Selenium biofortification of crops on a Malawi Alfisol under conservation agriculture

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Abstract

Biofortification with selenium (Se) may rely on rapid uptake by crops, following application, to offset progressive fixation into unavailable organic forms of Se in soil. A biofortification study was conducted on an Alfisol within a long-term conservation agriculture (CA) field trial at Chitedze Research Station, Malawi. The aim was to assess the dynamics of selenium bioavailability to a staple cereal *(Zea mays*) and a range of legumes (cowpeas, groundnuts, pigeon peas and velvet beans) under CA management, as well as residual Se effects in the year following biofortification. Isotopically labelled selenate (>99% enriched $^{77}Se^{VI}$) was applied to each plot, in solution, at a rate of 20 g ha^{-1}, at maize flowering (75 days after planting), in February, 2017. Samples of grain and stover from maize and legumes, and topsoil, were collected at harvest in May, 2017 and May, 2018. Plant and soil samples were analyzed by ICP-MS for selenium isotopes (^{77}Se and ^{78}Se). The concentration of ^{77}Se in the grain of maize and single-cropped legumes exceeded 200 µg kg^{-1} in all the treatments. This would contribute approximately 56 – 64 µg day^{-1} to the Malawi diet, as refined maize flour. The fertilizer derived Se concentration ratio of maize grain-to-stover Se were >1 in 2017 but <1 in 2018; which followed the same trend as the native soil-derived Se in the residual year. In legumes the grain-to-stover concentration ratio was consistently < 1 in both years, except for the velvet beans. Differences in CA management had minimal influence on ^{77}Se concentration in plant grain but the low yield in the single conventional treatment reduced ^{77}Se uptake. Residual ^{77}Se in the soil (35 % of the applied) measured at harvest in 2017 was still present at harvest in the residual year (2018) but was completely unavailable to any of the crops. Almost none of the remaining ^{77}Se was present in soluble or phosphate-extractable forms and virtually all was present in the 'organic' (TMAH-extractable) fraction. Thus, annual Se applications to maize would be necessary to maintain concentrations which could improve dietary supply and reduce current Se deficiency in Malawi.

Highlights.

Selenium at 20 g ha^{-1} raises dietary levels above the recommended dietary allowance

Selenium fixation into humus-bound forms eliminates residual availability

Conservation agriculture treatments have little effect on selenium uptake by maize

Legumes (e.g. groundnuts) accumulate greater concentrations of selenium than maize

Keywords.

Maize, Malawi, conservation agriculture, selenium biofortification, micronutrient deficiency.

1. Introduction

Selenium (Se) is a trace element with an essential nutritional role in human and animal health. Selenium deficiency in humans has been linked to thyroid gland dysfunction, irreversible brain damage, peripheral vascular diseases, chronic and degenerative osteoarthropathy (Kashin–Beck disease), impaired immune response to viral infections, male infertility, pre-eclampsia in women, heart diseases and higher risks for several types of cancers (Cardoso et al., 2015; Fairweather-Tait et al., 2011; Riaz and Mehmood, 2012). According to the Institute of Medicine of the USA National Academy, the Se recommended dietary allowance (RDA) for Se is 55 µg day^{-1} for adults while the tolerable upper intake for adults is 400 µg day^{-1} (Bendich, 2001).

Dietary Se intake can be strongly related to the availability of Se in soil (Fairweather-Tait et al., 2011; Mehdi et al., 2013; Rayman, 2008), especially where populations depend on local food production in Se-deficient regions. The bioavailability of selenium in soil depends on supply factors, such as parent material and atmospheric inputs, and on soil factors that affect the strength of Se sorption such as pH and the concentration of soil organic matter and hydrous oxides of Fe, Al and Mn (Fordyce et al., 2000; Lopez et al., 2017; Rayman, 2008). It is recognized that selenite ions ($HSeO_3^-$, SeO_3^{2-}) are adsorbed strongly on hydrous oxides at low pH (3.5 – 6.5). However, most Se in soils is usually organically bound in humus and there is evidence that different forms of humus can give rise to differences in Se bioavailability (Qin et al., 2012). It has also been shown that soil microbial processes have some involvement in the control of inorganic Se availability (Tolu et al., 2014). In highly weathered, acidic, oxide-rich tropical soils, as found in Malawi, low bioavailable Se presents a serious restriction to dietary Se supply with estimates of 70 % of the population eating insufficient Se with an average daily intake range of 27 – 45 µg $capita^{-1}$ day^{-1} (Chilimba et al., 2011; Hurst et al., 2013; Joy et al., 2015) which is in close agreement with national plasma Se data for adult women (Phiri et al., 2019).

Dietary intake of Se can be increased through agronomic 'biofortification' of crops (Broadley and White, 2006; Chilimba et al., 2012a; Mathers et al., 2017) with application as Se-containing fertilizers applied to soil or as foliar sprays (Lopez et al., 2017). In particular, the success of agronomic biofortification of staple cereal crops is well recognized (Broadley et al., 2010; Chilimba et al., 2012a; Chilimba et al., 2014). The use of selenium-enriched fertilizers was the public-health solution successfully adopted in 1984 by Finland, that resulted in an increase in Se intake from 38 µg d^{-1}, before fortification, to 80 µg d^{-1} in 2000, assuming a daily energy intake of 10 MJ (Broadley et al., 2006; Eurola and Hietaniemi, 2000; Hartikainen, 2005).

The use of enriched Se isotopes as tracers in field experimentation permits discrimination between soil-derived and fertilizer-derived Se in crops and, potentially, allows the examination of residual effects of Se application in soil and crops. The approach has been made possible by advances in ICP-MS technology, the commercial availability of several enriched stable Se isotopes and the fact that comparatively small Se additions are required on a field plot scale (c. 1 mg m^{-2}) for realistic biofortification results. To date, there have been relatively few studies that have utilized this approach in field experiments. Chilimba et al. (2012b) studied uptake of ^{74}Se in maize in Malawi; Mathers et al. (2017) used ^{77}Se to audit the fate of Se applied to wheat in U.K. soils. More recently, Ligowe et al. (2020) used enriched ^{77}Se to characterize uptake and residual availability to green vegetables grown in an Oxisol, Alfisol and Vertisol from Malawi (Ligowe et al., 2020).

Conservation agriculture (CA) that focuses on minimum soil disturbance, the retention of crop residues and crop diversification, is one of the cropping systems which has been heavily promoted in recent years in southern Africa (Thierfelder and Wall, 2010; Thierfelder et al., 2017; Kassam et al., 2009). In addition to providing a coping strategy for climate change (Branca et al., 2011; Lipper et al., 2014; Steward et al., 2019), conservation agriculture is widely reported to improve soil health and crop yield through partly increased soil organic matter (Ligowe et al., 2017; Ngwira et al., 2012; Powlson et al., 2016).

The aim of this study was to examine the viability of Se biofortification in an Alfisol, managed under CA, and representing typical agronomic circumstances in Malawi and, more broadly, in sub-Saharan countries. We used application of ^{77}Se-enriched selenate ($^{77}Se_{Fert}$) to sub-plots of maize and selected legumes (cowpea, ground nuts, pigeon peas and velvet beans) within an established CA rotational and intercropped trial. The objectives were (i) to assess Se availability, uptake and translocation within crops and (ii) to quantify residual effects of Se application in the following season.

2. Materials and Methods

2.1. Study location

The study was carried out within a long-term CA trial situated at Chitedze Research Station (CRS), Malawi. The CRS is located on the Lilongwe-Kasungu plains (13.973 S, 33.654 E) at 1145 m above sea level. The soils are Ferruginous Latosols, classed as Alfisols under the USDA Soil Taxonomy (USDA, 1975) which are deep and free-draining with a well-developed structure. In the first year of the trial establishment (2007), baseline topsoil (0-10 cm) analysis showed the following average values for the site: pH (H_2O) = 5.2 ± 0.02, soil organic matter content = 3.3 ± 0.13 %, total C content = 2.1 ± 0.07 %, total N content = 0.17 ± 0.01 %, available P = 14.2 ± 0.6 µg g^{-1} and available K = 0.44 ± 0.03 cmol$_C$ kg^{-1} (Ligowe et al., 2017).

2.2. Conservation agriculture (CA) trial design and treatments

The CA trial was designed with ten management treatments including the conventional approach to maize cultivation (‘control’) in a randomized complete block design, with four replicates per treatment. The trial covers an approximate area of one hectare (Plate 1). The long-term CA experiment treatments are described in Table 1.

2.3. Fertilizer application

A uniform recommended fertilizer rate of 69 kg ha^{-1} N, 21 kg ha^{-1} P_2O_5 and 4 kg ha^{-1} S is applied, yearly, to all the treatments. The application comprised 23 kg ha^{-1} N, 21 kg ha^{-1} P_2O_5 and 4 kg ha^{-1} S as a basal fertilizer dressing at planting and 46 kg ha^{-1} N, in the form of urea, 21 days after planting.

2.4. Sub-plots for isotopically labelled selenate ($^{77}Se_{Fert}$) biofortification treatment

Within the middle of each plot of the CA trial, sub-plots (2 m x 2 m), were marked. Each sub-plot included 3 maize rows, with 24 maize plants, in the case of plots in which maize was the sole crop (T1, T2, T3 and T5C and T5G). For the intercropped plots (T6, T7, and T8 in Table 1) the main plot was the middle 1 m^2 of the plot, consisting of one maize row and 2 side-by-side rows of legumes. In rotational legume plots (T4C and T4G), having 27 legume plants per sub plot.

2.5. Application of $^{77}Se_{Fert}$

Isotopically enriched selenate ($^{77}Se^{VI}$) was prepared from an elemental stock of enriched $^{77}Se^{0}$ (50 mg; 99.2% atom %) from Isoflex, USA, following the method described by Mathers et al. (2017). Isotopically enriched selenate ($^{77}Se_{Fert}$) was then applied to the sub-plots at a single rate of 20 g ha^{-1} of Se, (2 mg m^{-2}), as a potassium selenate solution, 75 days after planting, at the maize crop-tasseling stage. Aliquots of the ^{77}Se stock solution containing 8 mg ^{77}Se were diluted to 500 mL with irrigation water then applied evenly to the sub-plot (4 m^2) using a watering can. After Se application, 500 mL of water were applied to each sub-plot to aid Se distribution and wash Se solution off the leaves.

2.6. Soil sampling and preparation

Soil samples were taken from all the sub-plots soon after harvest (May 2017), approximately 80 days after Se application, and again a year later (May 2018). Topsoil samples (0 – 20 cm), using an Edelman soil auger (Dow AgroSciences Limited, U.K.) were taken within the middle row of the sub-plots, leaving a discard of 0.25 m from each end of the row. Eight sampling points per plot were used, four from each side of the maize rows. Similarly, in legume plots, soil samples were taken within the two central rows, at eight sampling points within the plot. The soil samples from the eight sampling points were mixed by hand in a bucket in the field shortly after collection. Using the quartering method, a homogenized composite sample, c. 500 g, was collected from each plot. The soil samples were air-dried, under shade, in the laboratory and sieved to <2 mm.

2.7. Plant sampling and preparation

Samples of maize and legumes were collected within the sub-plot rows. Two maize plants from each side of the middle row were discarded and the four middle maize plants were harvested. Eight legume plants were sampled from the two middle rows, four plants from each row. For the intercropped legumes, four plants were sampled from each side of the middle maize row, next to where the maize plants were sampled. Maize cobs and legume pods were removed from the harvested crop samples; the maize grain was de-husked and the legume grain shelled. A composite sample from each sub-plot was dried to constant weight in an oven (Scientific Laboratory Supply Ltd) at 40 ºC. The crop stover was carefully cut into small portions (5 - 10 cm), washed using tap water and distilled water to remove soil dust and dried in perforated bags at 40 ºC. Representative sub-samples (c. 200 g) of the biomass and grain were ground to <40 µm using an ultra-centrifugal mill (Model ZM-200, Retsch, Haan, Germany).

The remaining crop residues were retained on the soil surface in the CA plots (T2 -T8) while in the conventional plot (T1), residues were removed soon after harvest. The ^{77}Se demarcated, 2 m x 2 m, sub plots were fenced to prevent residue mixture and blowing off to the treatment area.

2.8. Soil analysis

2.8.1. Total soil elemental analysis

Finely ground soil samples (~0.2 g DW) were digested with 2 mL 68 % Primar Plus™ HNO_3, 1 mL 60 % $HClO_4$ and 2.5 mL 70 % HF, (Fisher Scientific Ltd., U.K.) in PFA digestion vessels using a 48-place Teflon-coated graphite heated block digester (Model A3, Analysco Ltd, Chipping Norton, U.K.), as described in Mathers et al. (2017). Digested samples were initially diluted to 50 mL and then 1-in-10, both using Milli-Q water (18.2 MΩ cm), prior to analysis by a single-quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (Thermo-Fisher Scientific icap-Q) operating in He and H_2 cell modes. To measure 'residual' $^{77}Se_{Fert}$, the samples were analysed using a triple quadrupole ICP-MS (Thermo-Fisher Scientific icap-TQ) in O_2-cell mode.

2.8.2. 'Soluble' soil selenium - potassium nitrate (0.01 M KNO_3) extraction

Potassium nitrate extraction followed the procedure described by Chilimba et al. (2012a). Air dried < 2 mm sieved soil (4.0 g) was shaken end-over-end in 20 mL of 0.01 M KNO_3 for 2 hr. The suspension was then centrifuged for 30 minutes at 2500 g and the supernatant filtered to < 0.22 µm using a syringe filter (Millipore, Cork, Ireland). Nine mL of the filtered solution was matrix-adjusted with 1 mL of 10 % tetramethyl ammonium hydroxide (TMAH) prior to analysis for 'Soluble Se' by ICP-MS operating in H_2-cell mode.

2.8.3. 'Adsorbed' soil selenium - potassium phosphate (0.016 M KH_2PO_4) extraction

Centrifuge tubes containing the (wet) soil plug remaining after the KNO_3 extraction were weighed and 20 mL of 0.016 M KH_2PO_4 (pH 4.8) was added. The soil suspensions were shaken again for 1 hr, then centrifuged and filtered before retaining an aliquot, preserved in 1% TMAH, for Se analysis by ICP-MS in H_2-cell mode.

2.8.4. 'Organic' soil selenium – Tetramethyl ammonium hydroxide (10 % TMAH) extraction

After weighing the tubes again, the remaining soil plug was re-suspended in 10 mL of 10 % (v/v) TMAH. The tubes were heated in an oven at 70 °C, for 3 hr, and gently shaken by hand at 1.5 hr. The tubes were then centrifuged at 3500 rpm and the supernatant was filtered to < 0.22 µm using a Millex syringe driven filter unit (Millipore, Cork, Ireland). One mL of the supernatant was diluted with 9 mL Milli-Q water and analyzed by ICP-MS in H_2 cell mode.

2.9. Plant analysis

2.91. Crop (grain and stover) elemental analysis

The elemental composition of the crops was determined following microwave digestion (Multiwave 3000; Anton Paar, U.K.) of 0.2 g finely ground material in 6.0 mL 68% HNO_3 (Primar Plus™ grade; Fisher Scientific, U.K). Digested samples were initially diluted to 20 mL with Milli-Q water and with a further 1-in-10 dilution step prior to analysis by ICP-MS.

2.10. Data processing and interference correction

Raw intensity data, counts-per-second (CPS), for ^{77}Se and ^{78}Se analyzed in H_2-cell mode using single quadrupole ICP-MS were processed to correct for drift and for the formation of Se hydrides ($^{76}SeH^+$, $^{77}SeH^+$) that interfere with determination of ^{77}Se and ^{78}Se respectively, as described by Mathers et al. (2017). Triple-quadrupole ICP-MS analysis employing O_2 as a cell gas to mass-shift ^{77}Se and ^{80}Se to m/z 93 and 96 respectively was necessary to reduce interference from ^{76}GeH hydride (m/z = 77) from soil-derived germanium. In addition, a small correction for $^{76}GeOH$ (m/z 93) was also implemented based on measurement of ^{72}Ge and using a Ge standard to determine the m/z ratio (72/93).

2.11. Estimation of dietary Se intake

Dietary Se intake was estimated for maize flour and legumes assuming daily consumption of either refined (276 g capita^{-1} day^{-1}) or unrefined (249 g capita^{-1} day^{-1}) maize flour and an average legume consumption of 71 g capita^{-1} day^{-1}, derived from a legume consumption range of 55 to 87 g capita^{-1} day^{-1}, as estimated by Joy et al. (2015b) in Malawi. The product of the crop Se concentration (μg kg^{-1}) and the daily estimated flour/grain intake (kg) provides an estimated Se daily intake (μg day^{-1}).

2.12. Statistical data analysis

Analytical data for soil and plant analysis were statistically analyzed using linear mixed models fitted with the lme command in the nlme library (Piheiro et al., 2017) for the R platform (R Core Team, 2017). For analyses in which observations from the two seasons were combined, the linear mixed model was used to accommodate, in its random effects structure, correlations between observations on the same plots in successive seasons, and the correlation between subplots in the rotation treatments (where the plots in different phases of the rotation are split plots of the original design, and are not allocated independently to plots within the blocks). In the case of measurements on crop Se (legume and maize, grain and stover) there were marked differences in the variability between the 2017 and 2018 harvests, so analyses were done separately for these two seasons. The combined analysis was used for soil measurements

The effects of treatments and seasons, and their interaction, were analyzed as fixed effects. Specific orthogonal contrasts among treatments were identified a priori. These were as shown in Table 2.
Because the main effect of season has just one degree of freedom, the interaction of season and treatment can also be partitioned into single degree of freedom-contrasts, for which the null hypothesis in each case is that any contrast is the same in the two seasons.

Note that contrasts C3, C5, C6 and C7 could be used to examine differences in measurements on legume crops, corresponding specifically to (C3) a comparison between legume in monocrop (in rotation with maize) and legume in intercrop with maize, (C5) a comparison between cowpea and groundnut monocrop, (C6) a comparison between velvet bean and cowpea and pigeon pea (sown at the same time as the maize) in intercrop and (C7) a comparison between cowpea and pigeon pea both in intercrop.

3. Results and discussion

3.1. Concentration of $^{77}Se_{Fert}$ and Se_{Soil} in maize and legumes

Maize

In the year of $^{77}Se_{Fert}$ biofortification (2017) the concentrations of $^{77}Se_{Fert}$ in maize grain had a restricted range across all CA treatments (217±27 µg kg^{-1}), including the conventionally cultivated maize plots, and showed no difference between treatments (Tables 3 and 4). The results confirm the potential of fertilizer to increase Se in staple grain as has been demonstrated in previous studies (Broadley et al., 2010, 2006; Chilimba et al., 2012a; Mathers at al., 2017).

In the following year, 2018, the concentration of $^{77}Se_{Fert}$ in maize grain was much lower, 0.7 - 3 µg kg^{-1}, with the largest values recorded in the maize grown in rotation with cowpea (Table 3). Thus, the applied $^{77}Se_{Fert}$ was mainly unavailable for plant uptake beyond the single year of application (Fig. 2). Very similar results for a second crop were reported by Mathers et al. (2017) for wheat grown with applied ^{77}Se on a site in the U.K. and Chilimba et al., (2012b) for maize with ^{74}Se applied grown at two field sites in Malawi. Non-isotopically labelled studies by Stroud et al. (2010) and Gupta et al. (1993), with wheat and barley respectively, found no evidence of residual Se in crops the year following application, regardless of application rate.

In the first harvest after biofortification (2017) the concentration of $^{77}Se_{Fert}$ was greater in the crop grain than in the stover whereas, in the following year (2018) $^{77}Se_{Fert}$ concentration was greater in the crop stover (Table 3). The concentration ratio of $^{77}Se_{Fert}$ (grain:stover) was between 2 and 3 in 2017 but was <1 (0.11 - 0.94) in 2018 (EA Table 1). Therefore, the residual $^{77}Se_{Fert}$ (in 2018) was translocated less efficiently to the grain compared to the year of application (2017). This may reflect a change in speciation in inorganic Se between the two years: selenate was applied in 2017 but selenite is more likely to be the main inorganic form of $^{77}Se_{Fert}$ in 2018 given the high solubility of selenate and that the soil pH of the trial treatments was 4.2 to 6.0 (Ligowe et al., 2017). Stroud et al. (2010) noted that selenite is the dominant inorganic form under 'moderate redox conditions' and is adsorbed strongly by soil, especially at low pH. They measured the speciation of phosphate-extractable Se one year after applying selenate fertilizer and found only selenite and soluble organic-Se species (Stroud et al., 2010).

In the 2017 season there was a difference in the mean concentrations of soil-derived selenium (Se_{Soil}) in the maize grain between the mono-cropped and mix-cropped CA maize treatments (contrast C2, Table 4), and in 2018 there was a significant difference in this variable between the rotation and intercropped CA systems (C3, Table 4). However, in 2017 the concentration of (Se_{Soil}) was 39 % greater than the grain Se concentration in 2018. This might be due to the inter-seasons climatic, c. rainfall, variations. In the maize stover the concentration of Se_{Soil} differed between treatments (Table 4, C1 (both years); C3, C4, C7 (2018)) and also between years (EA Table 1). However, the Se_{Soil} grain-to-stover concentration ratios in 2017 and 2018 were approximately equal (both < 1), while the grain-to-stover ratio of $^{77}Se_{Fert}$ in 2017 ranged between 1.9 and 3.2 (AE Table 2). The timing of the $^{77}Se_{Fert}$ application to plots, at maize tasseling, may also have contributed to rapid translocation to the developing seed in 2017. Sankaran and Grusak (2014) and Sperotto (2013) suggested that continued root uptake and translocation of minerals to the flower tissues during grain filling is more important than the remobilization of previously stored minerals within the plant cells.

Legumes

The concentration of $^{77}Se_{Fert}$ in the legume grains differed significantly with cropping pattern (Table 5, contrast C3, both seasons); in the year of $^{77}Se_{Fert}$ application, single-cropped legumes had greater $^{77}Se_{Fert}$ concentrations, 400 and 711 μg kg^{-1} in cowpea and groundnuts, respectively than in legumes intercropped with maize, with an average mean $^{77}Se_{Fert}$ concentration of 78 μg kg^{-1}. The reduced $^{77}Se_{Fert}$ concentration in the intercropped legumes as compared to sole cropped, might be attributed to poor development and growth of intercropped legumes due to the shielding effect (of maize) but also damage to the legumes planted in between the maize rows, caused by manual weeding of the plots. Partitioning of $^{77}Se_{Fert}$ between the 2 crops (maize and legume) grown in close association is likely to have contributed to the low $^{77}Se_{Fert}$ concentration in the intercropped legume grain. For example, 10 % and 0.44 % of the applied $^{77}Se_{Fer}$ was taken up by maize and cowpeas, respectively, grown in association in 2017 showing the significance of the intercropped legume in recovering the applied Se. In sole cropped legumes, groundnut $^{77}Se_{Fert}$ grain concentration was 43 % more compared to the cowpea sole cropped grain in the 2017 season (Table 5, contrast 5, P=0.004). The variation of $^{77}Se_{Fert}$ concentration between these sole cropped legumes might have been attributed to the variation in maturity and hence variation in the crop development stage at the time of $^{77}Se_{Fert}$ application. The legume grain $^{77}Se_{Fert}$ in the following year after biofortification was negligible, with an average mean of not more than 1.31μg kg^{-1} suggesting the non-accessibility of the residual $^{77}Se_{Fert}$ beyond the cropping season in which it is applied.

The concentrations of Se_{Soil} in the same mono-cropped grain legumes of cowpea and groundnuts were indistinguishable (30 to 33 μg kg^{-1}, respectively), implying that the legume type and differences in maturity, did not affect the accessibility of native soil Se.

Dietary consequences

In Malawi, more than 83% of the population live in rural areas and depend on locally produced crops (Erick et al., 2009). Cereals, legumes and vegetables constitute the main dietary components (Ferguson et al., 1989; Donovan et al., 1991) for rural people not living close to Lake Malawi. In the current study, the contribution to Se dietary intake from Se_{Soil} was very small. Using values for the mean per-capita daily consumption of maize flour which is refined (*ufa oyera*; 249 g) and unrefined (*mgaiwa*; 276 g) (Joy et al. 2015a), Se intake was estimated as < 6 μg day^{-1} from both sources (EA Table 3). This is far below the minimum recommended Se intake of 40 μg day^{-1} required to prevent Se deficiency (Winkel et al., 2012). These results substantiate similar findings in a national survey of Se maize grain concentration of 22 μg kg^{-1} Chilimba et al. (2011) and an estimated Se dietary contribution of < 7 μg day^{-1} (Hurst et al., 2013) from maize grown on non-calcareous soils that cover the majority (c. 95 %) of the cultivated land in Malawi (Joy et al., 2015b). Joy et al., (2015b) suggested that food crops produced on non-calcareous soils in Malawi contribute approximately 19 μg day^{-1} to Se intake, presenting a very high risk of Se deficiency. By contrast, maize grain biofortified with Se_{Fert} at an application rate of 20 g ha^{-1}, would contribute 51 to 58 µg day^{-1} when using unrefined maize flour and 56-64 µg day^{-1} for refined maize flour in the year of Se fertilizer application. The CA maize grown in rotation with cowpea would have provided the greatest intake in both cases of maize flour processing (EA Table 3). Thus, possible dietary intakes of Se would be within the daily recommended intake range of 50 - 70 µg day^{-1} (IMFNB, 2000; WHO, 2009; Malagoli et al., 2015) and well below the chronic toxicity level of 400 µg day^{-1} (Winkel et al., 2012). The implication is that, even in combination with other dietary components from the region, biofortification of maize with Se could not lead to excessive Se intake and would provide clear nutritional benefits.

The equivalent contribution of the legumes to Se dietary intake, assuming daily consumption of 71 g of legumes, Joy et al. (2015a), are reported in EA Table 4. Single-cropped cowpea and groundnuts would provide 29 and 50 µg day^{-1}, respectively, whereas intercropped cowpea would contribute only 13 µg day^{-1}and intercropped pigeon peas and velvet beans would provide < 2 µg day^{-1}. The restrictions imposed on legumes by the intercropped maize appear to negate the viability of intercropped legumes within a Se biofortification program.

3.2. Offtake of $^{77}Se_{Fert}$ in the grain and stover of maize and legumes

Maize

The uptake of $^{77}Se_{Fert}$ was >95 % larger in 2017 than in 2018, the second season after $^{77}Se_{Fert}$ application (Fig. 1). As hypothesized, based on the crop Se concentration data, the $^{77}Se_{Fert}$ retained in the soil after the harvest of the first crop was largely in a form which was not available for plant uptake in the following year. Uptake of $^{77}Se_{Fert}$ differed between the treatment combinations (Table 4, C1 and C3, 2017; C3, 2018). The conventionally cultivated maize (T1) produced the smallest $^{77}Se_{Fert}$ uptake among all the treatments (Table 4, C1, P=0.01 in 2017) but the effect was not significant in 2018 (Table 4, C1, P=0.536 and Fig. 1). Similarly, maize grain $^{77}Se_{Fert}$ uptake was slightly greater in the CA legume rotation treatments than in the CA legume intercropped plots, contrast C3, (P=0.035 in 2017; P=0.0002 in 2018). The trend in $^{77}Se_{Fert}$ uptake in the grain was also seen in the maize stover (Table 4).

The differences between treatments in $^{77}Se_{Fert}$ uptake in maize arose mainly because of differences in yield (rather than concentration) in grain and stover (EA Table 5). Yields of maize grain in the conventional (T1) $^{77}Se_{Fert}$ sub-plots in 2017 and 2018 (2180 and 3510 kg ha^{-1} respectively) were significantly smaller than from the CA plots (T2-T8) with average yields of 7820 and 7810 kg ha^{-1} in 2017 and 2018 respectively, which drives the significantly higher offtake on the CA treatments (Table 4; C1, 2017). This difference reflects the improved soil fertility and physical characteristics in CA plots (Ligowe et al., 2017; Thierfelder and Wall, 2009). Uptake of Se_{Soil} was also less in conventional (T1) with a mean concentration of 0.032 g ha^{-1} while the CA treatments recorded a mean Se_{Soil} uptake range of 0.083 - 1.53 g ha^{-1}, hence the significance differences in C1 (Table 4). As with the $^{77}Se_{Fert}$, differences in Se_{soil} uptake were due to the depletion of soil nutrients due to yearly soil tillage and residue removal that lead to the differences in crop yield (EA Table 5).

In the year of $^{77}Se_{Fert}$ application (2017), the grain:stover ratios of $^{77}Se_{Fert}$ uptake were 1.5 in the conventional cultivation plot and an average of 3.7 in CA plots. However, in the residual year (2018) the ratios were less < 1.0 in all treatments (EA Table 6). The difference between 2017 and 2018 may reflect differences in (i) timing and concentration of available $^{77}Se_{Fert}$ within the growing season or (ii) the speciation of $^{77}Se_{Fert}$ in soil. In particular, the (residual) available $^{77}Se_{Fert}$ in 2018 was clearly much smaller and it is likely that it was present as inorganic Se^{IV} (selenite) and humus-bound Se rather than the Se^{VI} (selenate) originally applied. An additional factor may have been some unavoidable foliar application of $^{77}Se_{Fert}$ when the Se was originally sprayed on the plots – this might have contributed to the greater ratio seen for the CA plots where ground cover by the retained crop residues was greater than in conventionally cultivated maize plots. Whatever the combination of mechanisms, the data suggests that application of a large concentration of selenate at maize flowering promotes transfer into the grain. By contrast, a greater proportion of Se taken up throughout the growing season appears to remain deposited in the crop stover. The 2018 grain:stover ratios followed a trend similar to that of Se_{Soil} in that more of the native (soil-derived) Se was in the maize stover than in the maize grain (EA Table 3).

Legumes

Uptake of both $^{77}Se_{Fert}$ and Se_{soil}, by legume grain and stover, differed markedly between the two years, with negligible $^{77}Se_{Fert}$ uptake in 2018 (Fig 1). In both years, $^{77}Se_{Fert}$ uptake under single-crop rotations (T4C and T4G), was significantly greater than for intercropped legumes (Table 5; C3). As found for $^{77}Se_{Fert}$ concentrations, the uptake of $^{77}Se_{Fert}$ in intercropped plots, was hindered by the vigorous growth of maize that suppressed the root and leaf development of the intercropped plants and thereby limited $^{77}Se_{Fert}$ uptake. Among the single-crop rotation legumes (contrast C5), groundnuts grain and stover (T4G) recorded the greatest mean (2.76 and 2.34 g ha^{-1} in grain and stover, respectively) offtake in 2017 – significantly greater than cowpea (0.805 and 0.950 g ha^{-1} in grain and stover) under single-crop rotation (Fig. 1a). This may partly have arisen due to differences in maturity between the two crops. At the time of $^{77}Se_{Fert}$ application, 75 days after planting, cowpea was at the post-blossoming stage whereas groundnuts were still at the flowering stage. Cowpea matures in 60-90 days (Abadassi, 2015) and groundnuts take between 110-120 days to mature (Nautiyal et al., 2010). This might have contributed to the lower uptake of $^{77}Se_{Fert}$ in cowpea than in groundnuts. Hence the timing of $^{77}Se_{Fert}$ application in the maize legume intercropping cropping system is crucial to maximize Se uptake and translocation to edible parts of both crops.

3.3. Fractionation of $^{77}Se_{Fert}$ in soil for the application year (2017) and residual year (2018)

A sequential extraction procedure to measure 'Soluble', 'Adsorbed', 'Organic' and 'Total' concentrations of $^{77}Se_{Fert}$ and Se_{Soil} was undertaken on soil samples collected at the end of the two growing seasons: 2017 and 2018. The main aim was to assess how much of the soil-retained $^{77}Se_{Fert}$ was fixed into organic matter (TMAH-extractable) and what proportion of the $^{77}Se_{Fert}$ applied was retained in a bioavailable form (Soluble and Adsorbed) after the harvest of the biofortified crop in 2017 and after the harvest of the following crop in 2018. The $^{77}Se_{Fert}$ results are presented in Fig. 2.

3.3.1. Soluble and adsorbed $^{77}Se_{Fert}$

Soluble $^{77}Se_{Fert}$ after crop harvest was 94 %, greater in 2017 as compared to 2018 (Figs 2a and 2b; Table 6; p=<0.0001). The concentration of soluble $^{77}Se_{Fert,}$ in 2017 was similarly greater in conventional (T1), 0.357 unlike in CA treatments with a mean of 0.156 (p = 0.002). Among the CA treatments the 'groundnuts in rotation after maize' (T4 G) plots gave higher values. The greater soluble $^{77}Se_{Fert}$ in T1 and T4G suggests greater retention of the soluble $^{77}Se_{Fert}$ after the first crop.

The same trend was observed for adsorbed $^{77}Se_{Fert}$ where more was retained in the conventional and the rotation plots planted with legumes, (contrast p values of 0.03 and 0.04, for contrast C1 and contrast C3, respectively; Table 6). Generally, it was observed that both soluble and adsorbed $^{77}Se_{Fert}$ were retained to a greater extent in plots with single-cropped maize and legumes compared to rotational and intercropped treatments (Figs 2a - 2d). This might be partly attributed to differences in uptake of $^{77}Se_{Fert}$ (Fig. 1) where total uptake of $^{77}Se_{Fert}$ was greater in maize under rotation (T5C and T5G) and in intercropped plots (T6, T7 and T8) with a combined uptake by both maize and legumes, as compared to the sole maize treatments (T1, T2 and T3). The results are consistent with Chilimba et al. (2014) who investigated agronomic biofortification in intercropped systems and observed a greater recovery of Se in maize-groundnut intercropped treatments compared to single cropped plots. Additionally, these findings may suggest improved $^{77}Se_{Fert}$ efficiency use when two or more crops are grown in association, as reported by Salehi et al. (2018) for other plant nutrients (N). Thus, the reduced soluble and adsorbed $^{77}Se_{Fert}$ in the maize grown after cowpea and groundnuts rotation (T5C and T5G) plots is consistent with greater uptake of the applied $^{77}Se_{Fert}$ (Fig. 1), facilitated by greater yields of both maize grain and stover as a result of improved soil fertility (EA Table 5).

Over the course of the residual year (after harvest 2017 until harvest 2018), there was a loss of soluble and adsorbed $^{77}Se_{Fert}$, (Figs 2a - 2d) representing average percentage reductions of 93 % and 18 % respectively. This was not due to uptake by the crops, because concentrations of $^{77}Se_{Fert}$ in the crops from 2018 were very small. It was also not due to leaching or volatilization because there was very little loss of 'total' or 'organic' $^{77}Se_{Fert}$ between harvests in 2017 and 2018 (Figs 2e – 2h). The losses of soluble and adsorbed $^{77}Se_{Fert}$ therefore, must have been due to a change in Se fractionation in the soil – essentially conversion into organic forms.

Correlation between Adsorbed and Soluble soil $^{77}Se_{Fert}$ following harvest.

The relationship (slope) between adsorbed and soluble $^{77}Se_{Fert}$ (Fig. 3) is effectively a dimensionless distribution coefficient (kd) which reflects the strength of adsorption of 'available' Se in the soil. At harvest in 2017 the kd value was 0.89 and showed remarkable consistency across all the treatments despite values of soluble $^{77}Se_{Fert}$ varying by an order of magnitude (Fig. 3). However, in 2018 the available Se appeared to be nearly 7 times more strongly adsorbed with a kd value of 6.8 (Fig. 3). The relationships shown in Fig. 3 indicates that in 2018 the soluble $^{77}Se_{Fert}$ was very low and restricted to between 0.00 to 0.025 µg kg^{-1}, suggesting occupancy of more strongly adsorbing sites. Comparing the two trends in Fig. 3 (data from 2017 and 2018), there does appear to have been a systematic increase in the strength of available $^{77}Se_{Fert}$ adsorption during the residual year.

3.3.2. Total (HF digest) and Organic (TMAH extractable) $^{77}Se_{Fert}$

The organic and total $^{77}Se_{Fert}$ data are shown in Fig. 2e - 2h. There were no statistically significant differences in the two fractions between treatments and across the two years (Table 6). A strong correlation between organic and total $^{77}Se_{Fert}$ was seen for both years, with a slope of 0.99 (Fig. 4). The data strongly suggest that the $^{77}Se_{Fert}$ that remains in the soil is in an organic (humus-bound) form rather than, for example, occluded within an intractable mineral phase. TMAH (pH c. 12 at 10%) would also solubilize selenite bound to Fe oxide surfaces so such a conclusion relies on complete solubilization of oxide-bound Se by the phosphate extraction (Adsorbed Se) prior to TMAH extraction. Previously, measurement of ^{77}Se in an HF soil digest would be problematic due to (for example) interference from germanium hydride (^{76}GeH) (Mathers et al, 2017). The similarity of the TMAH-extracted Se and HF-digested therefore validates the use of oxygen mass shifting with a triple-quadrupole ICP-MS as a preferred technique.

Soil-derived $^{77}Se_{Soil}$ was determined on acid digestions of the soil as a check on possible systematic errors in the assay between the two harvest years; the values in 2017 and 2018 would not be expected to change. However, there was a small significant difference, < 7 %, between the two years ($p<0.001$) (Fig. 5; Table 5). It is likely that this arose due to a small systematic error in the year-on-year sampling or analysis and probably reflects the difficulty in maintaining the quality of analysis rather than a real process.

Whilst 35 % of the Se fertilizer is stored in a residual fraction in the soil before the following year's crop, the accumulation of fertilizer Se to the existing pool of soil Se is small. At an application rate of 20 g ha^{-1} only 0.0072 mg kg^{-1} of fertilizer Se would be retained annually, in addition to the natural total soil Se concentration of 0.332 mg kg^{-1} at this site. Taking a soil toxicity level of 20 mg kg^{-1} (Walsh and Fleming, 1952), yearly application of fertilizer Se would take > 3000 years to reach this concentration

4. Conclusions

A single application of ^{77}Se to crops grown either under CA or conventional cropping systems, at the grain filling stage, provides a viable approach to Se biofortification. Application of 20 g ha^{-1} Se produced sufficient grain Se enrichment in maize and legumes to provide the recommended dietary Se requirement. The additional organic inputs to the soil through CA cropping systems has no apparent influence on the rapid fixation of applied ^{77}Se. The transformation of applied selenate was almost entirely into humus-bound forms which completely negates any residual agronomic value of the applied Se despite its retention in the soil. Within the CA trial, low yield in the conventional maize cultivation treatment, caused by loss of soil fertility due to crop residue removal, reduced the recovery of applied Se uptake compared to the higher-yielding CA treatments. In particular the greatest overall uptake occurred in a maize-legume rotation cropping system. By contrast, the concentration and uptake of Se by intercropped legumes was affected by poor crop and root development as a consequence of competition for resources by the tall maize crop and possibly disturbances during manual weeding.

Given that applied Se remaining in the soil is not available for plant uptake beyond the growing season in which it is applied, seasonal application of Se would be required in order to continuously maintain recommended dietary Se intake. This could be achieved through adoption of Se fertilizer amendments to existing fertilizers. Further work should investigate (i) the speciation of residual Se to assess the effects of long-term Se biofortification and accumulation in the soil and (ii) the effects of timing (of application) on plant uptake and, particularly, translocation to the edible crop grain.

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List of Tables

Table 1. Treatment descriptions of the Chitedze long-term Conservation Agriculture trial.

Treatment	Description
T1	Check plot: using traditional farmers' practice using a hand hoe (ridge and furrow system); maize is the sole crop and no crop residues are returned to the soil
T2	Basin: maize planted as the sole crop in basins with dimensions of 0.15 x 0.15 x 0.15m, that are re-constructed yearly; crop residues are retained.
T3	Direct Seeding: maize as a sole crop, planted with a dibble stick; crop residues are retained.
T4C	Crop rotation: direct seeding, with a dibble stick, of the rotation sequence 'cowpea–maize–cowpea'; crop residues are retained; split plot with T4G.
T4G	Crop rotation: direct seeding, with a dibble stick, of the rotation sequence 'groundnut–maize–groundnut'; crop residues are retained; split plot with T4C.
T5C	Crop rotation: direct seeding, with a dibble stick, of the rotation sequence 'maize-cowpea-maize' (in contrast to T4C); crop residues are retained; split plot with T5G.
T5G	Crop rotation: direct seeding, with a dibble stick, of the rotation sequence maize-groundnut-maize (in contrast to T4G); crop residues are retained; split plot with T5C.
T6	Intercropping: direct seeding, with a dibble stick, of maize intercropped with pigeon pea; crop residues are retained.
T7	Intercropping: direct seeding, with a dibble stick, of maize intercropped with cowpea; crop residues are retained
T8	Intercropping: direct seeding, with a dibble stick, of maize intercropped with velvet beans at 8 weeks; crop residues are retained

Table 2: List of treatment contrasts

Contrast number	Contrast code	Contrast Description
1	C1	Check plots - conventional cultivation (T1) compared with the mean for all CA treatments (T2-T8) - an assessment of the general impact of CA practices.
2	C2	Mono-cropped maize compared (T2 and T3) with mixed-cropped maize (T4C, T4G, T5C, T5G, T6, T7 and T8) - an assessment of the impacts of diversification by rotation or intercropping.
3	C3	Maize under legume rotation (T5C and T5G) compared with maize intercropped with legumes (T6, T7, and T8)
4	C4	Maize planted in basins (T2) compared with monocrop maize planted directly into residues with a dibble stick (T3).
5	C5	A comparison between maize rotation with cowpea (T5C) and with groundnut (T5G).
6	C6	A comparison between intercropping with a late-sown legume -velvet bean (T8) and intercropping with cowpea or pigeon pea-sown with the maize (T6 and T7)
7	C7	A comparison between the cowpea (T7) and pigeon pea intercrop (T8)

Table 3: Concentration of $^{77}Se_{Fert}$ ($\mu g\ kg^{-1}$) in the crops (maize and legume, stover and grain) in 2017 and 2018. Treatments are fully described in Table 1 and include: T1 = conventional cultivation; T2 = CA basins; T3 = CA sole maize; T4C = CA rotation cowpea after maize; T4G = CA rotation groundnuts after maize; T5C = CA rotation maize after cowpea; T5G = CA rotation maize after groundnuts; T6 = CA intercropped: maize + Pigeon peas; T7 CA intercropped: maize + cowpeas T8 = CA intercropped: maize + velvet beans.

Crop part	**Maize Grain $^{77}Se_{Fert}$ ($\mu g\ kg^{-1}$)**		**Maize Stover $^{77}Se_{Fert}$ ($\mu g\ kg^{-1}$)**		**Legume Grain $^{77}Se_{Fert}$ ($\mu g\ kg^{-1}$)**		**Legume Stover $^{77}Se_{Fert}$ ($\mu g\ kg^{-1}$)**	
Treatment	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
T1	209	1.89	111	5.17				
T2	210	1.24	65.5	1.32				
T3	203	0.77	108	2.77				
4C		2.16		10.5	404		305	
4G		2.78		6.83	711		427	
5C	232		119			2.49		4.08
5G	228		102			1.32		7.93
T6	226	1.11	96.4	4.36	16.5	1.37	11.6	0.46
T7	223	0.72	72.9	0.85	188	0.79	199	2.92
T8	206	1.35	67.3	2.20	28.4	0.54	52.0	0.27
SE±	**33.6**	**0.45**	**18.8**	**2.75**	**60.6**	**0.48**	**45.5**	**0.52**

Table 4: Statistical contrasts of maize grain and stover $^{77}Se_{Fert}$ and Se_{soil} concentrations and plant uptake. Contrasts (C) numbers are described in Table 2.

		P-values for Maize Grain				P-values for Maize Stover			
Contrast number	Denominator DF	$^{77}Se_{Fert}$ Concentration	$^{77}Se_{Fert}$ Uptake	Se_{Soil} Concentration	Se_{Soil} Uptake	$^{77}Se_{Fert}$ Concentration	$^{77}Se_{Fert}$ Uptake	Se_{Soil} Concentration	Se_{Soil} Uptake
2017									
C1	21	**0.790**	**<0.0001**	**0.584**	**0.0002**	**0.308**	**0.010**	**0.006**	**0.043**
C2	21	**0.572**	**0.057**	**0.013**	**0.412**	**0.772**	**0.196**	**0.101**	**0.555**
C3	21	**0.718**	**0.035**	**0.201**	**0.009**	**0.078**	**0.005**	**0.448**	**0.058**
C4	21	**0.882**	**0.589**	**0.206**	**0.489**	**0.121**	**0.214**	**0.985**	**0.937**
C5	21	**0.942**	**0.918**	**0.972**	**0.957**	**0.533**	**0.398**	**0.941**	**0.964**
C6	21	**0.665**	**0.787**	**0.741**	**0.834**	**0.459**	**0.311**	**0.717**	**0.392**
C7	21	**0.944**	**0.316**	**0.775**	**0.368**	**0.384**	**0.197**	**0.272**	**0.181**
2018									
C1	21	**0.370**	**0.085**	**0.741**	**0.129**	**0.718**	**0.536**	**0.013**	**0.050**
C2	21	**0.112**	**0.032**	**0.238**	**0.109**	**0.226**	**0.217**	**0.069**	**0.408**
C3	21	**0.002**	**0.0002**	**0.010**	**0.003**	**0.021**	**0.019**	**0.039**	**0.0007**
C4	21	**0.459**	**0.431**	**0.609**	**0.642**	**0.713**	**0.805**	**0.021**	**0.031**
C5	21	**0.337**	**0.274**	**0.347**	**0.307**	**0.352**	**0.340**	**0.281**	**0.195**
C6	21	**0.433**	**0.360**	**0.036**	**0.042**	**0.929**	**0.903**	**0.733**	**0.970**
C7	21	**0.540**	**0.502**	**0.667**	**0.698**	**0.351**	**0.458**	**0.037**	**0.022**

Table 5: Statistical contrasts for legume grain and stover $^{77}Se_{Fert}$ and Se_{soil} concentration and plant uptake. The contrasts are: C3 = legume in monocrop (in rotation with maize) and legume in intercrop with maize, C5 cowpea and groundnut monocrop in rotation with maize, C6 = velvet bean and cowpea and pigeon pea (sown at the same time as the maize) in intercrop and C7 = comparison between cowpea and pigeon pea both in intercrop (see Table 2).

		P-values for Legume Grain				P-values for Legume Stover			
Contrast number	Denominator DF	$^{77}Se_{Fert}$ Concentration	$^{77}Se_{Fert}$ Uptake	Se_{soil} Concentration	Se_{soil} Uptake	$^{77}Se_{Fert}$ Concentration	$^{77}Se_{Fert}$ Uptake	Se_{soil} Concentration	Se_{soil} Uptake
2017									
C3	12	**<0.0001**	**<0.0001**	**0.367**	**0.157**	**<0.0001**	**<0.0001**	**0.506**	**<0.0001**
C5	12	**0.004**	**0.0001**	**0.478**	**0.0006**	**0.080**	**<0.0001**	**0.375**	**0.067**
C6	12	**0.338**	**0.670**	**0.007**	**<0.0001**	**0.359**	**0.810**	**0.755**	**0.025**
C7	12	**0.068**	**0.985**	**0.045**	**0.0005**	**0.013**	**0.952**	**0.187**	**0.001**
2018									
C3	12	**0.042**	**0.008**	**0.012**	**0.042**	**<0.0001**	**<0.001**	**0.039**	**<0.0001**
C5	12	**0.113**	**0.763**	**0.814**	**0.142**	**0.0002**	**<0.001**	**0.169**	**0.001**
C6	12	**0.380**	**0.685**	**0.004**	**<0.0001**	**0.045**	**0.696**	**0.249**	**0.060**
C7	12	**0.415**	**0.091**	**0.150**	**0.010**	**0.006**	**0.701**	**0.227**	**0.004**

Table 6: Statistical contrasts for the soil $^{77}Se_{Fert}$ fractionation (Soluble, Adsorbed, Total) and Se_{Soil} for both year (2017 and 2018). Contrasts are: C1= Check plot (conventional) vs all CA treatment; C2 = Mono-cropped maize vs mixed-cropped maize; C3 = Maize under legume rotation vs maize in legume intercrop; C4 = Basin planted maize vs dibble stick planted maize; C5 = Maize under cowpea rotation vs maize under groundnut rotation; C6 = Maize after groundnuts in rotation vs groundnuts after maize rotation; C7 = Maize after cowpea in rotation vs cowpea after maize rotation; C8 = Maize velvet bean intercropped (late sown) vs maize cowpea or pigeon peas intercropped; C9 = Maize cowpea intercropped vs maize pigeon peas intercropped (see Table 2).

Contrast number	Denominator DF	P-values for Soluble $^{77}Se_{Fert}$	P-values for Adsorbed $^{77}Se_{Fert}$	P-values for Total TMAH $^{77}Se_{Fert}$	P-values for Total HF $^{77}Se_{Fert}$	P-values for Se_{Soil}
C1	22	0.016	0.027	0.070	0.065	0.318
C2	22	0.4081	0.631	0.707	0.532	0.942
C3	22	0.034	0.041	0.831	0.219	0.001
C4	22	0.894	0.074	0.143	0.749	0.913
C5	7	0.411	0.082	0.662	0.864	0.856
C6	28	0.001	0.003	0.513	0.351	0.256
C7	28	0.08	0.086	0.511	0.24	0.388
C8	22	0.618	0.32	0.885	0.202	0.077
C9	22	0.986	0.712	0.311	0.329	0.557
Year (Yr)	28	<0.0001	0.05	0.115	0.856	<0.0001
C1 x Yr	28	0.004	0.013	0.068	0.177	0.387
C2 x Yr	28	0.268	0.723	0.442	0.639	0.773
C3 x Yr	28	0.006	0.114	0.79	0.745	0.297
C4 x Yr	28	0.9	0.91	0.976	0.992	0.457
C5 x Yr	28	0.469	0.91	0.458	0.417	0.38
C6 x Yr	28	0.041	0.003	0.407	0.238	0.033
C7 x Yr	28	0.147	0.036	0.714	0.621	0.236
C8 x Yr	28	0.548	0.039	0.582	0.059	0.121
C9 xYr	28	0.828	0.097	0.01	0.146	0.891

Plates.

Plate 1: Aerial photograph of the long-term conservation agriculture trial (c. 1 ha) at Chitedze, Malawi (February 2017).

List of Figures.

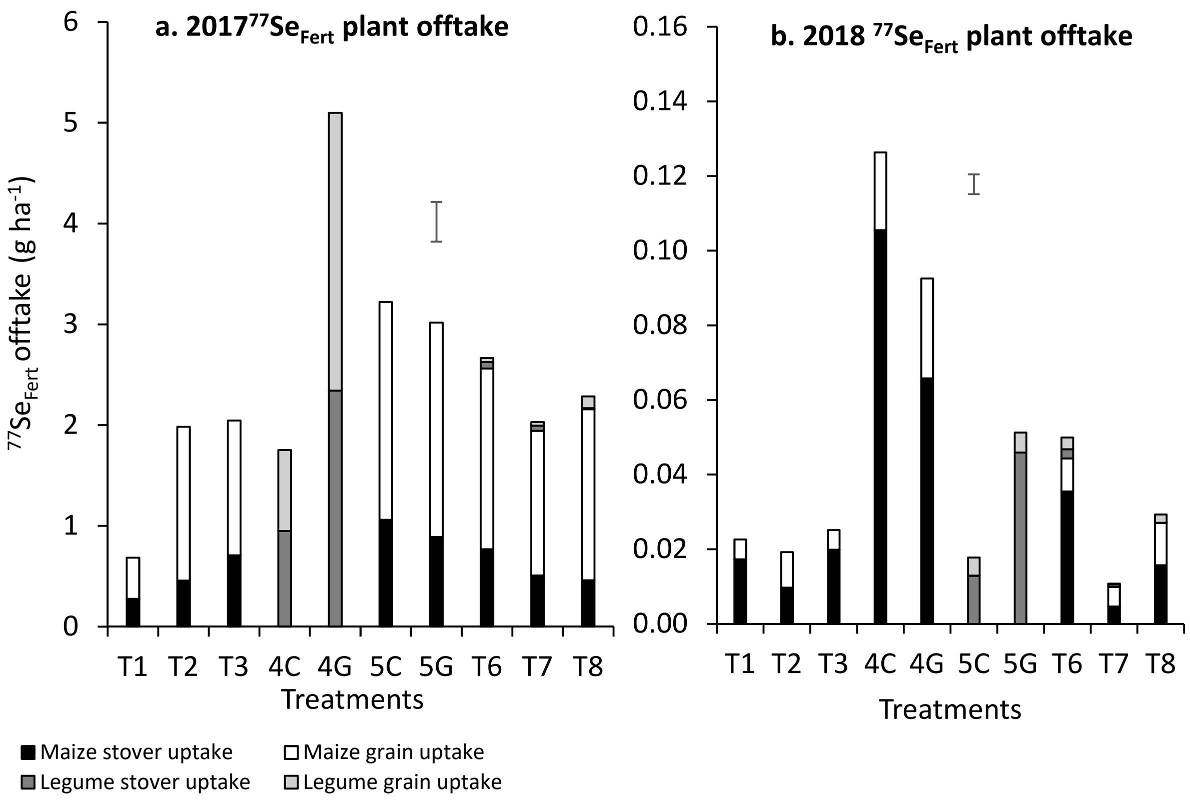
Figure 1: Offtake of $^{77}Se_{Fert}$ (g ha^{-1}) in maize and legume stover and grain in 2017 and 2018. Treatments included: T1 = conventional cultivation; T2 = CA basins; T3 = CA sole maize; T4C = CA cowpea rotation after maize; T4G = CA groundnuts rotation after maize; T5C = CA maize rotation after cowpea; T5G = CA maize rotation after groundnuts; T6 = CA intercropped maize + pigeon peas; T7 CA intercropped maize + cowpeas; T8 = CA intercropped maize + velvet beans. Note the change in Y-axis scale from 2017 to 2018. Error bars = ±SEM.

Figure 2: Soil fractions of fertilizer-derived ^{77}Se ($^{77}Se_{Fert}$) in 2017 and 2018, including: 'Soluble' (a, b), 'Adsorbed' (c, d), 'Organic' (e, f) and 'Total' (g, h). Note the difference in y-axis scales for a-d and e-h. Values presented are the average of four replicate plots; error bars are ± standard error of means. Treatments include: T1 = conventional; T2 = CA basins; T3 = CA sole maize; T4C = CA rotation cowpea after maize; T4G = CA rotation groundnuts after maize; T5C = CA rotation maize after cowpea; T5G = CA rotation maize after groundnuts; T6 = CA intercrop: maize + Pigeon peas; T7 CA intercrop: maize + cowpeas T8 = CA intercrop: maize + velvet beans. Error bar = ±SEM.

Figure 3: Correlation between Adsorbed and Soluble soil $^{77}Se_{Fert}$ at harvest of the bio-fortified crop in 2017 (open circle; slope = 0.89) and in 2018 (closed circle; slope = 6.8).

Figure 4: Correlation between total Organic and Total soil $^{77}Se_{Fert}$ at harvest of the bio-fortified crop in 2017 (open circle; slope = 0.99) and in the following year, 2018 (closed circle; slope = 1.15). The line represents a 1:1 relationship.

Figure 5: Soil-derived ^{77}Se in 2017 and 2018. Error bars = ±SE for 4 replicates



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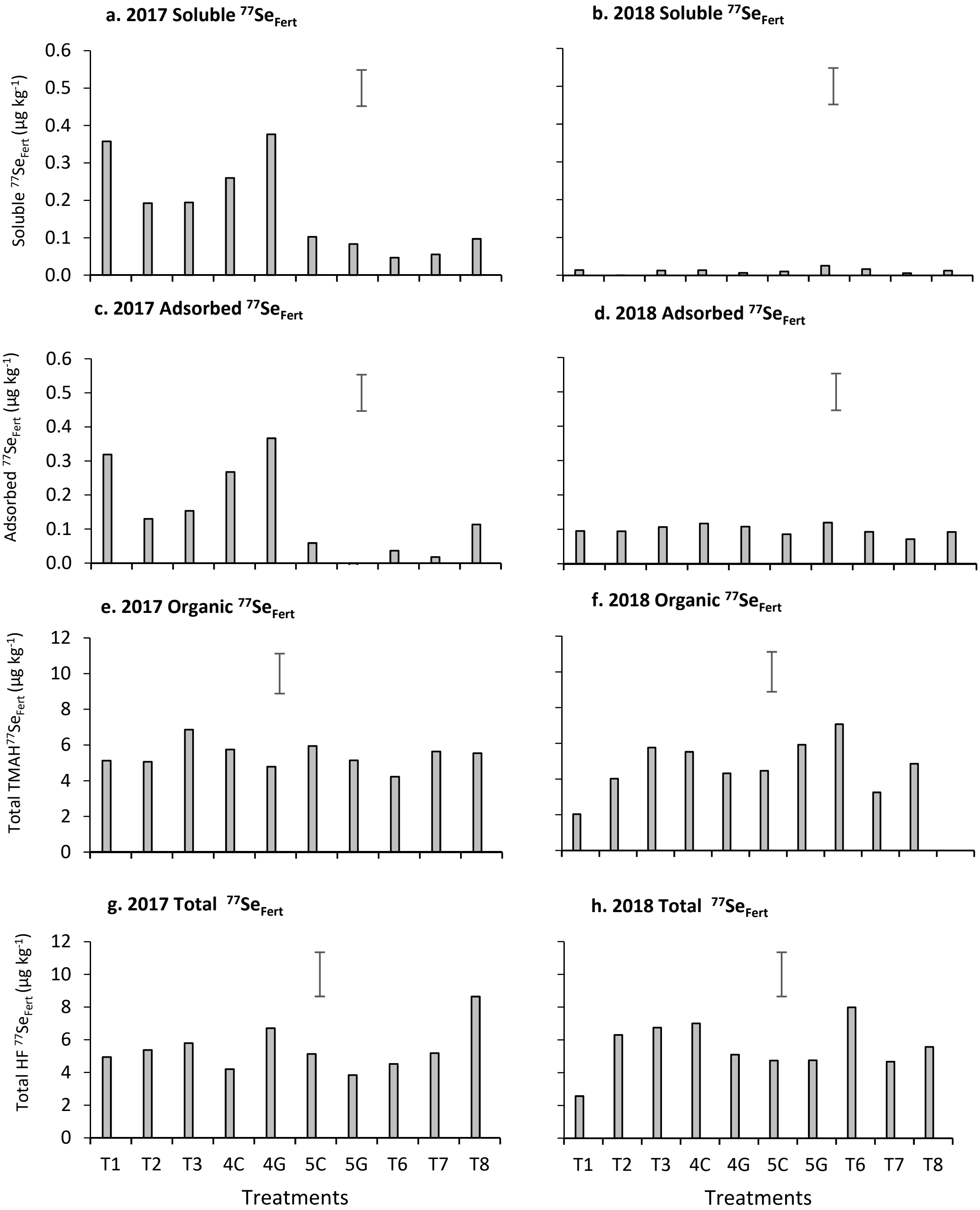

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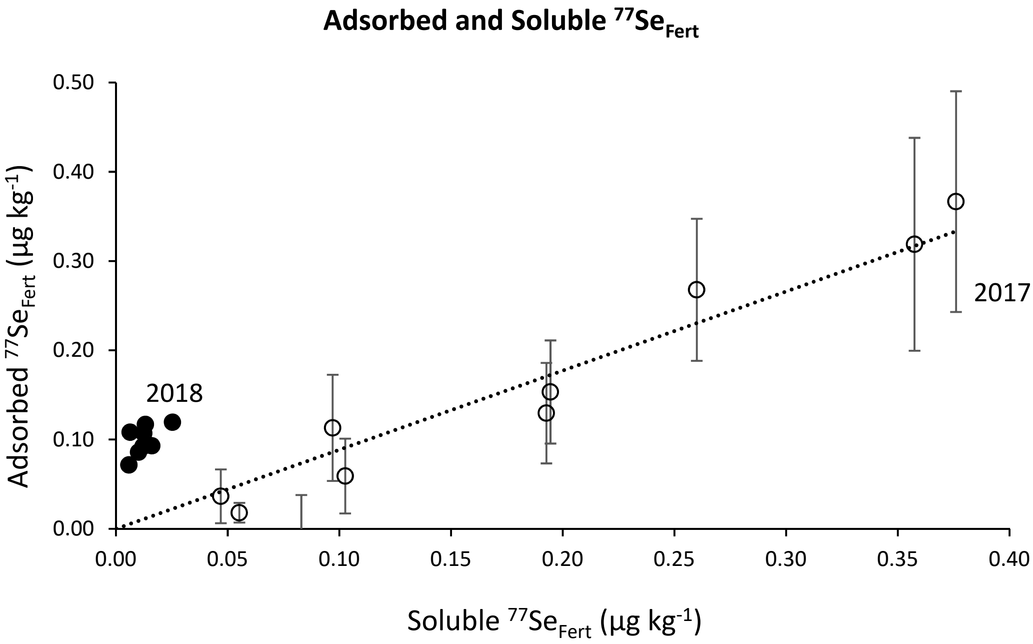

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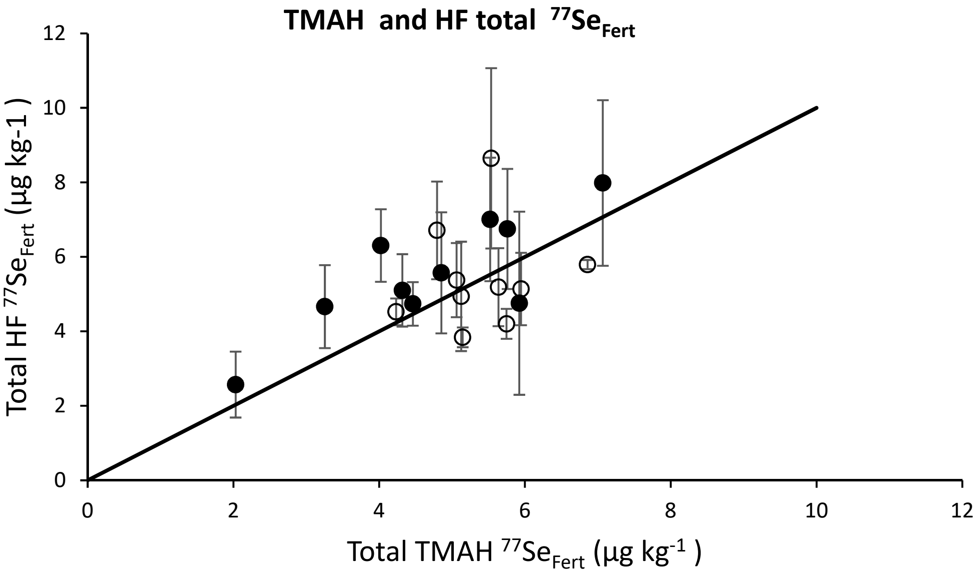

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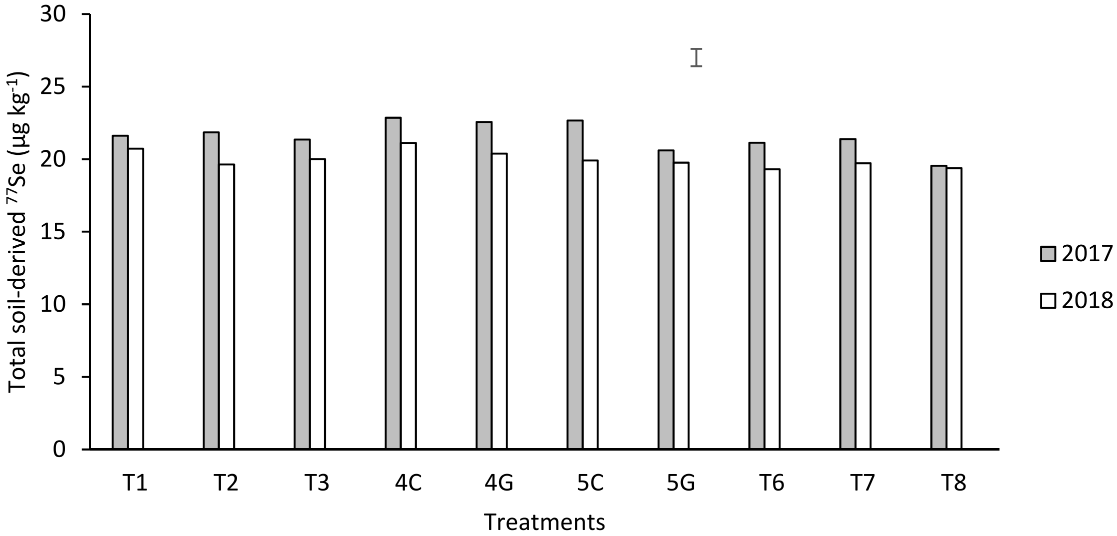

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