Assessing plant-available glyphosate in contrasting soils by Diffusive Gradient in Thin-films technique (DGT)

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Abstract

Glyphosate represents one quarter of global herbicide sales, with growing interest in both its fate in soils and potential to cause non-target phytotoxicity to plants. However, assessing glyphosate bioavailability to plants from soil residues remains challenging. Here we demonstrate that the diffusive gradient in thin-films technique (DGT) can effectively measure available glyphosate across boundary conditions typical of the soil environment: pH 4-9, P concentrations of 20-300 $\mu\text{g P L}^{-1}$ and NaHCO_3 concentrations of 10-1800 mg L^{-1} . In this study, four soils with different glyphosate sorption properties were dosed with up to 16 mg kg^{-1} of glyphosate and phytotoxicity to wheat and lupin was measured against the DGT-glyphosate concentrations. An improved dose response curve was obtained for root elongation of wheat and lupin across soil types when DGT-glyphosate was used instead of alkaline-extractable (*i.e.*, total extractable) glyphosate. Total extractable glyphosate concentrations of 2.6 and 5.0 $\text{mg glyphosate kg}^{-1}$ in the sandy Tenosol, equivalent to 2.9 and 6.5 $\mu\text{g L}^{-1}$ DGT-extractable glyphosate, reduced the root length of lupins (but not wheat) were by 32-36% compared with the untreated control. DGT is therefore a promising method for assessing phytotoxic levels of glyphosate across different soils.

Key words: phytotoxicity, *Triticum aestivum*, *Lupinus angustifolius*, soil-glyphosate-DGT, sorption desorption isotherm, XRD

1. Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum, non-selective systemic, post-emergent herbicide used for weed control in agricultural and urban environments. The commercialisation of glyphosate-tolerant crops in 1996 (Benbrook 2012) has resulted in increased glyphosate use, and glyphosate now represents one-quarter of global herbicide sales (GSBR 2011). Because of its widespread application, a growing body of research has focused on assessing the fate and risks of glyphosate residues in soil, water and food (Williams et al., 2000; Borggaard and Gimsing, 2008; Helander et al., 2012; Kaniserry et al., 2015).

Glyphosate has a propensity to bind to soil particles, which reduces its mobility, bioavailability and risk to non-target organisms. The dominant binding mechanism involves the phosphonic acid moiety interacting with positively charged sites on clay minerals, notably aluminium and iron (hydr)oxides (Sprankle et al., 1975; Shushkova et al., 2010). Because glyphosate is zwitterionic, interaction of the amine group in glyphosate with negatively charged soil/colloidal particles and functional groups in soil organic matter can also contribute to sorption, albeit to a lesser extent (Borggaard and Gimsing, 2008). The influence of soil organic carbon content on glyphosate sorption is not consistent (Farenhorst et al., 2009), with some studies reporting that soil organic carbon (SOC) content decreased glyphosate sorption (Arroyave et al., 2016; Day et al., 1997), while others suggest that SOC increased glyphosate sorption (Piccolo et al., 1996; Albers et al., 2009). Glyphosate can also bind with negatively charged organic matter through di/trivalent cation bridging (dissolved cations, Ca^{2+} , Al^{3+} and Fe^{2+} , Barrett and McBride, 2007) and also with divalent cations in 2: 1 clay minerals (Glass, 1987; Piccolo et al., 1994).

Nevertheless, in certain soils or under particular environmental conditions, glyphosate sorption can be limited, leaving a significant fraction of labile glyphosate that is more amenable for biological uptake or off-site movement (Barja and dos Santos Afonso, 2005; Borggaard and Gimsing, 2008; Al-Rajab and Schiavon, 2010). Light-textured soils with low iron or aluminium (hydr)oxide mineral

content generally have a lower capacity for glyphosate sorption (Piccolo et al., 1994) which can increase the risk of crop seedling damage relative to heavier-textured soil types (Bott et al., 2011). These soils are also more susceptible to leaching and runoff losses of glyphosate (Piccolo et al., 1994). The presence of anions which compete for soil sorption sites, particularly inorganic phosphate, is another factor that can lower glyphosate sorption (Dion et al., 2001; Gimsing et al., 2004a) leading to increased bioavailability and potential phytotoxicity (Bott et al., 2011; Cornish, 1992). Soil pH regulates the charge density of both glyphosate and clay minerals, such that neutralisation of soil pH has a tendency to decrease glyphosate sorption (Gimsing et al., 2004b). Generally, at high soil pH (> 7), the negative-charged soil colloids increased and glyphosate molecules existed as HG^{2-} (~100%, Munira et al., 2016). It has also been shown that water soluble humic substances found in soil solution can facilitate glyphosate leaching to lower soil layers (Piccolo et al., 1994).

It is therefore clear that a number of soil characteristics influence the bioavailability and transport of glyphosate residues in soil. To date, there has been a focus on predicting glyphosate fate and availability through empirical and mechanistic modelling, with varying success (Barrett and McBride, 2007; Glass, 1987; Gimsing et al., 2004b). However, direct assessment of glyphosate bioavailability to date has been limited, due to a lack of an appropriate methodology. Current analytical methods generally aim to measure total residue loads, through exhaustive extraction with or without correction for recovery by spiking with internally-labelled isotopic standards (Botero-Coy et al., 2013; Koskinen, et al., 2016). A key limitation in assessing the bioavailable glyphosate lies in the small volume of pore water in soil and the low amount of glyphosate remaining in the pore water (Vereecken, 2005). A potential solution to this is the application of diffusive gradient in thin-films (DGT), an analytical technique that measures the diffusive supply of elements and compounds by acting as an infinite sink (Degryse, 2009). The technique differs from other extraction techniques by responding to kinetics of release from the soil rather than relying on a pseudo-equilibrium between extractant and soil, so that the analyte present in the soil solution and the fraction resupplied from

the solid phase are measured. As such, DGT has been shown to effectively predict plant-available P (Tandy et al., 2011). DGT has been identified as a valid passive sampling technique for total glyphosate in aquatic environments (Fauvelle et al., 2015), but as far as we are aware, the potential for DGT to effectively assess bioavailable glyphosate across different soil types has not yet been tested.

A number of potential challenges may arise in applying DGT in the assessment of bioavailable glyphosate in soil. The competition of common soil anions, especially phosphate (Tandy et al., 2011; Mason et al., 2010) with glyphosate for the DGT binding layer, could provide a misleading account of the true glyphosate availability.

To address these questions, we aimed to establish the boundary conditions for the use of DGT with glyphosate and to test the potential for the DGT technique to quantify glyphosate availability in different soils. We hypothesize that DGT-available glyphosate will provide a useful estimate of plant available glyphosate than currently available analytical methods. This was tested by spiking four soils, differing in their capacity to bind/fix glyphosate, with a range of glyphosate concentrations and concurrently measuring root growth inhibition of two different plant species, wheat (*Triticum aestivum*) and narrowleaf lupin (*Lupinus angustifolius*).

2. Material and methods

The overall experimental design is shown in Figure 1.

2.1 Soil characterisation

Four contrasting soils were used for these experiments. Soils were taken from Australian grain cropping fields from locations shown in Table 1. The soils were air-dried, sieved to <2 mm, and stored in sealed containers at ambient temperature until use. Soil properties were measured on all soils: pH (1:5 0.01 M CaCl₂), plant available P (Colwell P), texture, total organic C content, calcium carbonate content, maximum water holding capacity, CEC at soil pH, and exchangeable cations,

using methods described in Rayment and Lyons (2010). The physicochemical characteristics and glyphosate residue concentration of each soil are given in Table 1.

Soils were further characterized by X-ray diffraction (XRD) and mid-infra-red (MIR) reflectance spectrometry. X-ray diffraction (XRD) patterns were collected using a Bruker D8 Advance Diffractometer, operating at 40kV with a copper lamp. Scans were collected between 5° and 70° in steps of 0.019°. Qualitative assessment of the diffraction patterns was conducted using DIFFRAC-EVA. Mid-infrared (MIR) analysis of the soils for predictions of particle size and mineralogy were conducted with VIC-DGET using the method and model outlined in the literature (Robinson and Kitching, 2016). All soils were air dried and ground with a mortar and pestle to a fine homogenous texture prior to analysis.

2.2 DGT boundary conditions: effect of pH, P and bicarbonate on DGT accumulation of glyphosate

2.2.1 Experimental setup

The effect of different solution chemistries (pH, phosphate and bicarbonate concentrations) on glyphosate accumulation by DGT was tested to assess the boundary conditions of this technique. Elution recovery of glyphosate for the DGT was measured using a published method (Fauvelle et al., 2015). In brief, three polypropylene tubes were filled with 12 mL of 1 ng mL⁻¹ ¹⁴C-labelled glyphosate (specific activity 7.5 kBq L⁻¹; 95% purity, specific activity 3.7 MBq mL⁻¹, American Radiolabeled Chemicals, Saint Louis, USA) stock solution and one TiO₂ binding gel (see below for specifications) was added to each tube. There was no sorption of glyphosate onto the wall of polypropylene tubes. Sample volumes of 5 mL were taken from the stock solution before and after 24 h shaking to determine the glyphosate concentration. The binding gel was eluted in 1mL of NaOH for 24 h.

For the pH and competition experiments (orthophosphate and bicarbonate), all solutions were made up in 0.01 M NaCl to minimise variability in solutions of low ion strength (Mason et al., 2010) and solution temperature was maintained at 20 ± 2 °C for the duration of the experiments. Solution

samples (2 L) contained a total glyphosate (99% purity, Nufarm Australia) concentration of $50 \mu\text{g L}^{-1}$, which approximates the soil solution concentration derived from a soil concentration of $1000 \mu\text{g kg}^{-1}$ (Farenhorst et al., 2015) and an 'average' equilibrium sorption coefficient (K_d) of 100 L kg^{-1} . Each solution was also spiked with ^{14}C -labelled glyphosate at a rate of 7.5 kBq L^{-1} (Al-Rajab and Schiavon, 2010) to enable quantification of DGT-glyphosate via scintillation counting. To assess the effect of pH on the glyphosate sorption by the DGT binding layer, solution pH was adjusted using 1% HCl and/or NaOH to give five different pH values covering the range of soil pH used in subsequent experiments. These were pH 4, 5.5, 7, 8.5, and 9.

For solution P-competition experiments, we tested a range of phosphate concentrations based on the values of DGT-measured P concentrations (C_{DGT}) in agricultural soils for grain production across Australia: 0, 20, 40, 80, 160, and $300 \mu\text{g P L}^{-1}$ (Mason et al., 2010). For the bicarbonate competition experiment, six NaHCO_3 concentrations (0, 10, 20, 100, 350, 1800 mg L^{-1}), based on the range of NaHCO_3 concentrations used by Mason et al. (2005), were tested for their influence on glyphosate accumulation by DGT. For the P and competition experiments, solution pH was set to 7. For the HCO_3^- competition experiment, solution pH was dictated by the concentration of carbonate.

2.2.2 DGT deployment in solution

DGT samplers with 0.91 mm diffusive layer thickness (diffusive gel plus prefilter) and a TiO_2 binding layer were used (Fauvelle et al., 2015). A poly (ether sulfone) prefilter ($0.45 \mu\text{m}$, 25 mm diameter) was placed on top of the diffusive gel. The cylindrical DGT piston devices had an exposure window of 2.54 cm^2 . The gel solution used for making diffusive gels as well as for the mixed binding layer was composed of 15% (v/v) acrylamide and 0.3 % (v/v) agarose derived cross linker.

The exposure window of the DGT samplers (three replicates) was fully submerged in each solution (2 L) for 24 h while stirring vigorously. Blank DGTs were carried out by submerging DGTs in 0.01 M NaCl (pH 6). At retrieval after 24 h, the DGTs were removed from solutions and washed by a gentle

stream of deionized water. The DGTs were opened, and the binding gel was removed and eluted in 1.9 mL of 0.5 M NaOH for 24 h. The eluates (1.3 mL of NaOH) of each sample received 10 mL Ultima Gold scintillation cocktail (LSC-cocktail) from Packard (USA) in a glass scintillation vial. The radioactivity in the eluates was measured using a liquid scintillation analyzer (Perkin-Elmer-Tri-carb 2910 TR).

2.3 Glyphosate sorption-desorption isotherms

Glyphosate sorption-desorption isotherms were established to provide an estimate of the dissolved fraction of soilborne glyphosate residues at equilibrium. 1 g of each soil was immersed in 5 mL of ^{14}C -enriched glyphosate solution and mixed on an end over end shaker at 25 °C for 24 hours to equilibrate. Soils were UVC irradiated to minimize microbial activity. Six different concentrations of glyphosate (0.5, 1, 2, 4, 8 and 16 mg glyphosate/kg soil) were used to construct isotherms. After equilibration, the soil-glyphosate mixtures were centrifuged at 2600 x g for 20 minutes, and 2 mL of the supernatant removed and passed through a 0.2 µm filter (Merck hydrophilic syringe filter). No adsorption of glyphosate on the filters was found. Each supernatant sample was made up to 20 mL with Opti-Fluor scintillation cocktail (Perkin-Elmer, Melbourne, Australia) in a scintillation vial. The radiation present in each sample was then counted using a scintillation counter (Perkin-Elmer Tri Carb 3110, Australia) and compared to the initial solution concentration to determine sorption. These results were then used to determine the Freundlich sorption coefficients describing the sorption process for each soil, using the equations (Mamy and Barriuso, 2007):

$$x/m = K_{f(a)} C_e^{n(a)}$$

where x/m is the amount of ^{14}C -glyphosate sorbed on the soil (mg glyphosate kg⁻¹ soil), calculated as the difference in concentration difference between the initial glyphosate solution and the corresponding supernatant after equilibration, C_e is the herbicide concentration in the supernatant solution (mg glyphosate L⁻¹), and $K_{f(a)}$ and $n(a)$ are empirical Freundlich adsorption coefficients.

Following the sorption step, the supernatant for each sample was removed and a fresh aliquot of deionized was added, by weight, to re-establish a 1:5 soil : solution ratio. A single desorption step was then conducted at each concentration by shaking for 24 h and then analyzing the supernatant as described above. The amount of herbicide still sorbed on the soils at each desorption stage was calculated as the difference between the initial sorbed amount and the desorbed amount. This single-step 24 h desorption isotherm is referred to as the pseudo-desorption isotherm and was calculated according to Mamy and Barriuso (2007) as:

$$x/m = K_{f(24)} C_e^{n(24)}$$

where $K_{f(24)}$ and $n(24)$ are Freundlich coefficients of the pseudo-isotherm after the first 24 hours desorption. All isotherms were fit in the R software environment (R Core Team, 2016) using the package

2.4 Soil incubation and glyphosate analysis

2.4.1 Experimental setup

Two sets of glyphosate-spiked soil were established, both covering the same range of glyphosate concentrations (0, 0.5, 1, 2, 4, 8 and 16 mg kg⁻¹ soil), covering and extending the range of glyphosate levels detected in agricultural soils (Peruzzo et al., 2008; Primost et al., 2017; Silva et al. 2018). One set was spiked with ¹⁴C-labelled glyphosate for the DGT-glyphosate experiment and the other for glyphosate phytotoxicity bioassay. For DGT-glyphosate experiment, glyphosates-spiked soils were prepared by dissolving unlabeled glyphosate (30 mg L⁻¹) in 0.001 M CaCl₂, spiked with sufficient ¹⁴C-labelled glyphosate (stock 3.7 MBq mL⁻¹) to achieve 30 KBq per treatment per 18 g of oven-dried equivalent soil (Al-Rajab and Schiavon, 2010). To homogenize the glyphosate-soil mixture, 20 g of soil was placed in a polyethylene bag and glyphosate was gradually added to soil by pipetting appropriate volumes of the diluted stock solution to achieve the required soil concentration at 60% of water holding capacity (WHC) of each soil. After each addition, glyphosate-soil mixture was

thoroughly mixed within the plastic bag. Unamended control samples were treated in the same way using 0.001 M CaCl₂ only. Glyphosate-treated soils were incubated at a moisture content of 60% WHC for 28 days before extraction and phytotoxicity bioassays. At this time a subsample (6 g) was analysed for DGT-glyphosate and another subsample (5 g) analysed for total OH-extractable glyphosate and estimation of dissolved OH-extractable glyphosate, as described below

2.4.2 DGT- glyphosate

DGT soil samplers identical to those used for the competition experiment were used for glyphosate sampling. Soil (~ 6 g of dried equivalent soil) was weighed and placed in a 50-mL centrifuge tube cap in triplicate. Water was added to bring the moisture level to 80% of water holding capacity of each soil. The containers were equilibrated for 24 h before deployment of DGT. The exposure window of the samplers was smeared with soil (in order to ensure contact) and pushed gently into the surface of moist soils. DGTs were deployed in soils for 24 h. At retrieval, the DGTs were removed from the soil and any adhering soil washed away by a gentle stream of deionized water. Blank DGTs were also analyzed to confirm the absence of background contamination of glyphosate. The DGTs were opened and the binding gel removed and eluted in 1.9 mL of 0.5 M NaOH for 24 h. The mass of glyphosate, *M*, accumulated in the binding gel was calculated using the following equation:

$$M = C * (V_{\text{acid}} + V_{\text{gel}}) / f_e$$

where *C* is the concentration of glyphosate in the eluted samples (measured by scintillation counting), *V_{acid}* is the volume of acid used for elution and *V_{gel}* is the volume of the binding gel, *f_e* is the elution factor, 0.8, which was determined using an elution recovery test described above. From *M*, the time averaged concentration of glyphosate at the interface of the DGT and the soil, (*C_{DGT}*) can be calculated:

$$C_{\text{DGT}} = M * \Delta g / (D * A * t)$$

where A is the surface area of the DGT sampling window (cm^2), t the deployment time (s), Δg the total thickness of the diffusive gel layer and filter membrane (cm), and D is the diffusion coefficient of the particular analyte in the diffusive gel ($\text{cm}^2 \text{s}^{-1}$). In this case, D is the diffusion coefficient for glyphosate, $2.95 \times 10^{-10} \text{ m}^2 \text{s}^{-1}$ (Fauvelle et al., 2015).

2.4.3 Total and dissolved glyphosate

Isotopically-labelled glyphosate (^{13}C , ^{15}N ; Novachem, Australia) was added to soil (5 g) prior to extraction as a surrogate for calculation of extraction efficiency, and used to calculate total soil concentrations of glyphosate. Extraction was performed by addition of 20 mL 0.6 M KOH, followed by shaking for 45 minutes on an automatic shaker and sonicating for 30 minutes. Samples were subsequently centrifuged at 2000 x g for 10 minutes and the supernatant analysed for glyphosate and aminomethylphosphonic acid (Zhang et al., 2017). Briefly, cleanup was performed on a strong-anion exchange column (Bond Elut SAX, Agilent) before filtering (0.2 μm) into glass vials. Underivatised glyphosate and AMPA were separated on a porous graphitic carbon column (Hypercarb, Thermofisher) and quantified via MS/MS (Micromass, Waters) after comparison with an analytical glyphosate (Novachem, Australia) standard curve, using isotopically-labelled (^{13}C , ^{15}N) glyphosate as an internal standard.

A proxy measure for the equilibrium dissolved glyphosate concentration in soil was estimated from the total glyphosate concentration using previously determined Freundlich pseudo-isotherm coefficients, such that:

$$C_e = ((x/m)/K_{f(24)})^{-n(24)}$$

Although this is not strictly a true estimate of the soil porewater concentration of glyphosate because of the low experimental soil: solution ratio used to calculate sorption coefficients, it provides a relative estimate for comparing the dissolved fractions in different soil types.

2.5 Glyphosate phytotoxicity bioassay

2.5.1 Experimental setup

For the root elongation study, a second batch of soil (100 g per soil type) was prepared in the same way as in the soil DGT study, but was not spiked with ^{14}C -labelled glyphosate. All treatments were equilibrated for 28 d before plant bioassays under the exact experimental conditions as described above for the analytical subsamples. A root elongation assay was performed based on the International Organization for Standardization (ISO) 11269-1 (ISO, 2012). *Triticum aestivum* (cv. Diamond) and *Lupinus angustifolius* (cv. Mandelup) were used in the assay. Soil moisture content was maintained at 60% water holding capacity. Four pre-germinated wheat or one lupin seeds with radicle <2 mm in length were planted in each of three replicated 50-mL centrifuge tubes (35 mm diameter) of each glyphosate treatment for all four soils. Soil was packed into the tubes to the respective bulk density of each soil to a depth of 100 mm. To reduce evaporation from the soils, tubes were loosely capped initially to allow air exchange. All tubes were arranged randomly. After 4 d, plants were removed intact, and the length of the longest root on each plant was recorded. For wheat each replicate value represented mean root lengths of four plants per tube.

2.5.2 Statistical analysis

One-way ANOVA was initially applied to determine the significance of the factor 'glyphosate dose' on lupin or wheat root length for individual soil types. A one-way ANOVA was used (rather than a two-way incorporating 'soil type' as an additional factor) because the incubation of glyphosate in soil for 28 d resulted in different final concentrations of glyphosate in each soil, regardless of the fact that initial spiking doses were the same. Least significant differences at 5 % critical value (LSD) were calculated using the error mean square from each one-way ANOVA. Assumptions of data normality and heterogeneity of residuals were checked and met for each ANOVA.

Subsequently, dose-response data were fitted to a log-logistic curve for each of the soils tested using the following equation:

$$y = c + (c-d) / (1 + \exp\{b(\log(x)-\log(ED_{50}))\})$$

where y = wheat/ lupin root length, x = soil concentration of added glyphosate using LC/MS-MS or DGT-accumulated glyphosate concentration, and b , c , d and ED_{50} are parameters to be fitted. In this case, the lower limit c was set to 0.1, reflecting the fact that all germinated seeds had a measurable root length; and the ED_{50} is the effective concentration of added glyphosate that reduces the root length in the control by 50%. A very small value ($1 \times 10^{-3} \text{ mg kg}^{-1}$) was attributed to the control soil to allow log transformation before curve fitting. Data were analyzed in the R software environment (R Core Team, 2016) using the packages '*agricolae*' (de Mendiburu, 2015) '*drc*' (Ritz et al., 2015) and were graphed using the package '*ggplot2*' (Wickham, 2009).

3. Results and discussion

3.1 Validation of DGT methodology under varying pH, P and bicarbonate concentrations

The elution recovery of glyphosate from DGT was $80 \pm 6\%$ for a 24 h deployment at 25 °C, which aligned with a recent solution study that showed a recovery of 86 % using the same mixing binding layer (Fauvelle et al., 2015). Given the large volume of the donor solution used (*i.e.* 2L) there was no measurable change in activity of ^{14}C -glyphosate in the solutions with varying pH either before or after 24-hour DGT deployment. The accumulation of glyphosate in DGT was not significantly affected by solution pH, indicating that this device is robust across a wide range of potential soil solution pH values (Figure 2, $P > 0.05$). This is consistent and extends previously reported results over a narrower pH range (pH 5-8.5, Fauvelle et al., 2015). For the assessment of the influence of P on DGT-glyphosate binding, the solution was standardised to pH 7.

The accumulation of glyphosate in DGT was not significantly affected by solution P concentrations (Figure 2). These results indicate that DGT can effectively work as an infinite sink for glyphosate when P ranged between 20 and $300 \mu\text{g P L}^{-1}$, supporting the possibility of using DGT for the assessment of both P and glyphosate under the same deployment conditions.

For bicarbonate competition, no effect on DGT-glyphosate retention was detected at lower HCO_3^- concentrations, *i.e.* 10-100 mg L^{-1} (Figure 2). There was a 10% reduction in glyphosate retention in the solution with 350 or 1800 $\text{mg HCO}_3^- \text{L}^{-1}$ ($P < 0.05$, Figure 2). It has been reported that there is a 7 % and 18% decrease in DGT-measured P at 1800 $\text{mg HCO}_3^- \text{L}^{-1}$ when using Ca and Mg as the associated cations, respectively (Mason et al., 2005). Because of the phosphonate moiety on glyphosate, which gives glyphosate the similar binding properties as phosphate, the mechanism behind the competition for binding sites between anions and phosphate may explain the decrease in glyphosate recovery in the presence of bicarbonate (Mason et al., 2010). It should be noted however that in our study we used Na bicarbonate as salt and the solubility of bicarbonate will be much lower in the presence of Ca or Mg as typical in calcareous soils (e.g. the Calcarosol, Table 1). Considering that the range of HCO_3^- in most soils will be lower than the extremes tested here, and that high concentrations of HCO_3^- only lowered glyphosate binding by 10%, we believe any impacts on estimates of glyphosate availability will be negligible.

3.2 Soil properties regulate sorption-desorption and DGT accumulation of glyphosate

Glyphosate sorption was strongest to the clay Vertosol, followed by the Chromosol, Calcarosol and the sandy Tenosol, which displayed the lowest glyphosate binding affinity (Table 2). Pseudo-sorption isotherms, accounting for a single desorption, were similar or lower for the Vertosol, Chromosol and Tenosol, however the Calcarosol displayed a higher $K_{f(24)}$ value than the corresponding sorption coefficient, representative of lower desorption potential (Table 2, Figure S1 of Supporting Information).

The Freundlich sorption coefficients for glyphosate on the soil here fall within the range of those reported for other soils, including $K_f=20-450$ for 16 Swedish soils (Ghafoor et al., 2011) and 32-540 for 17 French soils (Sidoli et al., 2015). Although glyphosate sorption is negatively associated with pH (Gimsing et al., 2004a; Ghafoor et al., 2011; Sidoli et al., 2015) and available P (Sidoli et al., 2015), texture appeared to play an equally important role in regulating sorption in the soils tested here.

For example, although the Tenosol and Chromosol had a lower pH soil than the Vertosol, the sorption capacity was also lower. The dominance of 2:1 clay minerals in the clay Vertosol relative to the mineralogy of the other soils (Table S1) would contribute to the greater glyphosate sorption capacity in this soil type (Waiman et al., 2016), particularly compared to the quartz-dominated Tenosol. Significant levels of Al and Fe oxides, protonated at low pH, would have contributed to the moderate binding capacity of the Chromosol (Waiman et al., 2016; Gerritse et al., 1996). Of particular interest is the sorption-desorption behaviour of the Calcarosol. This soil had a relatively low sorption capacity, concomitant with a high pH, but demonstrated an elevated pseudo-sorption isotherm, suggesting reduced desorption. Of the four soils, the Calcarosol has the highest Ca content ($33 \text{ cmol (+) kg}^{-1}$) and exchangeable calcium (78%) and X-ray diffraction analysis (Table S1) showed that calcite is the most dominant mineral. The glyphosate desorption pattern is somewhat similar to the behaviour of P in calcareous soils, where the poor solubility of the precipitated calcium phosphates can restrict P release into solution (Delgado et al., 2002). The possibility of glyphosate precipitation in calcareous soils, in addition to surface sorption, has not to our knowledge been directly investigated despite being alluded to previous research (Bott et al., 2011; Munira et al., 2016) and may deserve more attention.

Following the 28d incubation under warm moist conditions, total glyphosate concentrations, measured via hydroxide extraction and IS-corrected LC-MS/MS quantification, were significantly lower than the initial corresponding concentrations directly following spiking (Figure 3A). Recoveries were highest for the Calcarosol, followed by the Tenosol, Chromosol and Vertosol. The low recovery for the Chromosol and Vertosol (< 50% of the applied concentration) implies considerable degradation in these soils. Indeed, other experiments in our laboratory (Rose et al., 2018) have demonstrated higher baseline microbial activity in these two soils, concomitant with their higher organic carbon levels, compared with the Calcarosol and Tenosol. Nevertheless, sorption reactions would also play a role in regulating both bioavailability (for degradation) and chemical extractability of the remaining glyphosate.

To account for this, we estimated the relative dissolved concentration in each soil by adjusting total measured concentrations using the specific soil pseudo-adsorption coefficients previously calculated. In contrast to the total glyphosate concentrations measured, a greater amount of dissolved glyphosate was predicted for the Tenosol than the Calcarosol, whilst lower relative dissolved concentrations remained for the Chromosol and Vertosol (Figure 3B). This pattern of glyphosate availability in each soil was similar to that of the DGT-glyphosate: the time-averaged concentration of glyphosate at the interface of the DGT and the soil was consistently higher in the Tenosol than in the other soils at all spiking doses of glyphosate, with DGT-glyphosate ranging broadly between 0.13-6.52 $\mu\text{g L}^{-1}$ (Figure 3C). For the rest of the soils, the DGT -glyphosate concentrations decreased in the following order: Calcarosol > Chromosol > Vertosol, and ranged between 0.05-1.65, 0.13-3.83 and 0.02-0.89 $\mu\text{g L}^{-1}$ for each soil, respectively (Figure 3C)

3.3 Glyphosate phytotoxicity relates to DGT-glyphosate concentrations across soil types

It is generally accepted that glyphosate is deactivated when it reaches the soil and so poses little risk to crops (Duke and Powles, 2008). However, under certain circumstances phytotoxicity from soilborne glyphosate residues has been observed (Bott et al., 2011; Cornish, 1992). In our root elongation assay, glyphosate dose only had a significant effect ($P=0.04$) on lupin root length in the Tenosol, with the two highest doses (2.6 and 5.0 mg kg^{-1} glyphosate) reducing root elongation by approximately 32-36%. Glyphosate dose did not significantly affect wheat root elongation in any of the soil types (Table 3).

The difference in sensitivity between the two crops is in agreement with a recent study which established dose response curves for wheat and lupin shoot biomass (42 d growth) in a sandy soil treated with glyphosate one month before sowing (Rose et al., 2018). In that study, the effective dose for 10% shoot biomass reduction (ED10) for lupin was estimated to occur at 6.8 $\text{mg glyphosate kg}^{-1}$ soil, whereas concentrations up to 14.8 mg kg^{-1} had no significant effect on wheat shoot biomass (Rose et al., 2018).

Based on the significant effect of soilborne glyphosate dose on lupin root length detected by ANOVA, log-logistic regressions for both crop species were fit by plotting normalized root length data against total extractable glyphosate or DGT-extractable glyphosate. Although normalization of root length data obstructs the determination of log-logistic parameter standard error estimates (Knezevic et al., 2007), this analysis was performed to compare the utility of DGT-extraction versus conventional ‘total’ extraction for estimating dose-response relationships across soil types.

Model fits of normalized lupin root length against DGT-extractable glyphosate provided lower root mean square error than model fits against total extractable glyphosate (Figure 4A and 4B), suggesting that DGT is able to assess the glyphosate bioavailability and toxicity to these crops across a range of soil types. This was supported by the fact that collapsed dose-response curves for wheat across four soils could not be derived using total extractable glyphosate concentrations or spiked glyphosate concentrations as the explanatory variable (Figure 4C; Figure S1); whereas the hypothesis of a log-logistic response fit against DGT-extractable glyphosate (Figure 4D) was not rejected by lack-of-fit F test (Ritz et al., 2015). Importantly, the lowest glyphosate recovery by DGT was 100 ng glyphosate per DGT sampler over 24 hours, recovered from the Vertosol spiked at 0.5 mg kg^{-1} . Following elution with 2 mL of NaOH, the sample concentration provided by DGT extraction was 50 ng mL^{-1} , which is well above the limit of detection of most current routine analytical methods (Koskinen et al., 2016). Thus the DGT method presented here will be suitable for detecting agronomically relevant soilborne glyphosate concentration greater than 0.05 mg kg^{-1} .

3.4 Implications for assessment of bioavailable glyphosate in the presence of P fertiliser

We have demonstrated the potential for the DGT technique to quantify glyphosate availability in different soils, and that the technique is robust across a range of soil pH, soil P and bicarbonate concentrations.

The ability of DGT-glyphosate to estimate the soil solution glyphosate concentration was thus supported by a strong correlation with the predicted dissolved fraction (adj. $r^2 = 0.95$), compared to

a weaker correlation with the total extractable glyphosate concentrations (adj. $r^2 = 0.61$) across soil types (Figure 5). Presumably the greater (pseudo)sorption capacity of glyphosate in the Calcarosol than the Tenosol soil resulted in lower accumulation by DGT, even though higher total concentrations were present.

The thresholds for significantly impaired root growth in this study were generally higher than the maximum concentrations of soilborne glyphosate of 2-8 mg kg⁻¹ recently measured under field conditions (Peruzzo et al., 2008; Primost et al., 2017; Silva et al. 2018), except for lupin growing in the sandy Tenosol, supporting the view that under most conditions glyphosate residues pose a low risk to plant growth (Duke and Powles, 2008). However, it is known that certain circumstances, such as P fertilisation, can result in remobilisation and increased glyphosate availability in soil (Delgado et al., 2002; Sasal et al., 2015; Bott et al., 2011; Beltrano et al., 2013). Rose et al. (2018) observed that superphosphate banded under lupin seeds at sowing reduced lupin seedling shoot growth by over 60% in soil containing 1.3 mg kg⁻¹ of residual glyphosate in the top 0-10 cm soil profile. Based on these findings, more research is required to assess the validity of DGT methodology for estimating bioavailable glyphosate in the presence of P fertilizer, and the consequences for crop growth on sandy soils. In addition, there is some evidence that the presence of inorganic phosphate inhibits degradation of glyphosate by some bacteria (Newman et al., 2016). The World Health Organisation (WHO) recommends that the effects of phosphate fertilisers on the binding of glyphosate to soils should be investigated (Gomes et al., 2016). The results presented here suggest that DGT can have the potential to predict the phytotoxicity of glyphosate as a result of the interactions between glyphosate and P fertiliser in soil (Tejedor-Tejedor and Anderson, 1990; Persson et al., 1996; Rahnemaie et al., 2007).

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Figure captions

Figure 1: Schematic representation of the experimental design.

Figure 2: Effect of solution pH, P and bicarbonate concentration on the ratio of DGT measurements (C_{DGT}) with direct measurements of solution concentrations (C_{Sol}). Solution was sampled before and after 24 hour incubation and no difference in ^{14}C activity in solution was found. Solution pH was fixed at pH 7. Grey points are actual data, black points are average values, and error bars are standard error.

Figure 3: Total (A), dissolved (B) and DGT-extractable (C) glyphosate concentrations in different soils, 28 d after spiking with a range of glyphosate concentrations.

Figure 4: Dose-response of wheat and lupin to measurable glyphosate concentrations across four contrasting soil types. (A) and (C) show the response of lupin and wheat, respectively to total measured glyphosate concentrations; whereas (B) and (D) show the response to DGT-measured glyphosate concentrations. Each point represents the mean of three replicates. Solid lines show best-fit sigmoidal response curves, with the shaded area representing 95% confidence regions. RMSE = root mean squared error. Note that no fit could be obtained for wheat response against total measured glyphosate concentrations.

Figure 5: Correlation of DGT-extractable glyphosate with the A. total OH-extractable (adj. $r^2=0.61$) and dissolved OH-extractable (adj. $r^2=0.95$) glyphosate. Figure S1: Dose-response of wheat and lupin to the spiked glyphosate concentration across four contrasting soil types. (A) shows the response of lupin and (B) shows the response of wheat. Solid lines show best-fit sigmoidal response curves, with the shaded area representing 95% confidence regions. RMSE = root mean squared error. Note that no fit could be obtained for wheat response against total measured glyphosate concentrations.

Table 1: Soil physical and chemical properties

Sample ID	Unit	Tenosol	Chromosol	Vertisol	Calcarasol
EC	dS/m	0.036	0.34	0.12	0.25
pH (CaCl ₂)	pH units	5	4.7	5.7	7.9
pH (Water)	pH units	5.8	5.2	6.7	8.6
Sulfur (KCl40)	mg/kg	5.2	30	8.4	12
Colwell Phosphorus	mg/kg	5.8	150	83	39
Phosphorus Buffer Index _{+Col P}	L/kg	15	60	110	210
Organic Carbon	%	0.24	2.3	1.5	2.8
Total Nitrogen	%	0.03	0.25	0.15	0.16
Total Organic Carbon	%	0.3	2.8	2	3
Texture					
Sand	%	85.1	45.3	38.8	44.9
Silt	%	2.74	26.3	13.8	7.86
Clay	%	12.5	24.1	40.0	15.4
Exchangeable Cations					
Aluminium	cmol(+)/kg	0.15	0.15	<0.1	<0.1
Calcium	cmol(+)/kg	0.98	6.8	23	21
Potassium	cmol(+)/kg	0.15	2.1	0.99	1.7
Magnesium	cmol(+)/kg	0.33	1.5	17	3.9
Sodium	cmol(+)/kg	0.071	0.14	1.5	0.51
CEC	cmol(+)/kg	1.7	11	42	27
Calcium/Magnesium Ratio		3	4.5	1.3	5.4
Aluminium Saturation	%	9.2	1.4	<0.1	<0.1
Exchangeable Calcium	%	58	64	54	78
Exchangeable Potassium	%	8.8	20	2.3	6.2
Exchangeable Magnesium	%	19	14	40	14
Exchangeable Sodium	%	4.2	1.4	3.7	1.9
DTPA Micronutrients					
Copper	mg/kg	0.11	2.1	1.9	0.15
Iron	mg/kg	79	140	33	6.7
Manganese	mg/kg	0.76	61	53	5
Zinc	mg/kg	0.3	4.2	2.5	1.1

Table 2: Freundlich sorption and pseudo-sorption coefficients for glyphosate on various soils. Values in brackets are 95% confidence intervals

Soil	Sorption			Pseudo-sorption		
	$K_{f(a)}$	$n(a)$	r^2	$K_{f(24)}$	$n(24)$	r^2
Calcarosol	45 (26-76)	1.00 (0.86-1.21)	0.99	69 (41-119)	0.97 (0.84-1.14)	0.99
Chromosol	89 (58-135)	0.95 (0.85-1.07)	0.99	77 (38-159)	0.95 (0.79-1.18)	0.98
Tenosol	26 (13-51)	0.96 (0.77-1.30)	0.97	34 (7-164)	1.00 (0.66-2.06)	0.88
Vertosol	227 (97-531)	0.88 (0.74-1.08)	0.99	90 (23-352)	0.96 (0.7-1.51)	0.94

Table 3: Root length of wheat and lupin exposed to increasing concentrations of glyphosate in different soil types.

Calcarosol			Chromosol			Tenosol			Vertosol		
Total Gly. (mg kg ⁻¹)	Root length (cm)		Total Gly. (mg kg ⁻¹)	Root length (cm)		Total Gly. (mg kg ⁻¹)	Root length (cm)		Total Gly. (mg kg ⁻¹)	Root length (cm)	
	Lupin	Wheat		Lupin	Wheat		Lupin	Wheat		Lupin	Wheat
0.0	4.5	4.7	1.8	4.9	3.5	0.0	4.1	4.7	0.0	6.4	8.5
0.6	4.4	4.5	2.2	4.2	3.4	0.2	4.0	4.6	0.4	7.2	8.6
0.9	4.5	4.3	2.4	4.0	3.5	0.6	4.2	4.6	0.6	6.5	8.6
1.6	4.4	4.3	2.7	4.1	3.6	0.8	3.3	4.4	1.0	6.0	8.3
3.7	3.8	4.2	3.2	4.1	3.6	1.5	3.3	4.0	1.0	6.3	8.5
6.9	3.9	4.0	3.5	4.3	3.5	2.6	2.6	3.9	1.3	6.2	8.3
7.7	3.9	3.8	4.5	4.0	3.4	5.0	2.8	3.2	2.7	5.9	8.4
LSD	1.2	1.3	LSD	1.3	1.3	LSD	1.1	1.5	LSD	1.2	1.3
Pr(>F)	0.659	0.83	Pr(>F)	0.75	1	Pr(>F)	0.04	0.38	Pr(>F)	0.36	0.99

Highlights

- A DGT method was developed to assess the available glyphosate
- A threshold toxicity of glyphosate to wheat and lupin was quantified across four soils
- This technique can be used in the risk assessment of herbicide residues in soils.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Accepted Manuscript

Assessing plant-available glyphosate in contrasting soils by diffusive gradient in thin-films technique (DGT)

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