± 0.22‰ for soybean (average ± 2s, n = 4), −0.40 ± 0.17‰ for rice (average ± 2s, n = 4) and −0.74 ± 0.36 (average ± 2s, n = 3) with an overall average $\delta^{41}K$ of −0.55 ± 0.24‰ 2s. For Ca, the roots gave $\delta^{44}Ca$ of −0.50 ± 0.26‰ for soybean (average ± 2s, n = 4), −0.67 ± 0.4 for rice (average ± 2s, n = 3), −0.82 ± 0.24 (average ± 2s, n = 3) for wheat with an overall average $\delta^{44}Ca$ of −0.67 ± 0.44. The average K isotopic compositions of leaves are −1.216 ± 0.095‰ (n = 4) for soybean, −0.62 ± 0.18‰ (n = 4) for rice, and −1.11 ± 0.46‰ (n = 4) for wheat with an overall average of −0.97 ± 0.4‰, compared to an overall average leaf $\delta^{44}Ca$ of −0.83 ± 0.09‰. The root-leaf difference in $\delta^{41}K$ for each of the plants is small with the average difference for soybean of 0.57 ± 0.37‰, for rice of 0.26 ± 0.28‰ and for wheat 0.59 ± 0.3‰. The average root-leaf $\delta^{41}K$ difference for all the plants is resolvable from zero at 0.47 ± 0.16‰ however the average root-leaf difference in $\delta^{44}Ca$ of 0.03 ± 0.25‰ is essentially zero. For the soybean plants, where stems were analyzed in addition to roots and leaves, the lightest K occurs in the stems ($\delta^{41}K$ avg = −1.31 ± 0.40‰ 2s) as does the lightest Ca ($\delta^{44}Ca$ avg = −1.20 ± 0.19 ‰ 2s), with average root-stem differences of 0.64 ± 0.26 ‰ for $\delta^{41}K$ and 0.70 ± 0.23% for $\delta^{44}Ca$. A similar root-stem difference in $\delta^{44}Ca$ of 0.45 ± 0.09 ‰ has been observed in experiments with French beans under similar growth conditions.(18) A small or zero root-leaf difference in $\delta^{44}Ca$ is also consistent with the observations in Cobert et al.(18)

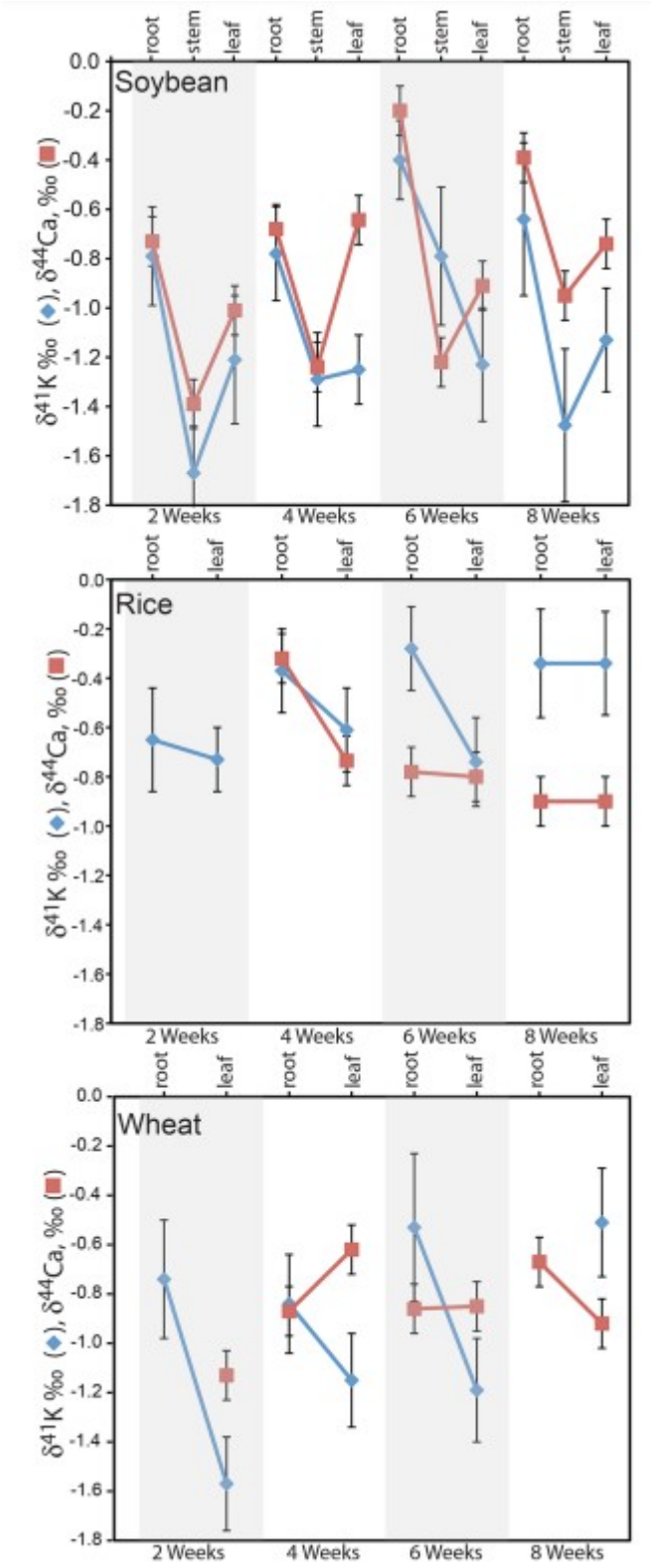


Figure 1. K and Ca isotopic compositions of plants grown hydroponically in this study, and harvested after 2, 4, 6, and 8 weeks. Uncertainties shown are ±2s. Top: soybean (roots, stems, leaves). Middle: rice (roots, leaves). Bottom: wheat (roots, leaves). The $\delta^{41}K$ and $\delta^{44}Ca$ of the K and Ca used in the hydroponic solution are both essentially 0.

Table 1

weeks	sample	$\delta^{41}K$	±2s	$\delta^{44}Ca$	±2s
2	#1 wheat root	−0.74	0.24		
2	#1 soy root	−0.79	0.20	−0.73	0.1
2	#1 soy stem	−1.67	0.19	−1.39	0.1
2	#1 soy leaf	−1.21	0.26	−1.01	0.1
4	#2 soy root	−0.78	0.19	−0.68	0.1
4	#2 soy stem	−1.29	0.19	−1.24	0.1
4	#2 soy leaf	−1.25	0.14	−0.64	0.1
6	#3 soy root	−0.4	0.16	−0.2	0.1
6	#3 soy stem	−0.79	0.28	−1.22	0.1
6	#3 soy leaf	−1.23	0.23	−0.91	0.1
8	#4 soy root	−0.64	0.31	−0.39	0.1
8	#4 soy stem	−1.48	0.31	−0.95	0.1
8	#4 soy leaf	−1.13	0.21	−0.74	0.1
2	#1 rice root	−0.65	0.21		
2	#1 rice leaf	−0.73	0.13		
4	#2 rice root	−0.37	0.17	−0.32	0.1
4	#2 rice leaf	−0.61	0.17	−0.74	0.1
6	#3 rice root	−0.28	0.17	−0.78	0.1
6	#3 rice leaf	−0.74	0.18	−0.8	0.1
8	#4 rice root	−0.34	0.22	−0.9	0.1
8	#4 rice leaf	−0.34	0.21	−0.9	0.1
2	#1 wheat root	−0.74	0.24		
2	#1 wheat leaf	−1.57	0.19	−1.13	0.1
4	#2 wheat root	−0.84	0.20	−0.87	0.1
4	#2 wheat leaf	−1.15	0.19	−0.62	0.1
6	#3 wheat root	−0.53	0.3	−0.86	0.1
6	#3 wheat leaf	−1.19	0.21	−0.85	0.1
8	#4 wheat root			−0.67	0.1
8	#4 wheat leaf	−0.51	0.22	−0.92	0.1
	K and Ca nitrate	0	0.18	−0.03	0.10

The overall pattern of isotopic fractionation of K and Ca is similar for each of the plants, but there is a difference in the relative size of the fractionation. Since different mass differences are involved in the measured isotopic compositions of K (^{41}K and ^{39}K, a difference of 2 amu) and Ca (^{44}Ca and ^{40}Ca, a difference of 4 amu), to directly compare the isotopic fractionation of these two elements by plants it is useful to compare them on a per amu basis (Table 3). The average ratio of the per amu fractionation of K isotopes to Ca isotopes is 2.05 ± 0.50 suggesting an approximate factor of 2 larger discrimination by plants of the light K isotope compared to the light Ca isotope, though they have similar atomic masses.

Discussion

K and Ca Transport Flux Model and Fractionation Factors

We have measured differences in isotopic ratios between different parts of the plants and between the plants and the source K and Ca in the hydroponic

solution (our analog for “soil porewater”). We would, however, also like to say something about the isotopic fractionation that accompanies the movement of K and Ca between “soil porewter” and roots and then within the plant. This fractionation may not be simply the difference in isotopic ratio, because each part of the plant has fluxes of K and Ca entering and exiting. To address this issue we have constructed a fairly simple box model that treats each of the major parts of the plant (roots, stem, leaves) as a separate reservoir and evaluate how K and Ca isotopes would need to be differentially transported to explain the observations.

The model and the associated equations (Figure 2) are explained in more detail in the Supporting Information. Isotopic fractionation is assumed to occur during transfer of K and Ca between reservoirs. The isotopic fractionation factors (ε) represent the difference in $\delta^{41}K$ (or $\delta^{44}Ca$) between the K (or Ca) transported out of a reservoir, and the average value in the reservoir. The accumulation rate of K in each reservoir is assumed to be proportional to the (mass) growth rate of that reservoir. For example, the growth rate of roots is proportional to $F_1 + F_4 – F_2$, and the growth rate of stems is proportional to $F_2 – F_3$. The ratio of the growth rate of root to the growth rate of shoots (=stem + leaves) is $[F_1/(F_2 – F_4)] – 1$. The proportionality between K content and mass is consistent with the generally constant concentration of K in plant biomass, although what differences there are would need to be factored in for relating the model flux values to mass changes.

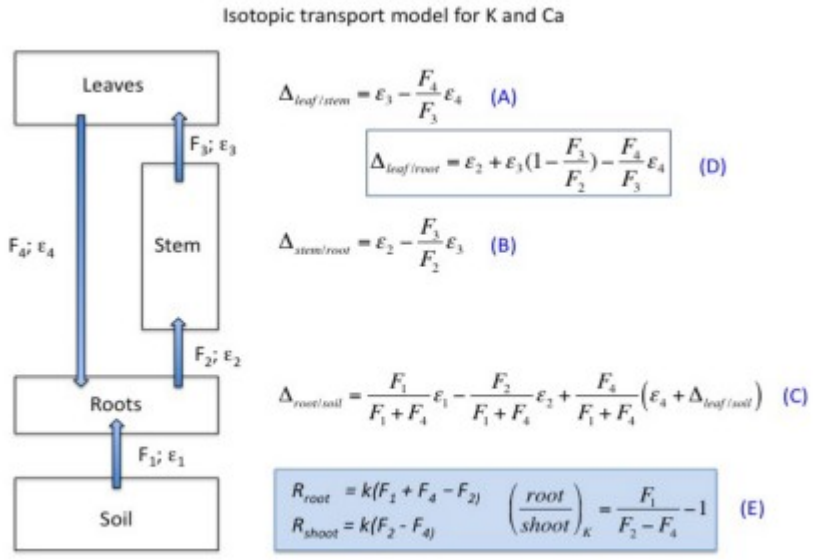


Figure 2. Simple box model for the transport of K and Ca in plants, where F_i are fluxes of K or Ca, ε_i are isotopic fractionation factors, and Δ_i are isotopic differences (e.g., between stem and root). Seethe text for explanation and use of the model, as well as the Supporting Information for details of its derivation.

The observed difference between root K and hydroponic solution K (“soil porewater” K referred to as “soil”) is in the range of $\Delta_{root/soil} = -0.5$ to -0.9‰.

This observed difference is a function of various K fluxes (F_i) and isotopic fractionation factors (ε_i) as shown in Figure 2. The model and data do not allow for much latitude with regard to the fractionation factors, but we work through the analysis to show how the model can be used to deduce the ε_i values. We begin with the Δ values (difference between $\delta^{41}K$ values) between leaf and stem, or between stem and root. For example, the typical values of $\Delta_{stem/root}$ are -0.5 to -0.8‰ (Table 2). Since leaves are typically heavier than stems, and can be affected by recycling of K back to the roots, it may be reasonable to set $\varepsilon_3 = 0$‰ as a first guess, which then requires that $\varepsilon_2 \approx -0.5$ to -0.8‰ (fractionation between the root-to-stem flux and roots; Figure 2, eq B). Because the bean leaves typically have $\delta^{41}K$ that is similar to or slightly higher than that of stems, it suggests that ε_4 has a small negative value, which we estimate as -0.2‰ (or slightly larger because $F_3/F_4 < 1$ if there is leaf growth; Figure 2, eq A).

Table 2. Averages of the Data from Table 1

	avg $\delta^{41}K$	±2s	avg $\delta^{44}Ca$	±2s
soybean root	−0.65	0.22	−0.5	0.26
soybean stem	−1.31	0.4	−1.2	0.19
soybean leaf	−1.216	0.095	−0.83	0.18
rice root	−0.4	0.17	−0.67	0.4
rice leaf	−0.62	0.18	−0.81	0.11
wheat root	−0.74	0.36	−0.82	0.24
wheat leaf	−1.11	0.46	−0.88	0.22

Table 3. Comparison on a per amu Basis of the Fractionation of K and Ca Isotopes by Plants in This Study

	K isotopic fractionation per amu	±2s	Ca isotopic fractionation per amu	±2s	K/Ca isotopic fractionation per amu	±2s
soy root	−0.33	0.11	−0.13	0.07	2.6	1.6
soy stem	−0.66	0.15	−0.30	0.05	2.2	0.6
soy leaf	−0.61	0.05	−0.21	0.05	2.9	0.7
rice root	−0.20	0.09	−0.17	0.10	1.2	0.9
rice leaf	−0.31	0.09	−0.20	0.06	1.5	0.6
wheat root	−0.37	0.18	−0.21	0.06	1.8	1.0
wheat leaf	−0.56	0.23	−0.22	0.06	2.5	1.2
ave. of all	−0.43	0.14	−0.21	0.05	2.05	0.5

Given that we have values for ε_2 (-0.6 ‰) and ε_4 (-0.2 ‰) we can substitute them into the equation for the (measured) effective root/soil fractionation (Figure 2, eq C):

$$\Delta_{root/soil} = \frac{F_1}{F_1+F_2}\varepsilon_1 + \frac{F_2}{F_1+F_2}\varepsilon_2 + \frac{F_4}{F_1+F_2}(\varepsilon_4 + \Delta_{soil/soil}) \quad (1)$$

We have measured $\Delta_{root/soil}$ to be -0.5 to -0.8‰, so by the above reasoning we now have values for all of the parameters except ε_1 and the fluxes.. To go farther we need approximate values for the K flux ratios, and we use $F_1:F_2:F_3:F_4 = 1:0.8:0.6:0.4$ based on published experiments on lupin. (43) With these values the stems and leaves are growing at the same rate, and 40% of the K being taken up by the plant is being returned to the roots

from the leaves. The root/shoot mass ratio would be 1.5, which is a typical value.(44) Substituting into equation 1, and estimating $\Delta_{leaf/soil} = -1.2‰$ (Table 2), gives $\varepsilon_1 = -0.7$ to $-1.0‰$ (fractionation between root and soil pore water). Using this value and the flux ratios given above we can then deduce a self-consistent set of isotopic Δ values:

$$\Delta_{root/soil} = -0.6‰; \quad \Delta_{stem/root} = -0.6‰; \quad \Delta_{leaf/stem} = +0.1‰ \quad \text{and} \quad \Delta_{leaf/soil} = -1.1‰$$

Thus, given a plausible set of fluxes, and the fractionation factors (ε's) deduced above, we can reproduce the observed general pattern and magnitudes of K isotopic distribution. Our analysis suggests that the isotopic discrimination associated with K uptake from the hydroponic solution is the largest ($\varepsilon_1 = -0.7$ to $-1.0‰$), the discrimination associated with transfer of K from root to stem is the next largest ($\varepsilon_2 \approx -0.6‰$), and the other two discrimination factors are small (0 to $-0.2‰$). For the example given, if the hydroponic solution has $\delta^{41}K = 0$, then the $\delta^{41}K$ values for the roots, stems, and leaves would be -0.6, -1.2, and $-1.1‰$, respectively, similar to those observed (Table 2). As a check, the bulk plant $\Delta^{41}K$ value relative to the solution must be equal to ε_1. We do not have measurements of the relative masses of the plant parts, but it is evident from the data that the bulk plant has a $\delta^{41}K$ somewhat lower than the roots, and hence is likely to be in the same range as inferred for ε_1 (i.e., $\Delta^{41}K_{plant\text{-}solution} \approx -0.7$ to -1.0)

The model can help evaluate isotopic fractionation in the plants under different growth conditions, because the observed isotopic differences between plant components depend on the flux ratios, even if the fractionation factors are constant. For example, if roots are not growing significantly in comparison to shoots ($F_2/F_1 \rightarrow 1$), then the observed root/soil fractionation becomes smaller even though the fractionation factor (ε_1) remains the same. If roots are growing much faster than shoots, then the observed root/soil fractionation becomes larger. These flux ratios are likely to vary over the lifetime of the plant, and also depend on soil conditions and nutrient availability.

The transport model for Ca is somewhat simpler than that for K in that there is virtually zero return of Ca to the roots from leaves.(43) However, there is a complication in that for Ca the leaves are more consistently higher in $\delta^{44}Ca$ than stems compared to the pattern for $\delta^{41}K$. In their Ca-replete experiments Cobert et al.(18) also found stems to have lower $\delta^{44}Ca$ than roots or leaves. The only way to accommodate these observations in the context of our model is to have the parameter $\varepsilon_3$have a positive value for Ca isotopes. This would tend to make the stems lighter than both the leaves and roots. The adsorption of light Ca (low $\delta^{44}Ca$) into cell walls lining xylem pathways has been suggested as the mechanism for isotopic fractionation between roots and shoots,(45,18,19)and would be consistent with a positive ε_3. This light Ca loss during transport would cause the Ca carried in the xylem to become progressively enriched in heavy isotopes, making the leaves generally higher in $\delta^{44}Ca$ than stems. Excess Ca sequestered as Ca-oxalate nodules could

enhance this fractionation, as Ca-oxalate derived from leaves has been shown to have lower $\delta^{44}Ca$ than the free-Ca in leaves.(18,19)

Mechanisms of K and Ca Transport in Plants

The model described above is nonspecific with regard to the transport mechanisms and source of isotopic fractionation. A number of different mechanisms have been called upon to explain isotopic fractionation within plants(17−19,28−32,45,46) including adsorption or ligand binding, transport processes across cellular membranes some of which involve transporter proteins, and diffusion within plant fluids involving differences in diffusivity between isotopes. This last mechanism, diffusion within the vascular fluids of plants, has been proposed for Zn by Moynier et al.,(31) which has been also taken up as on mechanism for Cu isotopic fractionation in plants.
(28) Moynier et al.(31) in considering this mechanism calculate over a 10 cm length scale the fractionation of Zn isotopes by diffusion and derive a value for the ratio of the diffusivities of ^{66}Zn to ^{64}Zn within a factor of 10 of a published value for Zn diffusion in water and conclude that this mechanism could explain, at least in part, their observation of increasing Zn isotopic fractionation with leaf height. However, their diffusion model assumes a stagnant fluid, and does not take into account fluid movement within the plant. To resolve whether diffusive isotopic fractionation can be a significant mechanism, we calculate the Péclet (Pe) number (eq 2), a dimensionless number that compares the relative importance of fluid advective transport to diffusion in the fluid:

$$\mathrm{Pe} = vL/D \quad (2)$$

where v is the fluid velocity, L is the length scale, and D is the diffusivity. For Pe < 1 diffusive transport dominates, for Pe > 1 advective transport dominates. Using a length scale of 10 cm, a fluid velocity in the xylem of between 0.25 mm/s (night conditions) and 0.4 mm/s (day conditions) from measurements in castor bean plants(47) and a diffusivity of K in water of 1.8×10^{-9} m^2/s(48)yields a Pe number of on the order of 10^4, indicating that diffusive effects within xlyem and phloem are completely negligible. This is likely true for other nutrient elements in plants, since they likely have diffusivities that are within an order of magnitude of 10^{-9} m^2/s.

Plant cellular cytoplasmic resting concentrations of Ca^{2+} are on the order of 100 nM, a factor of $\sim 10^6$ less than cytoplastic K^+ concentrations, and can rise to ~1 μM in its cellular role in molecular signaling.(2,6,36) This requirement of extremely low Ca^{2+} concentration in the cytoplasm is a result of its tendency to form insoluble salts with sulfates, phosphates and other compounds that would be detrimental for the cell. Instead, Ca^{2+} is stored in cell organelles such as vacuoles at ~1 mM levels, or in the apoplast. Unlike K^+, Ca^{2+} has key structural roles in providing binding in cellular membranes, as well as in cellular walls and other rigid structures of plants. Based on this structural role for Ca^{2+} and Ca experimental isotopic observations, Cobert et al.(18) and Schmitt et al.(19) call on a solid/fluid ion exchange process (akin

to ion chromatography) to explain plant Ca isotopic fractionation, both during Ca^{2+} uptake by plant roots, as well as during apoplastic and xylem transport, and precipitation in leaves of Ca-oxalate (this latter process is emphasized by both(19) and by Moynier and Fujii(49) for root-leaf fractionation). In contrast to Ca^{2+}, the bulk of which in plants participates in solid/liquid exchange processes, K^+ participates mostly as a dissolved ion in plants.(4)

In order to maintain the ~100 mM cellular cytoplasm K^+ concentration, K^+ must be driven up a concentration gradient from the lower K^+ concentration external solution, either from the soil solution or from extracellular fluids.(4) For K transport into plant cells, two broad mechanisms operate: a high affinity K transport system (HATS) under low K^+ external concentration ($[K^+] < 1$ mM, and a low affinity K transport system (LATS) under high K^+ external concentration ($[K^+] > 1$ mM), reflective of its key and multifaceted role in plant nutrition.(9) The LATS uses highly selective K-specific ion channels across the cellular membrane, while the HATS uses transporter proteins and synporter systems. For our experiments with $[K^+] = 2.5$ mM, the LATS is likely the dominant mode of K^+ influx from the hydroponic solution to root cells and throughout the plant, the energetically intensive HATS mode being saturated and down-regulated. After uptake by root cells, K^+ follows a variety of routes (symplastic and apoplastic) to the xylem fluid for distribution throughout the plant, delivery to symplastic and apoplastic pathways for trans-membrane uptake by cellular tissues. (11) Transport of solute-bearing fluids through the xylem is hydraulically driven by transpiration from the leaves, and root pressure arising from osmotic processes.(50) Thus, the most likely processes affecting K^+ isotopic fractionation are the various means of transport across cellular membranes.

K^+ specific channels for transport across cellular membranes (favored in LATS) can be highly selective; Na^+ for example can be selected against by a factor of 10 000 compared to K^+,(51) that is for every 10 000 K atoms entering a K^+ specific channel only one Na ion does. It has been suggested that the 25% difference in ionic radius between Na^+ and K^+ is a key factor in this selectivity.(51,52) Figure 3 shows a plot of estimated selectivities relative to K^+ as a function of ionic radius. With ionic radii differences of 9% for Rb^+ and 19% for Cs^+, selectivity against those ions is also fairly significant,(51,53) further suggesting a partial role for ionic radius in the selectivity of K^+ channels. This apparent sensitivity to ionic radius suggests a possible relationship to K^+ isotopic fractionation. Ionic radius is affected by isotopic mass, with smaller radii trending with increasing nuclear mass (i.e., number of neutrons) for an element. For instance, ^{22}Ne is ~0.2% smaller than ^{20}Ne(54) (Figure 4). Using the difference in ionic radius between Na^+ and $K^+$to scale the selectivity against Na^+ by K^+ specific channels we extrapolated to a size difference between ^{41}K and ^{39}K ions of 0.0035% required to produce a 1‰ selectivity against ^{41}K relative to ^{39}K, that would be sufficient to account for our K isotopic observations. This estimated radius difference between ^{41}K and ^{39}K is consistent with the trend shown in Figure 4.

The K+ specific channel may be optimized to the size of $^{39}K^{+}$, the more common isotope of K (^{39}K is about 93.3% while ^{41}K is about 6.7% of K).

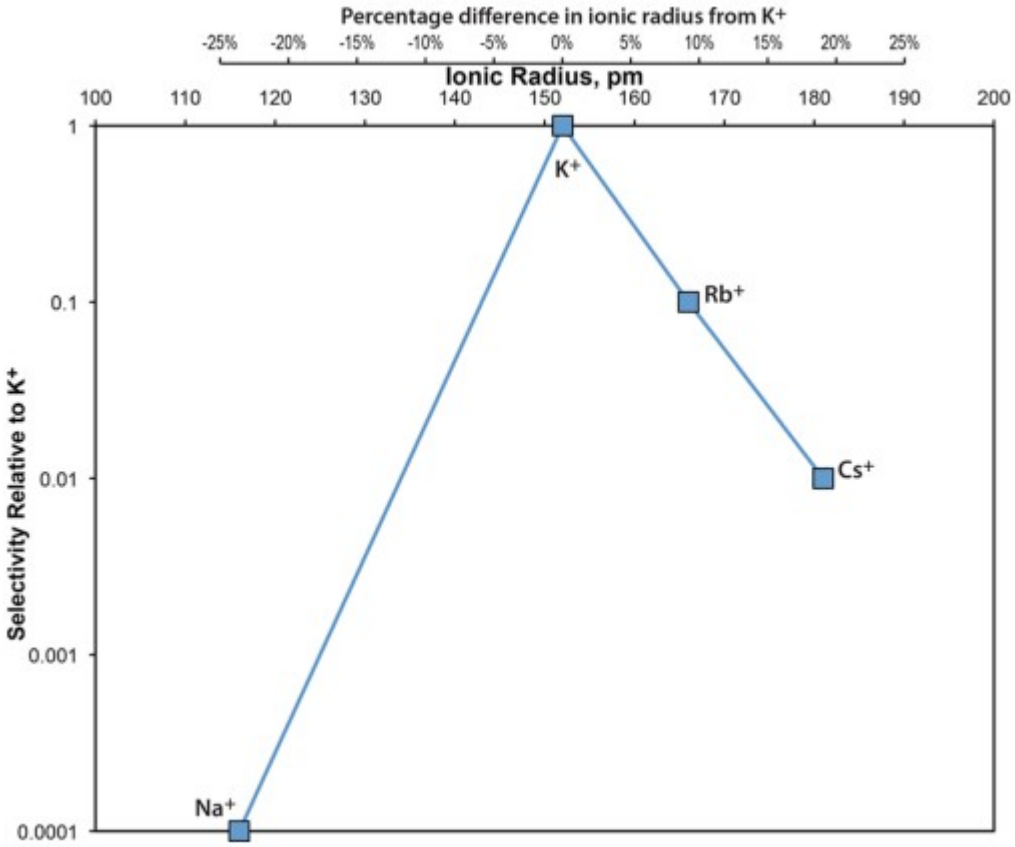


Figure 3. Plot of plant K^{+} channels estimated uptake selectivity of Na^{+}, Rb^{+}, and Cs^{+} relative to K^{+}(51,53) under potassium replete conditions vs ionic radius and the percentage difference in ionic radius relative to K^{+}.

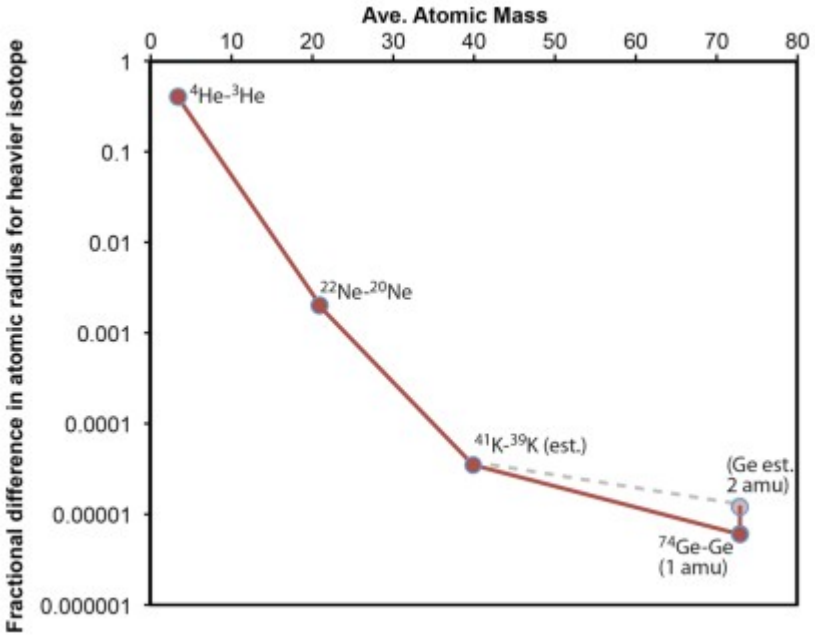


Figure 4. Average atomic mass of the element vs the fractional difference in atomic radius for the heavier isotope. He,(63) Ne,(54) K as estimated in the text, and Ge.(64) The faded symbol for Ge represents an estimated extrapolation to 2 amu of the fractional difference in size.

Another possible effect to consider with transport across cellular membranes is that ions would need to be desolvated before their passage. Hoffman et al. (55) suggest through molecular dynamics simulations, that water exchange rates for solvated ions has a rate dependence on the mass of the solvated ion such that the lighter isotope is favored in desolvation, resulting in a predicted ~2‰ fractionation in $^{41}K/^{39}K$ between a precipitating solid and the solution. However, a further feature of K^+ specific channels lies in the process of desolvation/solvation of K^+ during transmembrane transport. Solvated K^+ enters the channel where the K^+ is handed off to eight protein bonded oxygen atoms replacing the role of the solvating H_2O molecules, minimizing the energy required for desolvation, with resolvation occurring with the exit of K^+ from the channel.(56)Therefore, we suggest that isotopic fractionation due to dehydration of K^+ accompanying K^+channel transport may be minimal. Instead we hypothesize that their size selectivity as argued above is the main source of fractionation of K isotopes.

We propose that the HATS, operative under low external K^+, would fractionate K isotopes less that the LATS, which in large part depends on highly selective K^+ ion channels. This suggestion is supported by experiments involving cells of a marine diatom where under low external Zn concentration (HA, high affinity transport), the observed isotopic fractionation of Zn is four times less than under high Zn external concentration (LA, low affinity transport).(57) A difference between HA vs LA transport has also been called upon by Deng et al.(58) to explain differences in Zn isotopic fractionation between Zn nonaccumulating and Zn

hyperacumulating plants. For isotopic fractionation of K in plants, a test of this would be examining K isotopic fractionation as a function of external solution K^+ concentration. Another possible test would be experiments involving genetically modified plants that are capable of only either HATS or LATS K^+ transport to look for contrasts in K isotopic fractionation.

Conclusions

We have shown through hydroponic growth experiments that soybean, wheat and rice can fractionate the isotopes of potassium relative to the nutrient source, with all portions of the plant lighter than the source K. While the pattern of root to leaf K isotope fractionation is similar to that of the isotopic fractionation Ca, the magnitude of the fractionation on a per amu basis is about 2 times greater for K than for Ca, and may be related to different mechanisms of fractionation- in the case of Ca mainly association with solid/liquid exchange(18) and precipitation of oxalates in leaves (as proposed by refs (19) and (49)) versus K isotopic fractionation associated mainly with trans-membrane transport.

The box-model proposed here may be a useful framework for using K and Ca isotopes in plants to constrain the relative transport fluxes and mechanisms for these elements, and how they change with nutrient availability, light levels, plant maturity, and other factors.

Since K is a significant nutrient for plants, and is often added as fertilizer in agriculture,(59,60)there may be advantage in being able to isotopically distinguish between resident soil K, the K added as fertilizer, the K in the plants, and how it is recycled back (or lost) in fallow periods. Drought and salt stress is a significant challenge in agriculture, and understanding the roles and mechanism of K and Ca in the response of plants to stress is an active area of research.(61) From our discussion, as Ca and K appear to be fractionated by different processes in plants, the comparisons between their isotopic behaviors with variation of nutrient availability and salt loading may prove illuminating. In particular, conducting experiments with mutant plant varieties with different trans-membrane transport processes shut off may provide insights into the operation of K^+ transport channels and their ion selectivity.

Our results also bear on the global geochemical cycling of K. Seawater K is nearly 0.6‰ heavier than BSE,(39,24) and based on K isotopic analyses of porewater from oceanic sediments and modeling of that data Santiago Ramos et al.(62) suggest that weathering of sediments on the seafloor is a sink for ^{39}K, driving seawater K to a heavy $\delta^{41}K$ value. From our experiments, it would appear that plants would be either lighter than the BSE, or about the BSE value depending on the K source. With up to 40–70% of the dissolve K load of rivers coming from plant decay,(33)the light K of plants should be a driver of seawater K toward lighter values, with the best test being a K isotopic survey of major rivers. This emphasizes the need identified by Santiago Ramos et al.(62) for an oceanic sediment sink for light K.

Acknowledgments

This research was supported by from the US Department of Energy, Office of Science, Office of Basic Energy Sciences under Award No. DE-AC02-05CH11231 to Lawrence Berkeley National Laboratory. We wish to thank Barbara Rotz of the Oxford Greenhouse for her assistance in setting up and tending the hydroponic experiments. We also thank three anonymous reviewers whose comments helped us improve the manuscript.

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