Short-term iodine dynamics in soil solution

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ABSTRACT

Assessing the reactions of iodine (I) in soil is critical to evaluating radioiodine exposure and understanding soil-to-crop transfer rates. Our mechanistic understanding has been constrained by method limitations in assessing the dynamic interactions of iodine between soil solution and soil solid phase over short periods (hours). We use microdialysis to passively extract soil solution spiked with radioiodine ($^{129}I^-$ and $^{129}IO_3^-$), to monitor short-term (≤40 hours) *in-situ* fixation and speciation changes. We observed greater instantaneous adsorption of $^{129}IO_3^-$ compared to $^{129}I^-$ in all soils and the complete reduction of $^{129}IO_3^-$ to $^{129}I^-$ within 5 hours of addition. Loss of ^{129}I from solution was extremely rapid; the average half-life of $^{129}I^-$ and $^{129}IO_3^-$ in soil solution was 4.06 and 10.03 hours, respectively. We detected the presence of soluble organically bound iodine (org-^{129}I) with a low molecular weight (MW) range (0.5-5 kDa) in all soils and a slower (20 to 40 hours) time-dependent formation of larger MW org-I compounds (12-18 kDa) in some samples. This

20 study highlights the very short window of immediate availability in which I from rainfall or

21 irrigation remains in soil solution and available to crops, thus presenting significant challenges to

22 phytofortification strategies in soil-based production systems.

23 **Introduction**

24   Iodine (I) is an essential micronutrient for mammalian life, required for the synthesis of thyroid

25 hormones, approximately 1.9 billion people worldwide are estimated to be at risk of developing

26 an iodine deficiency disorder.[1, 2] Radioiodine (^{129}I; $t_{1/2} = 1.57 \times 10^7$ yr and ^{131}I; $t_{1/2}$ 8.02 d) released

27 as a consequence of anthropogenic activities poses a major ecological and health concern due to

28 its biophilic properties and relatively high environmental mobility.[3] Once ^{129}I enters the

29 environment, it behaves similarly to ^{127}I, capable of bioaccumulating through the food chain and

30 subsequently stored in the thyroid where it can induce tumors.[4-6] Understanding the

31 biogeochemical processes and mechanisms that affect I cycling in the terrestrial environment is

32 critical to evaluating the cause of I deficiency and potentially harmful exposure to radioiodine

33 isotopes.

34   Soil properties including pH, redox potential, concentration of soil organic matter (SOM) and

35 Fe/Al/Mn hydrous oxides affect I fixation, mobility and speciation.[5, 7-11] Following natural and

36 anthropogenic I addition events (e.g. rainfall, irrigation, nuclear weapons testing), soluble

37 inorganic-I species, iodide (I^-) and iodate (IO_3^-), can be converted to soluble organic compounds

38 (org-I) and/or incorporated into the solid soil phase, bound to SOM or adsorbed to metal oxides.[5-

39 7, 9, 12] It was demonstrated previously that IO_3^- and org-I are less mobile and have a greater affinity

40 for solid-soil components compared to I^-.[12-14] Metal hydrous oxides can influence iodine dynamics

41 in soils. Whilst ferric and aluminum oxides adsorb IO_3^- more strongly than I^-,[13] the presence of

42   manganese oxide birnessite (δ-MnO_2) can oxidize I^- to IO_3^-.[15] However, in the presence of organic

43   matter (OM) the oxidation of I^- to IO_3^- is limited as the intermediate product, I_2, is incorporated

44   into OM (pH <7).[16] Whilst the pH and redox potential of soils affects I speciation and mobility,

45   SOM appears to be the dominant factor controlling I retention in soils.[12, 17, 18]

46   Microdialysis is an established method for neuroscience and pharmacokinetics and has been

47   used to investigate the mobility and bioavailability of macro-nutrients and metal ions in soil[19-22];

48   its applicability for following I reaction dynamics was previously evaluated by the authors.[23] The

49   passive sampling conditions of microdialysis enable the extraction of the soil solution phase with

50   high temporal resolution and minimal disturbance to the soil structure. Unlike conventional soil

51   solution sampling methods which are destructive (e.g. centrifugation, high-pressure squeezing) or

52   require soil to be close to saturation (e.g. Rhizon™ samplers), microdialysis can perform

53   continuous extractions of the soil solution at a wide range of water contents (as low as ~50% water

54   holding capacity (WHC)). Evaluating soluble I without disturbing the ambient soil conditions,

55   which could result in changes in I speciation, is vital for a more detailed understanding of I

56   biogeochemistry in soil.

57   In this study, we investigated short-term soil soluble I dynamics in three soils with contrasting

58   physicochemical properties. Microdialysis was used to sample an isotopic I tracer (^{129}I) under

59   ambient soil conditions to monitor changes in concentration and speciation following an addition

60   event at 2.5 hour (hr) intervals over a 40 hr period. Iodine in the sampled dialysate was analyzed

61   and speciated to determine: (i) the rate at which inorganic-I is removed from soil solution and

62   adsorbed to the soil solid phase; and, (ii) the molecular weight (MW) range of soluble org-I

63   compounds which are formed.

64

Materials and Methods

Soil sampling and physiochemical characteristics

Three topsoils (0 – 10 cm), from adjoining arable, grassland and woodland sites, were collected from a location in Nottinghamshire, UK. The soils were sieved to <2 mm, homogenized and air-dried. Soil pH was determined using an Orion pH meter after equilibrating 5 g of soil in 12.5 mL of 0.01 M CaCl$_2$ for 30 min. Loss-on-ignition (LOI), as an estimate of SOM content, was determined after heating in a muffle furnace at 450 °C for 16 hr, after an initial drying period. The water holding capacity (WHC) of the soil was determined gravimetrically in triplicate according to Grace, et al. [24]. Total soil ^{127}I was extracted with 5% tetramethylammonium hydroxide (TMAH) at 70 °C for 3 hr (with shaking after 1.5 hr); once cooled, 5 mL of Milli-Q water was added and the bottles were centrifuged (20 min at 3500 rpm) and the supernatant solutions retained for analysis by ICP-QQQ.[25] To determine the concentrations of reactive iron, aluminum, and manganese hydrous oxides, soils were extracted with dithionite-citrate-bicarbonate (DCB) solution, centrifuged (20 min at 2500 rpm), filtered (<0.22 µm) and the supernatant solutions retained for analysis by ICP-QQQ.[26] Particle size analysis was determined using the method described in Rawlins, et al. [27], using a laser diffraction particle size analyzer.

Soil incubation and microdialysis sampling

The radioiodine tracer (^{129}I) was obtained from the American National Institute of Standards as NaI (NIST, Gaithersburg, Maryland, USA; CRM 4949C, 0.004 mol L^{-1} Na^{129}I, 3451 Bq mL^{-1}). The primary stock (^{129}I$^-$) was made up to 100 mL with 0.01 M NaOH, as recommended by the suppliers. Iodate (^{129}IO$_3^-$) was prepared from the ^{129}I$^-$ stock by oxidation, using a method adapted from Yntema and Fleming [28]. To 50 mL of the ^{129}I$^-$ stock, 5 mL of 0.1 M HCl was added in an

4

88   initial neutralization step, followed immediately by 5 mL of 0.2 M sodium chlorite for oxidation.

89   Successful oxidation to $^{129}IO_3^-$ was confirmed by anion exchange LC-ICP-QQQ. The water

90   content of the air-dried soils (~90 g dry weight (dw)) was raised to 50% of WHC with Milli-Q

91   water (18.2 MΩ cm; Millipore) and allowed to equilibrate for 10 days at 20 ± 0.5 °C in the dark.

92   The moist soils were then homogenized with equivalent volumes of $^{129}I^-$ or $^{129}IO_3^-$ in solution to

93   bring the soils to 80% WHC with a final ^{129}I concentration of 0.2 mg kg^{-1} (dw basis). The soils

94   were then distributed between triplicate 50 mL Corning® polypropylene centrifuge tubes (~30 g

95   dw of soil per replicate).

96   The microdialysis system consisted of a syringe pump (KD Scientific Legato 200 Series),

97   equipped with four plastic syringes (BD Plastipak; 20 mL) used to deliver the perfusate solution.

98   Syringes were attached to microdialysis probes CMA 20 (10 mm length, 500 µm outer and 400

99   µm inner diameter) with a polyethersulfone (PES) membrane (100 kDa molecular weight cut-off

100 (MWCO). Within two minutes of ^{129}I addition to soil, incision holes were made, using an

101 introducing needle, and the microdialysis probes were inserted ~1.5 cm beneath the soil surface.

102 Probes were perfused with Milli-Q water at a flow rate of 5 µL min^{-1} and dialysate solutions

103 collected at 2.5 hr intervals in glass microvials over a 40 hr extraction period. Samples were

104 initially stored in the refrigerated micro-fraction collector (6 °C; CMA 470) before freezing at −20

105 °C until defrosting immediately before analysis. Throughout the extraction, soils were kept in the

106 dark at a constant temperature of 20 ± 0.5 °C. The probes and micro-fraction collector were from

107 CMA Microdialysis AB (Kista, Sweden). In order to assess the concentration (µg L^{-1}) of ^{129}I in

108 the soil solution a correction factor was applied to the measured dialysate solutions as $C_{soil\_solution}$

109 $= C_{dialysate}/(1- e^{-PS/F})$. Where $C_{soil\_solution}$ and $C_{dialysate}$ are the concentrations of the target analyte in

110   the external soil solution and the dialysate solution respectively, *PS* in the surface area of the

111   microdialysis membrane and *F* is the perfusion flow rate, adapted from Clough [29].

112

Total iodine analysis

114   Total ^{127}I concentrations were determined in single MS, no-gas mode (Agilent 8900 ICP-QQQ,

115   Agilent Technologies). All ^{129}I concentrations were measured using an ICP-QQQ (Agilent 8900,

116   Agilent Technologies). Analysis was conducted using both quadrupoles and the reaction cell, to

117   remove isobaric and polyatomic interferences, a full description of ^{129}I analysis can be found in

118   Humphrey, et al. [30]. Due to the small sample volume, we used an Agilent 1260 Infinity Bio-inert

119   Quaternary LC pump and high performance autosampler to act as a low volume autosampler: vials

120   were also equipped with flat bottomed glass inserts to further reduce the sample volume

121   requirement. Throughout the analytical run, an in-house quality control sample with a known

122   concentration of 5 µg L^{-1} ^{129}I was measured with an average recovery of 101% (*n =28*) and a limit

123   of detection (LOD) (3 x SD blanks) of 0.0075 µg L^{-1} for ^{129}I.

124

Iodine speciation analysis

126   Inorganic-I (^{129}I$^-$ and ^{129}IO$_3^-$) concentrations were measured by ICP-QQQ following on-line

127   chromatographic separation in isocratic mode using a Hamilton PRP X-100, anion exchange

128   column (250 mm × 4.6 mm, 5 µm) and a samples injection volume of 25 µL. The mobile phase

129   was 100 mM NH$_4$NO$_3$, adjusted to pH 9.5 with TMAH (25%), at a flow rate of 1.5 mL min^{-1}.

130   Quality control samples with a known I concentration of 5 µg L^{-1}, ^{129}I$^-$ and ^{129}IO$_3^-$, were measured

131   with an average recovery of 97% and 96% (*n =22*), respectively. The LOD for ^{129}I$^-$ and ^{129}IO$_3^-$

132   was 0.007 and 0.006 µg L^{-1}, respectively.

133   The MW distribution of soluble org-I was determined using size exclusion

134   chromatography (SEC) hyphenated with UV absorbance detection and ICP-QQQ. An Agilent

135   1260 Infinity Bio-inert Quaternary LC pump and high performance autosampler in isocratic mode

136   with an AdvanceBio SEC 130 Å (2.7 µm, 4.6 x 300 mm column, MW range 100-120,000 Da,

137   Agilent) was used as the sample introduction system. Samples (10 µL) were injected onto the

138   column with a mobile phase (flow rate of 0.35 mL min^{-1}) consisting of 150 mM sodium phosphate

139   buffer, pH 7.0. Platinum tip sampler and skimmer cones were used on the ICP-QQQ due to the

140   high sodium concentration in the mobile phase. The UV detector scanned at 220 nm to measure

141   the calibration protein standards, and 254 nm to detect dissolved organic carbon.[9, 31-35] Protein

142   standards of known MWs: Ovalbumin (45 kDa); Myoglobin (17 kDa); Aprotinin (6.7 kDa);

143   Neurotensin (1.7 kDa); and Angiotensin II (1 kDa) (AdvanceBio SEC 130Å protein standard,

144   Agilent) were used to calibrate the column.

145

146   **Results and Discussion**

147   *Soil characteristics*

148   The soil physiochemical characteristics in Table 1 reflect their land use. Compared to the arable

149   and grassland soils the acidic woodland soil had a much greater LOI, extractable Fe/Al/Mn

150   hydrous oxide content and total I concentration. Given that all three soils were collected with 2.5

151   km of each other the greater I concentration in the woodland soil may reflect a greater ability to

152   retain I from precipitation and the absence of annual crop removal.

153

154   **Table 1** Summary of soil physiochemical characteristics. Values expressed as mean ± standard

155   error (SE) (n=3)

	Units	Arable	Grassland	Woodland
Location (long, lat decimal degrees)		52.9001, -1.0884	52.8890, -1.0877	52.8986, -1.0744
Elevation	m	95	83	121
pH ($CaCl_2$)		6.9	5.9	3.6
LOI	%	5.1	7.0	50.6
WHC	%	36.8	41.5	70.8
^{127}I	mg kg^{-1}	3.38 ± 0.05	3.63 ± 0.05	12.9 ± 0.10
^{129}I	µg kg^{-1}	<DL	<DL	<DL
Fe_2O_3	%	0.180 ± 0.02	0.0131 ± 0.001	0.696 ± 0.06
$Al(OH)_3$	%	0.0379 ± 0.003	0.0523 ± 0.007	0.925 ± 0.05
MnO_2	%	0.0594 ± 0.005	0.0226 ± 0.003	0.622 ± 0.07
Texture (clay/silt/sand)	% mass	41/41/18	17/19/64	24/35/41
Texture classification		Clay	Sandy Loam	Clay Loam

156

157 *Total ^{129}I dynamics in soil solution*

158   Total concentrations of soluble ^{129}I sampled from the soils spiked with $^{129}I^-$ or $^{129}IO_3^-$ are shown

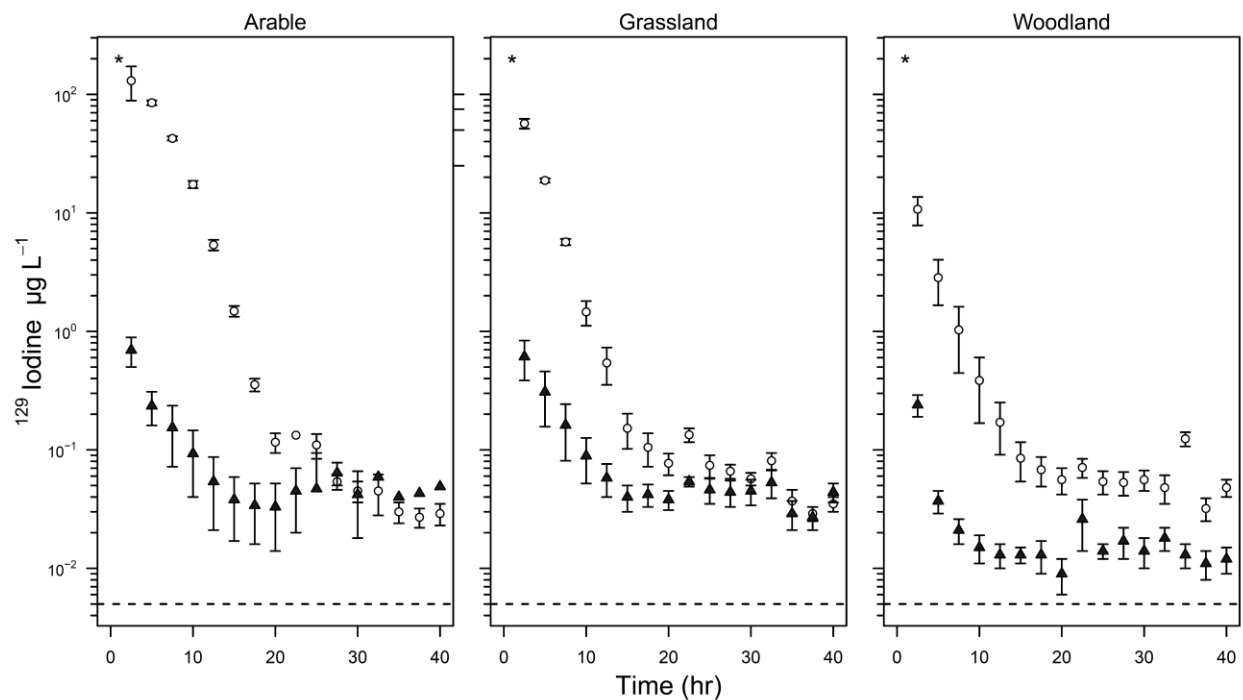159 in Figure 1.

160

Figure 1 Soluble ^{129}I (µg L^{-1}) sampled using microdialysis from arable, grassland and woodland soils at 2.5 hour intervals over 40 hours. Symbols represent mean values obtained in triplicate, error bars indicate ± S.E. An asterisk at the top left of each chart denotes the initial spike concentration; white circles (○) represent soils spiked with ^{129}I$^-$; black triangles (▲) represent soils spiked with ^{129}IO$_3^-$. Note the log scale on the Y-axis. The dashed line indicates the detection limit. Each microdialysis dialysate sample was sampled at 5 µL min^{-1} for 2.5 hours.

Losses of ^{129}I from soil solution were observed in all the soils within 2.5 hr, irrespective of the initial spike speciation (^{129}I$^-$ or ^{129}IO$_3^-$). The concentration of ^{129}I in the first dialysate samples (0-2.5 hr) were significantly higher in soils treated with ^{129}I$^-$ compared to ^{129}IO$_3^-$. The significantly larger initial loss of ^{129}IO$_3^-$ from solution compared to ^{129}I$^-$ observed in this study may be a combination of adsorption on inorganic soil phases or, rapid immobilization within SOM.[5]

9

175 Volatilization has previously been reported to occur from soils, but is suppressed by the presence

176 of SOM[6] and the likelihood of it meaningfully contributing to total loss rates is low.[36] Therefore,

177 adsorption onto inorganic soil phases and rapid immobilization by SOM are far more likely reasons

178 for the losses of ^{129}I from soil solution; both of these processes are pH-dependent. Prior to the

179 incorporation of IO_3^- with OM studies have shown that a reduction to an electrophilic intermediate

180 species, such as HOI or I_2 occurs, this reduction occurs much faster under acidic conditions (pH<

181 6).[16, 37-40] Clays, hydrous oxides and SOM can absorb I^-, where sorption generally increases with

182 decreasing pH, as observed in this study.[41-43] In previous studies it has been shown that when soil

183 pH >6.0 I^- sorption is predominantly influenced by OM, however, under more acidic conditions

184 the role of specific adsorption to iron and aluminum oxides becomes increasingly important.[44, 45]

185 The faster immediate sorption of $^{129}IO_3^-$ compared to $^{129}I^-$ highlights the different adsorption

186 mechanisms of inorganic-I in soils.

187 The significant proportion of apparent instantaneous loss of $^{129}IO_3^-$ from solution in all

188 experimental soil and the highest loss rates of $^{129}I^-$ from soil solution is consistent with previous

189 results.[5] First-order kinetic equations were used to assess the rate at which total ^{129}I was removed

190 from soil solution as: $C_t = C_oe^{-kt}$, where C_t is the ^{129}I concentration at time t (hr) (mg L^{-1}), C_o is

191 the initial concentration of ^{129}I in soil solution (mg L^{-1}), and k is the first-order rate constant (hr^{-1}).

192 The half-life in soil solution was then calculated from ln(2)/k. The average half-life of soils spiked

193 with $^{129}I^-$ and $^{129}IO_3^-$ was 4.06 ± 0.96 and 10.03 ± 0.78 hr, with r^2 values of 0.77 and 0.45,

194 respectively. Shetaya, et al. [5] investigated I dynamics in soils and reported that the loss of I^- from

195 solution was extremely rapid, reaching completion over minutes–hours, whereas IO_3^- loss from

196 solution was slower, typically occurring over hours–days, which is consistent with the results in

197 the present study. However, in the current study the use microdialysis has enabled far greater

198   sampling resolution compared to previous studies at time intervals within a similar range to the

199   reaction times reported by Shetaya, et al. [5]. The results presented in this paper indicate that iodine

200   adsorption occurs more rapidly in soils under realistic conditions than previously observed using

201   batch sorption experiments and chemical extraction approaches.

202

203   *^{129}I speciation in soil solution*

204   The concentrations of soluble inorganic ($^{129}I^- + ^{129}IO_3^-$) and organic (total ^{129}I minus the sum of

205   inorganic species) ^{129}I in the three soils spiked with either $^{129}I^-$ or $^{129}IO_3^-$ are shown in Figure 2. A

206   combined inorganic fraction was calculated due to the concentrations of $^{129}IO_3^-$ present in the soils

207   treated with either $^{129}I^-$ or $^{129}IO_3^-$ falling below the LOD (shown in Figure S1).

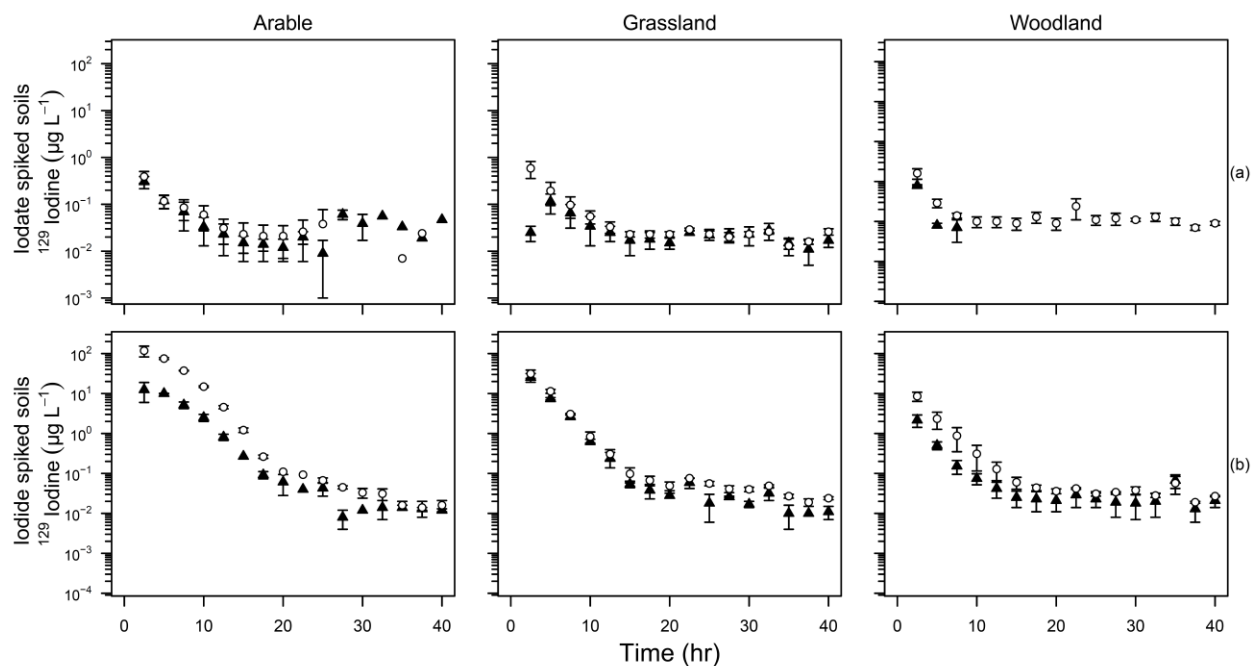208

209

210   **Figure 2** Soluble ^{129}I speciation of (a) iodate and (b) iodide spiked arable, grassland and woodland

211   soils over 40 hours. Symbols represent the mean values obtained in triplicate, bars indicate ±SE.

212   White circles (○) represent inorganic-^{129}I concentrations; black triangles (▲) represent soluble

213   org-^{129}I concentrations. Note the log scale on the primary Y-axis. Samples below the detection

214   limit were omitted from the plot. Microdialysis dialysate was sampled at 5 µL min^{-1} for 2.5 hours.

215

216   The proportion of inorganic-^{129}I present in soil solution was greater compared to org-^{129}I in soils

217   spiked with I$^-$, however, this trend was not observed consistently in IO$_3^-$ spiked soils. In the arable

218   soil spiked with ^{129}IO$_3^-$, where the org-^{129}I became the dominant fraction in samples after 25 hr,

219   when inorganic-^{129}I concentrations were below the LOD. In contrast, in woodland soil spiked with

220   ^{129}IO$_3^-$ the soluble org-^{129}I fraction was below the LOD 10 hr after addition, and only inorganic

221   species were detected.

222   The speciation of stable and radioiodine present in soil systems has been the focus of several

223   studies[9, 46-48] with I$^-$ and org-I identified as the dominant species present. When ^{129}IO$_3^-$ was added

224   to the soils we observed an immediate reduction to ^{129}I$^-$ in soil solution, with very low

225   concentrations of ^{129}IO$_3^-$ remaining in soil solution beyond 2.5 hrs (Figure S1, d). Reduction of

226   IO$_3^-$ to I$^-$ in soils has previously been observed[49, 50] and may arise from biotic processes. However,

227   it seems more likely that reduction is caused by abiotic interactions with solid or dissolved OM as

228   the reaction rates appear to be too rapid for biological processes.[5] The rapid reduction reported in

229   this study is much faster compared to previous work, where the reduction of IO$_3^-$ occurred over

230   days.[51] Possible reasons for the discrepancies between the studies include the significantly lower

231   concentrations of ^{129}I used in this study and the lower soil pH used in this study as the reduction

232   of IO$_3^-$ to the reactive iodine species is thermodynamically more favorable at lower pH.

233   In contrast, when soils were spiked with ^{129}I$^-$, a very low concentration of ^{129}IO$_3^-$ was detected

234   in the arable and grassland soils within 7.5 hr, indicating that limited ^{129}I$^-$ oxidation to ^{129}IO$_3^-$ had

235   occurred (Figure S1). We only observed I$^-$ oxidation in the arable and grassland soils; there was

236   no evidence for oxidation in the organic-rich acidic woodland soil (Figure S1). Factors controlling

237     $^{129}I^-$ oxidation in soil remain unclear.[52] The conversion of I^- to org-I requires I^- to be initially

238   oxidized to an intermediate such as I_2 or HOI.[16, 37] Iodide-oxidizing bacteria in soils have

239   previously been observed to influence I^- sorption/incorporation into SOM and oxidation to IO_3^-

240   and to produce volatile org-I compounds such as CH_3I.[53, 54] In addition, metal oxides (Al, Fe, Mn)

241   and humic substances can act as oxidizing agents. In batch kinetic experiments pH and metal oxide

242   concentrations significantly influence reaction rates, with faster oxidation occurring at lower pH

243   and greater metal oxide concentrations.[55, 56] However, in our *in-situ* experiments, we did not

244   observe I^- oxidation in the acidic, Fe/Al/Mn rich woodland soil. Figure 2 shows that the greatest

245   initial loss of I^- occurred in the woodland soil, which had the largest metal oxide concentrations,

246   an order of magnitude higher than the arable and grassland soils, highest organic matter and the

247   lowest pH. The rapid loss of I^- from solution in organic-rich soils has been described as a first-

248   order reaction.[57] Previous studies have demonstrated that I^- can easily be remobilized as it is only

249   bound by weak electrostatic attraction to solid soil media,[57] therefore, further studies assessing the

250   speciation of iodine during dynamic processes such as rainfall events or plant uptake should be

251   investigated to assess iodine mobility in soils.

252

253   *Molecular weight distribution of dissolved organic ^{129}I*

254   The presence of org-^{129}I was confirmed using SEC-ICP-QQQ; the presence of multiple peaks

255   indicates that soluble org-I exists at a range of MWs. Time-dependent changes in speciation and

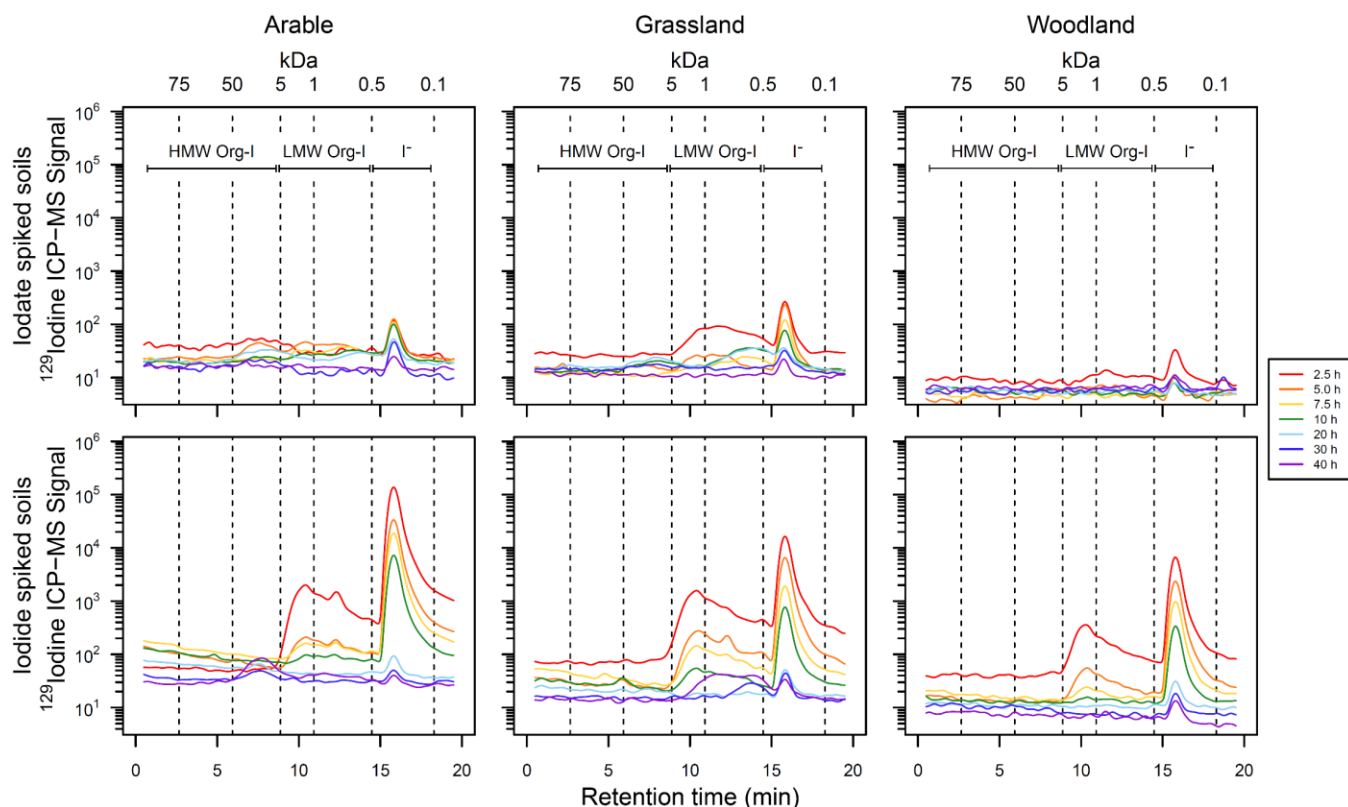256   molecular weight of ^{129}I in the soil solution phase are shown in Figure 3.

257

Figure 3 Molecular weight distribution of soluble isotopically labeled ^{129}I in arable, grassland and woodland soils spiked with ^{129}IO$_3^-$ or ^{129}I$^-$ sampled over 40 hours, separated by size exclusion chromatography. Note the log Y-axis. High molecular weight organically bound I (HMW org-I), low molecular weight organically bound I (LMW org-I) and ^{129}I$^-$ retention times labeled.

Within all three experimental soils, for addition of both ^{129}I$^-$ and ^{129}IO$_3^-$, chromatographic peaks were observed for ^{129}I$^-$ between 15 and 18 min that decreased over the 40 hr extraction period. The intensity of the ^{129}I$^-$ peak was considerably less in the ^{129}IO$_3^-$ treated soils due to the higher rates of instantaneous adsorption on inorganic soil phases and rapid immobilization by SOM. All soils spiked with ^{129}I$^-$, had a broad peak representing LMW org-^{129}I between 9 and 15 min; the overall intensity of the org-I peak decreased over the 40 hr sampling period. There was a shift in the MW distribution in the arable soil spiked with ^{129}I$^-$ between 20 and 40 hr when the signal intensity of

14

271 the LMW org-I peak decreased and a HMW org-I peak, with a retention time between 5 and 10

272 min emerged. In contrast, within the grassland soil spiked with ^{129}I$^-$, dissolved org-^{129}I had a lower

273 MW distribution; no org-I compounds >5 kDa were present throughout the sampling period. The

274 emergence and rapid decline of LMW org-^{129}I between 0.5–5 kDa in the woodland soil further

275 illustrates how quickly initially soluble I is removed from solution in organic rich soils. However,

276 within the arable and grassland soils, the MW distribution of the org-I underwent various changes

277 within the 40 hr sampling period. The UV absorbance results confirmed that soluble organic

278 compounds had a broad MW range (Figure S2).

279   The transport behavior of inorganic-I in soils may be significantly impacted by the fixation of

280 dissolved I into macromolecular humic substances, however, many of the mechanisms controlling

281 the rate of fixation remain unknown.[33, 38, 58, 59] Xu, et al. [3] demonstrated that higher proportions of

282 ^{125}I$^-$ and ^{125}IO$_3^-$ were associated with organic compounds <3 kDa, 16 and 20% compared to 6 and

283 3% in the >3 kDa fraction, respectively. In addition, they found that binding sites became saturated

284 and less available when the treatment concentration increased.[3] In resuspension experiments, Xu,

285 et al. [18] found that mobile ^{129}I was associated with amphiphilic organic compounds with an average

286 MW between 13.5 and 15 kDa. Figure 3 shows that higher proportions of soluble inorganic-I were

287 incorporated into organic compounds with a low MW (<5 kDa). However, there was evidence of

288 larger dissolved org-I compounds with a MW between 12 and 18 kDa 20 hr after ^{129}I addition, in

289 agreement with Xu, et al. [3].

290   The time-dependent formation of these larger dissolved org-I compounds could be due to smaller

291 dissolved compounds binding together via weak chemical forces or the influence of microbial

292 activity. The iodination of SOM in acidic conditions was shown to be predominantly an abiotic

293 process, however, in less acidic conditions (pH ≥5), microbial assisted iodination of SOM was

294   observed.[3] Whilst microbial activity is generally not deemed essential for the transformation of

295   IO_3^- into org-I (which seems to be primarily controlled by abiotic processes[6]), microbial laccases

296   have been shown to enhance I^- sorption in soils.[53] Microbiological activities have previously been

297   reported to incorporate I^- with humic acids and soils with a relatively HMW and low mobility.[33]

298   Heumann, et al. [60] identified that in the presence of microorganisms the production HMW org-I

299   compounds significantly increased over an 8 week period; in contrast Figure 3 demonstrates that

300   formation of relatively HMW compounds occurred over 20-40 hrs.

301   It has previously been suggested that the rapid rate of reaction of I with SOM could be beneficial,

302   as humus may act as a natural barrier, accumulating any radioiodine released from nuclear waste

303   repositories as immobile org-I compounds.[18] Following a radioiodine contamination event limited

304   soil-to-crop transfer would be beneficial for reducing the risk and duration of potentially harmful

305   exposure.[18, 61, 62] The short-term experiments presented in this paper show that a relatively small

306   proportion of inorganic-^{129}I is rapidly incorporated into LMW organic compounds (<2.5 hr) which,

307   over time, can be sorbed to the solid soil phase or bind together to form HWM organic compounds

308   (~30 hr).

309   Evaluating the short-term transport, speciation and fate of I in soil is critical to assess the

310   environmental mobility and plant availability, as ^{127}I is an essential micronutrient for which dietary

311   intake often depends on the transfer of I from soil-to-crops.[63] Soil I concentrations are dependent

312   on the deposition of volatized I compounds from seawater.[11] Specific rainfall characteristics

313   influence the concentration of I in rainfall and soil properties affect the volume of I retained by

314   soils that would be accessible for plant uptake.[5] Passive uptake is the predominant pathway for I

315   absorption in plants,[30] as such, plant uptake is highly dependent upon the concentration in the soil

316   solution phase. By using ^{129}I as a proxy for ^{127}I, it is possible to predict how much soluble I is

317 available for plant uptake following an addition event. We demonstrate that I only remains in the

318 soil solution phase for a very limited period, before being sorbed on to inorganic soil phases or

319 immobilized by SOM. Consequently, there is a severely limited window of opportunity for plants

320 to absorb I from the soil solution immediately following rainfall or irrigation and limits the transfer

321 of I to animal and human diets. However, this study did not investigate the impact of rewetting

322 and remobilization of I bound to the solid soil phase, and this is should be the subject of further

323 research. Considering that there are an estimated 1.9 billion people at risk of I deficiency

324 worldwide,[64] the findings in this paper may contribute to better understanding of the efficacy of

325 phytofortification strategies.

326

327 **Supporting Information**

328 Additional information on analytical methods and supplementary results.

329   TOC GRAPHIC FOR MANUSCRIPT
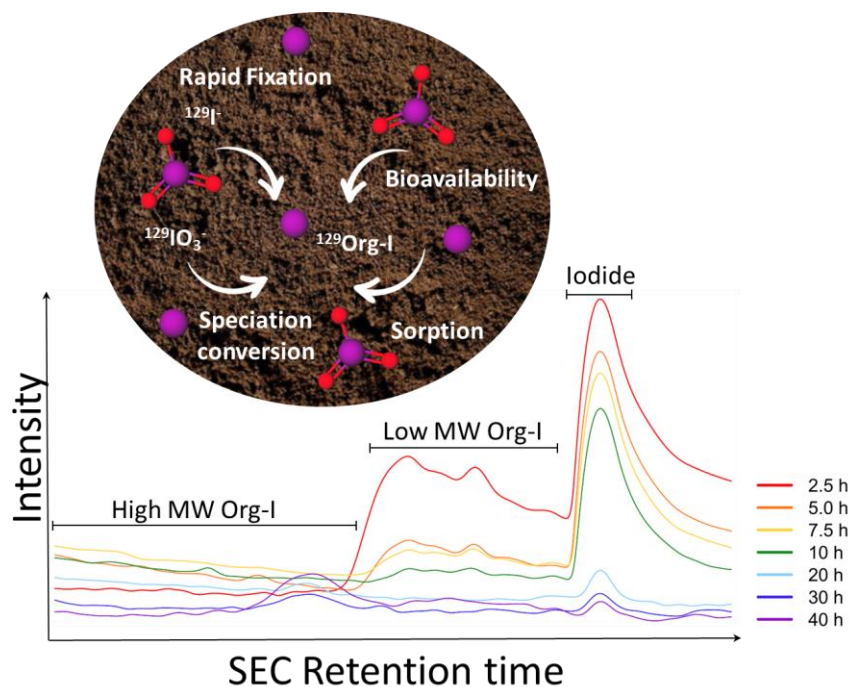
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339   **Author Contributions**

340   The manuscript was written through contributions from all authors. All authors have given

341   approval to the final version of the manuscript.

Conflict of Interest

The authors declare no competing financial interest.

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