

Using chemical fractionation and speciation to describe uptake of technetium, iodine and selenium by *Agrostis capillaris* and *Lolium perenne*.

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1 **Abstract**

2 To understand the dynamic mechanisms governing soil-to-plant transfer of selenium (Se),
3 technetium-99 (^{99}Tc) and iodine (I), a pot experiment was undertaken using 30 contrasting soils after
4 spiking with ^{77}Se , ^{99}Tc and ^{129}I , and incubating for 2.5 years. Two grass species (*Agrostis capillaris* and
5 *Lolium perenne*) were grown under controlled conditions for 4 months with 3 cuts at approximately
6 monthly intervals. Native (soil-derived) ^{78}Se and ^{127}I , as well as spiked ^{77}Se , ^{99}Tc and ^{129}I , were assayed
7 in soil and plants by ICP-MS. The grasses exhibited similar behaviour with respect to uptake of all
8 three elements. The greatest uptake observed was for ^{99}Tc , followed by ^{77}Se , with least uptake of ^{129}I ,
9 reflecting the transformations and interactions with soil of the three isotopes. Unlike soil-derived Se
10 and I, the available pools of ^{77}Se , ^{99}Tc and ^{129}I were substantially depleted by plant uptake across the
11 three cuts with lower concentrations observed in plant tissues in each subsequent cut. Comparison
12 between total plant offtake and various soil species suggested that $^{77}\text{SeO}_4^{2-}$, $^{99}\text{TcO}_4^-$ and $^{129}\text{IO}_3^-$, in
13 soluble and adsorbed fractions were the most likely plant-available species. A greater ratio of $^{127}\text{I}/^{129}\text{I}$
14 in the soil solid phase compared to the solution phase confirmed incomplete mixing of spiked ^{129}I
15 with native ^{127}I in the soil, despite the extended incubation period, leading to poor buffering of the
16 spiked available pools. Compared to traditional expressions of soil-plant transfer factor (TF_{total}), a
17 transfer factor ($\text{TF}_{\text{available}}$) expressed using *volumetric* concentrations of speciated 'available' fractions
18 of each element showed little variation with soil properties.

19 **Keywords:** Selenium; Technetium; Iodine; Bioavailability; Enriched stable isotope; Soil-to-plant
20 transfer.

21 **Highlights:**

- 22 - Grass rapidly takes up the oxyanions pertechnetate, iodate and selenate from soil
- 23 - Extraction with KNO_3 and KH_2PO_4 provides an estimate of available soil Tc, Se and I
- 24 - Soil pH and organic carbon largely control fixation and speciation of Tc, Se and I
- 25 - Transfer factors are consistent using speciation of available Tc, Se and I oxyanions

26 **Introduction**

27 Selenium (Se) and iodine (I) are essential micronutrients for human beings and animals, with critical
28 roles in antioxidant defence and immune functioning. Overt Se deficiency has been associated with
29 dilated cardiomyopathy (Keshan disease; Ge and Yang, 1993), osteoarthropathy (Kashin-Beck disease;
30 Guo et al., 2014) and cretinism (coupled with I deficiency; Vanderpas et al., 1990); more marginal
31 deficiencies may contribute to reduced immune function, cancers, cardiovascular disease, thyroid
32 disease, diabetes, and viral diseases (Rayman, 2012; Navarro-Alarcon et al., 2008). Iodine deficiency
33 diseases (IDDs), have multiple adverse effects on growth and development in children, and can result
34 in goitre in adults due to inadequate thyroid hormone levels (Zimmermann et al., 2008). Combined
35 Se and I deficiencies may exacerbate the effect of individual deficiencies considering their collective
36 roles in antioxidation and maintenance of thyroid function (Schomburg et al., 2008; Brauer et al.,
37 2006). Recommendations for adult daily Se and I intake are 55-75 $\mu\text{g d}^{-1}$ and 150 $\mu\text{g d}^{-1}$ respectively
38 (Stoffaneller et al., 2015; WHO, 2007); however people living in regions where there is limited
39 availability of Se and I from dietary sources are often unable to meet these requirements.
40 Biofortification of staple food crops through the addition of enriched fertilizers (Se), irrigation water
41 (I) or foliar sprays (Se and I) is therefore increasingly being employed with the aim of increasing
42 dietary intake (e.g. Alfthan et al., 2011; Broadley et al., 2010; Jiang et al., 1997; Mathers et al., 2017).

43 The isotopes ^{79}Se , ^{99}Tc and ^{129}I are also important fission products that can be released as a
44 consequence of severe nuclear accidents and radioactive waste disposal. With long half-lives and a
45 significant likelihood of mobilization in the geosphere they have the potential to contribute
46 significantly to long-term radiation exposure and risk (IAEA, 2012; Icenhower et al., 2010; NDA, 2010;
47 Shi et al., 2012). Therefore, for both deliberate applications of stable Se and I and accidental release
48 from nuclear facilities of radioactive isotopes of Se, Tc and I there is a need to increase our
49 understanding of the Se, I and Tc soil-to-plant transfer processes, particularly how added Se, I and Tc
50 behave in soil-plant systems.

51 Shetaya et al. (2012) observed that ^{129}I added to soil was rapidly transformed from inorganic to
52 organic forms in both soil solid and solution phases, with iodide being fixed more rapidly than iodate.
53 Di Tullo et al. (2016) obtained similar results for Se in a 2-year incubation experiment, observing that
54 redistribution among soil solid phases controlled Se fate in soils leading to stronger retention. Se and
55 I sorption is greatest in soils with high organic carbon content, high Fe/Mn oxide content and low pH
56 (Shetaya et al., 2012; Chilimba et al., 2011). By contrast, Tc is expected to be mobile in the surface
57 soil environment as pertechnetate (TcO_4^-), the most stable form under aerobic conditions (Tagami et
58 al., 2005; Druteikiene et al., 2014). Pertechnetate is highly soluble in water, binds very weakly to most
59 sediment and soil mineral phases, and is highly available to plants (Begg et al., 2007; Li et al., 2014).
60 All of these studies indicate that partitioning of Se, Tc and I between soil phases controls their mobility
61 and bioavailability, thereby regulating soil-to-plant transfer. Several studies have investigated
62 transfer of Se, ^{99}Tc and I from soils to plants (e.g. Wang et al., 2017; Sakizadeh et al., 2016; Weng et
63 al., 2009; Bennett et al., 2003). However, most studies focus on the distribution of these elements in
64 the soil and various parts of the plant, with limited attention directed towards the behaviour and
65 bioavailability of added Se, ^{99}Tc and I. This information is essential for the prediction of Se and I fate
66 in agronomic biofortification, and for the management of radioactive waste and areas contaminated
67 by nuclear accidents.

68 To express bioavailability of trace elements and radionuclides and their relative accumulation in
69 plants, transfer factors (TF) are often defined as the ratio of the concentration of an element in a
70 plant to its concentration in the soil. Several studies have calculated TF values for Se, ^{99}Tc and I for a
71 range of soil-plant systems (e.g. Uchida et al., 2011; Sakizadeh et al., 2016; Tagami et al., 2005).
72 Uptake differs from one plant species to another, between elements and soil types. This makes it
73 difficult to predict plant available Se, ^{99}Tc and I since the number of reliable TF datasets for these
74 elements remains limited. Development of a 'universal' TF value for Se, ^{99}Tc and I that is applicable

75 to a wide range of soil types would significantly improve prediction of their concentrations in plants
76 and estimates of radiation dose to humans.

77 The aim of the current study was to investigate soil-to-plant transfer of Se, ⁹⁹Tc and I in 30 soils of
78 contrasting properties and land use that had been collected and incubated in a moist, aerobic
79 condition for 2.5 years after spiking with ⁷⁷Se, ⁹⁹Tc and ¹²⁹I. The plant availability of the added ⁷⁷Se,
80 ⁹⁹Tc and ¹²⁹I was subsequently established in a pot experiment. The objectives of the study were: (i)
81 to investigate the plant availability of added ⁷⁷Se, ⁹⁹Tc and ¹²⁹I after 2.5 years incubation in soils and
82 to compare this to the availability of native Se and I and (ii) to identify the chemical *species* of ⁷⁷Se,
83 ⁹⁹Tc and ¹²⁹I that appears to drive plant uptake and may thereby provide 'universal' TF values for each
84 element.

85 **Materials and methods**

86 **Soil sampling and incubation**

87 Twenty topsoil samples (0-15 cm) were collected from a range of sites in the UK. A further ten soils
88 were sampled from relatively uncontaminated locations within the Chernobyl Exclusion Zone,
89 Ukraine, as part of a study examining the kinetics of radioisotope kinetics in soils (see Table 1 with
90 soils, locations and main soil properties). Sampling sites were selected to cover a broad range of
91 relevant soil characteristics including pH, texture, organic matter content, land use and parent
92 material. When possible, soils from the same parent material and texture but under different land
93 uses were collected (e.g. arable, grassland and woodland). Following collection, field moist soil was
94 homogenised, spread in trays and air dried at room temperature until just dry enough to be sieved to
95 < 4 mm. The soils were not allowed to dry completely so as to maintain microbial activity. After sieving,
96 soils were stored at 4 °C, prior to use, to preserve their remaining moisture content without allowing
97 anaerobic conditions to develop.

98 The moisture content of the partially dried soils (< 4 mm) was adjusted by gently mixing each soil in
99 a food processor and adding Milli-Q water. No attempt was made to apply a prescribed water content
100 or adjust the soil to a fixed water potential. Instead the primary objective was to achieve a friable,
101 aerobic but moist consistency so that the soil could be mixed and sub-sampled without forming clods.
102 The amount of water added to each soil was recorded. The soils were pre-incubated at 10 °C for 2-3
103 days prior to addition of the isotopes to avoid the short-lived flush of microbial activity which occurs
104 when dry soils are moistened. After this period, a portion of ca. 250 g of each moist soil was removed
105 and transferred to 0.5 L Duran bottles as a control. The remaining soil was spiked with a small volume
106 (c. 3 mL) of isotope stock solution to provide the equivalent of 100 µg kg⁻¹ of ⁷⁷Se as selenate, 108 µg
107 kg⁻¹ of ⁹⁹Tc as pertechnetate, and 201 µg kg⁻¹ of ¹²⁹I as iodate whilst the samples were mechanically
108 stirred for 4 minutes to ensure uniform mixing. Details of the spiking solutions and their preparation
109 is given in Table A in the EA. The spiked soil was distributed across three Duran bottles of 1 L capacity
110 providing individual microcosms. The bottles had one hole (c. 3 mm diameter) in the lid to allow gas
111 exchange and prevent reducing conditions whilst avoiding excess water loss during incubation. The
112 weights of each microcosm (ca. 500 g moist soil) were recorded so the dry weight equivalent of
113 periodic subsamples taken for fractionation and speciation analysis could be determined and the
114 moisture content monitored gravimetrically throughout the experiment. The spiked and control
115 microcosms were incubated in darkness at 10.0 ± 1.0 °C for 2.5 yr following which the soils were
116 potted up and sown with grass.

117 **Soil characterization**

118 Soil pH was determined after shaking 10 g of sieved soil in 25 mL 0.01 M CaCl₂ for 30 minutes. Total
119 carbon contents were determined on finely ground samples using a FLASH EA1121 CNS analyser.
120 Organic carbon was determined using a Shimadzu TOC-VCPH analyser after acidification of the soils
121 with HCl to pH 2-3 to liberate inorganic carbon. The remaining organic carbon was determined as
122 CO₂ after heating to 720°C in the presence of a platinum coated alumina catalyst using a non-

123 dispersive infra-red detector. Total free iron oxides in soils were extracted by shaking ca. 0.25 g finely
124 ground soil with 20 mL of 0.3 M Na-citrate in 1 M NaHCO₃ and 0.07 M Na-dithionite for 24 h in a 20 °C
125 water bath followed by centrifugation and filtering to < 0.22 µm. Estimates of amorphous and poorly
126 crystalline oxides in the soil were obtained following extraction in 0.2 M ammonium oxalate and 0.125
127 M oxalic acid and shaking in darkness for 2 h, following a method adapted from (Schwertmann, 1973).
128 All filtered solutions (< 0.2 µm) were then acidified to 2% HNO₃ and diluted 1-in-100 before analysis
129 by inductively coupled plasma mass spectrometry (ICP-MS; Model iCAP-Q; Thermo Fisher Scientific,
130 Bremen, Germany).

131 **Fractionation and speciation of I, Se and Tc in soil after incubation**

132 Fractionation and speciation of Tc, I and Se was determined for each soil prior to the pot trial using
133 sequential extraction with (i) 0.01 M KNO₃, (ii) 0.016 M KH₂PO₄ and (iii) 10% tetra methyl ammonium
134 hydroxide (TMAH), to determine Se, ⁹⁹Tc and I fractions operationally designated as (i) 'soluble', (ii)
135 'adsorbed' and (iii) 'organic' (Table 2). Soil equivalent to 4 g DW (± 0.01 g) was equilibrated with 20
136 mL 0.01 M KNO₃ in 50 mL polypropylene centrifuge tubes. After shaking end-over-end for 2 h, soil
137 solutions were centrifuged at 3500 rpm for 30 min and filtered using a < 0.22 µm Millex syringe filter
138 unit into a universal tube. This extraction was intended to mimic soil pore water composition and
139 provide estimates of soluble Se, ⁹⁹Tc and I. The remaining soil plug was then dispersed in 20 mL 0.016
140 M KH₂PO₄ shaking end-over-end for 1 h, and the sampling procedure repeated. The third step
141 involved heating the remaining soil with 10 mL of 10% TMAH at 90 °C for 14 h to dissolve humic and
142 fulvic acids and so solubilise organically bound I, Se and ⁹⁹Tc. The suspensions were again centrifuged
143 and a 1 mL aliquot of the supernatant was further diluted (1-in-10) to give a final concentration of 1%
144 TMAH for analysis. The supernatant solutions from KNO₃ and KH₂PO₄ extractions were dispensed (1.5
145 mL) into HPLC vials for immediate speciation analysis by ICP-MS operating in hydrogen cell mode. An
146 additional 4.8 mL of the supernatant from both extractions was combined with 0.2 mL of 50% HNO₃
147 for total ⁷⁷Se, ⁷⁸Se and ⁹⁹Tc analysis and a further 4.8 mL combined with 0.2 mL of 25% v/v TMAH for

148 total ^{127}I and ^{129}I analysis. The samples were weighed between extractions to account for carry-over
149 of analytes.

150 **Pot trial with *Agrostis capillaris* and *Lolium perenne***

151 After 900 days (c. 2.5 yr) incubation, a pot trial was carried out, using the incubated soils (30 soils \times 3
152 replicates) to assess the bioavailability of ^{77}Se , ^{99}Tc and ^{129}I . Two grass species, *Agrostis capillaris* (AC)
153 and *Lolium perenne* (LP), which are adaptive to a wide range of soil types and common on nutrient-
154 poor soils in the UK, were selected as the experimental plants. Incubated soils were gently packed
155 into 6.5 cm diameter pots (6 cm deep) with filter papers in the base. Seeds (c. 0.5 g) were spread
156 evenly on the surface of the soil, covered with perlite and watered with 20 mL water to assist
157 germination. All the pots (a total of 180) were then placed in a shallow tray and randomized within
158 three blocks in a constant temperature glasshouse (15 °C). Water was applied twice a day to maintain
159 the water content of the pots slightly below field capacity throughout the duration of the experiment.
160 In addition, 10 mL of KNO_3 solution was applied to all pots at a rate equivalent to 100 kg N ha^{-1} 1 week
161 after germination and following each cut.

162 **Sample collection and processing**

163 After four weeks of growth, the first batch of samples was harvested by cutting the grasses at
164 approximately 1 cm above the surface of soil. Samples were transferred to paper bags and wet weight
165 recorded before being dried in an oven at 40 °C for 4 days to achieve a constant weight. After
166 recording the dry weight, each sample was then chopped into <2 mm pieces with stainless steel
167 scissors, and stored prior to chemical extraction. This process was repeated for two subsequent
168 harvests at approximately four week intervals. Irrigation water was also sampled for ^{127}I analysis.

169 **Determination of total I, Se and Tc in plant tissue**

170 Total ^{77}Se , ^{78}Se , ^{99}Tc ^{127}I and ^{129}I concentrations in grass were determined following extraction with 5%
171 TMAH. TMAH has been shown to extract quantitatively the I and over 90% of total Se content from
172 plant material, thus it can provide a reasonable estimate of Se and I in plant samples (Watts and
173 Mitchell, 2009; Bowley et al., 2017). Approximately 0.3 g (\pm 0.1 g) of oven-dried, chopped grass was
174 suspended in 5 mL of 5% TMAH in pressurised PFA vessels and extracted via microwave heating
175 (Model Multiwave PRO fitted with a 24-place rotor; Anton Paar). Digested samples were diluted to
176 25 mL with Milli-Q water and transferred to 50 mL centrifuge tubes. After centrifuging at 3000 rpm
177 for 30 min, 10 mL of the supernatant was pipetted into ICP sample tubes and stored at 4 °C before
178 analysis.

179 **ICP-MS analysis and data processing**

180 Determination of total selenium (^{77}Se and ^{78}Se), ^{99}Tc and iodine (^{127}I and ^{129}I) concentrations in the
181 soil solutions, grass extracts, and irrigation water was undertaken by ICP-MS operating in hydrogen
182 cell mode for Se, kinetic energy discrimination (KED; He gas) mode for ^{99}Tc and standard mode for I.
183 An internal standard solution containing 5 $\mu\text{g L}^{-1}$ ^{185}Re in a 1% TMAH matrix with 4% methanol was
184 used to correct for instrumental drift. Samples were introduced from an autosampler (Cetac ASX-520)
185 incorporating an ASXpressTM rapid uptake module. The instrument was externally calibrated with all
186 five isotopes (^{77}Se , ^{78}Se , ^{99}Tc , ^{127}I , ^{129}I). For Se analysis, discrimination between soil- and spike-derived
187 ^{77}Se , and elimination of errors due to mass bias and hydride generation were dealt with as described
188 in detail by Mathers et al., (2017).

189 Speciation of KNO_3 and KH_2PO_4 soil fractions (soluble and adsorbed fractions) was undertaken
190 following in-line chromatographic separation using a Dionex ICS-3000 fitted with a 50 mm length PRP-
191 X100 Hamilton anion exchange column (250 \times 4.6 mm; 5 μm particle size). The mobile phase
192 consisted of an isocratic flow of 1.5 mL min^{-1} of a 0.05 M NH_4ClO_4 solution for ^{99}Tc and 0.05 M NH_4NO_3 ,

193 1×10^{-5} M $(\text{NH}_4)_4\text{EDTA}$, 2% v/v methanol adjusted to pH 9.4 with TRIS base for I and Se. Sample
194 processing was undertaken using QtegraTM software (Thermo-Fisher Scientific) with manual
195 integration of peaks. Instrumental drift was addressed by analysing calibration standards after every
196 12 samples and applying a linear drift correction.

197 **Statistical analysis**

198 All statistical analyses (*T* test and Spearman correlation analysis) were performed in SPSS (version
199 23.0). Coefficients of variation (CV) were calculated for the concentrations of ⁷⁷Se, ⁹⁹Tc and ¹²⁹I in
200 grass grown in the different soils.

201 **Results and discussion**

202 **Soil characteristics**

203 Selected properties of the soils are presented in Table 1. The soils covered a wide range of pH (3.32-
204 8.00), organic carbon concentrations (0.2-42.6%), and Fe, Mn and Al oxide concentrations (0.01-22.7
205 g kg^{-1} , 0.001-3.53 g kg^{-1} and 0.14-5.99 g kg^{-1} , respectively). The fractionation and speciation of ⁷⁷Se,
206 ⁹⁹Tc and ¹²⁹I in soil fractions immediately prior to setting up the pot experiment are given in Table B
207 (Electronic Annex). Soil weights and grass yields are given in Table C (Electronic Annex).

208 **Distribution of Se, Tc and I in grass**

209 Decreasing concentrations of ⁷⁷Se, ⁹⁹Tc and ¹²⁹I in the shoot tissues across the three harvests, due to
210 rapid uptake of selenate, pertechnetate and iodate, was observed for both grass types (AC and LP,
211 Fig. 1) but the declining trend across the three cuts was less pronounced for ¹²⁹I (1st-2nd cut: $t_{AC} =$
212 2.724 , $P_{AC} = 0.008$; $t_{LP} = 2.258$, $P_{LP} = 0.026$; 2nd-3rd cut: $t_{AC} = -0.294$, $P_{AC} = 0.769$; $t_{LP} = 5.605$, $P_{LP} = 0.000$;
213 $N = 90$). Concentrations of ⁷⁷Se, ⁹⁹Tc and ¹²⁹I in the shoots varied greatly between soils for the first cut
214 (⁷⁷Se, $CV_{AC} = 1.203$, $CV_{LP} = 1.439$; ⁹⁹Tc, $CV_{AC} = 1.070$, $CV_{LP} = 1.810$; ¹²⁹I, $CV_{AC} = 2.063$, $CV_{LP} = 2.296$). The
215 concentrations of ⁷⁷Se, ⁹⁹Tc and ¹²⁹I in the two grass species were comparable (⁷⁷Se, $t = 0.879$, $P =$

216 0.381; ^{99}Tc , $t = -0.633$, $P = 0.528$; ^{129}I , $t = 1.241$, $P = 0.216$; $N = 90$) and followed the same order: $^{99}\text{Tc} >$
217 $^{77}\text{Se} > ^{129}\text{I}$. After three cuts, the average proportions of total ^{77}Se , ^{99}Tc and ^{129}I transferred to *Agrostis*
218 *capillaris*, respectively, were $13.8 \pm 8.5\%$, $39.3 \pm 16.2\%$ and $0.2 \pm 0.3\%$; equivalent values for *Lolium*
219 *perenne* were $16.7 \pm 12.2\%$, $53.8 \pm 30.0\%$ and $0.2 \pm 0.2\%$. For soil-derived Se and ^{127}I , there was broad
220 consistency across the first two cuts, suggesting that the supply of soil- and irrigation-derived Se and
221 I remained constant throughout the pot trial.

222 **Factors controlling plant uptake of ^{77}Se , ^{99}Tc and ^{129}I from soil**

223 The total offtake ($\mu\text{g pot}^{-1}$) of spiked ^{77}Se , ^{99}Tc and ^{129}I , after three cuts of grass was compared with
224 the total amount ($\mu\text{g pot}^{-1}$) of each element in the various soil fractions: (i) soluble inorganic species
225 (selenate, iodate and pertechnetate), (ii) total soluble, (iii) total soluble + adsorbed and (iv) total in
226 the soil (Fig. 2). Fig. 2 suggests that the mobility and bioavailability of ^{99}Tc was the greatest, followed
227 by ^{77}Se and ^{129}I . For ^{77}Se all plots showed a clear trend but fell below the 1:1 line suggesting that the
228 supply of ^{77}Se , exceeded plant uptake capacity, although this appears to contradict the observation
229 in Fig. 1 of a decline in Se uptake with subsequent cuts. Use of the 'soluble + adsorbed' fraction to
230 represent plant available Se seemed to provide the most coherent trend, suggesting a role for both
231 immediately available Se and re-supply from the adsorbed fraction. For the plots of soluble $^{99}\text{TcO}_4^-$,
232 total soluble ^{99}Tc and 'soluble + adsorbed' ^{99}Tc there was close to a 1:1 relation between offtake and
233 soil- ^{99}Tc . This reflects (i) the dominance of soluble $^{99}\text{TcO}_4^-$ as the main form of soil ^{99}Tc outwith fixed
234 organic forms and (ii) the highly bioavailable nature of the pertechnetate anion, leading to almost
235 complete depletion of reserves by both grasses over three harvests. In the case of ^{129}I , soluble *iodate*
236 fell close to a 1:1 relation with offtake, suggesting $^{129}\text{IO}_3^-$ was the most likely plant-available species.
237 For total soluble ^{129}I the trend fell well below the 1:1 line, possibly implying that soluble organic ^{129}I
238 was not available to plants. For the 'soluble + adsorbed' fraction and 'total soil ^{129}I ' the trend deviated
239 further from the 1:1 trend, highlighting the extent to which ^{129}I had been fixed within soil organic
240 pools in the latter case. It is also worth noting that a trend for uptake as a function of *total* ^{77}Se , ^{129}I

241 and ^{99}Tc was only evident because of expressing concentration on a *volumetric* (rather than
242 gravimetric) basis and thereby compensating for the differences in density of the potted soil;
243 expressing total concentration on a gravimetric basis would produce a single value on the X-axis for
244 all soils.

245 **Ratios of $^{127}\text{I}/^{129}\text{I}$ in soil and plant**

246 The ratio of $^{127}\text{I}/^{129}\text{I}$ in the soluble fraction was comparable to that in the adsorbed fraction (Fig. 3a),
247 confirming isotopic equilibrium between the two fractions. However, a consistently greater ratio was
248 observed in the organic iodine fraction (TMAH extractable) (Fig. 3b), indicating the existence of an
249 inaccessible fraction (of ^{127}I) in soil humus, into which ^{129}I had not yet been assimilated even after 900
250 days of contact. When compared with $^{127}\text{I}/^{129}\text{I}$ in the grasses by the time of the first cut, a slightly
251 higher ratio was observed when compared to $^{127}\text{I}/^{129}\text{I}$ in the soluble fraction (Figs 3c and 3d). This
252 probably reflects a substantial contribution of ^{127}I applied in irrigation water ($2.3 \mu\text{g L}^{-1}$ of ^{127}I) during
253 the pot trial. There were no other sources of wet or dry deposition of I during the growing of the grass.
254 Thus, the trends shown in Figs 3e and 3f, scattered around a 1:1 line, seem to suggest a broad
255 relationship between the isotope ratio in the grass and in the organic phase is simply the fortuitous
256 effect of two opposing factors. The intractable ^{127}I pool within the organic fraction raises the $^{127}\text{I}/^{129}\text{I}$
257 ratio in humus whereas the additional source of ^{127}I in irrigation water raises the ratio in the plant.

258 **Factors influencing the ^{129}I , ^{77}Se and ^{99}Tc soil-to-plant transfer**

259 The soil-to-plant transfer factors of ^{129}I , ^{77}Se and ^{99}Tc were calculated based on their total contents
260 and speciated available fractions respectively, using Eq. 1 and 2.

$$261 \quad TF_{total} = \frac{X_{GC}}{X_S} \quad (1)$$

$$262 \quad TF_{Avail} = \frac{X_{GC} \times V}{e^{[\ln X_0 - (\ln X_0 - \ln X_{GO})/2]}} \quad (2)$$

263 Where, X denotes ^{129}I , ^{77}Se or ^{99}Tc ; X_{GC} and X_{GO} are the concentration ($\mu\text{g kg}^{-1}$) and offtake ($\mu\text{g pot}^{-1}$)
264 of the spiked isotope (X) in grass at the first cut; X_{S} is the total concentration of the spiked X isotope
265 in soil ($201 \mu\text{g kg}^{-1}$ of ^{129}I , $100 \mu\text{g kg}^{-1}$ of ^{77}Se and $108 \mu\text{g kg}^{-1}$ of ^{99}Tc); X_0 is the initial content of soluble
266 and adsorbed inorganic species ($^{129}\text{IO}_3^-$, $^{77}\text{SeO}_4^{2-}$ and $^{99}\text{TcO}_4^-$) in the soil ($\mu\text{g pot}^{-1}$); V is the volume of
267 the pot occupied by the soil (cm^3). The denominator in Eq. 2 allows for depletion during the pot trial,
268 assuming an exponential decline. Fig. 4 shows the relationships between soil properties and soil-to-
269 plant transfer factors expressed in these two different ways.

270 When using the traditional approach based on total ^{77}Se ^{99}Tc , and ^{129}I contents in soil, TF_{total} values
271 varied greatly with soil properties. As shown in Fig. 4a, soil factors controlling availability to grasses
272 were similar for all three isotopes. The two grasses broadly presented coherent trends, with stronger
273 correlations in *Agrostis capillaris*. Highly significant negative correlations were observed between
274 organic carbon contents and TF_{total} values in both *Agrostis capillaris* (^{77}Se , $r = -0.625$; ^{99}Tc , $r = -0.801$;
275 ^{129}I , $r = -0.729$; $P < 0.01$, $N = 90$) and *Lolium perenne* (^{99}Tc , $r = -0.300$; ^{129}I , $r = -0.396$; $P < 0.01$, $N = 90$),
276 with the exception of ^{77}Se in *Lolium perenne* (^{77}Se , $r = -0.181$, $P = 0.088$, $N = 90$). Increasing organic
277 carbon contents, along with Fe, Mn and Al oxide contents, would all be expected to increase the
278 retention of ^{129}I , ^{77}Se and ^{99}Tc in soil through stronger binding, thus leading to reduced availability to
279 plants (Shetaya et al., 2012; Di Tullo et al., 2016). No significant correlation was observed between
280 soil pH and TF_{total} except for the ^{99}Tc TF_{total} in *Agrostis capillaris* ($r = 0.421$, $P = 0.000$, $N = 90$). Generally,
281 soil characteristics affect soil-to-plant transfer by changing the solid-solution equilibrium of different
282 species in soil and the kinetics of processes that lead to fixation (Kabata-Pendias, 2004). The great
283 variation in values of TF_{total} can be primarily ascribed to those soil characteristics (e.g. pH and organic
284 carbon, Shetaya et al., 2012) that strongly affect the solubility, speciation and fixation of ^{77}Se and ^{99}Tc
285 and ^{129}I .

286 When calculating soil-to-plant transfer factors based on soil available (soluble and adsorbed)
287 inorganic ^{129}I , ^{77}Se and ^{99}Tc species, the variation in TF_{Avail} across soils substantially decreased. As
288 shown in Figure 4b, TF_{Avail} of all three isotopes to both grasses was very conservative when correlated
289 with soil properties, suggesting that the soil-to-plant transfer of soluble inorganic anions was largely
290 independent of soil properties. No apparently coherent correlations were observed with TF_{Avail} and
291 organic carbon content. This might be because TF_{Avail} was defined using a volumetric rather than
292 gravimetric concentrations of speciated available fractions, which would effectively remove the effect
293 of soil properties associated with the density of the potted soil on the solubility and mobility of the
294 three elements. Similar results also applied to their correlations with Fe, Mn and Al oxide contents.
295 However, other soil factors may affect this transfer process leading to residual variation in TF_{Avail} .
296 Significant correlations were observed between soil pH and TF_{Avail} of ^{77}Se , ^{99}Tc and ^{129}I , in both grasses
297 (^{77}Se , $r_{AC} = -0.431$, $r_{LP} = -0.612$; ^{99}Tc , $r_{AC} = -0.409$, $r_{LP} = -0.602$; ^{129}I , $r_{AC} = -0.531$, $r_{LP} = -0.594$; $P < 0.01$, N
298 $= 90$), indicating that soil pH still had a major effect on the adsorption and conversion of inorganic ^{129}I ,
299 ^{77}Se and ^{99}Tc .

300 Comparison with TF_{total} , suggests that values of TF_{Avail} , with small variations, may provide a better
301 prediction of plant availability. By using the most likely plant-available species based on a volumetric
302 concentration, TF_{Avail} eliminated the influence of soil characteristics on the availability of all three
303 elements to a great extent. Thus, a single 'universal' TF value that applies to all soils can be estimated
304 that may be helpful for developing transfer models to predict the uptake of I, Se and Tc by plants
305 independently of the soil characteristics that control solubility. However, it should be acknowledged
306 that the practical difficulty in implementing this approach, in contexts such as spatial risk assessment,
307 then becomes prediction of the soil pore water concentrations of the inorganic oxyanions of I, Se and
308 Tc. This contrasts with the traditional approach using TF_{total} (e.g. Fv values; IAEA, 2009 and Gil-García
309 et al. 2009) which utilizes relatively robust estimates of total soil concentration of trace nutrients and

310 radionuclides but has to accommodate the influences of contact time and soil properties on
311 bioavailability by broadly classifying soils based on texture, organic matter content pH etc.

312 **Conclusions**

313 A better understanding of the added Se, ^{99}Tc and I behaviour in the soil-to-plant transfer is essential
314 for managing both soil fertilization or biofortification, and radioisotope contamination. This study
315 illustrates the complex behaviour of Se, ^{99}Tc and I soil-to-plant transfer by undertaking a pot trial with
316 two grass species and incubated spiked soils that covered a wide range of soil properties and land
317 use. In general, after 2.5 years of incubation, mobility and bioavailability followed the order $^{99}\text{Tc} >$
318 $^{77}\text{Se} > ^{129}\text{I}$. Unlike soil-derived I and Se, the available pools provided by the spiked ^{77}Se , and ^{129}I , were
319 strongly depleted by plants, as a result of incomplete mixing of the spiked isotopes with the native
320 soil pools and $^{77}\text{SeO}_4^{2-}$, $^{99}\text{TcO}_4^-$ and $^{129}\text{IO}_3^-$ in soluble and adsorbed fractions were identified as the
321 most important plant available species. Soil characteristics had a significant effect on transfer factors
322 calculated using total ^{129}I , ^{77}Se and ^{99}Tc but when volumetric concentrations of speciated available
323 fractions were used values of TF_{Avail} were shown to be largely independent of soil conditions and
324 properties. This may provide an alternative approach to more traditional descriptions of transfer
325 factors used in spatially co-ordinated risk assessment models.

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Table 1 Summary of soil properties

Soil	Code	pH	Org C (%)	Fe ₂ O ₃ (g/kg)	MnO ₂ (g/kg)	Al(OH) ₃ (g/kg)	Moisture* (%)
Meadow 1 subsoil	M1-S	7.19	0.2	0.36	0.054	0.24	1
Meadow 1 topsoil	M1-T	6.41	0.2	0.76	0.176	0.26	3
Meadow 2 subsoil	M2-S	5.06	2.6	11.5	0.050	1.11	10
Meadow 2 topsoil	M2-T	5.12	1.4	3.65	0.042	0.40	6
Forest 1 subsoil	F1-S	3.52	13.2	0.08	0.002	0.14	3
Forest 1 topsoil	F1-T	3.32	0.7	0.48	0.003	0.66	11
Forest 2 subsoil	F2-S	3.57	23.5	4.47	0.008	5.99	30
Forest 2 topsoil	F2-T	3.42	42.6	6.71	0.013	3.21	41
Forest 3 subsoil	F3-S	4.86	0.3	1.23	0.088	0.77	2
Forest 3 topsoil	F3-T	4.52	1.2	1.40	0.240	0.77	5
Budby Common moorland	BC-M	4.18	5.5	7.85	0.022	0.88	15
Breedon on the Hill grassland	BH-G	7.36	6.2	14.6	3.525	1.49	20
Breedon on the Hill woodland	BH-W	7.47	7.5	10.5	1.535	0.88	16
Bardsey woodland	BY-W	3.41	10.6	14.3	0.090	1.35	21
Cottam Arable	CO-A	6.37	2.3	9.37	0.356	0.63	9
Derbyshire grassland	DY-G	3.90	11.4	5.56	0.036	0.86	31
Derbyshire moorland	DY-M	3.46	38.6	3.94	0.024	2.43	36
Evesham arable	EV-A	6.04	2.6	14.2	0.736	1.24	12
Fladbury grassland	FD-G	6.19	5.7	18.4	0.679	1.63	24
Ives Head woodland	IH-W	3.88	9.5	9.50	0.095	1.60	20
Newport arable	NP-A	6.76	1.7	7.53	0.331	0.64	9
Plantation Edge woodland	PE-W	3.82	7.1	4.72	0.055	0.67	12
Sutton Bonington grassland	SB-G	6.02	5.0	10.1	0.331	1.18	15
Stoke Rochford arable	SR-A	7.77	3.8	12.4	0.364	1.33	9
Stoke Rochford grassland	SR-G	7.04	5.7	22.7	0.666	1.99	22
Stoke Rochford woodland	SR-W	8.00	5.2	0.01	0.001	1.81	13
Ticknal grassland	TK-G	5.32	6.3	11.5	0.165	2.04	20
Wick arable	WK-A	5.31	2.4	9.27	0.414	0.76	13
Wick woodland	WK-W	3.87	24.4	5.78	0.198	1.37	37
Worcester arable	WS-A	7.71	2.6	7.17	0.431	0.60	14

* Moisture content during incubation

Table 2:

Fraction	Reagent	Measurements
Soluble	0.01 M KNO ₃	<ul style="list-style-type: none">• Total soluble Se, Tc, I• Soluble selenate, soluble selenite, and soluble organic Se by difference (i.e. subtracting the sum of the soluble selenite and soluble selenite from the total soluble Se)• Soluble iodate, soluble iodide, and soluble organic I by difference (i.e. subtracting the sum of the soluble iodate and soluble iodide from the total soluble I)
Adsorbed	0.016 M KH ₂ PO ₄	<ul style="list-style-type: none">• Total adsorbed Se, Tc and I• Adsorbed selenate, adsorbed selenite, and adsorbed organic Se by difference (i.e subtracting the sum of the adsorbed selenite and adsorbed selenite from the total adsorbed Se)• Adsorbed iodate, adsorbed iodide and adsorbed organic I by difference (i.e. subtracting the sum of the adsorbed iodate and adsorbed iodide from the total adsorbed I)
Organic	10% TMAH	<ul style="list-style-type: none">• Total organically bound Se, Tc and I• Speciation was not possible as TMAH at pH > 12 can change the speciation in this fraction

Figures

Fig. 1. Box and whisker plots showing the concentrations of ^{77}Se , ^{99}Tc and ^{129}I on a fresh weight basis in shoot tissues of *Agrostis capillaris* and *Lolium perenne* across the three cuts. Selenium concentrations were corrected for soil ^{77}Se contributions. The arithmetic mean (\oplus) is also shown.

Fig. 2. Relationship between ^{129}I , ^{77}Se , ^{99}Tc offtake in plants and that in soil ($\mu\text{g pot}^{-1}$). Error bars show the average standard error of three replicates

Fig. 3. Ratios of $^{127}\text{I}/^{129}\text{I}$ in soil and plant. The dashed line represents the 1:1 relationship.

Fig. 4. Relationships between (a) $\log(\text{TF}_{\text{Total}})$, (b) $\log(\text{TF}_{\text{Avail}})$, and soil properties. Data are shown for *Agrostis capillaris* (\blacktriangle ; solid regression line) and *Lolium perenne* (\circ ; dashed regression line). Error bars show the average standard error of three replicate analyses.

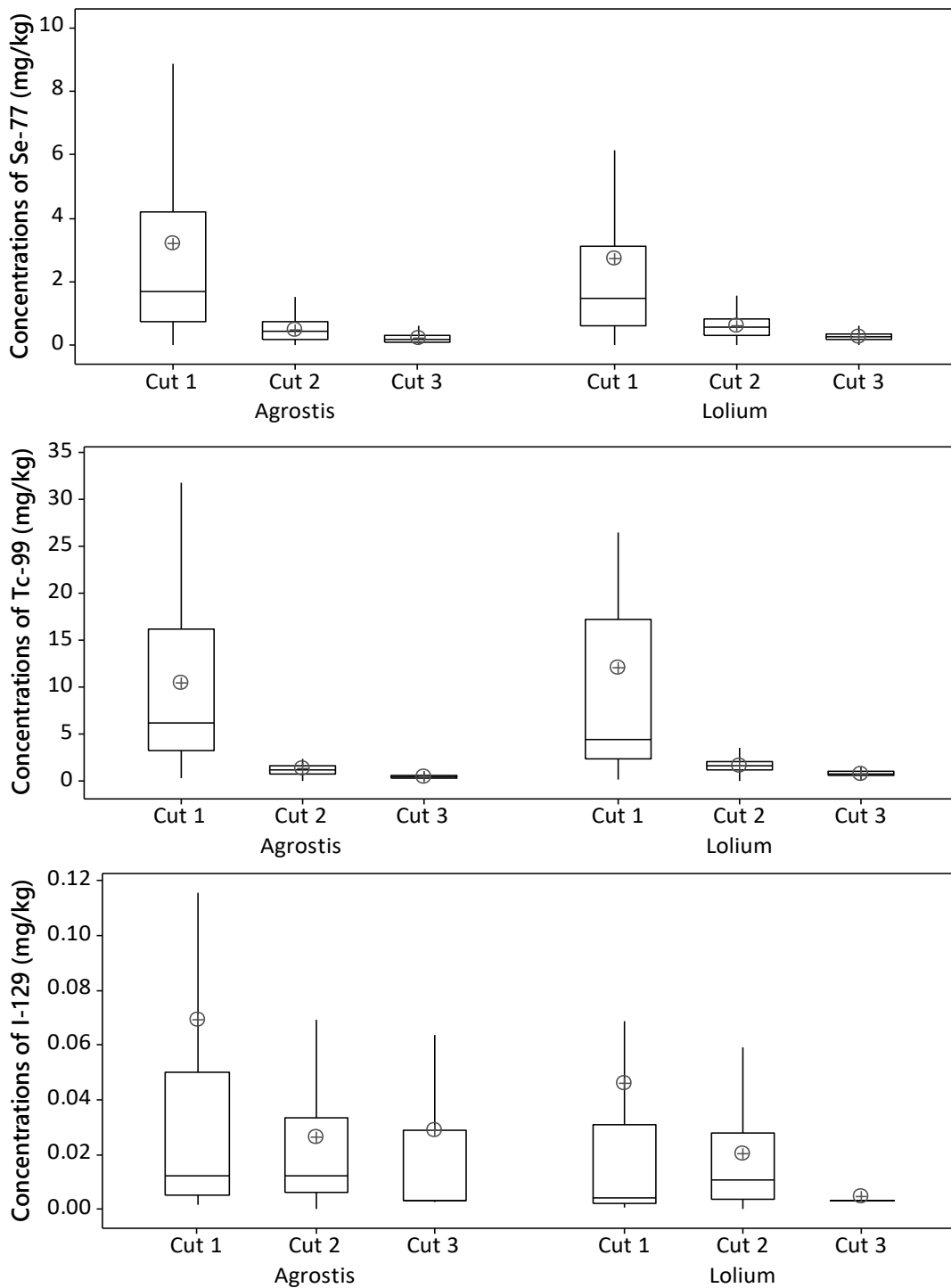


Fig. 1. Box and whisker plots showing the concentrations of ^{77}Se , ^{99}Tc and ^{129}I on a fresh weight basis in shoot tissues of *Agrostis capillaris* and *Lolium perenne* across the three cuts. Selenium concentrations were corrected for soil ^{77}Se contributions. The arithmetic mean (\oplus) is also shown.

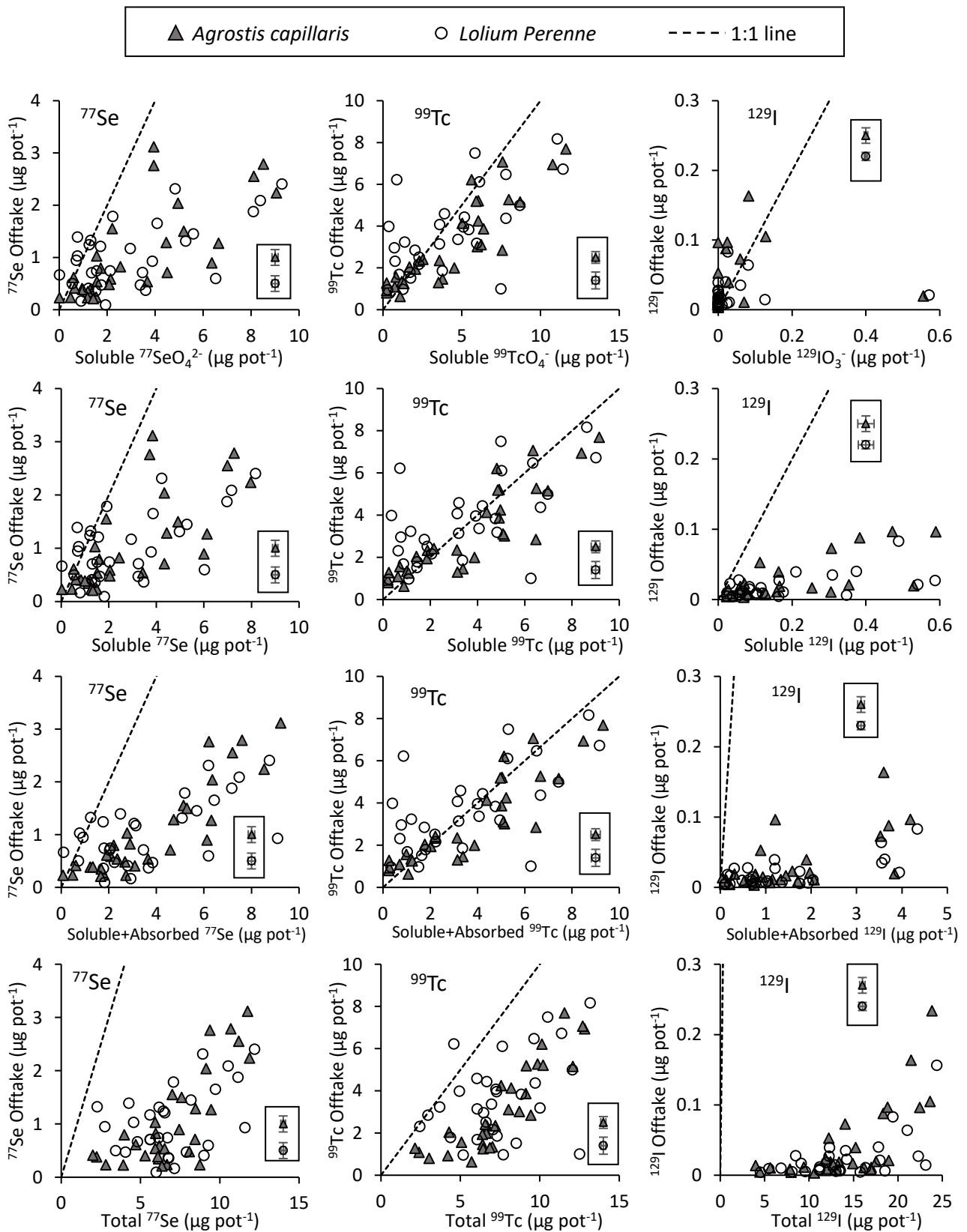


Fig. 2. Relationship between ^{129}I , ^{77}Se , ^{99}Tc uptake in plants and that in soil ($\mu\text{g pot}^{-1}$). Error bars show the average standard error of three replicates.

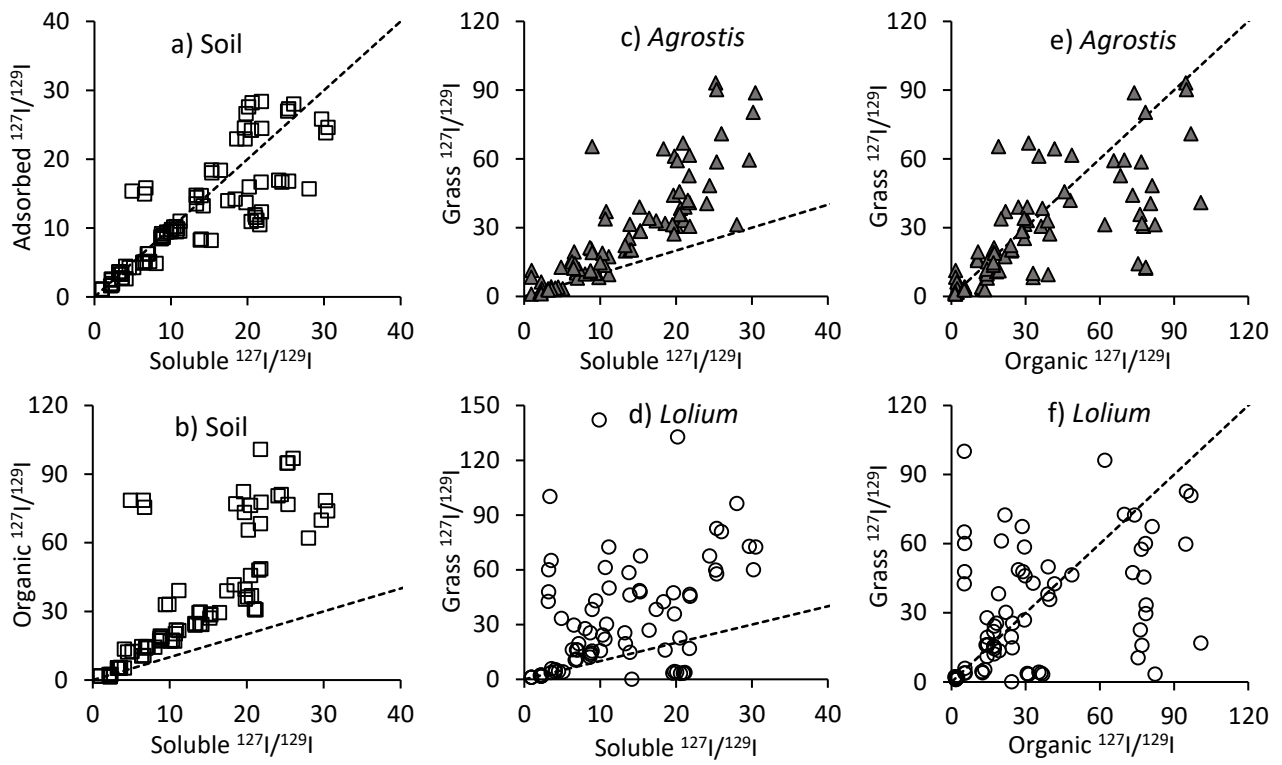


Fig. 3. Ratios of $^{127}\text{I}/^{129}\text{I}$ in soil and plant. The dashed line represents the 1:1 relationship.

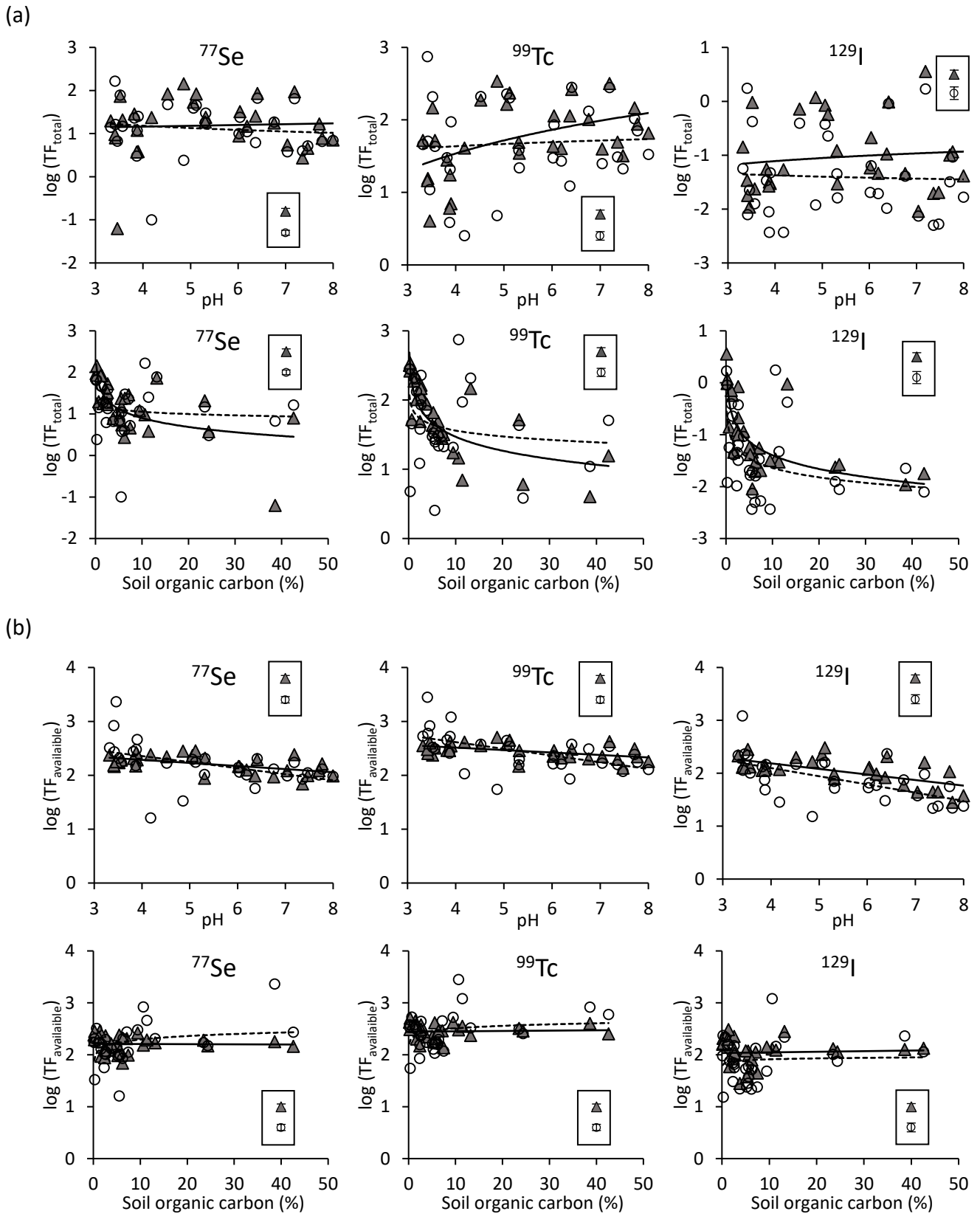


Fig. 4. Relationships between (a) $\log(TF_{Total})$, (b) $\log(TF_{Avail})$, and soil properties. Data are shown for *Agrostis capillaris* (\blacktriangle ; solid regression line) and *Lolium perenne* (\circ ; dashed regression line). Error bars show the average standard error of three replicate analyses.

Electronic Annex

Table A: Spike isotope preparation details.

Isotope	Preparation
⁷⁷ Se	A ⁷⁷ Se stock solution was prepared from an elemental stock of enriched ⁷⁷ Se ⁰ (50 mg; 99.2 atom %), purchased from Isoflex, USA that was oxidised according to the method of Collins et al. (2006)
⁹⁹ Tc	A ⁹⁹ Tc standard (3970 kBq, 5 mL of NH ₄ ⁹⁹ TcO ₄ in H ₂ O) was obtained from High Technology Sources Ltd. This primary stock was diluted with ultrapure water to produce a solution containing 62.6 mg L ⁻¹ ⁹⁹ Tc that was used to spike the soils.
¹²⁹ I	Iodine-129 was obtained as a solution of sodium iodide (¹²⁹ I ⁻) purchased from the American National Institute of Standards (NIST, Gaithersburg, Maryland, USA; CRM 4949C, 0.004 mol L ⁻¹ Na ¹²⁹ I, 1 Bq mL ⁻¹). Iodate-129 (¹²⁹ IO ₃ ⁻) was prepared by oxidation of ¹²⁹ I ⁻ with sodium chlorite using a method adapted from Yntema and Fleming (1939).

Table B: Fractionation of ^{77}Se , ^{99}Tc and ^{129}I in soils after 900 days incubation immediately prior to commencement of pot trial. 'nd = not detected'

	KNO ₃ Soil Extracts (Average)							KH ₂ PO ₄ Soil Extracts (Average)						
	^{129}I µg/kg	$^{129}\text{IO}_3^-$ µg/kg	$^{129}\text{I}^-$ µg/kg	^{77}Se µg/kg	$^{77}\text{SeO}_3^{2-}$ µg/kg	$^{77}\text{SeO}_4^{2-}$ µg/kg	^{99}Tc µg/kg	^{129}I µg/kg	$^{129}\text{IO}_3^-$ µg/kg	$^{129}\text{I}^-$ µg/kg	^{77}Se µg/kg	$^{77}\text{SeO}_3^{2-}$ µg/kg	$^{77}\text{SeO}_4^{2-}$ µg/kg	^{99}Tc µg/kg
M1-S	34.7	10.8	3.81	67.0	1.29	76.2	70.8	37.4	2.49	4.05	4.77	1.06	1.03	0.699
M1-T	10.1	0.766	1.05	68.0	0.617	79.7	85.3	23.6	0.865	3.11	3.13	0.444	0.141	1.48
M2-S	4.37	0.856	1.57	27.0	0.000	31.5	71.0	46.0	1.54	11.0	46.4	nd	40.8	3.57
M2-T	4.18	0.225	1.31	47.4	0.000	54.2	71.0	36.5	0.648	8.20	22.0	0.361	18.8	1.90
F1-S	5.27	0.000	1.19	62.5	0.397	72.5	62.5	5.59	0.705	1.10	1.86	0.063	0.761	4.11
F1-T	2.70	0.005	0.078	33.1	0.053	34.7	30.6	2.40	0.618	0.090	0.616	nd	0.264	5.24
F2-S	0.580	nd	nd	40.0	nd	43.3	35.6	4.70	0.284	0.672	15.3	0.226	15.7	9.02
F2-T	1.77	nd	nd	54.5	nd	57.6	28.5	2.60	0.308	0.013	nd	nd	0.000	2.91
F3-S	9.32	1.09	1.27	32.6	0.054	33.5	54.1	61.2	2.83	5.935	46.0	0.150	49.6	0.070
F3-T	5.04	0.265	1.086	39.7	nd	42.1	51.4	39.7	1.20	6.36	26.5	0.352	26.4	3.24
BC-M	0.444	0.006	nd	11.1	0.017	12.7	15.3	24.1	0.735	6.71	29.8	0.355	26.7	5.76
BH-G	0.894	nd	nd	22.4	0.538	23.2	47.0	10.7	0.804	1.18	4.47	3.285	nd	0.149
BH-W	1.31	nd	nd	18.6	0.854	19.3	63.1	9.89	0.842	1.04	8.70	7.549	nd	nd
BY-W	1.31	nd	0.001	15.7	0.032	17.9	16.7	12.9	0.611	2.14	42.8	0.090	43.1	3.56
CO-A	3.75	0.108	0.000	64.8	0.156	70.3	52.0	17.2	0.703	1.99	1.89	0.407	0.596	1.26
DY-G	2.54	nd	0.003	15.9	0.080	16.6	7.73	2.31	0.635	0.236	0.912	0.049	nd	1.08
DY-M	0.812	nd	0.007	0.533	0.074	0.063	5.23	1.56	0.462	nd	1.42	nd	0.607	1.79
EV-A	2.16	0.364	0.102	64.6	nd	68.4	57.9	22.8	0.832	2.30	5.03	0.273	3.67	nd
FD-G	2.15	nd	nd	57.9	nd	62.0	57.2	16.5	0.670	2.134	3.70	0.242	1.58	nd
IH-W	1.19	nd	nd	23.2	0.031	24.1	15.5	10.6	0.653	1.37	13.1	0.039	10.4	2.76
NP-A	1.87	0.014	nd	52.5	0.669	52.8	57.5	13.9	0.689	2.06	3.54	1.324	0.802	1.29
PE-W	2.08	nd	0.003	40.9	0.012	42.8	18.2	6.78	0.639	0.681	7.77	0.080	6.91	2.58
SB-G	2.64	nd	0.002	23.1	0.094	22.9	50.4	12.1	0.381	1.24	5.22	0.774	2.59	0.871
SR-A	6.08	6.34	nd	14.3	0.859	13.5	74.2	37.9	7.02	4.83	16.8	11.8	nd	nd
SR-G	0.876	nd	nd	20.9	0.101	22.4	49.8	13.9	0.616	1.93	5.19	1.340	1.68	nd
SR-W	3.78	0.867	0.066	25.3	0.600	25.8	63.8	21.8	1.14	2.00	7.95	4.899	0.078	nd
TK-G	1.19	0.029	0.001	23.9	nd	26.2	31.5	25.7	0.781	3.430	22.9	0.665	20.2	2.68
WK-A	3.42	nd	0.002	80.5	0.161	85.5	68.3	16.0	0.929	1.314	1.79	0.382	0.663	0.202
WK-W	1.42	nd	nd	30.3	0.059	32.2	12.6	1.27	0.777	0.006	0.031	nd	nd	0.235
WS-A	1.52	nd	nd	51.5	0.739	53.5	58.3	18.7	0.832	1.84	2.96	1.542	0.515	1.25

Table C: Weight of soil and yield of grass in each pot (both on a dry weight basis) for (a) *Agrostis capillaris* and (b) *Lolium perenne*.

(a)

Code	AC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
M1-S-A	119	0.150	0.455	0.771
M1-S-B	118	0.128	0.268	0.247
M1-S-C	120	0.208	0.608	0.520
M1-T-A	110	0.143	0.313	0.565
M1-T-B	102	0.256	0.677	0.797
M1-T-C	109	0.353	0.722	0.520
M2-S-A	67.3	0.178	0.321	0.679
M2-S-B	73.4	0.234	0.466	0.395
M2-S-C	69.3	0.130	0.498	0.470
M2-T-A	95.7	0.117	0.295	0.627
M2-T-B	86.7	0.146	0.820	0.501
M2-T-C	91.6	0.157	1.054	0.831
F1-S-A	114	0.261	0.362	0.197
F1-S-B	112	0.252	0.557	0.198
F1-S-C	110	0.308	0.338	0.236
F1-T-A	63.2	0.127	0.298	0.366
F1-T-B	60.3	0.159	0.513	0.577
F1-T-C	61.7	0.152	0.796	0.705
F2-S-A	39.1	0.189	0.442	0.308
F2-S-B	41.6	0.219	0.536	0.276
F2-S-C	38.4	0.251	0.212	0.500
F2-T-A	22.4	0.292	0.391	0.744
F2-T-B	22.2	0.263	0.657	0.672
F2-T-C	22.2	0.236	0.454	0.685
F3-S-A	119	0.176	0.323	0.204
F3-S-B	113	0.146	0.312	0.185
F3-S-C	121	0.183	0.586	0.743
F3-T-A	98.0	0.178	0.669	1.091
F3-T-B	93.2	0.314	0.788	1.006
F3-T-C	90.1	0.237	0.679	0.918

Code	AC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
BC-M-A	43.5	0.184	0.359	0.461
BC-M-B	46.0	0.159	0.232	0.291
BC-M-C	51.1	0.266	0.329	0.162
BH-G-A	68.6	0.347	0.49	0.865
BH-G-B	67.1	0.291	0.34	0.271
BH-G-C	64.2	0.378	0.348	0.709
BH-W-A	62.0	0.341	0.414	0.625
BH-W-B	61.5	0.307	0.775	0.679
BH-W-C	60.7	0.434	0.361	0.275
BY-W-A	52.3	0.086	0.25	0.185
BY-W-B	50.7	0.111	0.371	0.317
BY-W-C	55.6	0.149	0.273	0.266
CO-A-A	93.3	0.421	0.751	0.881
CO-A-B	93.6	0.216	0.557	0.501
CO-A-C	96.5	0.505	0.587	0.497
DY-G-A	27.6	0.220	0.459	0.616
DY-G-B	27.5	0.180	0.343	0.323
DY-G-C	29.1	0.196	0.806	0.812
DY-M-A	38.5	0.218	0.589	1.144
DY-M-B	40.6	0.156	0.615	1.183
DY-M-C	37.9	0.205	0.587	0.851
EV-A-A	74.9	0.226	0.471	0.614
EV-A-B	75.8	0.199	0.756	0.560
EV-A-C	77.0	0.366	0.765	0.548
FD-G-A	57.6	0.209	0.136	0.227
FD-G-B	61.9	0.186	0.222	0.454
FD-G-C	58.0	0.292	0.403	0.847
IH-W-A	63.6	0.192	0.808	0.904
IH-W-B	65.1	0.203	0.484	0.748
IH-W-C	66.4	0.180	0.733	0.782

Code	AC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
NP-A-A	86.0	0.365	0.477	0.719
NP-A-B	88.8	0.454	1.006	0.712
NP-A-C	79.1	0.373	0.856	0.453
PE-W-A	56.9	0.167	0.348	0.431
PE-W-B	58.9	0.192	0.305	0.322
PE-W-C	62.7	0.269	0.46	0.824
SB-G-A	64.3	0.232	0.542	0.652
SB-G-B	64.3	0.211	0.67	0.597
SB-G-C	53.9	0.401	0.664	0.527
SR-A-A	82.6	0.218	0.309	0.357
SR-A-B	90.8	0.279	0.386	0.304
SR-A-C	88.4	0.185	0.175	0.196
SR-G-A	62.4	0.229	0.235	0.131
SR-G-B	65.0	0.196	0.264	0.278
SR-G-C	62.7	0.227	0.33	0.332
SR-W-A	81.5	0.340		0.158
SR-W-B	82.4	0.213	0.565	0.343
SR-W-C	78.1	0.466	0.352	0.353
TK-G-A	59.0	0.200	0.353	0.845
TK-G-B	60.1	0.207	0.889	0.896
TK-G-C	58.4	0.138	0.824	0.622
WK-A-A	79.5	0.221	0.433	0.626
WK-A-B	74.2	0.373	0.508	1.024
WK-A-C	69.3	0.458	0.767	0.874
WK-W-A	19.9	0.222	0.324	0.640
WK-W-B	19.9	0.188	0.753	0.786
WK-W-C	19.8	0.311	0.925	1.156
WS-A-A	79.7	0.189	0.177	0.118
WS-A-B	84.3	0.190	0.305	0.278
WS-A-C	89.0	0.310		0.253

(b)

Code	LC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
M1-S-A	124	0.253	0.492	0.987
M1-S-B	124	0.139	0.651	0.890
M1-S-C	117	0.260	1.125	0.868
M1-T-A	108	0.101	0.427	0.631
M1-T-B	102	0.212	0.63	0.993
M1-T-C	106	0.219	0.778	0.982
M2-S-A	68.6	0.079	0.442	0.428
M2-S-B	71.9	0.339	0.62	0.481
M2-S-C	71.4	0.215	0.66	0.435
M2-T-A	93.2	0.147	0.38	0.605
M2-T-B	83.4	0.289	0.818	0.783
M2-T-C	90.7	0.194	0.862	0.813
F1-S-A	111	0.199	0.363	0.311
F1-S-B	113	0.188	0.421	0.330
F1-S-C	111	0.213	0.336	0.262
F1-T-A	60.5	0.059	0.523	0.697
F1-T-B	59.5	0.047	0.688	0.634
F1-T-C	63.9	0.065	0.637	0.678
F2-S-A	39.0	0.208	0.201	0.268
F2-S-B	41.4	0.210	0.183	0.218
F2-S-C	41.1	0.167	0.187	0.206
F2-T-A	23.1	0.180	0.525	0.786
F2-T-B	22.9	0.168	0.595	0.791
F2-T-C	21.9	0.125	0.48	0.858
F3-S-A	118	0.157	0.403	0.494
F3-S-B	113	0.186	0.624	0.429
F3-S-C	116	0.268	0.639	0.584
F3-T-A	97.5	0.229	0.519	0.641
F3-T-B	99.7	0.296	0.628	0.996
F3-T-C	94.3	0.191	0.870	1.030

Code	LC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
BC-M-A	68.7	0.194	0.344	0.403
BC-M-B	74.0	0.168	0.329	0.233
BC-M-C	71.2	0.190	0.303	0.276
BH-G-A	65.6	0.280	0.933	1.317
BH-G-B	69.5	0.274	0.847	0.970
BH-G-C	65.7	0.455	1.410	1.747
BH-W-A	62.9	0.289	0.797	0.849
BH-W-B	63.5	0.310	1.029	0.911
BH-W-C	66.8	0.359	1.225	0.976
BY-W-A	40.8	0.078	0.387	0.235
BY-W-B	41.2	0.107	0.274	0.214
BY-W-C	45.6	0.035	0.285	0.243
CO-A-A	90.4	0.306	0.935	1.448
CO-A-B	90.8	0.338	1.359	1.197
CO-A-C	96.9	0.161	1.008	1.111
DY-G-A	43.2	0.246	0.531	0.601
DY-G-B	44.8	0.199	0.754	0.526
DY-G-C	48.9	0.243	0.836	0.461
DY-M-A	48.9	0.099	0.384	0.806
DY-M-B	48.7	0.156	0.426	1.004
DY-M-C	46.3	0.117	0.411	0.988
EV-A-A	86.4	0.208	0.601	0.878
EV-A-B	84.2	0.306	0.836	0.964
EV-A-C	74.8	0.403	0.867	1.057
FD-G-A	57.6	0.346	0.591	0.992
FD-G-B	55.3	0.214	0.673	0.661
FD-G-C	55.2	0.223	0.718	1.406
IH-W-A	55.1	0.084	0.438	0.741
IH-W-B	56.4	0.197	0.456	0.626
IH-W-C	56.7	0.184	0.512	0.976

Code	LC Soil g pot ⁻¹	Cut 1 Yield g pot ⁻¹	Cut 2 Yield g pot ⁻¹	Cut 3 Yield g pot ⁻¹
NP-A-A	55.7	0.157	0.496	0.672
NP-A-B	55.5	0.282	0.779	0.789
NP-A-C	56.4	0.233	0.996	0.961
PE-W-A	77.1	0.159	0.408	0.348
PE-W-B	82.3	0.126	0.39	0.616
PE-W-C	76.5	0.037	0.235	0.662
SB-G-A	34.8	0.196	0.745	0.758
SB-G-B	35.0	0.330	1.104	0.922
SB-G-C	32.9	0.257	0.889	0.824
SR-A-A	85.8	0.274	0.486	0.699
SR-A-B	91.5	0.239	0.587	0.678
SR-A-C	92.3	0.386	0.764	0.851
SR-G-A	68.9	0.211	0.379	0.309
SR-G-B	68.7	0.214	0.431	0.372
SR-G-C	65.5	0.289	0.371	0.408
SR-W-A	28.2	0.280	0.504	0.616
SR-W-B	27.2	0.302	0.757	0.825
SR-W-C	26.8	0.408	0.629	0.767
TK-G-A	64.3	0.319	0.548	0.659
TK-G-B	64.1	0.192	0.473	0.613
TK-G-C	67.3	0.208	0.593	0.898
WK-A-A	64.4	0.191	0.789	1.424
WK-A-B	62.0	0.318	1.637	2.347
WK-A-C	58.4	0.212	1.087	1.141
WK-W-A	56.5	0.193	0.687	0.999
WK-W-B	62.1	0.191	1.084	0.798
WK-W-C	60.7	0.182	0.920	1.258
WS-A-A	65.1	0.161	0.393	0.633
WS-A-B	68.2	0.265	0.576	0.757
WS-A-C	68.6	0.394	0.338	0.694