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Natural potassium (K) isotope fractionation during corn growth and quantification of K fertilizer recovery efficiency using stable K isotope labeling

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ABSTRACT: An improved understanding of the potassium (K) cycle in the soil–plant system is scientifically and economically significant, but the conventional research based on K concentration measurements has several known limitations. The recent advent of high-precision stable K isotope analysis (reported as $\delta^{41}K$ values) can facilitate the use of both stable K isotope labeling and mass-dependent isotopic fractionation in studying the K nutrient cycle, including K fertilizer utilization, and plant–soil interactions. As a proof-of-concept, we conducted a pot study to quantify uptake of K fertilizer by corn. Three groups of treatment (50, 100, 200 mg K kg^{-1} soil) were conducted using soils pre-mixed with different amounts of ^{41}K-labeled fertilizer. A control group used the same soil without fertilizer treatment. Aboveground shoots and soils were sampled and analyzed after ~6 weeks. The control group showed preferential uptake of light K isotopes by corn with an estimated mass-dependent fractionation of ~-0.37‰ (±0.23‰) in $^{41}K/^{39}K$ between shoot and soil. In fertilized experiments using an enriched ^{41}K tracer, $\delta^{41}K$ data unambiguously quantifies fertilizer-derived K in corn shoots, yielding apparent fertilizer recovery efficiency of 59% to 81%. In comparison, the K concentration-based method underestimated fertilizer utilization at low K treatment and overestimated fertilizer utilization at high K treatment, because it cannot distinguish different K sources whose relative contributions to the bioavailable K pool in soil can vary in response to plant–soil interactions. Our study demonstrates the potential of stable K isotopes in improving the understanding of the K cycle in soil–plant systems.

KEYWORDS: potassium cycle, potassium isotopes, fertilizer, isotope labeling, nutrient utilization, potassium isotope fractionation; corn

1. INTRODUCTION

Potassium (K) is a macronutrient essential for plant growth. It is one of the most abundant cations in plants and is highly mobile between different cell compartments and within plants. The uptake and transport of K is mainly facilitated by membrane protein transporters and K channels.[1-4] Although the critical concentration of K (defined as the concentration at which 90% of maximum yield is achieved, ref 5) is typically less than 2% of dry matter for many plants, the actual concentrations found in plants vary over a wide range and can reach up to 10% of dry weight,[6] so-called luxury consumption. Because K has critical functions in many physiological processes during plant growth, such as protein synthesis, enzyme activation, photosynthesis, stomata movement, osmotic regulation, membrane electric potential regulation, and pH homeostasis,[2, 7-9] K deficiency can lead to retarded growth, and chlorotic and necrotic organs.

Although K contents in soil are typically large, the majority of K resides in minerals that are not readily available to plants.[10] Potassium fertilizers are, therefore, commonly needed in agriculture to ensure sufficient bioavailable K in soils that can sustain healthy plant growth and harvest.[11] The rate of K fertilization is critical because inadequate nutrient supply will restrict crop yield and quality while excess application may cause plant damage, nutrient leaching, and other potential environmental consequences.[12] Proper management of K fertilizers is desirable for economic profitability of agriculture and soil health.[13, 14] Such management for K is particularly crucial in the context of both population growth and ongoing climate change that is predicted to cause an increase in drought and heat events in many parts of the world in the near future, because K is a key nutrient influencing crop resistance to diseases and many environmental stresses, such as drought and salt.[8, 9, 11, 13, 15-18]

Optimization of fertilization strategies requires accurate quantification of fertilizer uptake. Conventionally, uptake of fertilizer-derived K by crops is estimated as apparent recovery efficiency (RE) using the difference method, which is based on the difference in K contents between fertilized and unfertilized crops relative to the quantity of fertilizer applied,[19-21] instead of a direct assessment on how much of K in plants is derived from fertilizer. However, crops can respond differently under different fertilization conditions,[22, 23] and conventional soil tests based on chemically extractable-K concentrations are often inadequate in accounting for these different crop responses. In addition, because K is present in different pools in soils (i.e., soluble, exchangeable, non-exchangeable, and minerals), varying plant–soil interactions under different fertilization conditions may alter the dynamics of K transfers among these different K pools, which typically cannot be accounted for by the concentration-based difference method. For example, it has been demonstrated that plants can utilize more non-exchangeable K in soils that was previously considered to be hardly accessible by plants.[24-26] A couple of recent studies also reported that the quantity of exchangeable K determined by an isotope dilution method in certain soils is larger than the quantify estimated by the conventional cation-exchange methods,[27, 28] implying the presence of significant labile K in soil that had been neglected previously. Further complexities in quantification of K fertilizer utilization arise from incomplete knowledge on the kinetics of soil K fixation and release as a function of the fertilization rate.[29-33] Therefore, development of an alternative method that can quantify K fertilizer utilization more accurately is of substantial scientific and practical significance.

The use of isotope-labeled fertilizer offers a direct means to study nutrient uptake by plants. The isotope labeling technique has been extensively employed to trace and quantify utilization of several nutrients, such as nitrogen (e.g., ^{13}N and ^{15}N, ref 34-37), phosphorus (e.g., ^{32}P and ^{33}P radioisotopes, ref 38, 39), and sulfur (e.g., ^{34}S or ^{35}S, ref 40, 41), providing critical information on detailed processes and kinetics associated with cycling of these nutrients in the soil–plant system. The use of stable isotopes over radioisotopes is generally preferred because of the absence of safety and regulatory controls attendant to their use in the laboratory, greenhouse, and field, and great strides have been made in studying several nutrient cycles, such as the N cycle using stable ^{15}N.[34, 42-44]

However, application of enriched K isotopes to study the K cycle in the soil–plant system is relatively rare, despite the availability of three naturally occurring K isotopes (^{39}K, ^{40}K, ^{41}K) and several radioactive isotopes that can be artificially produced (e.g., ^{38}K, ^{42}K, ^{43}K). Some early studies used the radioisotope ^{42}K to investigate exchangeable K in soil and K uptake related to plant physiology,[45, 46] but the timescale of the experiments is limited because of the short half-life of ^{42}K (~12.3 hours, ref 47). Radioactive ^{40}K is a naturally occurring isotope with a very long half-life (1.248×10^9 years, ref 48, 49), so, in principle, it can be used for longer-term agricultural research, such as quantification of fertilizer utilization. However, there were only a couple of studies in the literature that used enriched ^{40}K to trace K in the soil–plant system,[50, 51] likely because of the combined challenges associated with ^{40}K analysis and the use of radioactive material.

Stable ^{41}K isotope is an attractive target for isotopic labeling because: (1) the use of an enriched stable isotope avoids any hazards and regulatory challenges associated with radioactive material, (2) the natural abundance of ^{41}K is relatively low (~6.7%), and (3) highly enriched ^{41}K isotope (>95%) is commercially available. Despite these advantages, this potential application has long been hampered by difficulties in precisely analyzing $^{41}K/^{39}K$ ratios using mass spectrometry because of polyatomic interferences derived directly from the argon plasma (e.g., $^{40}ArH^+$ on $^{41}K^+$) and/or the difficulty in correcting for instrumental mass bias.[52, 53] These difficulties can be alleviated to some extent by using a ^{41}K tracer of high enrichment, so that changes in the ^{41}K tracer abundance in samples can be large enough to be resolved under an analytical precision of several parts per thousand (i.e., ‰ or "per mil") achievable by thermal ionization mass spectrometry (TIMS) or conventional quadrupole inductively coupled plasma mass spectrometry (ICP-MS) with a collision cell. Several studies have used these approaches and an enriched ^{41}K tracer to study K uptake and internal cycling in plants (particularly trees),[54-57] showing the potential of the stable K isotope labeling. These studies, however, generally used ^{41}K tracers with percent- to sub-percent-level enrichment, so the required amount of tracer (hence the cost) was relatively high.

Recent advance in multi-collector ICP-MS (MC-ICP-MS) has made it possible to achieve high-precision analysis of $^{41}K/^{39}K$ ratios to an external precision of ~0.1‰ or better (2 standard deviations), an improvement of more than an order of magnitude relative to TIMS- and quadrupole ICP-MS-based approaches. MC-ICP-MS analysis, therefore, allows for detection of subtle K isotope variations down to sub-per-mil level in natural samples for the first time.[58-64] This new analytical capability may revolutionize the study of the K nutrient cycle in two ways. First, a few studies have recently shown significant natural variations in $^{41}K/^{39}K$ in a variety of different plants and K isotope fractionation during plant (i.e., soybean, rice, and wheat) growth in hydroponic experiments.[61, 65-67] These natural $^{41}K/^{39}K$ variations, not resolvable previously, potentially provide a new research avenue that may improve our understanding of the K nutrient cycle in a way similar to how natural mass-dependent isotope variations for carbon and nitrogen have advanced the understanding of the carbon and nitrogen cycles.[68-70] Second, the improved analytical precision allows for the use of a ^{41}K tracer with considerably lower enrichment, making stable K isotope labeling a more financially feasible approach through reducing the total amount of enriched ^{41}K (hence the cost) needed for an experiment.

To the best of our knowledge, application of labeled stable K isotopes (^{41}K) to directly quantify K fertilizer recovery efficiency from soil, and a comparison of results to the conventional difference method, have not been explored previously. Here, as a proof-of-concept, we conducted a pot study that grew corn (*Zea mays* L.) using a ^{41}K-labeled fertilizer in a greenhouse. The primary goal of this study was to quantify K uptake specifically related to fertilizer using an enriched ^{41}K tracer, and then compare the K fertilizer recovery efficiency obtained using the isotope labeling method with that derived from the conventional K concentration-based difference method. Overall, our results demonstrate clear advantages of using ^{41}K tracers in determining K uptake as compared to the conventional concentration-based method. Furthermore, natural K isotope fractionation associated with corn growth has not been reported prior to this study, and our unfertilized control experiments determine the natural mass-dependent K isotope fractionation between plant and soil during corn growth.

2. MATERIALS AND METHODS

2.1. Pot experiments

A six-week pot study that grew corn (*Zea mays* L.) was conducted in a greenhouse at the University of Wisconsin–Madison to quantify K fertilizer utilization using labeled ^{41}K (Figure 1). A Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls) from a field at the Agricultural Research Station in Arlington, Wisconsin was used for this study. The field where the soil was taken is not known to have been fertilized, manured, or cultivated for several decades. The soil was dried and sieved to <4 mm aggregates. The soil is mainly composed of feldspar, quartz, and mica with lesser amounts of chlorite, interstratified chlorite-vermiculite and vermiculite in the fine silt fraction, and interstratified smectite-illite, kaolinite, quartz and illite in the clay fraction.

Four different treatments were established: a control with no added K (Group-A), 50 mg K kg^{-1} soil (Group-B), 100 mg K kg^{-1} soil (Group-C), and 200 mg K kg^{-1} soil (Group-D). These values here refer to K rather than KCl. Each treatment was repeated in triplicate pots lined with polyethylene bags to prevent drainage and leaching. Each pot contained the equivalent of 1.5 kg of oven-dried soil. Nitrogen and phosphorus were maintained at constant and adequate levels by addition of 200 mg kg^{-1} as urea-N and 100 mg kg^{-1} as monocalcium phosphate-P ("triple superphosphate") across all treatments. Urea-N is an ammonium-based fertilizer as it hydrolyzes with water to form NH_4^+ ions in soils. Although NH_4^+ and Ca^{2+} ions may compete with K^+ ions for exchange sites in soils, the use of N and P fertilizers is not expected to affect our intended K study, because (1) the Plano silt loam soil used in our experiments has high cation-exchange capacity, comparable to that reported for a similar Wisconsin soil (i.e., ~11.0 centimoles per kilogram),[71] which should provide exchange sites sufficient for K, NH_4, and Ca ions in our experiments; and (2) N and P fertilizers were kept at the same rates across all the pots. For K-labeled treatments, the ^{41}K labeled fertilizer was first weighed and then mixed thoroughly along with N and P fertilizers into a known quantity of dry soil, and the fertilized soil was then transferred into each pot. Six corn kernels were planted for each pot at a depth of ~1 cm, watered to field capacity, and covered for germination. After germination, the seedlings were thinned to leave the four strongest. Deionized water was added periodically by weight to keep the soils at field moisture capacity. Six weeks after planting, the aboveground plants were harvested and dried at 60°C for 48 hours. Plants from the same pot were then combined during further processing for chemical and isotopic analyses. Due to the well-known difficulty in separating and cleaning roots from soil, we did not sample roots in these experiments.

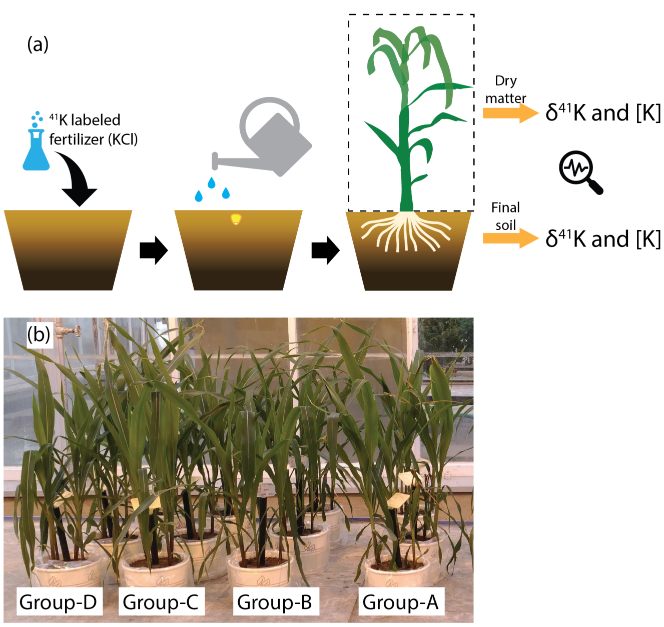

Figure 1. *(a) A schematic of the pot experiment in this study; (b) A photo of corn grown out of the experiments taken before harvest.*

2.2. Nomenclature and K isotope tracer

Fertilizer recovery efficiency (RE) can be defined in different ways.[12, 21] In this study, we adopted the definition from Fixen et al.,[12] which defines fertilizer recovery efficiency to be the percentage of fertilizer nutrient found in the plant biomass relative to the total amount of fertilizer nutrient applied to the system.

Stable K isotopes are expressed by the conventional δ-notation that describes parts-per-thousand deviation of the $^{41}K/^{39}K$ ratio in a sample relative to that in a standard:

$$\delta^{41}K = \left(\frac{^{41}K/^{39}K_{sample}}{^{41}K/^{39}K_{NIST\ SRM\ 3141a}} - 1\right) \times 1000$$

Currently, the high purity K solution, NIST SRM 3141a, is the recommended standard for reporting stable K isotope data.[60, 72] Natural mass-dependent K isotope fractionation between plant and soil can be quantified by the K isotope fractionation factor $\alpha_{plant/soil}$, following the standard definition:

$$\alpha_{plant/soil} = \frac{^{41}K/^{39}K_{plant}}{^{41}K/^{39}K_{soil}}$$

Because α is very close to unity, sometimes it is more convenient to use the enrichment factor ε, which is related to α through the following relation:

$$\varepsilon = 1000 \times (\alpha - 1)$$

Highly enriched ^{41}KCl (96.5% enrichment) was purchased from ISOFLEX USA (San Francisco, CA). The high precision of MC-ICP-MS analysis allowed us to decrease this enrichment to moderate cost, while still retaining the ability to sensitively trace K atom and mass transfer. The enriched ^{41}KCl was mixed with isotopically normal KCl with $\delta^{41}K$ close to 0‰ to produce a ^{41}K-labeled fertilizer with $\delta^{41}K = 5.56$‰ prior to use. A total of ~0.7 mg original ^{41}KCl tracer (96.5% enrichment) was consumed during this study. To ensure that the enriched KCl fertilizer was isotopically homogenous, the original ^{41}KCl tracer and normal KCl were dissolved in water and then mixed. This isotopically homogenous solution was then evaporated to precipitate the labeled KCl.

2.3. Soil and plant K concentration and isotope analyses

Dry plants were weighed to determine the total aboveground yield, ground and then ashed at 500°C. The ashed plants were fully dissolved in 2 M HCl for K concentration measurements using an atomic absorption spectrometer (AAS) (PerkinElmer Analyst 200) at the Department of Soil Science, University of Wisconsin–Madison. Aliquots were taken from each sample and saved for K isotope analysis. Initial soil and soil after harvest were dried and ground for soil tests. Plant-available K in soil was extracted by the Bray-1 solution,[73] which has been the standard soil K testing method used in laboratories certified by the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) since 1974.[74] The Bray procedure extracts ~90% as much K as the 1 N ammonium acetate procedure that is used as a standard soil test method by some other states in the US. Briefly, the Bray-1 solution is composed of 0.03 N NH_4F and 0.025 N HCl, and plant-available K from soil is extracted using a soil:extractant ratio of 1:10 (g:ml). Typically, 1.5 g soil and 15 ml Bray-1 solution were used. Upon mixing, the suspension was agitated continuously by a shaker for 5 min, and then filtered using 8 μm cellulose filter paper (9 cm Whatman No. 2 filter paper or equivalent). Aliquots of Bray extracts were taken for K concentration analysis by AAS, and the remaining solutions were used for K isotope analysis.

For high-precision stable K isotope analysis, it is critical to purify K from other elements in samples to avoid matrix effects during analysis by mass spectrometry.[58, 60] Separation of K from major matrix elements (e.g., Na, Mg, Ca, Si) was achieved through a single-stage manual chromatographic protocol using Bio-Rad Poly-Prep® columns packed with 2 ml of Bio-Rad

AG50W-X8 cation exchange resin (200–400 mesh, H^+ form) and 0.4 M HCl as an eluent.[75] Digested plant and Bray extract samples were evaporated to dryness and then dissolved in 0.4 M HCl, followed by cation-exchange separation. After loading samples in 0.4 mL 0.4 M HCl onto cation exchange columns, Na was eluted with 23 mL 0.4 M HCl. Potassium was subsequently collected in 28 mL 0.4 M HCl, and other cations in samples remained on resin. Potassium was quantitatively (>99%) recovered from the column purification. High purity reagents, including quartz-distilled HCl and 18.2 mΩ·cm H_2O, were used throughout sample preparation, and the total procedural blank was typically ~20 ng K, which is negligible compared to >100 µg K processed through the chromatography separation.

Stable K isotope ratios were measured on a Nu Plasma II multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at the Department of Geoscience, University of Wisconsin–Madison. High-precision stable K isotope ratio measurement is notoriously challenging because the main plasma gas Ar introduces significant interferences on K isotope masses (e.g., $^{40}Ar^{1}H^{+}$ on $^{41}K^{+}$). However, Ar-based interferences may be overcome by a combined use of "cold plasma" techniques,[76] and a high-mass-resolution source defining slit. In this study, "cold plasma" was achieved by increasing the distance between the torch and the plasma interface and reducing the radiofrequency (RF) forward power from its typical setting of 1300 W to 800 W. The use of a high mass resolution source defining slit increased mass resolution so that K isotope peaks can be resolved from the suppressed Ar-based interferences. Sample solutions were introduced to the MC-ICP-MS via an Aridus II desolvating unit with a ~50 µL min^{-1} Teflon nebulizer. A sample-standard bracketing protocol was used to correct for instrumental mass bias, with a pure K solution from High-Purity Standards used as the bracketing standard. This solution is well-calibrated against the NIST SRM 3141a K standard in our lab. Based on recent recommendations,[60, 72] all measured $\delta^{41}K$ data were reported on the NIST SRM 3141a scale in this study (Table 1). The external reproducibility is ≤0.15‰ (2SD), based on repeated measurements of two USGS rock standards (BCR-2 and BHVO-2), with a $\delta^{41}K$ value of -0.41 ± 0.15‰ (2SD) for BCR-2, and -0.40 ± 0.12‰ (2SD) for BHVO-2. As an additional data quality control, we also routinely analyzed a natural seawater sample that was collected from 500 m at the SEATS site in the South China Sea,[77] because previous studies have demonstrated that seawater has a homogeneous $\delta^{41}K$ value.[78, 79] We obtained a $\delta^{41}K$ value of 0.10 ± 0.12‰ (2SD) for seawater. Our $\delta^{41}K$ results for all three reference materials (i.e., BCR-2, BHVO-2, seawater) are in excellent agreement with results reported from other laboratories.[72, 75]

3. RESULTS

3.1. Potassium concentrations and crop yield

Potassium concentration and weight results for soil and plants are summarized in Table 1. Bray-extracted K concentration from the initial unlabeled soil was 71 mg K kg^{-1} soil. This value is lower than the critical level of K for Wisconsin soils for corn (~130 mg K kg^{-1}) based on the Bray extraction procedure, placing our soil at the boundary between Very Low (VL) and Low (L) categories for corn in loamy soil.[74] For the fertilized experiments (Groups B–D), initial soils were all fertilized to K levels close to, or above, the critical soil K level. For the unfertilized control experiments (Group-A), the average K concentration of the soil after harvest was 67.1 mg K kg^{-1} soil, slightly lower than that of the initial soil. For the fertilized experiments (Group B-D), average Bray-extracted K concentrations from soil after harvest ranged from 76.7 mg K kg^{-1} soil to 101.2 mg K kg^{-1} soil. The average K concentration in soil extracts after harvest in Group B was marginally higher than that in the control Group A, whereas the averages from Groups C and D were all higher than the control Group A (p < 0.05, one-way ANOVA). Overall, average K concentrations in soil extracts after harvest increased with increasing K fertilization from Groups A to D (Figure 2a). Average K concentrations in the dry aboveground shoot ranged between 10.1 mg g^{-1} and 40.4 mg g^{-1}, and they increased with increasing K fertilization from Groups A to D ($p \leq 0.05$) (Figure 2b). The average yield, as defined by the dry weight of aboveground shoot, was slightly higher in the three fertilized groups (Groups B–D) than the average yield from the control Group-A, but the differences among all 4 groups were not statistically significant (Figure 2c). Because the critical K concentration in corn is ~1.3% of dry matter,[6] which is close to, or lower than, those measured in experiments from Groups A–D, none of corn in our experiments had grown under K deficit conditions, even for Group A. The observed increase in K concentrations in plants with increasing K fertilization but similar plant dry weights from our experiments are consistent with luxury K consumption.

Our K concentration results for soils and plants revealed an imbalance of plant-available K mass before and after the growth in all experiments. The total plant-available K mass in each pot before corn growth can be estimated using the K concentration measured in Bray-extracts of initial untreated soil, combined with the known soil weight, plus K mass from the fertilizer if used. The total plant-available K mass in each pot after the harvest can be estimated by the sum of K mass in corn shoots and the Bray-extractable pool in soil after the growth. Even without considering K mass in roots, varying excesses in plant-available K were observed in all experiments after the corn growth (Table 1), indicating recharge of plant-available K during the experiments.

Table 1. Results of plant dry weight, K concentrations and mass in plant and soil, and plant-available K excesses

		K treatment (mg K kg^{-1} soil)	Total K mass in fertilizer [1] (mg)	Shoot dry weight (g)	Shoot [K] (mg g^{-1})	Total K mass In shoot (mg)	Soil [K] (mg K kg^{-1} soil)	Apparent excess plant-available K [2] (mg)
Initial soil		-		-	-	-	71	
Group-A (control)	GA-1	0	0	6.50	11.3	73.1	63.3	61.6
	GA-2	0	0	8.50	9.1	77.7	60.9	62.6
	GA-3	0	0	7.00	9.9	69.2	77.7	79.3
Group-B	GB-1	50	75	8.43	11.9	100.6	75.9	32.9
	GB-2	50	75	8.15	13.7	111.8	69.9	35.2
	GB-3	50	75	8.87	11.8	104.6	84.3	49.6
Group-C	GC-1	100	150	9.07	16.6	150.4	90.9	30.2
	GC-2	100	150	8.83	17.7	156.1	83.1	24.3
	GC-3	100	150	8.07	25.7	207.1	87.9	82.4
Group-D	GD-1	200	300	8.13	41.0	333.5	108.9	90.4
	GD-2	200	300	9.25	39.0	361.1	96.0	98.6
	GD-3	200	300	7.43	41.0	304.7	98.7	46.3

[1] Each pot contained 1.5 kg soil

[2] Apparent excess plant-available K = (Bray-extractable K in soil after harvest + K in shoot) – (Bray-extractable K in initial soil + K from fertilizer)

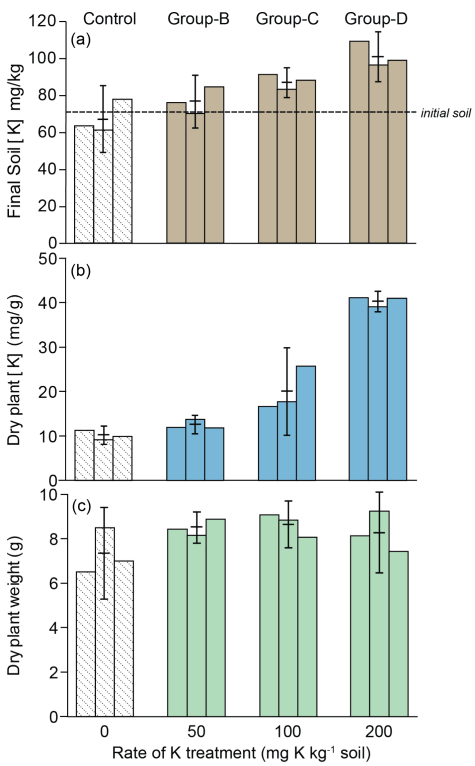

Figure 2. *(a) K concentrations of Bray extracts from the final soil after harvest in comparison to that of the initial soil (horizontal dashed line); (b) K concentrations in the harvested plant dry*

matter; (c) dry weight of the harvested plants. The mean (±standard deviation) is also shown for each group.

3.2. Potassium isotope composition in plant and soil

Potassium isotope compositions of fertilizer, corn shoot, and soil extracts were provided in Table 2. The $\delta^{41}K$ value of Bray extract from the initial soil was -0.11‰, falling in the range reported for natural samples.[72] Relative to the $\delta^{41}K$ value of the enriched K tracer (i.e., 5.56‰), the isotopic contrast between soil and fertilizer provided a nearly ~6‰ leverage for tracing K uptake. This is a factor of ~40 times greater than the analytical uncertainty (i.e., ~0.15‰, 2SD), offering a high level of sensitivity to tracing enriched ^{41}K. For the control group (Group-A), shoot $\delta^{41}K$ values from the triplicate pots displayed a narrow range between -0.34‰ and -0.22‰. Soil extracts after harvest from Group-A showed $\delta^{41}K$ values between 0.25‰ and 0.47‰, which were higher than the $\delta^{41}K$ value from the initial soil extract and the values measured in shoot ($p < 0.001$). In fertilized treatments (Groups B–D), $\delta^{41}K$ values of both shoot and soil Bray extracts after harvest were found to fall within the $\delta^{41}K$ range between the unlabeled initial soil and ^{41}K-labelled K fertilizer. The influence of ^{41}K-labelled fertilizer is easily discernible from high $\delta^{41}K$ values measured in plants and soil extracts (Figure 3), which increased with the increasing fertilization, as traced by the ^{41}K-labelled fertilizer from Groups B to D. Except for one experiment in Group D where the measured $\delta^{41}K$ values from the plant and soil extract were similar, $\delta^{41}K$ values of shoot in the three fertilized groups were always higher than $\delta^{41}K$ values measured in corresponding after-harvest soil extracts. This is in marked contrast to the unfertilized control Group-A where $\delta^{41}K$ values of plant from all three pots were found to be lower than those measured in Bray extracts of initial and final soils. In addition, although K isotope compositions of Bray-extracts of initial fertilized soils in Groups B–D were not directly measured, they can be estimated by a simple mass-balance calculation based on the K concentration and isotope composition of the Bray extract from the unfertilized soil and the K mass added from fertilizer and its $\delta^{41}K$ value, because the soluble KCl fertilizer can be surely extracted by the Bray solution. The calculation yielded 2.23‰ for Group B, 3.21‰ for Group C, and 4.07‰ for Group D. These estimated $\delta^{41}K$ values of plant-available K in initial fertilized soils were higher than $\delta^{41}K$ values measured in plants and soil Bray-extracts after the harvest from the same experiments, implying recharge of labile K carrying low $\delta^{41}K$ values during our experiments.

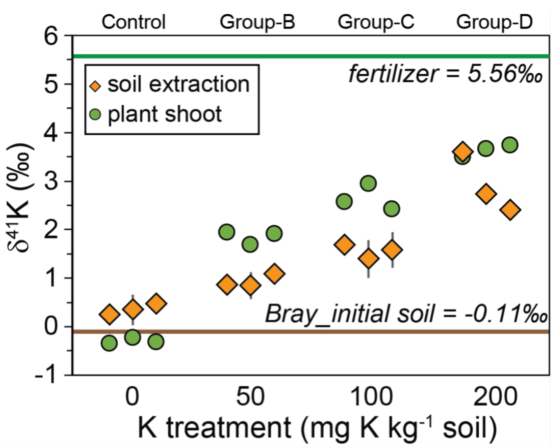

Figure 3. *K isotope composition of shoot and Bray extracts of the final soil. The $\delta^{41}K$ values of Bray extract of the initial unlabeled soil and the initial ^{41}K-enriched fertilizer are also shown.*

Table 2. Results of K isotope compositions for fertilizer, corn shoot, and soil extracts

		K treatment	Corn shoot			Soil Bray extract		
		(mg K kg^{-1} soil)	$\delta^{41}K$ (‰)	2SE	n	$\delta^{41}K$ (‰)	2SE	n
Group-A	GA-1	0	-0.34	0.04	7	0.25	0.09	5
	GA-2	0	-0.22	0.05	5	0.35	0.14	5
	GA-3	0	-0.32	0.07	4	0.47	0.08	6
Group-B	GB-1	50	1.94	0.03	6	0.86	0.09	5
	GB-2	50	1.69	0.04	6	0.85	0.14	4
	GB-3	50	1.91	0.04	6	1.09	0.04	5
Group-C	GC-1	100	2.57	0.04	5	1.69	0.07	4
	GC-2	100	2.94	0.06	5	1.40	0.20	4
	GC-3	100	2.42	0.07	4	1.58	0.19	4
Group-D	GD-1	200	3.50	0.07	4	3.61	0.09	5
	GD-2	200	3.67	0.07	4	2.74	0.07	5
	GD-3	200	3.74	0.10	4	2.40	0.05	4
Initial soil						-0.11	0.11	4
KCl fertilizer						5.56	0.10	5

4. DISCUSSION

Plant growth can induce resolvable isotope fractionation for not only conventional light elements including C, H, O, N, and S,[69, 80] but also many metal nutrient elements, such as Mg, Ca, Fe, Cu, and Zn.[81-86] Isotope fractionation of these elements during plant growth has proven useful in study of nutrient cycles in the soil–plant system. Currently, study of K isotope fractionation during plant growth are scarce (none for corn). Our unfertilized control group (Group-A) provided an opportunity to investigate and quantify potential natural mass-dependent K isotope fractionation during corn growth. In contrast, the fertilized groups (Groups B–D) are ideal for quantification of K fertilizer utilization through use of the enriched ^{41}K tracer; natural, mass-dependent K isotope fractionation in these groups will be only a second-order effect relative to the nearly ~6‰ contrast in the initial soil and ^{41}K-labeled fertilizer. Below, we first focus on results from Group-A and discuss their implications for natural fractionation of stable K isotopes during corn growth. Then, we quantify apparent K fertilizer recovery efficiency (RE) in the ^{41}K-labelled experiments and compare results to those calculated using the conventional difference method based on K concentrations alone.

4.1. Quantification of natural K isotope fractionation during corn growth

Our unfertilized control Group-A provided evidence for natural, mass-dependent K isotope fractionation that favors light K isotopes (i.e., ^{39}K) in corn during its growth, as indicated by lower $\delta^{41}K$ values measured in corn shoots relative to the $\delta^{41}K$ value of Bray extract from the initial unfertilized soil (Figure 3). Consistent with this observation, Bray extract from the soil after harvest showed $\delta^{41}K$ values higher than those measured in shoot and in the initial soil extract (Figure 3). The apparent $\delta^{41}K$ differences between corn shoots and soil extracts after harvest ($\delta^{41}K_{shoot} - \delta^{41}K_{soil}$) ranged from -0.79‰ to -0.57‰ in the three pots in Group-A (Table 2).

The apparent isotopic differences between plant and soil are the accumulative results of progressive removal of isotopically light K from soil during K uptake by corn, so they are dependent on the extent of uptake. The underlying intrinsic K isotope fractionation factor is more fundamental and applicable to other studies in future. Because all pots were lined with polyethylene bags to prevent drainage and leaching, each pot was a closed system for K. It is, therefore, reasonable to consider K isotope fractionation during corn growth to mimic a Rayleigh distillation process. The intrinsic K isotope fractionation factor ($\alpha_{shoot/soil}$) between shoot and soil can be quantified by the following Rayleigh equation (Mariotti et al., 1981):

$$\delta^{41}K_{shoot} = \delta^{41}K'_{soil} - \left(\frac{f}{1-f}\right) \varepsilon \ln(f) \qquad \textbf{(Equation 1)}$$

where ε is the isotope enrichment factor between corn shoot and soil, $\delta^{41}K_{shoot}$ is the measured $\delta^{41}K$ of corn shoot, $\delta^{41}K'_{soil}$ is $\delta^{41}K$ of the plant-available K pool in soil, and f denotes the fraction of plant-available K not taken up by corn shoot (i.e., plant-available K remaining in soil and K in root). Because the total plant-available K pool (K_{total}) in each pot can be estimated by the sum of K mass in shoot (K_{shoot}) and root (K_{root}), and K mass remaining in soil after harvest (K_{soil_t}), f can be calculated by the following K mass conservation equation:

$$f = 1 - K_{shoot} / (K_{shoot} + K_{root} + K_{soil\_t}) \qquad \textbf{(Equation 2)}$$

There are two major sources of uncertainty in calculating ε using the above equations. The first one is K mass in root (i.e., K_{root}). Although K_{root} was not measured directly, its possible range can be estimated based on our measured shoot dry weight and shoot K concentrations (Table 1), and reasonable assumptions for root to shoot (R/S) mass ratios and K concentration ratios reported in the literature.[15, 87-90] The second major uncertainty is the K isotope composition of the plant-available K pool in soil (i.e., $\delta^{41}K'_{soil}$ in Equation 1). The complexity arises from our observation that, even without considering roots, the sum of K mass in shoot and soil extract after harvest already showed ~58%–74% “excess” relative to the quantity of plant-available K pool estimated based on Bray extract of initial unfertilized soil (Table 1). This implies recharge of plant-available K in soil during corn growth in all three pots in Group-A. Consequently, it may not be appropriate to assume that $\delta^{41}K'_{soil}$ was the same as $\delta^{41}K$ measured in the Bray extract of initial unfertilized soil. The possible $\delta^{41}K$ range of the recharged plant-available K can be estimated based on studies of $\delta^{41}K$ variations in natural weathering profiles and soils, and associated K isotope fractionation during relevant processes,[66, 91-93] and our measured $\delta^{41}K$ values in soil extract after harvest. A complete description on how these two parameters were estimated is provided in the Supporting Information.

To account for the uncertainties associated with K_{root}, $\delta^{41}K'_{soil}$, and all other parameters in Equations 1 and 2, we used a Monte Carlo method to estimate the shoot–soil K isotope fractionation enrichment factor ε. A detailed description on our Monte Carlo method, including parameter selection and solution screening, is provided in the Supporting Information. A total of 100-million Monte Carlo simulations were conducted to test all possible parameter ranges, and these simulations yielded a best-estimate shoot–soil K isotope enrichment factor ε of -0.37‰ (±0.23‰, 1SD), which is the first-ever estimate available for plant-induced K isotope fractionation for corn.

The observed increase in plant-available K in soil in our experiments can be explained by root–soil interactions. Although early studies considered K in some non-swelling 2:1 layer silicates in soil, such as illite and mica, to be inaccessible by plants, a large body of later studies have shown that root–soil interactions, sometimes involving microorganisms, can cause significant release of soluble K from non-exchangeable and/or mineral K pools upon plant growth on the order of only a few days.[17, 94-106] It has been shown that non-exchangeable and/or mineral K pools can sometimes contribute to ~80-100% of the total K available to plants.[97] In our case, the Plano silt loam is a relatively young soil, with topsoil formed from loess and containing feldspars and micas in the silt fraction and interlayered smectite-illite in the clay fraction, so there are abundant mineral and non-exchangeable K sources in the soil.

4.2. Possible mechanisms for K isotope fractionation during corn growth

The observed $\delta^{41}K$ difference between corn shoot and soil in unfertilized treatments in Group-A (Figure 3) and our estimated enrichment factor for K isotope fractionation demonstrate that light K isotopes are preferentially utilized during corn growth. By analogy with stable isotope fractionation of other elements in soil–plant systems,[83-86, 107] two processes may contribute to the observed K isotope fractionation: (1) transport of K in soil solution to the root surface, and (2) K uptake by plant tissues.

First, K is mainly transported to the plant root surface through diffusion in soil solution, driven by K concentration gradient between root surface and adjacent soil solution.[96, 108, 109] Diffusion is known to cause kinetic isotope fractionation for many metal elements, including K.[110] Previous experiments have shown that light K isotopes diffuse faster than the heavy isotopes in both water and methanol, resulting in large kinetic K isotope fractionation of up to several per mil in $^{41}K/^{39}K$ ratios.[111, 112] It can be envisioned that plant-available K in soil near corn root was first utilized and then replenished by diffusion of soluble K from soil further away from the root. This diffusion process in soil may cause preferential supply of light K isotope to root, leading to the observed K isotope fractionation in plants.

Transport of K into and/or within plant cells may also cause K isotope fractionation. Potassium is taken up by plants primarily through two routes: the high-affinity transport system (HATS) by proton-coupled K^+ transporters under low external K concentrations, and the low-affinity transport system (LATS) by ion channels.[1, 4, 113-117] Potassium isotope fractionation has been previously suggested or observed in different plants and within a plant.[58, 61, 65-67] It is generally considered that K isotope fractionation can occur during K transport into and/or within plant cells, and this is particularly supported by hydroponic experiments that should be less influenced by diffusion processes in growth medium compared to measurements of plant samples from field.[67] This K uptake process may contribute to our observed K isotope fractionation in corn. However, the influence of the two different K transport mechanisms on K isotope fractionation remains unknown. For example, Li (2017) hypothesized that energy-consuming HATS could produce larger K isotope fractionation than LATS,[65] whereas Christensen et al. (2018) proposed the opposite.[67] Further study is clearly required to better characterize the nature of K isotope fractionation associated with different K transport mechanisms.

4.3. Quantification of K fertilizer recovery efficiency using stable ^{41}K isotope labeling

The use of the ^{41}K-enriched fertilizer in experiments in Groups B to D allows for direct tracing and quantification of fertilizer-derived K in corn shoot. This is evident from high $\delta^{41}K$ values measured in shoots that progressively approach the $\delta^{41}K$ value of initial ^{41}K-labeled fertilizer (i.e., 5.56‰) with increasing fertilization rate (Figure 3). We can quantify the relative contribution of K sources in shoot using a two-component mixing calculation:

$$\delta^{41}K_{shoot} = F\,(\delta^{41}K_{fert} + \varepsilon) + (1 - F)\,(\delta^{41}K'_{soil} + \varepsilon) \qquad \textbf{(Equation 3)}$$

where F indicates the percentage of K in shoot that is derived from the fertilizer, $\delta^{41}K'_{soil}$, $\delta^{41}K_{fert}$, and $\delta^{41}K_{shoot}$ are $\delta^{41}K$ values of the total plant-available K pool in soil, initial ^{41}K-labelled fertilizer, and shoot, respectively. Although the shoot–soil K isotope enrichment factor ε is considered in the calculation, its influence on F is minor because its magnitude is relatively small compared to the K isotope contrast between the fertilizer and soil. In Equation 3, $\delta^{41}K_{fert}$ and $\delta^{41}K_{shoot}$ were measured directly, and ε was estimated in Section 4.1 (i.e., -0.37±0.23‰). $\delta^{41}K'_{soil}$ is the same as in Equation 1 and has the same uncertainties as described in Section 4.1. To better estimate F, we applied the same Monte Carlo method used in Section 4.1 (see details in the Supporting Information) to propagate all uncertainties to F.

Average F values obtained from 100-million Monte Carlo simulations are reported in Table 3. Overall, the percentage of fertilizer-derived K in shoot generally increases with increasing amount of fertilizer. On average, ~42% of K found in shoot came from the fertilizer for the treatments in Group-B (50 mg K kg^{-1} soil), ~56% of shoot K came from the fertilizer for the treatments in Group-C (100 mg K kg^{-1} soil), and ~73% of K in shoot originated from the fertilizer for the treatments in Group-D (200 mg K kg^{-1} soil). The propagated uncertainty on F was found to be relatively small (i.e., ~5%) because of the large δ^{41}K difference between the labeled K fertilizer and soil.

We can now define the apparent K fertilizer recovery efficiency (RE_i) using an isotopic tracer approach with the following equation:

$$RE_i\ (\%) = \frac{F \times K_{shoot}}{K_{fert}} \quad \textbf{(Equation 4)}$$

where K_{shoot} is total K mass in shoot, K_{fert} is total K mass in fertilizer applied to each pot, F is defined by Equation 3, and the subscript i refers to isotope method. RE_i provides a measure of fertilizer utilization by plants relative to total fertilizer applied. In practice, this value is useful in evaluating fertilizer efficiency and potential fertilizer loss for various nutrient management practices. Using this ^{41}K labeling approach, we quantified average fertilizer recovery efficiency (RE_i) to be ~59%, ~63%, and ~81% for Group-B, -C, and -D, respectively (Table 3).

Table 3. Fertilizer recovery efficiency (RE) estimated using the isotope labeling and difference methods

		K treatment	K isotope labeling			Difference
			F	1 s.d.	RE_i	RE_d
		(mg K kg^{-1} soil)	(%)	(%)	(%)	(%)
Group-B	GB-1	50	43.4	4.7	58.2	36.3
	GB-2	50	39.0	4.8	58.2	51.3
	GB-3	50	42.9	4.8	59.8	41.7
Group-C	GC-1	100	54.4	4.6	54.6	51.3
	GC-2	100	60.9	4.5	63.4	55.2
	GC-3	100	51.9	4.8	71.6	89.1
Group-D	GD-1	200	70.7	4.4	78.6	86.7
	GD-2	200	73.6	4.3	88.6	95.9
	GD-3	200	74.8	4.3	76.0	77.1

4.4. Comparison between isotope tracer and concentration methods and implications

Our study provides the first opportunity to directly compare isotope-tracer-based fertilizer recovery efficiency with that estimated based solely on K concentrations. In soil nutrient studies, the apparent fertilizer recovery efficiency is often estimated based on a comparison between fertilized and unfertilized cropping systems, and this so-called difference method can be written by the following equation:[12, 21]

$$RE_d\ (\%) = (K_{shoot} - K'_{shoot})/K_{fert} \qquad \textbf{(Equation 5)}$$

where RE_d indicates apparent fertilizer recovery efficiency calculated based on the difference method, K_{shoot} is total K mass in shoot from fertilized groups (i.e., Group-B to -D in this study), K'_{shoot} is K mass in shoot from the unfertilized control group (i.e., Group-A in this study), and K_{fert} is the total amount of K in fertilizer. The calculated results using the difference method are tabulated in Table 3. Group-B experiments yielded an average RE_d of ~43%, Group-C experiments yielded an average RE_d of ~65%, and Group-D yielded an average RE_d of ~87%. Considerable scattering of sometimes up to ~20% in RE_d results among three different pots in each group was observed.

A detailed comparison of RE values calculated based on the two different methods is provided in Figure 4. Overall, the average RE value calculated based on the difference method (RE_d) is lower than the average value calculated based on our K isotope tracer method (RE_i) for Group-B that received the lowest amount of K fertilizer (50 mg K kg^{-1} soil), whereas average RE values calculated based on the two methods are comparable for Group-C and -D that received higher amounts of K fertilizer. However, it is obvious that the data scatter among the three pots in each group is considerably larger for RE_d as compared to RE_i, so a comparison of averages alone is likely inadequate in revealing significant differences in results from the two methods. It is more informative to make a pairwise comparison across all individual pots. For all three pots in Group-B, the difference method consistently yielded fertilizer recovery efficiency values (RE_d) lower than those estimated by the K isotope tracer method (RE_i) by ~7% to up to ~22%. For Group-C, two pots show lower RE_d values than RE_i values, and one pot shows the opposite. In contrast to Group-B, the difference method consistently yielded higher fertilizer recovery efficiency values than those estimated by the K isotope tracer method for all three pots in Group-D – the group that received the highest amount of K fertilizer (200 mg K kg^{-1} soil). This trend is obvious from a Bland–Altman plot (Figure 5). A Bland–Altman plot has been widely used to assess agreement between two measurement methods, and it examines the relation between mean and difference (i.e., bias) for paired measurements by the two methods subject to assessment.[118, 119] A linear correlation (r^2 = 0.74) can be seen in our results (Fig. 5); the K isotope tracer method quantifies a higher RE value relative to the value quantified by the difference method at low fertilizer recovery efficiency conditions, whereas it quantifies a lower RE value relative to the difference method at high fertilizer recovery efficiency conditions.

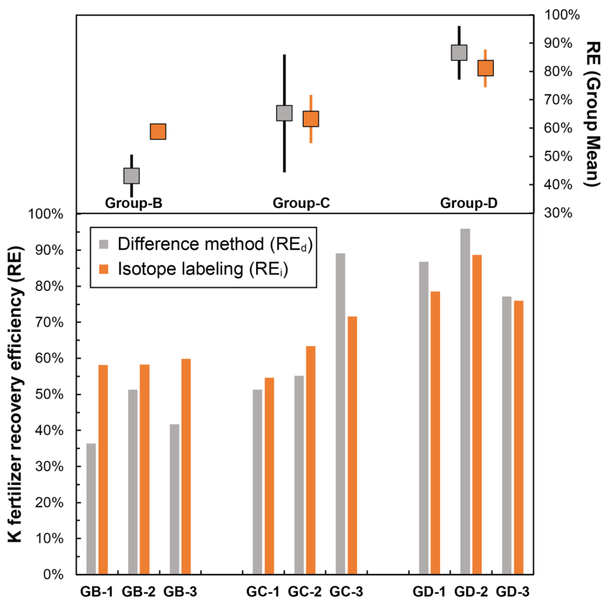

Figure 4. *A comparison of apparent K fertilizer recovery efficiency calculated using the difference method (RE_d) and the stable ^{41}K isotope labeling technique (RE_i). Group averages are shown in the upper panel and results of individual experiments are shown in the lower panel.*

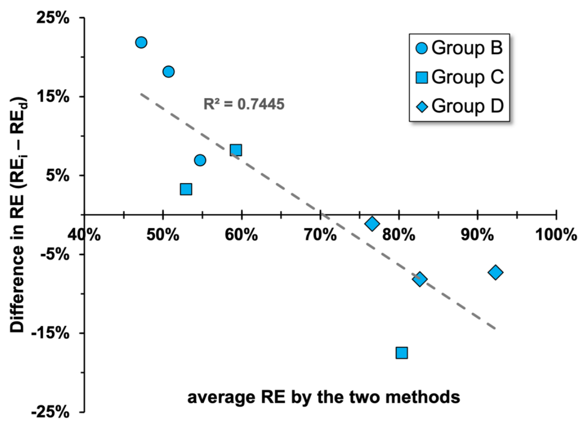

Figure 5. *A Bland–Altman plot that assesses agreement in RE results obtained by the K isotope method and the conventional difference method. It is apparent that the bias between the two method is correlated with fertilizer recovery efficiency that is broadly related to the fertilization rate in our experiments.*

We consider fertilizer recovery efficiency estimated based on the isotope tracer method (RE_i) to be more accurate, because our K isotope labeling approach allows for unambiguous differentiation of K from soil and fertilizer based on their K isotope compositions. In contrast, estimation biases may occur using the difference method, causing the observed discrepancies between RE_i and RE_d (Figure 4). The fundamental assumption of the difference method is that a soil–plant system behaves the same under unfertilized and fertilized conditions. This assumption neglects the dynamics of K exchange among different K pools in soil and possible different physiological responses of plants to nutrient uptake under different nutrition conditions. The possible influence of such negligence can be understood via detailed analysis of the RE_d calculation as described in Equation 5; because the total amount of added fertilizer (K_{fert}) is known without ambiguity, the biased RE_d estimate can only arise from the term ($K_{shoot}- K'_{shoot}$). In a fertilized experiment, K in shoot (K_{shoot}) is derived from two sources: fertilizer (K^{fert}_{shoot}) and soil (K_{shoot}^{soil}), whereas in the control experiment K in shoot only comes from unfertilized soil ($K_{shoot}^{\prime soil}$). Equation 5 then can be rewritten as:

$$RE_d\ (\%) = (K_{shoot}^{fert} + K_{shoot}^{soil} - K_{shoot}^{\prime soil})/K_{fert} \qquad \textbf{(Equation 6)}$$

Estimation of fertilizer recover efficiency RE_d is accurate only when K_{shoot}^{soil} is the same as $K_{shoot}^{\prime soil}$ (i.e., $K_{shoot}^{soil} = K_{shoot}^{\prime soil}$). Because K_{shoot} is measured and K concentration measurements cannot differentiate K_{shoot}^{fert} from K_{shoot}^{soil}, any actual difference between K_{shoot}^{soil} and $K_{shoot}^{\prime soil}$ (i.e., $K_{shoot}^{soil} - K_{shoot}^{\prime soil}$) would be wrongly associated with fertilizer, leading to over- or under-estimate of fertilizer utilization.

In the difference method, fertilizer recovery efficiency is underestimated if soil-derived K in shoot from a fertilized experiment is less than that in the unfertilized control (i.e., $K_{shoot}^{soil} < K_{shoot}^{\prime soil}$), whereas overestimation occurs under an opposite situation (i.e., $K_{shoot}^{soil} > K_{shoot}^{\prime soil}$). Soil-derived K in shoot is related to plant-available K in soil. In our experiments, all pots had the same type of soil of the same mass at the beginning, so the initial size of plant-available K pool in unfertilized soil should be the same for all the treatments. However, as mentioned before, even without considering K in roots, excesses in plant-available K were observed in all treatments (Table 1), indicative of release of non-exchangeable K that recharged the plant-available K pool in soil during corn growth. Variations in the amount of this "recharged" plant-available K in soil in different treatments relative to the control are the key to explain biases on RE_d estimates using the difference method.

Release of non-exchangeable K is controlled by dynamic soil–plant–fertilizer interactions. Previous studies have shown that K release from the non-exchangeable pool in soil is dependent on K concentrations in soil solution, and the release rate typically increases with decreasing soil-solution K concentrations.[25, 120-124] In turn, K concentrations in soil solution can be affected by the plant K uptake rate, and a higher K uptake rate should cause lower K concentrations in soil solution near plant roots. Moreover, plant K uptake rate is affected by K nutrient levels, and it has been observed that the overall K uptake rate often increases with increasing amount of plant-available K.[25, 125] This relation between K uptake rate and nutrient level is broadly consistent with our observation that shoot dry mass remained largely the same in Group-B to -D, but K concentrations in shoot increased with higher fertilization rate (Figure 2), implying higher K uptake at higher K nutrient levels in soil.

Based on these previous studies, the increased fertilization rate from Groups-B to -D in our experiments should increase K concentrations in soil solution, which should cause decreased K release from non-exchangeable pool in soil relative to the control Group-A. This can explain the observed underestimation of fertilizer recovery efficiency by the difference method (RE_i) in all pots in Group-B and two pots in Group-C (Figure 4). In the fertilized experiments, because the amount of recharged plant-available K in soil was smaller than that in the control group, more fertilizer K was in fact utilized by plants (i.e., the situation of $K_{shoot}^{soil} < K_{shoot}^{\prime soil}$). The difference method that assumed $K_{shoot}^{soil} = K_{shoot}^{\prime soil}$, therefore, led to an underestimate of fertilizer recovery efficiency.

However, although the increased fertilization rate increases K concentrations in soil solution from Groups-B to -D, they should also increase the plant K uptake rates. When the plant K uptake reaches a sufficiently high rate under a certain high K fertilization rate, removal of K from soil solution by plant uptake should outpace the amount of soluble K added from fertilizer. In addition, K fixation into soil minerals, such as aluminosilicate layers of vermiculites and interstratified smectite/illite, may also occur, and field experiments indicate that the fixation can increase with increasing K fertilization rates.[29, 32, 105, 126, 127] As a result, as K fertilization rate increases, the combined effect of plant K uptake and K fixation would eventually overcome the initial increase in soil-solution K concentrations brought about by fertilizer, and the extent of recharged plant-available K in soil should increase again. This may explain the observed overestimates of fertilizer recovery efficiency by the difference method (RE_i) in all three pots in Group-D and one pot in Group-B (Figure 4). In these fertilized experiments, because the amount of recharged plant-available K in soil was larger than that in the control group, less fertilizer K was in fact utilized by plants (i.e., the situation of $K_{shoot}^{soil} > K_{shoot}^{\prime soil}$). The difference method, therefore, led to an overestimation of fertilizer recovery efficiency. Despite the uncertainty on K mass in roots, our above interpretations are consistent with apparent excesses in plant-available K (Table 1); apparent K excesses in Group-B are lower than those found in the control Group-A, whereas K excesses are the highest in Group-D.

The RE values obtained using our K isotope tracer method were more consistent for different pots in each group (Figure 4), relative to RE values obtained by the difference method. This may result from the fact that the difference method does not account for soil–plant–fertilizer interactions that may vary slightly across individual pots in each group. In contrast, our K isotope tracer method can "see through" soil–plant–fertilizer interactions to directly trace K from soil and fertilizer in each pot. Consequently, RE_i estimates obtained by the isotope tracer method tend to be more consistent within each group. The ^{41}K-tracer experiment conducted here as proof-of-concept is an invitation to continue similar investigations in other settings, for example, in more weathered soils without such abundant supplies of non-exchangeable K, and, eventually, in field soils where plant roots are free to mine soil K more extensively and less intensively than in a pot experiment, and over longer time scales to maturity and harvest. In this initial phase, fertilizer efficiency derived by the ^{41}K-tracer will have obligatory comparisons to efficiency by difference to control pots and plots, and then at some later stage, fertilizer efficiency by the stable K isotope tracer can be of value for guiding K usage by itself.

5. CONCLUSIONS

Understanding the fate of K fertilizer during crop growth and quantifying K fertilizer utilization is not an easy task but of considerable agronomic and economical significance. The conventional difference method based on K concentrations alone does not adequately account for dynamic soil–plant interactions at different fertilization conditions, thereby potentially leading to biased results. Enabled by recent analytical advances, here we demonstrate the utility of a novel K isotope tracer technique based on stable K isotopes in improving quantification of K fertilizer utilization through pot experiments. In addition, based on the unfertilized experiments, we show that corn growth preferentially utilizes light K isotopes, with an estimated shoot–soil isotope fractionation factor of -0.37‰ (±0.23‰) in $^{41}K/^{39}K$. Although the K isotope tracer method may not become a routine soil test because of the cost of enriched K tracers and analytical challenges associated with high-precision K isotope analysis as compared to the cheaper and simpler K concentration analysis, the K isotope tracer method can be utilized for pot, mesocosm, and small-scale field experiments. Moreover, the K isotope tracer method shows great potential for improving our fundamental understanding of the dynamics of K exchange among different soil pools and many complex soil–plant–fertilizer interactions that are conventionally difficult to investigate. In addition, as demonstrated by several other well-studied stable isotope systems, such as carbon and nitrogen isotopes, natural K isotope variations in soil–plant systems clearly deserve further investigations in laboratory- and field-based studies, and they are expected to provide valuable information on the K nutrient cycle that is not visible from K concentrations alone.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at

A Monte Carlo method used to estimate shoot–soil K isotope enrichment factor (ε) and the percentage of fertilizer-derived K in corn shoot (F).

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Notes

The authors declare no competing financial interest.

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Supporting Information for:

Natural potassium (K) isotope fractionation during corn growth and quantification of K fertilizer recovery efficiency using stable K isotope labeling

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Estimating the plant–soil K isotope fractionation factor using a Monte Carlo method

There are two major sources of uncertainty in calculating ε using Equations 1 and 2 in the main text: K mass in root (i.e., K_{root}) and the $\delta^{41}K$ value of the plant-available K pool in soil (i.e., $\delta^{41}K'$). For K_{root}, although this parameter was not directly measured, it can be estimated based on shoot dry weight and shoot K concentrations, both of which were measured in this study (Table 1 in the main text), and reasonable assumptions for root to shoot (R/S) mass ratios and K concentration ratios based on literature. Amos and Walters (2006) compiled R/S dry mass ratios from 45 published field and experimental studies for corn as a function of days after emergence. Based on this extensive data compilation, the R/S mass ratio for our ~6-week experiments should fall within a range between ~0.1 and ~1. This range should be representative for our experiments, because we previously conducted root separation and cleaning for similar corn growth experiments that used the same soil and pot, over a comparable duration, and R/S mass ratios of ~0.65 were obtained. In addition to a R/S dry mass ratio, a K concentration ratio between root and shoot is also needed to estimate total K mass in root, because K concentrations in root and shoot can be different. Previous studies showed that R/S K concentration ratios could vary between ~45% and ~80% for corn grown under a wide range of field and laboratory conditions (e.g., Warncke and Barber, 1974; Claassen and Barber, 1977; Zeng and Brown, 2000; Turan et al., 2010), and this large range should cover R/S K concentration ratios expected in our experiments.

The second major uncertainty for ε estimation is the K isotope composition of the plant-available K pool in soil ($\delta^{41}K'_{soil}$ in Equation 1 in the main text). A logical initial assumption for $\delta^{41}K'_{soil}$ is the $\delta^{41}K$ value measured in the Bray extract of initial unfertilized soil. However, even without considering K mass in root, the sum of K mass in corn shoots and Bray-extractable K in soil after harvest is already ~58%–74% higher than the total amount of K mass based on measurement of Bray extract of initial unfertilized soil (Table 1 in the main text). This implies recharge of plant-available K in soil during corn growth in all three pots in Group-A. It may be not appropriate to assume that $\delta^{41}K'_{soil}$ was the same as $\delta^{41}K$ measured in the Bray extract of initial soil. Because of the excess of plant-available K, $\delta^{41}K'_{soil}$ depends on the amount of the newly available soluble K and its $\delta^{41}K$ value. The amount of recharged plant-available K can be calculated by the mass difference between initial plant-available K estimated by measurement of the Bray extract of initial soil and the sum of K mass in root, shoot, and Bray-extractable K in soil after harvest. Only K mass in root (K_{root}) is unknown, and the rest can be calculated based on measurements of dry mass and K concentrations of soil and shoot. The amount of recharged plant-available K is then dependent on estimates of K_{root}. The $\delta^{41}K$ value of the recharged plant-available K in soil is more challenging to know without direct measurement, but an approximate range can be estimated. The average $\delta^{41}K$ value of the upper continental crust is ~ -0.45‰ (Huang et al., 2020; Wang et al., 2021), representing the starting value of primary silicates that are later transformed into various minerals in all soils during weathering. Weathering produces resolvable K isotope fractionation, and $\delta^{41}K$ values reported for natural weathering profiles ranged between ~ -0.45‰ and ~ -1‰ with the lower limit associated with highly weathered products (e.g., kaolinite or bauxite) (Chen et al., 2020; Teng et al., 2020). Assuming the bulk soil used in this study has an extreme $\delta^{41}K$ value of -1‰ similar to the highly weathered material observed in nature, and release of soluble K was caused by rapid dissolution of soil silicates, accompanied by no K isotope fractionation (Li et al., 2021a), the resultant soluble K should carry a similar $\delta^{41}K$ value of ~ -1‰, which sets a lower limit for $\delta^{41}K$ expected in the recharged plant-available K in our experiments. Alternatively, if the recharged soluble K has a similar $\delta^{41}K$ value to those of Bray extracts of final soil, we consider the upper limit of $\delta^{41}K$ for the recharged soluble K to be 0.5‰ based on the highest $\delta^{41}K$ value measured in soil Bray extracts after harvest from Group-A experiments. A $\delta^{41}K$ range from -1‰ to 0.5‰ is large for natural variations in K isotopes, and this range is taken as a maximum for the $\delta^{41}K$ values of plant-available K in natural soil. For example, a recent study reported $\delta^{41}K$ values of ~ -0.6‰ and ~0.5‰ for plant-available K in natural humid and arid soils from Hawai‘i (Li et al., 2021b).

A Monte Carlo approach that considered all the uncertainties noted above was used to estimate the shoot–soil K isotope fractionation enrichment factor ε based on Equations 1 and 2 in the main text. Random input values were generated within the prescribed ranges for R/S mass ratio (0.1–1), R/S K concentration ratio (0.45–0.80), and $\delta^{41}K$ value of recharged plant-available K (-1‰ – 0.5‰). For input parameters that were directly measured or can be calculated from direct measurements (i.e., dry mass, K concentrations, and $\delta^{41}K$ values of soil extracts and corn shoot), random numbers for each parameter were generated within the range bounded by the mean and standard deviation of this parameter. Because standard deviations of direct measurements for these parameters from the three pots are typically larger than corresponding analytical uncertainties, they may reflect subtle physiological differences in plant responses in these experiments. By using standard deviations of direct measurements from the triplicate pots, rather than analytical uncertainties, our Monte Carlo approach may have also accounted for true physiological differences in different pots. A compilation of all model input parameters and their prescribed ranges used in our Monte Carlo simulations is provided in **Table S1**, and a schematic diagram showing relations of different parameters in the soil–plant system considered here is provided in **Figure S1**.

A total of 100-million Monte Carlo simulations were conducted to calculate ε. Because each pot was a closed system with respect to K, each Monte Carlo simulation also allowed for calculation of root δ^{41}K ($\delta^{41}K_{root}$) based on the chosen input values and K isotope mass balance. Although $\delta^{41}K_{root}$ is not measured, it must fall within a reasonable natural range, making it a useful internal constraint to screen simulation results. If no *a priori* assumption on the limit of $\delta^{41}K_{root}$ was made, our 100-million Monte Carlo simulations would yield $\delta^{41}K_{root}$ values that spread between 14.36‰ and -78.93‰. This range greatly exceeds the δ^{41}K variability observed in any natural samples on the Earth. This indicates that not all 100-million Monte Carlo simulations could provide solutions that are realistic for the K isotope system, although all solutions numerically fulfil Equation 1 and 2 in the main text. The reported δ^{41}K values for roots of different plants (grasses, soybean, rice, and wheat) are typically ~0.4–0.8‰ lower than those of plant-available K in growth medium (Christensen et al., 2018; Li et al., 2021b). Although δ^{41}K values and potential K isotope fractionation specific to corn root have not been studied, there is no reason to believe corn root would behave fundamentally different from other plant types. As a result, we prescribed a range between -2‰ to 0‰ for $\delta^{41}K_{root}$ as a criterion used to screen Monte Carlo simulation results. Any simulation that yielded a $\delta^{41}K_{root}$ value outside this prescribed $\delta^{41}K_{root}$ range was considered to be unrealistic and then rejected. Without relying on detailed knowledge about root K isotope compositions, this prescribe range is very large for K isotope variations measured to date, making it likely that it covers the true $\delta^{41}K_{root}$ values expected in our experiments.

Our best-estimate of shoot–soil K isotope enrichment factor ε is -0.37‰ (±0.23‰, 1SD), based on 100-million Monte Carlo simulations that considered all possible combinations of input parameter values randomly selected from the prescribed ranges and an additional screening step based on a realistic $\delta^{41}K_{root}$ range as described above. About 40% of the solutions passed the screening. A smaller number (e.g., 1 million) of Monte Carlo simulations yielded the same result, indicating that the ε estimate provided here is numerically stable.

The same Monte Carlo approach was also used to estimate F in Equation 3 of the main text, using prescribed ranges tabulated in **Table S1**. In this case, the estimated ε along with its uncertainty (i.e., -0.37‰ ± 0.23‰) was used in Monte Carlo simulations based on the Equation 3. The F results reported in the main text were based on a total of 100 million simulations.

Table S1. Parameters used in the Monte Carlo simulations

Parameter	Definition	Range tested in Monte Carlo simulations
Measured		
M_{soil}	Total soil mass	1.5 kg
M_{shoot}	Shoot dry mass	7.33 ± 1.04 g
$[K]_{soil\_i}$	K concentration in Bray-extract from initial soil	71 mg K kg^{-1} soil
$[K]_{soil\_f}$	K concentration in Bray-extract from final soil after harvest	67.3 ± 9.1 mg K kg^{-1} soil
$[K]_{shoot}$	K concentration in corn shoot	10.1 ± 1.1 mg g^{-1}
$\delta^{41}K_{soil\_i}$	K isotope composition in Bray-extract from initial soil	-0.11 ± 0.11‰
$\delta^{41}K_{soil\_f}$	K isotopic composition in Bray-extract from soil after harvest	0.36 ± 0.11‰
$\delta^{41}K_{shoot}$	K isotope composition of shoot	-0.29 ± 0.06‰
Prescribed		
R/S mass ratio	Root to shoot dry mass ratio	0.1 ~ 1
R/S K concentration ratio	Root to shoot K concentration ratio	0.45 ~ 0.8
$\delta^{41}K_{recharge}$	K isotope composition of recharged plant-available K	-1 ~ 0.5‰
$\delta^{41}K_{root}$	Root K isotope composition used for result screening	-2 ~ 0‰

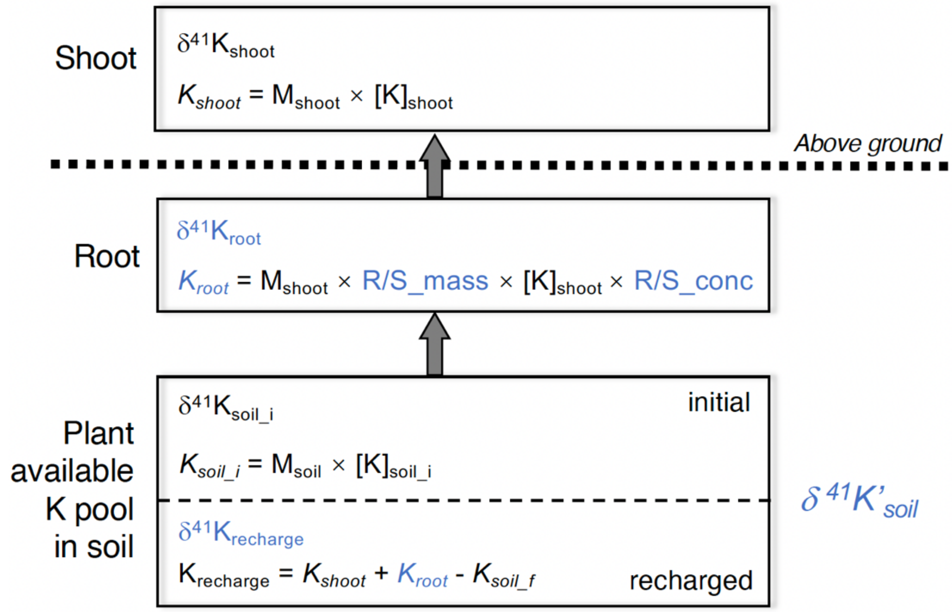

Figure S1 A schematic of the box model and parameters considered in our Monte Carlo simulations intended to estimate natural K isotope fractionation during corn growth. Directly measured parameters are in black, and parameters with prescribed values are in blue. Derived parameters based on measured or prescribed values are in italics. Parameters shown here are defined in Table S1.

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