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Use and analysis by thermal ionisation mass spectrometry of ^{26}Mg and ^{41}K to assess mineral uptake in Scots pine (*Pinus sylvestris* L.)

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Use of ^{26}Mg and ^{41}K as tracers allows the quantification of the uptake and internal cycling of Mg and K in plants. Application of thermal ionisation mass spectrometry (TIMS) used for a plant experiment is discussed here. Firstly, the sensitivity of the $^{26}Mg/^{24}Mg$ ratio to the amount of Mg loaded onto the mass spectrometer filament was assessed. Using NIST SRM-980 and amounts of Mg from 0.2 to 1.2 μg, no significant difference in the $^{26}Mg/^{24}Mg$ value after correction for isotope fractionation was observed. Analysis of SRM-980 produced a corrected mean $^{26}Mg/^{24}Mg$ value of 0.13960 ± 0.00006 (n = 10) close to the certified range (0.13932 ± 0.00026). Control of fractionation during K analysis by TIMS is important for accurate isotope determinations. Fractionation profiles for NIST SRM-985 using filament loadings of 1 and 5 μg K were plotted and, with the higher loading, produced a more stable $^{39}K/^{41}K$ value. Conversion of K from a chloride to an iodide had no significant effect on the measured ratio. The SRM-985 mean $^{39}K/^{41}K$ value was 13.916 ± 0.034, higher than the certified range (13.8566 ± 0.0063). Analysis of natural ^{26}Mg and ^{41}K levels in needles, stem wood, stem bark, fine roots and coarse roots from Scots pine allowed the precision of the analysis to be defined. This information, in conjunction with a simple model, was used to discuss the design of a tracer study in plants using ^{26}Mg and ^{41}K. Predicted whole tree $^{39}K/^{41}K$ and $^{26}Mg/^{24}Mg$ values from a 95 day experiment were then calculated and compared with the actual values measured using TIMS.

Introduction

Trees of economic significance, such as pine and spruce, can suffer from mineral deficiencies which can have a serious impact on growth. The dynamics of mineral uptake and the partitioning of macronutrients such as Mg and K over a growing season in these trees are not well understood. Whilst nutrient budget experiments can quantify uptake, quantifying internal cycling and the translocation of minerals within a plant over a growing season is more difficult. Isotopic labelling of Mg or K offers an opportunity to quantify both uptake and internal cycling and may allow a better understanding of these processes. Although radioactive isotopes exist for both Mg and K, none would be suitable for long term labelling studies. Radioactive isotopes of K have half lives ranging between 0.95 s ($_{19}K^{38m}$) and 22.4 h ($_{19}K^{43}$); $_{19}K^{40}$ is a long lived radiogenic nuclide with a half-life of 1.28 × 10^9 years. Half-lives of radioactive Mg range from 0.12 s ($_{12}Mg^{21}$) to 21 h ($_{12}Mg^{28}$). Also, from a practical aspect, the use of the radioisotopes is less attractive since it requires growth of the plants in designated radioactive laboratories or greenhouses, and precludes the possibility of field experiments.

Relatively few studies have used stable isotopes in this type of application. A microprobe analysis technique has been used to qualitatively trace the uptake of Mg, Ca and K isotopes from the roots to the stems of spruce trees over a 7 day period.[1,2] Högberg *et al.*,[3] using quadrupole, inductively coupled plasma-mass spectrometry (ICP-MS), have also assessed Mg uptake in excised Scots pine (*Pinus sylvestris* L.) roots over 4 h using ^{24}Mg, and Becker and Dietze[4] recently described the analysis of ^{26}Mg, ^{25}Mg, ^{41}K, ^{42}Ca and ^{44}Ca from a plant study using high resolution ICP-MS. None of these experiments, however, used isotopes on a whole plant basis for a time period of more than a few days, or considered the internal cycling of nutrients. The primary reason for this is the high cost of the isotopes, which necessitates the use of restricted amounts and, in turn, places a high demand on the analytical procedures used. The most accurate and precise means of analysing isotopes such as Mg and K is by thermal ionisation mass spectrometry (TIMS).[5] This technique has advantages over other mass spectrometry techniques, such as ICP-MS, in that there are no intrinsic problems of polyatomic interferences, or, for example, fast atom bombardment mass spectrometry, where hydride formation and low sensitivity make accurate isotope ratio measurements difficult.[6] TIMS has rarely been applied to plant studies. Analysis of Mg isotopes by TIMS has in the past been restricted to cosmology[7–9] and clinical investigations,[10–12] whilst K isotope analysis has been largely restricted to geochronometric measurements exploiting ^{40}K–^{40}Ar and ^{40}K–^{40}Ca radioisotope decay schemes.[13,14] We report here firstly on the development of ^{26}Mg and ^{41}K analysis by TIMS, tailored to plant tissues. For ^{26}Mg, specifically, we discuss the use of internal normalisation to correct for fractionation and investigate the sensitivity of the ratio measurements to the Mg loading on the filament. For K, filament loading is again discussed and, to reduce fractionation, the use of an iodide salt is assessed. The validity of the analytical procedures adopted is then demonstrated by long term quality control data generated from the analysis of the NIST standards SRM-980 for Mg and SRM-985 for K. Finally, we discuss the establishment of appropriate isotope dosing levels for a plant experiment designed to trace mineral uptake in Scots pine and present the results from a 95 day experiment.

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Experimental

Materials

Isotopic standards, SRM-980 and SRM-985, were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, USA). Isotope spikes (99.41 at.% ^{26}Mg and 99.04 at.% ^{41}K) were obtained from CK Gas Products (Finchampstead, Berkshire, UK). All acids used were of Aristar grade and were obtained from BDH Chemicals (Poole, Dorset, UK). A silica gel suspension for filament loading was prepared by shaking 1 g of finely ground silica gel in 15 ml of 0.66 M orthophosphoric acid overnight. The suspension decanted after a settling period of 28 min was used. High purity water was used throughout with a metered resistivity of ≥ 18 MΩ.

Mass spectrometry

Isotope analyses were carried out using a VG354 magnetic sector, thermal ionisation mass spectrometer (Micromass UK Ltd.,Wythenshawe, Manchester, UK). This instrument is equipped with a 5 Faraday cup detection system, isotope ratio measurements can be made in both a static mode using the multiple collectors or by using a single collector in conjunction with a peak jumping routine.

^{26}Mg analysis. For Mg analyses, 1 μl of silica gel/phosphoric acid suspension was added stepwise (0.3 μl portions) using a microsyringe to a heated, single, high purity (99.97%) Re filament (0.76 mm × 0.03 mm) which had been cleaned by boiling in 0.5 M HCl and ultrasonic washing (2×) with high purity water; 1 μl of the Mg sample solution (0.5 M HCl) was applied directly to the dry silica gel/phosphoric acid spot, in the same manner. A current of 1.2 A was used to heat the filament for loading, on completion of loading, the current was gradually increased to 2.5 A and held at this current, momentarily, to fuse the sample to the filament. The loaded filaments were located in a 16 place carousel, and enclosed in the source chamber of the mass spectrometer which was evacuated to $< 3 \times 10^{-7}$ mbar. The analysis was started after preheating each filament at 2.25 A. The ratios 25/24, 26/24 and 26/25 were monitored using a collective ion beam intensity (^{26}Mg + ^{25}Mg + ^{24}Mg) of 3.0×10^{-11} A and a single collector, peak jumping procedure. Between 100 and 160 ratio measurements were obtained in blocks of 20 for each analysis.

Magnesium is susceptible to isotope fractionation when undergoing evaporation in the source of a thermal ionisation mass spectrometer. To account for this, the measured (m) isotope ratio $(^{25}Mg/^{24}Mg)_m$ was normalised to the reference value (o) for terrestrial magnesium $(^{25}Mg/^{24}Mg)_o$ of 0.12663.[15] A correction factor, $\alpha = (^{25}Mg/^{24}Mg)_m/(^{25}Mg/^{24}Mg)_o$, was then used to calculate the fractionation corrected (c) isotope ratio, $(^{26}Mg/^{24}Mg)_c = (^{26}Mg/^{24}Mg)_m/\alpha^2$, assuming a power law correction as described by Stegmann *et al.*[12]

To evaluate the effect of the amount of Mg loaded on each filament on the measured isotope ratio, a range of solutions was prepared using SRM-980 dissolved in 0.5 M HCl. Filaments were loaded with 0.2, 0.6, 1.0 and 1.2 μg Mg, and the analysis was carried out as described above. This experiment was conducted for two reasons: (i) to define an optimal amount for loading, if any; and (ii) plant tissues vary markedly in Mg content, and whilst every attempt was made to ensure the same concentration was loaded onto the filaments, slight variations were inevitable and the significance of this on the measured ratio was established. Based on these results, all subsequent ^{26}Mg analyses were based on a 1 μg loading, and the performance of the mass spectrometer was monitored by analysing SRM-980 with each turret of samples.

^{41}K analysis. For ^{41}K analysis, a single, high purity (99.95%) Ta filament (0.760 mm × 0.03 mm) was loaded using a procedure similar to that described for Mg. However, the K was loaded either in the form of KCl or KI. After preheating the filaments at a current of 1.25 A, the ratios 39/41 and 40/41 were monitored using a single collector, peak jumping procedure. The ion beam intensity (^{41}K + ^{40}K + ^{39}K) was increased stepwise from 1.0×10^{-12} A to a maximum of 1.0×10^{-10} A. Between 300 and 500 ratio measurements were then obtained in blocks of 25 for each analysis at the maximum beam current.

Potassium, like Mg, is a relatively light element and undergoes marked fractionation within the source of a thermal ionisation mass spectrometer. Due to the low natural abundance of ^{40}K (0.012%), K is generally treated as a two isotope system. As discussed by Fletcher *et al.*,[16] this restricts the options available to correct for fractionation and ^{39}K/^{41}K cannot be reliably corrected by normalisation of ^{40}K/^{41}K in the manner analogous to that described for Mg. Two options remain: to operate the mass spectrometer in such a way as to produce consistent fractionation patterns between samples and to load the K in a chemical form which minimises the fractionation.[16]

An initial experiment was conducted to determine if a consistent fractionation profile could be obtained for K and whether the concentration of K loaded on the filament influenced this pattern and the overall mean ratio. Five filaments were loaded with the NIST standard SRM-985 dissolved in 0.5 M HCl; two filaments were loaded with 1 μg K and three with 5 μg K. The solution concentrations were adjusted to allow the same volume of sample (1 μl) to be loaded in each case. The samples were then analysed as above collecting 500 ratios in total.

It has been suggested that, by loading filaments with K present in the form of KI instead of KCl, mass fractionation can be reduced, since iodide has a higher mass, lower boiling point (at atmospheric pressure) and dissociation energy than chloride.[16] To investigate this, we compared ^{39}K/^{41}K derived from five pine trees which had been divided into five component parts (needles, stem wood, bark, fine roots and coarse roots); K derived from acid digestion of the plant tissues (described below) was analysed in the form of KCl and KI. The digestion procedure produces KCl, so that it was necessary to convert an aliquot of this to KI, by taking each sample to dryness, and treating with 2 × 25 μl aliquots of concentrated HI, each of which was evaporated to leave an iodide salt. Finally, the sample was dissolved in 0.2 M HI and the volume adjusted to provide 5 μg K μl^{-1} for loading. A total of 300 ratios was collected from each sample from which an overall mean was calculated.

As a result of these experiments, all subsequent ^{41}K analyses were based on 5 μg of KI, from which 300 ratios were collected and an overall mean calculated. To ensure that the instrument performance was consistent, each turret of samples analysed contained the standard SRM-985, which had also been converted to the KI form.

Tree labelling study

Twenty 2 year old Scots pine trees were planted in sand in April 1997 and supplied with all essential nutrients (K, Mg, N, P, Ca, Mn, Zn, Cu, B and Fe). At the start of spring the following year, 10 trees were harvested and separated into five component parts: needles, stem wood, stem bark, fine roots and coarse roots. The ^{26}Mg/^{24}Mg and ^{39}K/^{41}K ratios were then measured in these tissues. The remaining 10 trees were split into two groups and supplied with ^{26}Mg and ^{41}K spikes at two levels, either in balance with a generous supply of nutrients (HIGH) or with a restricted supply of nutrients (LOW). Full details of tree maintenance are described by Proe *et al.*[17]

Due to the high cost of the ^{26}Mg and ^{41}K isotopes, it was essential, prior to dosing, to establish how much to apply to each tree so that the excess isotope could be not only detected, but would also allow flux rate measurements to be made between different tissues (see Proe *et al.*[17]). A key factor in the dose calculations was the cost of the spike isotopes. This constraint was used to establish the maximum amount of ^{26}Mg and ^{41}K spike which could be used. An arbitrary desirable minimum excess isotope level was taken to be equal to the tissue natural abundance mean isotope ratio $\pm 5\sigma$ (where σ is one standard deviation). Statistically, this would allow a clear distinction to be made between unenriched and enriched material with a confidence level well in excess of 99.99%. A simple model was then used to calculate the expected isotope ratio given a particular dose and pool size. The initial Mg and K pool sizes used in the model were based on 2 year old Scots pine. This modelling process not only allowed decisions to be made about the amount of dose to use, but in turn also the number of trees and nutrients to be supplied over the course of the study in the HIGH and LOW treatments.

We designed the HIGH treatments such that the initial Mg concentration in the trees was trebled and the K concentration doubled. The LOW treatment provided a third of the Mg and a third of the K with respect to the HIGH treatment. The HIGH treatment supplied 100 mg Mg spiked with 1.2 mg ^{26}Mg and 300 mg K spiked with 2.16 mg ^{41}K, with all other essential nutrients including N at a matched elevated level. The LOW treatment supplied 30 mg Mg spiked with 0.8 mg ^{26}Mg and 100 mg K spiked with 1.44 mg ^{41}K, again with all other nutrients at a matched level. The dose was divided between these treatments in a 60 : 40 split, to ensure that the final ratios in the whole tree were approximately the same. The aim of this was to change the isotope content to the same extent with respect to the natural abundance level and equalise the level of precision in quantifying uptake.

Sample preparation. At harvest, the trees were divided into several tissue types and all of a particular tissue type was collected. This plant material was then dried and ground to pass through a 1 mm screen. After mixing thoroughly, 0.5 g was ashed at 450 °C over 12 h and digested in an open tube using 10 ml of *aqua regia* (40% HCl : 25% HNO_3) for 10 h at 140 °C. The dry residue was taken up in 12.5 ml of 50% HNO_3, filtered through Whatman No. 542 filter paper and made up to 50 ml in a volumetric flask. Mg and K concentrations were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Although direct loading onto the mass spectrometer filament of sample digests has been used for ^{26}Mg analysis in the past,[12] such an approach did not prove successful in this work due to the presence on the filament of a significant Ca residue, which interfered with the Mg ionisation. Mg was, therefore, isolated from the digested plant material using cation exchange resin (AG 50W X8, 200–400 mesh, H form) packed into 3 × 150 mm glass chromatography columns. An aliquot of digest was taken to dryness, redissolved in 1 M HCl and the Mg eluted from the columns using 1 M HCl. K was analysed by loading the tissue digest directly onto a filament, without further purification.

Results

^{26}Mg analysis

The $^{26}Mg/^{24}Mg$ isotope ratio was largely insensitive to the amount of element loaded on the filament (Fig. 1). During the preheat period, the ratio was erratic, but, as the ion beam intensity was increased to 3.0×10^{-11} A, the ratio became more stable. There was no significant difference ($p = 0.505$) in the ratio with respect to the amount of Mg loaded over the range 0.2 to 1.2 μg. Overloading the filament with up to 20 μg of Mg was found to produce very weak and unstable ion beams (data not shown).

Analysis of the NIST standard SRM-980 using a filament loaded with 1.0 μg Mg yielded an overall mean ratio of 0.13960 ± 0.00006 ($n = 10$), where the uncertainty is equivalent to the 95% confidence interval. These data were collected over a period of approximately 1 year, over which time the fractionation correction term α ranged from 0.980 to 0.983. The certified mean value for $^{26}Mg/^{24}Mg$ SRM-980 is 0.13932 ± 0.00026,[15] and the results observed here lie within this range. This was also the case when analysing SRM-980 using variable concentrations of Mg (Fig. 1); the overall mean ratio for these data was 0.13979 ± 0.00013 ($n = 4$), consistent with the long term average data. Stegmann *et al.*[9,12] have reported $^{26}Mg/^{24}Mg$ values for a chemical standard ($MgCl_2$) from separate mass spectrometers of 0.139733 and 0.14016. The difference observed has been attributed by these authors to instrument specific fractionation effects within the mass spectrometer source, which presumably are not accounted for entirely by the normalisation procedure. Becker and Dietze[4] reported a value of 0.13940 ± 0.0007 obtained using a double focusing ICP-MS system, which is also within the range of values published for SRM-980.

^{41}K analysis

Comparison of $^{39}K/^{41}K$ isotope ratios after loading either 1 or 5 μg K from the NIST standard SRM-985 produced results which were not significantly different ($p = 0.383$) from each other (Fig. 2). During the sample preheat period, the ratio declined markedly as a result of thermally induced isotopic fractionation, with the lighter isotope (^{39}K) preferentially leaving the filament in accordance with the established Rayleigh distillation theory. However, throughout the 7 h measurement period, the ratio for both loading amounts declined more gradually. Towards the end of this period, the $^{39}K/^{41}K$ ratio increased slightly for the two filaments loaded with 1 μg K and may have been an indication that little of the sample remained after this period. This increase in the ratio is the complement of the decline observed at the start of the analysis as discussed by Fletcher *et al.*[16] Based on these results future analyses were undertaken using 5 μg K and 300 ratio measurements, to ensure that the data were obtained from the plateau region of the partial fractionation profile shown in Fig. 2.

Analysis of various tree tissues indicated that, with the exception of bark, the mean $^{39}K/^{41}K$ values of the samples in the iodide form tended to be slightly lower than those obtained from

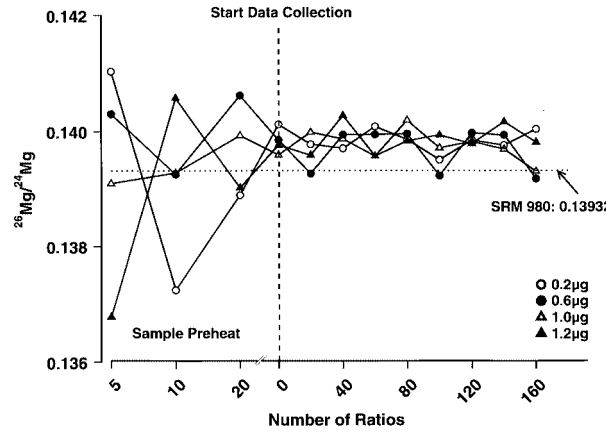

Fig. 1 $^{26}Mg/^{24}Mg$ variation with amount of Mg loaded onto the filament.

the same samples in the chloride form (Table 1). There was, however, no significant difference ($p = 0.145$) between the two forms of salt. It has been shown elsewhere that the use of KI reduces fractionation;[16] the absence of a marked effect here may have been due to the amount of K loaded on each filament (5 μg K *vs.* 1 μg K). The samples in this study were converted from a chloride form into the iodide prior to analysis, from these results, the benefits of this process may have been minimal.

Analysis of the NIST standard SRM-985 as KI using 5 μg K over a period of 1 year produced a long term mean value of 13.916 ± 0.034 ($n = 10$) where the uncertainty is at the 95% confidence interval; the range for the standard means was 14.0060 to 13.8372 (Fig. 3). The results obtained in this laboratory are slightly higher than the certified range of values which have a mean of 13.8566 ± 0.0063. The difference may be a result of instrument/filament fractionation effects. Fletcher *et al.*[16] reported a mean value of 13.911 ± 0.004 (where the error is 2σ) for SRM-985, which is consistent with that observed in this work. Becker and Dietze[4] reported a mean value of 13.5446 ± 0.0936 obtained using double focusing ICP-MS, lower than the certified range of values.

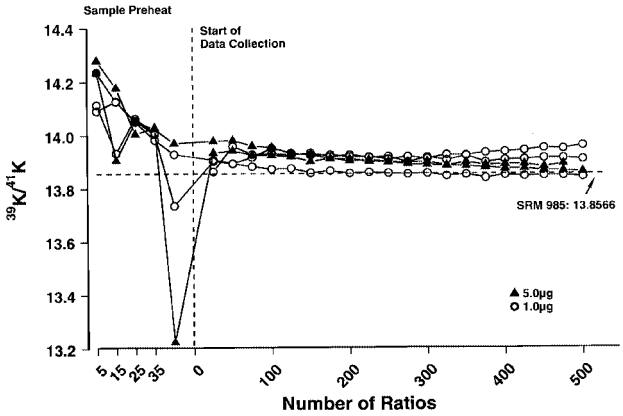

Fig. 2 $^{39}K/^{41}K$ variation with amount of K loaded onto filament over a prolonged measurement period.

Table 1 Comparison of $^{39}K/^{41}K$ analyses when K was loaded as KI and KCl. Values presented are means $\pm 2\sigma$ ($n = 5$)

Tissue	$^{39}K/^{41}K$, K loaded as KCl	$^{39}K/^{41}K$, K loaded as KI
Needles	13.891 ± 0.109	13.852 ± 0.123
Stem wood	13.903 ± 0.126	13.658 ± 0.168
Stem bark	14.028 ± 0.040	14.077 ± 0.082
Fine roots	13.989 ± 0.054	13.968 ± 0.070
Coarse roots	14.014 ± 0.021	13.930 ± 0.109
Overall mean	13.965 ± 0.093	13.907 ± 0.170

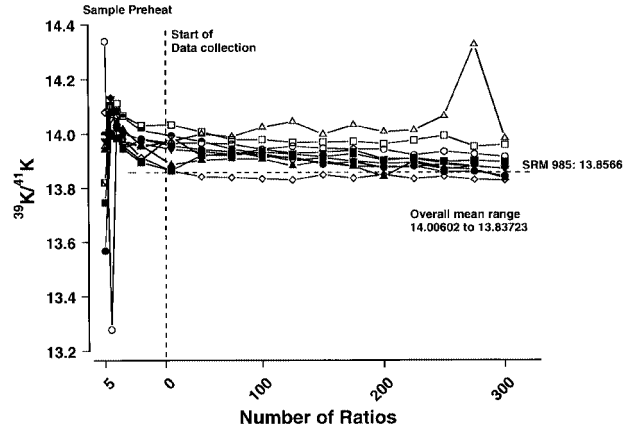

Fig. 3 $^{39}K/^{41}K$ determinations of NIST-985 over a 12 month period, when loaded as KI.

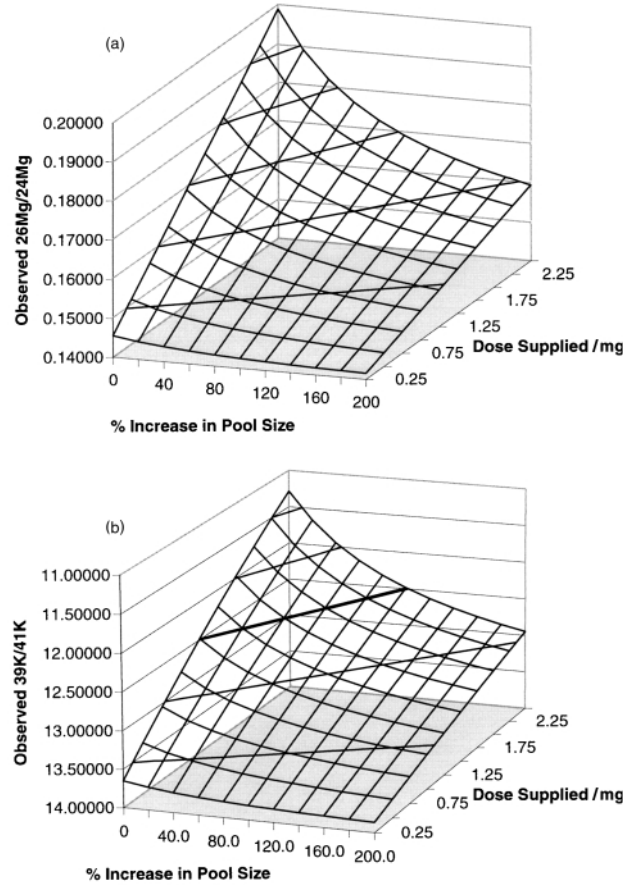

Fig. 4 Modelled changes in the measured isotope ratio after dosing with (a) ^{26}Mg and assuming an initial pool size of 50 mg, or (b) ^{41}K and assuming an initial pool size of 300 mg. Pool sizes are based on 2 year old Scots pine.

Table 2 Comparison of natural isotope ratios measured in various tissues, with the NIST standards for Mg (SRM-980) and K (SRM-985), together with the elemental composition of the tissues (% dry mass). Uncertainty on the isotope measurements is at the 95% confidence interval and for the elemental compositions is $\pm 2\sigma$ ($n = 10$)

Tissue	%Mg	$^{26}Mg/^{24}Mg$	%K	$^{39}K/^{41}K$
Needles	0.106 ± 0.010	0.13964 ± 0.00004	0.710 ± 0.146	13.926 ± 0.060
Stem wood	0.027 ± 0.008	0.13955 ± 0.00007	0.086 ± 0.019	13.873 ± 0.084
Stem bark	0.129 ± 0.018	0.13956 ± 0.00014	0.516 ± 0.082	14.058 ± 0.039
Fine roots	0.091 ± 0.011	0.13971 ± 0.00009	0.791 ± 0.264	13.969 ± 0.023
Coarse roots	0.034 ± 0.005	0.13960 ± 0.00007	0.263 ± 0.059	13.978 ± 0.036
Overall mean	—	0.13961 ± 0.00004	—	13.963 ± 0.022
NIST SRM-980/985	—	0.13960 ± 0.00006	—	13.916 ± 0.034

Table 3 Predicted and measured isotope ratios in trees receiving either the HIGH or LOW nutrient treatment

				$^{26}Mg/^{24}Mg$	
Treatment	Supplied Mg/mg	Final pool/mg	^{26}Mg dose/mg	Predicted[a]	Measured[b]
Mg LOW	33	83	0.8	0.15103	0.14388 ± 0.0070
Mg HIGH	100	150	1.2	0.14909	0.14273 ± 0.0082

				$^{39}K/^{41}K$	
Treatment	Supplied Mg/mg	Final pool/mg	^{41}K dose/mg	Predicted[a]	Measured[b]
K LOW	150	450	1.44	13.3634	13.3676 ± 0.102
K HIGH	300	600	2.16	13.2907	13.3949 ± 0.088

[a] Calculated using simple model. [b] Average $\pm 2\sigma$ measured in five trees.

Tree labelling

Analysis of Mg in the trees harvested prior to label administration (Table 2) produced $^{26}Mg/^{24}Mg$ values which were not significantly different from the SRM-980 ($p = 0.805$) values measured in this laboratory and there were no differences ($p = 0.093$) between the tissues sampled. The overall mean $^{26}Mg/^{24}Mg$ value was 0.13961 ± 0.00004, almost identical to the SRM-980 value of 0.13960 ± 0.00006. Similarly, K analysis of the tree tissues produced an overall mean $^{39}K/^{41}K$ value of 13.963 ± 0.022 (Table 2), and again there was no significant difference ($p = 0.335$) from the SRM-985 value of 13.916 ± 0.034, or between tissues ($p = 0.061$). Although not significant, the stem bark did appear to have a slightly elevated $^{39}K/^{41}K$ value in all of the analyses (Tables 1 and 2). The reason for this is not clear, but may be evidence of a small degree of natural fractionation.

From the precision of the baseline isotope analyses (Table 2), a minimum target isotope enrichment which could be measured with confidence was calculated from the overall mean, and estimated as the mean ± five times the standard deviation of that mean. For Mg, this implied a minimum $^{26}Mg/^{24}Mg$ value of 0.14036 and, for K, a minimum $^{39}K/^{41}K$ value of 13.583. Modelled changes in the whole tree isotope ratios following the uptake of different amounts of isotope spike (0.25–2.5 mg) and supplied nutrient, resulting in pool size changes of between 0 and 200%, are shown in Fig. 4. These plots clearly illustrate, as might be expected, that the greatest change in the observed isotope ratio arises from a minimal change in pool size and high isotope dose. A less obvious observation is the effect of the changing dose levels, which produce a linear change in the expected isotope ratio, whilst changes in the pool size produce a curvilinear response. The greatest change in the ratio is observed when the pool size starts to increase; however, as the pool size continues to increase, the extent of change in the observed ratio reduces. This has important implications for experimental planning, in that pool size changes can be manipulated in plant experiments by the level of nutrient supplied and the duration of the study. The response of the ratio to the supplied dose is dependent on the initial pool size so that, for K with an initial pool in our model of 150 mg, a 2.5 mg dose produced a ~20% change in the ratio, whereas with Mg the pool size was taken to be 50 mg so that a 2.5 mg dose produced a ~40% change in the ratio.

Using the simple model, the expected $^{26}Mg/^{24}Mg$ and $^{39}K/^{41}K$ values in the trees were predicted (Table 3). The actual $^{39}K/^{41}K$ ratios measured in the trees at the end of the study agree well with the predicted values, with differences of just 0.03% and 0.8% for the HIGH and LOW, respectively. However, there was poor agreement between the $^{26}Mg/^{24}Mg$ predicted and measured values, where the differences of 4.97% and 4.46% were much greater. This may have been due to an error in the initial pool size estimate used in the model or that the supplied label was not taken up by the trees. Elemental balance data suggest that the pool size estimates were accurate.[17] The reason for the discrepancy is thought to have been the presence of Mg in the sand used to grow the trees, as the presence of Mg has been noted elsewhere in acid washed sand.[3] Fortunately, the dilution effect of this extraneous Mg was insufficient to drop the measured $^{26}Mg/^{24}Mg$ value below our defined minimum ratio of 0.14036 based on the SRM-980 or 0.14111 based on the natural isotope ratios measured in the tissues.

Conclusion

Magnesium isotope analysis by TIMS is insensitive (in the range 0.2–1.2 μg Mg) to the amount of Mg loaded on the filament and, using internal normalisation, accurate isotope ratios may be obtained. In pine trees there is no evidence of natural fractionation of ^{26}Mg. Accurate K isotope ratios can be obtained using 5 μg K and a relatively long analysis period. No discernible beneficial effects were observed after conversion of the samples to an iodide salt as a means of minimising fractionation, but this may have been due to the amount of K loaded. We found no conclusive evidence for natural fractionation of ^{41}K in pine trees. The precision and accuracy offered by TIMS allows financially viable stable isotope tracer studies to be conducted in young Scots pines. Such experiments can be used to quantify uptake and importantly the remobilisation/translocation of minerals between different plant compartments. This approach will enhance the level of understanding, particularly relating to the mobility and use of key macronutrients in plants.

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