1 Online microdialysis-high performance liquid chromatography-inductively coupled plasma mass

2 spectrometry (MD-HPLC-ICP-MS) as a novel tool for sampling hexavalent chromium in soil

3 solution.

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10 Abstract

11 Conventional soil solution sampling of species-sensitive inorganic contaminants, such as hexavalent 12 chromium (Cr^{VI}), may induce interconversions due to disruption of system equilibrium. The temporal 13 resolution that these sampling methods afford may also be insufficient to capture dynamic 14 interactions, or require time-consuming and expensive analysis. Microdialysis (MD) is emerging as a 15 minimally invasive passive sampling method in environmental science, permitting the determination 16 of solute fluxes and concentrations at previously unobtainable spatial scales and timeframes. This 17 article presents the first use of MD coupled to HPLC-ICP-MS for the continuous sampling and 18 simultaneous detection of Cr^{VI} in soil solution. The performance criteria of the system were assessed using stirred solutions; good repeatability of measurement (RSD < 2.5%) was obtained for Cr^{VI}, with a 19 20 detection limit of 0.2 µg L⁻¹. The online MD-HPLC-ICP-MS setup was applied to the sampling of native 21 Cr^{VI} in three soils with differing geochemical properties. The system sampled and analyzed fresh soil 22 solution at 15-minute intervals, offering improved temporal resolution and a significant reduction in 23 analysis time over offline MD. Simple modifications to the chromatographic conditions could resolve 24 additional analytes, offering a powerful tool for the study of solute fluxes in soil systems to inform 25 research into nutrient availability or soil-to-plant transfer of potentially harmful elements.

26 Introduction

The separation and quantification of trivalent (Cr^{III}) and hexavalent (Cr^{VI}) chromium (Cr) in soil is a 27 burgeoning area of research motivated by the significant differences in toxicity and mobility 28 29 between the two oxidation states.¹ Due to the negative charge of its compounds, typically chromate 30 (CrO_4^{2-}) and dichromate $(Cr_2O_7^{2-})$,² Cr^{VI} is more mobile and bioavailable in soil-water systems than Cr^{III}, ³ and is therefore more likely to be transferred from contaminated soil to drainage water and 31 32 into plants.⁴ The measurement of total Cr^{VI} in solid matrices presents a metrological challenge due to the potential for species interconversions during extraction and analysis,⁵ which is 33 further compounded when measuring changes in the bioavailable pool of Cr^{VI} in soil-pore water systems. 34 35 This usually involves specialized extractions and/or separation steps which not only cause significant 36 disruption to the equilibrium of the system,⁶ but also produce large numbers of samples which are

37 more susceptible to artefactual errors.

The kinetics of Cr species interconversions in soil-pore water systems are of particular importance when there is the potential for transportation of Cr^{VI} into groundwater and/or sediment systems.⁷ Attenuation of Cr in these systems is dependent on the physiochemical properties of the water/soil and can be attributed to the formation of Cr^{III} following reduction of Cr^{VI}. Trivalent Cr is significantly limited in solubility due to its adsorption to mineral phases or co-precipitation with iron (Fe) (oxy-)hydroxides.⁸ The adsorption of Cr^{VI} in soil-pore water systems is a slower process than for Cr^{III},

resulting in order-of-magnitude lower partition coefficients (K_d) .⁹ However, this process is 44 accelerated through decreases in pH and increases in concentrations of soil organic carbon (SOC) 45 and reducing inorganic components such as Fe(II) and sulfides.¹⁰ Previously, the exchange kinetics 46 47 between soil solution and mineral phases have been measured using diffusive gradients in thin-films (DGT).¹¹ This passive sampling technique involves the chelation of labile analytes on a resin 48 49 implanted onto saturated soil, causing depletion around the DGT device and a shift in system equilibrium to resupply the soil solution from the solid phase.¹² This allows for the measurement of a 50 51 range of kinetic parameters, including distribution coefficients (K_{dl}), remobilization fluxes and adsorption/desorption rate constants.¹³ Despite its advantages, the technique suffers from spatial 52 53 limitations (a large sampling area, of the order of cm², is required for successful device deployment), 54 induces significant disruption to the equilibrium of the sampled soil and requires time-consuming 55 offline sample preparation and analysis.¹⁴ Depending on the temporal resolution required, a number 56 of devices may need to be deployed which adds to the processing time, analytical requirements and 57 overall cost.¹⁵

Microdialysis (MD) is another passive sampling technique that has been garnering increasing interest 58 59 within the field of soil science¹⁶⁻¹⁹ due to its high spatial and temporal resolution, and its 60 preservation of the *in situ* dynamics of the system undergoing sampling.²⁰ Microdialysis uses a probe 61 containing a semipermeable membrane with a specific molecular weight cut off (MWCO); the pumping of a perfusate solution into the probe creates a diffusion gradient within the sampled 62 medium causing solutes to diffuse across the membrane.²¹ The solution exiting the probe (dialysate), 63 containing the sampled solutes, can then be analyzed using a suitable analytical technique.²² The 64 65 minimal disruption to the soil, coupled with the ability of the technique to sample soil solution at representative water contents (~50% water holding capacity (%WHC) and higher)²³ makes MD a 66 67 very attractive tool to increase understanding of small-scale inorganic solute availability in soil.

68 The majority of MD sampling is undertaken offline through the collection of discrete samples over varying timescales (typically in the order of minutes to hours),²⁰ although recent articles have 69 demonstrated the potential for hyphenating MD with analytical detectors such as electrothermal 70 71 atomic absorption spectrometry (ETAAS)²⁴ and high performance liquid chromatography (HPLC).²⁵ 72 Online MD sampling and simultaneous analysis, depending on the analytical technique being used, 73 has the potential to overcome one of the biggest compromises in MD sampling- relative recovery 74 (RR) versus perfusate flow rate.²⁶ The RR of a system can be defined as the ratio of the solute 75 concentration in the dialysate to the solute concentration in the medium undergoing MD sampling, 76 and is a function of the resistances that impede solute transport imposed by the external 77 environment (R_{ext}), the MD probe membrane (R_m), the dialysate (R_d) and the perfusate flow rate 78 (Q_p) .²⁷ Therefore the lower the Q_p , the greater the likelihood of reaching a steady-state between the solute concentration in the external solution and the probe membrane, leading to RR values close to 79 100 % depending on the analyte being studied.²⁸ However, flow rates less than 5 µL min⁻¹ are not 80 81 practical for the majority of MD applications due to the increased time required to collect sufficient 82 sample for analysis, and the subsequent impact on temporal resolution. Online systems, with careful optimisation of liquid handling steps, can allow for lower Q_p, increased RR and immediate analysis 83 without adding additional time or cost constraints. The coupling of MD sampling to mass 84 spectrometry was first conceived over 25 years ago,²⁹ but has seen limited application in soil since 85 with no significant focus on inorganic solutes.³⁰ 86

The aim of this study was to couple MD to HPLC-ICP-MS (henceforth referred to as online MD-HPLC-ICP-MS) for continuous passive soil solution sampling and simultaneous analysis of Cr^{VI}. The objectives were:

- 90 (i) to undertake online MD calibration using stirred solutions of Cr^{VI};
- 91 (ii) to assess common performance characteristics (linearity, precision, limit of detection);
- 92 (iii) to apply the MD-HPLC-ICP-MS method to the sampling of Cr^{VI} in soils with differing
 93 geochemical characteristics.

94 Materials and Methods

95 Reagents.

96 All solutions were prepared in 18.2 M Ω cm ultrapure water (DDW, Merck Millipore, UK). Standards 97 for Cr^{VI} were prepared through dilution of a commercially available solution (Greyhound 98 Chromatography, UK). Ethylenediaminetetraacetic acid (di-ammonium salt, NH₄-EDTA), 99 trisaminomethane (TRIS) and ammonium nitrate (NH₄NO₃) (Sigma Aldrich, UK) were used for the 100 preparation of the chromatographic mobile phase.

101 Instrumental Apparatus and Analysis.

Separation and identification of Cr^{VI} in sampled soil solution was undertaken using a Dionex GP50 Gradient Pump (Dionex Corporation, USA) and a PRP-X100 anion exchange column (Hamilton Company, USA) coupled to an Agilent 8900 Triple Quad inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, Tokyo, Japan). A Rheodyne 7125 injector/switching valve (IDEX Corporation, USA) equipped with a 20 µL loop was used to interface the dialysate flow from the microdialysis probe with the column. The column was connected directly to the nebulizer of the ICP-MS instrument using a single piece of 0.18 mm internal diameter (ID) PEEK tubing.

109 The separation and identification of Cr^{VI} was achieved through isocratic elution using a mobile phase 110 consisting of 40 mM NH₄NO₃, 50 mM TRIS buffer and 5 mM NH₄-EDTA, adjusted to pH 7.0 using 111 concentrated nitric acid (HNO₃, Romil, UK). Mobile phase was introduced into the injector/switching 112 valve at a flow rate of 1.2 mL min⁻¹, resolving Cr^{VI} within 5 min. The ICP-MS instrument was operated 113 in collision cell mode, with the cell pressurized using helium (He) gas at a flow rate of 5.1 mL min⁻¹, to 114 reduce the impact of polyatomic interferences on m/z 52 (e.g. ⁴⁰Ar¹²C⁺).

115 Soil Sampling.

116 The physicochemical properties of the soil samples used to demonstrate the efficacy of the MD-

117 HPLC-ICP-MS setup are summarized in Table 1. Samples were primarily chosen due to their total Cr^{VI}

118 content, but also to provide a range of physicochemical properties to ensure a robust assessment of

119 the MD-HPLC-ICP-MS setup.

Soil ID	Country of Origin	Texture	рН	ТОС (%)	LOI (%)	Total Cr (mg kg⁻¹)	Cr ^{∨ı} (mg kg⁻¹)	Mn (mg kg⁻¹)	Fe (mg kg⁻¹)
1	U.K. (Glasgow)	Silty Sand	7.37	3.1*	n/a	1750*	28.5	790*	41663*
2	U.S. (NJ)	N/A	9.33	0.1*	n/a	1055*	15.3	60.0*	5950*
3	Kenya	Sandy Clay Loam	5.77	n/a	6.4	329	2.0	2636	69449

120 **Table 1.** Soil physicochemical properties.

121 * denotes a parameter that was not measured within author's laboratories.

Soil 1 was a sample of silty sandy soil from eastern Glasgow, held by the British Geological Survey (BGS) from a soil chemistry survey in 2018. Soil 2 (SRM2700) was purchased from NIST (National Manuscript: "es-2020-081408"

124 Institute of Standards and Technology, U.S.), and was a soil matrix reference material intended for 125 use in validating Cr^{VI} speciation data for soils and sediments. Soil 3 was a sample of sandy clay loam 126 soil collected in Kakamega County, Kenya and retained by BGS. The methods used for the 127 determination of total Cr, loss-on-ignition (LOI) and Cr^{VI} in these soils have been outlined 128 previously.^{31, 32} The presence of Cr^{VI} in soils 1 and 2 can be attributed to anthropogenic sources; the 129 Cr^{VI} in soil 3 is of geogenic origin, possibly derived from ophiolitic parent material.^{33, 34}

Maximum percentage water holding capacity (% WHC) was determined on 50 g subsamples of each soil, according to previously-outlined methods.³⁵ For each soil sampled by MD-HPLC-ICP-MS (n = 3 per soil), 10 g of soil was moistened to 70% WHC before being packed into polypropylene (PP) tubes

133 (Sarstedt, UK), henceforth referred to as microcosms, for online microdialysis sampling.

134 Calibration of MD-HPLC-ICP-MS System in Stirred Solutions.

135 Prior to application of the online MD-HPLC-ICP-MS setup to soil solution sampling, the RR for a range

136 of perfusate flow rates was calculated in stirred solutions to determine the optimum perfusate flow

rate for the system. The Cr^{VI} solution (100 μ g L⁻¹) was perfused with distilled deionized water (DDW,

138 18.2 M Ω cm at 25 °C, Millipore Merck, UK) at flow rates of 1, 3, 5, 7.5 and 10 μ L min⁻¹, with each flow

rate replicated 5 times. Solutions were stirred to remove the resistance contribution from the

- 140 external environment (R_{ext}).²⁷
- 141 The RR for each perfusate flow rate was calculated using Equation 1:

142
$$RR(\%) = 100 \times \frac{C_{dlul}}{C_{std}}$$

(1)

143 where C_{dial} is the concentration ($\mu g L^{-1}$) of the analyte in the dialysate and C_{std} is the concentration 144 ($\mu g L^{-1}$) of the analyte in the perfused solution.

145 Online Microdialysis Sampling.

The MD system consisted of a CMA 4004 syringe pump (CMA, Stockholm, Sweden) delivering 146 147 perfusate (DDW) through a 10 mL syringe (BD Plastipak, US) into a CMA 20 microdialysis probe with 148 a polyethersulfone membrane (10 mm length, 0.5 mm OD, 100 kDa MWCO). The dialysate flow was connected to the needle port of the injector/switching valve using a tubing adaptor (CMA, 149 150 Stockholm, Sweden) and a #22 gauge square-cut end syringe needle. New probes were perfused at 10 µL min⁻¹ with DDW. Prior to installation in soil microcosms, the online MD-HPLC-ICP-MS system 151 152 was calibrated through perfusion and injection of stirred calibration standards containing ⁵³Cr^{VI} at 1, 10, 25, 50 and 100 µg L⁻¹. A more concentrated calibration standard (4 mg L⁻¹) was perfused after 153 154 sampling each replicate for Soil 2, to extend the linear dynamic range whilst mitigating washout 155 issues that could arise when considering the low perfusate flow rate and narrow diameter of the 156 inlet and outlet probe tubing.

157 Fifteen minutes after probe installation- the total time taken to fill the volume of the outlet tubing and the 20 µL loop- the valve was switched to "inject" and the time resolved analysis (TRA) sequence 158 159 was initiated on the ICP-MS software to begin data acquisition. The injector remained in this position 160 until ten sample volumes of mobile phase had been pumped through the loop (equivalent to 10 161 seconds with a 20 µL loop), before being switched back to "load" to collect freshly-sampled dialysate 162 for the next injection. These steps were repeated over a period of 2 hours, giving a total of 8 163 injections of passively-sampled soil solution. Each rewetted soil was sampled in triplicate using the 164 online MD-HPLC-ICP-MS setup, with a new microcosm prepared for each replicate to minimize the 165 potential for solute depletion associated with continuous sampling.²⁰

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166 **Results and Discussion**

167 **Relative recovery of Cr^{VI} in solution.**

168 The RR of Cr^{VI} (stirred solutions) displayed a non-linear decrease with increasing flow rate (Fig. 1); 169 perfusate was delivered into the MD probe at flow rates of 1, 3, 5, 7.5 and 10 μ L min⁻¹ with 170 collection and injection of the dialysate from each flow rate replicated 5 times.



171

Figure 1. The effect of perfusate flow rate (μ L min⁻¹) on relative recovery (%) of Cr^{VI} (100 μ g L⁻¹) in stirred solutions (n = 5 for each flow rate). Exponential trendline and correlation coefficient (R² = 0.9737) are displayed within the chart. Error bars indicate ± standard error from 5 replicates.

Subsequent solution optimization and soil sampling were undertaken using a perfusate flow rate of 3 μ L min⁻¹. This flow rate represented the best compromise between RR (approximately 55%) and the frequency with which freshly sampled soil solution could be injected into the HPLC column, otherwise known as the temporal resolution. The temporal resolution of the online MD-HPLC-ICP-MS system was 15 minutes, representing a significant improvement in sampling capability compared to conventional offline MD which can usually only sample in hour increments to ensure sufficient volume is collected for analysis.³⁶

Previous studies have reported variability in RR due to inherent differences in probe structure arising from the manufacturing process.^{17, 37} A significant advantage of the online MD-HPLC-ICP-MS method is that, once flow rate calibration has been carried out for the analyte of interest, a single injection of a perfused calibration standard prior to soil sampling can identify any variability or reduction in the performance of the probe before time-consuming and potentially expensive soil sampling and analysis is undertaken.

188 Analytical Figures of Merit.

189 The method detection limit (DL) was determined according to previously-outlined methods.³⁸ Briefly, 190 5 replicate injections of a perfused 0.5 μ g L⁻¹ Cr^{VI} standard were undertaken using the online MD-191 HPLC-ICP-MS setup. The DL was calculated using Equation 2: Manuscript: "es-2020-081408"

192
$$DL = \frac{(f)(RSD)(C_{SLG})}{100\%}$$
 (2)

193 where *t* is a confidence factor using Student t-distribution with $\alpha = 0.99$ and n-1 degrees of freedom,

194 RSD is the relative standard deviation of the peak areas for the Cr^{VI} standard and C_{std} is the nominal 195 concentration of the injected Cr^{VI} standard; details of the precision of the injections are given in 196 Table 2. The DL was calculated as 0.2 µg L⁻¹ Cr^{VI}. A similar exercise was previously undertaken to 197 establish the Cr^{VI} DL for the HPLC-ICP-MS setup without MD sampling; the DL for the HPLC-ICP-MS 198 setup was calculated as 0.05 µg L⁻¹ Cr^{VI}.

Table 2. Precision of replicate injections of 0.5 μ g L⁻¹ Cr^{VI} standard (n = 5) used to calculate detection limit for the online MD-HPLC-ICP-MS setup.

Spike Replicate	Peak Area Counts
1	3190
2	2779
3	2594
4	2785
5	2939
Standard Deviation	222
Average	2857
RSD (%)	8

201

The precision of the online MD-HPLC-ICP-MS setup was assessed at the same time as the RR. Across each flow rate (1, 3, 5, 7.5, 10 μ L min⁻¹), the 5 replicate injections displayed good precision, demonstrating the repeatability of the technique for solution sampling (Table 3).

Table 3. Average RR and relative standard deviation (RSD, n = 5) for replicate injections at perfusate flow rates of 1, 3, 5, 7.5 and 10 μ L min⁻¹.

207	Flow Rate (µL min ⁻¹)	Average RR (%)	RSD (%)
208	1	89	2.2
200	3 55		1.3
209	5	40	1.3
210	7.5	27	2.0
210	10	22	1.4
244			

211

The linearity of response was determined through injections of perfused Cr^{VI} standards at nominal concentrations of 1, 10, 25, 50 and 100 µg L⁻¹. There was a strong positive linear correlation between

214 peak area counts and Cr^{VI} concentrations in stirred solutions (Fig. 2).



Figure 2. Calibration curve ($R^2 = 0.9994$) for online MD-HPLC-ICP-MS system at nominal concentrations of 1, 10, 25, 50 and 100 µg L⁻¹. MD probes were immersed in 50 mL plastic beakers containing Cr^{VI} solution, stirred and perfused at 3 µL min⁻¹.

219 Soil Solution Sampling.

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The online MD-HPLC-ICP-MS setup was applied to the sampling and analysis of Cr^{VI} in soil solution from the previously detailed microcosms, using the perfusate flow rate (3 μ L min⁻¹) and injector timings established during solution calibration (Fig. 3). The system was sensitive enough to sample Cr^{VI} in all microcosms at a temporal resolution of 15 minutes, a significant improvement compared to recent studies examining soil solution dynamics using offline microdialysis sampling and



225 analysis.³⁹

Figure 3. Soluble Cr^{VI} sampled using online MD-HPLC-ICP-MS technique. Markers represent mean values from triplicate measurements, and error bars indicate ± standard error (SE). The blue hatched

line in "Soil 3" graph is the detection limit for the online technique (0.2 μ g L⁻¹), the red hatched line is the Cr^{VI} detection limit for the HPLC-ICP-MS system (0.05 μ g L⁻¹).

The relevance and/or applicability of this temporal sampling resolution for monitoring the reduction 230 231 of Cr^{VI} in the environment is dependent on the geochemical conditions of the system under 232 investigation. From a solely-abiotic perspective, the presence of common electron donors (e.g. ferrous iron, soil organic matter (SOM)) will cause rapid (<5 minutes) reduction of Cr^{VI} up to pH 10, 233 whereupon the ferrous iron will be oxidized by dissolved oxygen faster than by Cr^{VI,40} The rate of 234 reduction of Cr^{VI} by SOM is also pH-dependent, decreasing with increasing pH but potentially 235 236 occurring over timeframes of several weeks at neutral pH (depending on both the SOM and initial Cr^{ν_l} concentrations in the system). 41 In addition, microbial reduction of Cr^{ν_l} (both aerobic and 237 anaerobic) can occur depending on both pH and the tolerance of the microorganism to Cr^{VI.42} These 238 mechanisms are not as well-defined as abiotic pathways of reduction, but could occur over several 239 hours at mg L⁻¹ concentrations of Cr^{VI} depending on the bacterium and concentration of electron 240 donors within the system.⁴³ Therefore, the temporal sampling resolution of the reported online MD-241 242 HPLC-ICP-MS setup should be sufficient to monitor these diverse processes, although specific studies 243 may require modifications to be implemented if rapid turnover is expected.

The differing trends in sampled Cr^{VI} concentrations can be attributed to a combination of the 244 physical particle size and the geochemical properties of each soil, as opposed to artefacts associated 245 246 with the online MD-HPLC-ICP-MS setup. Soil 1 and Soil 3 had been sieved to ≤2 mm prior to 247 sampling, whilst Soil 2 was used as received (milled material, packaged by NIST). The wider error bars for each sampled time point in Soil 2 are therefore due to increased Rext, with the finer particle 248 size of the material reducing the ability of Cr^{VI} to diffuse across the MD probe membrane.⁴⁴ This is 249 also reflected in the trend of decreasing sampled Cr^{VI} over the 120-minute sampling period, due to 250 the formation of a depletion zone around the MD probe arising from a combination of continuous 251 252 sampling and impeded solute diffusion.³⁰ Similar depletion profiles have been reported for offline 253 MD studies employing continuous sampling, and could be an informative artefact as nutrient uptake 254 by plant roots is also governed by depletion and formation of diffusion gradients within the soil.⁴⁵ 255 The majority of MD studies thus far have reconciled depletion zones in this way, due to their primary 256 focus being the assessment of diffusive flux of high-turnover soil nutrients (e.g. plant-available nitrogen (N)).⁴⁶ However, further assessment of these depletion trends- through targeted studies 257 258 into the significance of Rext, alongside additional MD probe calibration strategies such as retrodialysis 259 and/or no-net-flux techniques- are required to ensure wider adoption of MD by inorganic soil 260 scientists. Due to the requirement of predictive solution metal speciation models (e.g. Windermere 261 Humic Aqueous Model (WHAM), Visual MINTEQ) to be supplied with accurate estimates of labile pools of metal ion concentrations for site-specific bioavailability measurements,⁴⁷ the determination 262 of free metal ion concentrations in dialysate samples will need to account for inherent changes in 263 264 solute recovery due to the resistances imposed by R_{ext} and Q_p.

Sampling of Soil 1 was the most reproducible, with an average Cr^{VI} concentration of 19.8 ± 0.1 µg L⁻¹ 265 between 45 and 120 minutes, indicating that the sampled available pool of Cr^{VI} was resupplied 266 267 consistently by diffusion within the microcosm towards the MD probe. The decrease from 27.5 ± 3.4 268 to 19.9 \pm 1.6 µg L⁻¹ between 15 and 45 minutes could indicate the sampling of a short-lived pool of immediately exchangeable Cr^{VI} following rewetting, although the online MD-HPLC-ICP-MS setup 269 lacked the temporal resolution to confirm this. In comparison, Soil 3 had the lowest initial sampled 270 Cr^{VI} concentration of 0.63 ± 0.11 µg L⁻¹, which decreased to below the online technique DL after 45 271 minutes of sampling; the sampled Cr^{VI} concentration remained below this for the duration of 272 273 sampling for all 3 microcosm replicates. Due to the geochemical properties of this soil sample (high

Fe/Al/organic matter content, low pH), the observed rapid decrease is possibly due to Cr^{VI} adsorption to mineral solids⁴⁸ or reduction and subsequent precipitation as Cr^{III} compounds;⁴⁹ the precision of the microcosm replicates, combined with the inherently low sampled Cr^{VI} concentration, do not suggest that this quick temporal decrease is solely due to a depletion zone forming around the probe membrane.

279 Overall, the results of this study confirm that the online MD-HPLC-ICP-MS setup can be used to reproducibly sample and analyze soluble Cr^{vl} from a range of soils with different physicochemical 280 properties. Differences in the efficacy of Cr^{VI} sampling between soil microcosms in this study were 281 282 limited to particle size and/or geochemical factors influencing Cr^{VI} solubility, as opposed to artefacts 283 associated with the MD-HPLC-ICP-MS system. Assessing the performance of the setup at different 284 %WHC, alongside further method development to increase the temporal sampling ability and resolve more immediate pools of available Cr^{VI}, will contribute to the widespread adoption of the 285 reported online MD-HPLC-ICP-MS technique for short-term nutrient availability studies. 286

287 Future Prospects for Optimization and Implementation of Online MD.

288 The use of MD for soil solution sampling is still an emerging technique (the first comprehensive review was published in early 2020) and so, to a certain extent, the future prospects and discussion 289 points for online and offline MD are complementary.²⁰ One important consideration in the 290 291 continuous sampling of soil solution is the increased likelihood of depletion zones forming around 292 the probe due to removal of solute from solution.⁴⁶ The recharge of this zone is dependent on a 293 number of factors, including the ability of the solute to diffuse from un-sampled areas within the 294 medium, the concentration of solute within the medium and the diffusive resistance imposed by the soil. These factors may have significant ramifications for the interpretation of solute diffusive flux 295 296 data when multiple MD probes are deployed. The proximity of one MD probe to its neighbor could 297 create competing diffusion gradients and lead to a situation where probes with reduced 298 permeability- due to manufacturing defects, continued use or implantation in heterogeneous 299 portions of soil- would sample lower solute concentrations (Fig. 4). Depletion zones are a well-300 known component of MD, but a more empirical investigation is required to fully understand how 301 they impact both diffusive flux measurements and the efficacy of probes that are in close proximity 302 to each other.



Figure 4. Diffusion of solutes (blue circles) towards probes implanted in soil matrix. Reduced
 permeability (represented by diagonal fill) in right MD probe shifts the diffusion gradient towards
 the left MD probe, therefore the right MD probe will sample lower concentrations of solute.

306 The temporal resolution of the online MD-HPLC-ICP-MS setup in this article represents a significant 307 improvement over offline MD; further optimization of the instrumental setup could reduce this to sub-minute sampling frequencies. In recent years, the use of total consumption nebulizers has 308 309 allowed sample volumes in the order of microliters to be introduced in to ICP-MS instruments.⁵⁰ The 310 flow rates commonly used in MD are ideally suited to these sample introduction systems, with the 311 potential for the outlet tubing from the MD probe to be interfaced directly with the nebulizer to 312 monitor transient signals in real-time. Such a setup would also reduce the level of operator 313 supervision required, as the only 'hands-on' task would be the installation of the probe into the 314 microcosm prior to time-resolved analysis.

The spatial resolution of MD, combined with the greater sampling frequency afforded by online 315 316 coupling to analytical systems, would allow for the investigation of solute turnover/removal at rootand microbe-relevant scales in near real-time. Microbial reduction of Cr^{VI} has been reported on 317 numerous occasions, with incubation times varying from 45 min to 42 days⁵¹ due to the significant 318 319 variation in Cr^{VI} reduction efficiency between different strains.⁵² Undertaking online MD-HPLC-ICP-320 MS on sterile and non-sterile soil could provide more information on the impact of microbial communities on Cr^{VI} reduction, with the ability to investigate parameters such as temperature, pH 321 322 and soil type with greater replication than through batch experiments. Online monitoring would also allow for termination of the experiment once passively-sampled analyte concentrations reached DL, 323 324 potentially saving days of time-consuming and costly experimentation and analysis.⁵³

325 Understanding the mechanisms governing rapid soil fixation and speciation changes, for important 326 redox-active micronutrients such as iodine (I) and selenium (Se), previously limited in terms of temporal resolution, may now be possible.^{54 55} The use of stable and radio-isotope trials have 327 328 confirmed that the removal of I and Se from soil solution, primarily through incorporation into the 329 solid phase or immobilization by soil organic matter (SOM), is a rapid process which significantly reduces the bioavailability of these micronutrients.⁵⁶⁻⁵⁸ Humphrey, et al.⁵⁴, in (at the time of the 330 writing) the only application of offline MD to investigate I dynamics in soil solution, showed that 331 adsorption was more rapid than previously reported. Increased frequency of sampling through 332 online MD could further refine knowledge of the period over which soluble forms of I and Se are 333 334 available for uptake by crops, leading to improvements in biofortification strategies intended to 335 alleviate the prevalence of deficiency diseases.

The online MD-HPLC-ICP-MS system was only evaluated for Cr^{VI}, but through simple modification of 336 the chromatographic conditions (column, mobile phase composition) the setup could be applied to 337 338 the sampling and determination of other common inorganic species of interest, including compounds of arsenic, thallium and mercury, to better inform hazard assessment investigations. The 339 soil solution dynamics of inorganic nutrients essential to human health (e.g. iodine, selenium) could 340 341 be investigated at unprecedented temporal and spatial scales, allowing for more thorough 342 assessments of the efficacy of staple crop biofortification strategies that are essential for the billions 343 of people at risk of micronutrient deficiencies worldwide.

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The manuscript was written through contributions from all authors. All authors have given approvalto the final version of the manuscript.

362 Conflict of Interest

363 The authors declare no competing financial interest.

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