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Development and Evaluation of a New Colorimetric DGT Technique for the 2D Visualisation of Labile Phosphate in Soils

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Abstract

A new colorimetric technique for the measurement of labile phosphate in soils using the diffusive gradients in thin films (DGT) technique was developed in this study. This technique can determine the mass of phosphate accumulated on the precipitated Zr-oxide based binding gel by forming the blue colour following the standard molybdate-ascorbic acid method. The optimal reaction temperature and coloration time were 20ºC (room temperature) and 26 minutes. After determining a well-fitted calibration equation, the technique was able to measure phosphate concentration up to 2.5 mg/L for 24 h deployment with a detection limit of 10.1 µg/L. Two-dimensional quantitative visualisation of phosphate diffusion in three phosphorus (P) fertilised soils were obtained using the colorimetric technique. The results from the colorimetric DGT technique were compared to the elution DGT technique and Colwell P extraction. The DGT techniques (colorimetric and elution) and Colwell P measurements demonstrated similar patterns of phosphate diffusion in soil. Both DGT techniques showed similar phosphate concentration along the concentric rings around the fertiliser application. A new, convenient, and fast DGT colorimetric technique was developed, and successfully used to measure the distribution of potentially available phosphate in soils. The new technique is less laborious than current techniques as it does not require any pre-treatment of the binding gel layers or heating during scanning, thus providing faster results. Therefore, the technique may be more suitable for in-field applications and can be used to investigate the *in situ* diffusion of potentially available phosphate from fertilisers, and relate this to the plant uptake of P.

Keywords: diffusive gradients in thin films; colorimetric staining technique; elution technique; fertilised soils; two-dimensional measurement

1. Introduction

Phosphorus (P) is often a limiting nutrient for crop production. In Australia, most soils are deficient in P, and farming therefore relies on the use of P fertilisers (Lombi et al., 2005; White et al., 2010). Granular fertilisers, such as superphosphates and monoammonium phosphates (MAP), are commonly used for improving crop growth (Australian Bureau of Statistices, 2018). However, the mobility, and thus plant availability, of fertiliser P can be significantly reduced by adsorption and various precipitation reactions in soil (Coleman et al., 1960; Murrmann and Peech, 1969; Penn and Camberato, 2019). In fact, less than 5% of applied P may be taken up in the year when it is applied (Dorahy et al., 2005). However, over longer time frames it has been suggested that the uptake of residual P can increase to over 70% (Syers et al., 2008; Dhillon et al., 2017), where climatic and soil factors influence fertiliser efficiency. Band application of P fertilisers is often preferred as it is more efficient than broadcast application, minimises the tie-up of P, provides early growth stimulation and eliminates the adverse effects from concentrating the fertilizer too near the seed (Randall and Hoeft, 1988). Determining the diffusion behaviour of banded P fertilisers in various soil types can be used to evaluate new fertiliser formulations and to help optimise soil management practices as higher P diffusion indicates that less fixation has occurred (e.g. Lombi et al., 2005).

To investigate fertiliser P diffusion in soil, past studies have typically collected and analysed soil at varying distances (and incubation times) from the point of P fertiliser application. Various methods have been used to make these measurements and are summarised by Degryse and McLaughlin (2014). The same authors developed a high-resolution visualisation technique for P fertiliser diffusion in soils using an iron (III) oxide impregnated paper. The iron (III) oxide impregnated paper (P_i test) was first developed by Sissingh (1983) and modified by Menon et al.

(1989). In this test, iron (III) oxide impregnated paper was added to the $CaCl_2$ soil extraction then eluted in an acidic solution and the amount of P measured. Degryse and McLaughlin (2014) modified this technique by placing the iron oxide impregnated paper in direct contact with the soil surface then visualising P distribution using a modified malachite green method. This method provided very useful insights into the diffusion of P from several fertiliser formulations across a range of soil types. The results were comparable to chemical extraction methods (0.5 mol/L $CaCl_2$) where the soils were sampled in concentric circles around the granule. However, the visualisation method produces images with varying colours due to the background colour of the malachite green method. Also, the iron (III) oxide impregnated paper may be stained by soil particles, limiting the precision of P measurements by this method. Other researchers have used this technique to visualise the P diffusion of different fertilisers in soils, but not to accurately quantify P concentrations at different distances from the granule (McLaren et al., 2016; Degryse et al., 2017; Everaert et al., 2017; Andelkovic et al., 2018; Lustosa Filho et al., 2019).

High-resolution visualisation techniques have been used in biogeochemical sediment studies for a number of years to interpret two-dimensional data obtained using passive sampler techniques (Teasdale et al., 1999; Robertson et al., 2008; Pagès et al., 2011; Ding et al., 2013; Bennett et al., 2015; Guan et al., 2015; Liu et al., 2020). The DGT (diffusive gradients in a thin-film) technique (Davison and Zhang, 1994), combines a hydrogel diffusional layer and a binding layer that uses mineral phases (including iron(III) oxide and zirconium hydroxide), resins or membranes to accumulate solutes of interest to allow sensitive determination (Zhang et al., 1998; Ding et al., 2013; Guan et al., 2015; Davison, 2016; Huang et al., 2016; Zhu et al., 2019). A related method, the DET (diffusive equilibrium in a thin-film) passive sampler technique uses a single hydrogel

layer (Davison et al., 1991). Both passive sampler techniques colorimetric DET and DGT techniques have been described for labile phosphate (PO_4-P), iron(II), sulfide and ammonium for sediment studies (Teasdale et al., 1999; Pagès et al., 2011; Ding et al., 2013; Bennett et al., 2015; Kankanamge et al., 2017; Metzger et al., 2019; Kankanamge et al., 2020; Liu et al., 2020). The first study of two-dimensional DGT in soil was presented by Santner et al. (2012) using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to determine the distribution of PO_4-P around plant roots on a hydrogel containing ferrihydrite. However, using LA-ICP-MS can be quite expensive ($50-100 per hour), time-consuming (measuring 8 cm^2 of a sample in 4 hour with a scanning speed of 200 µm s^{-1}) (Santner et al., 2010; Kruse et al., 2015) and, the PO_4-P binding capacity of a ferrihydrite gel layer is lower than other binding materials (Bennett et al., 2010; Ding et al., 2010; Guan et al., 2015). More recently, Ding et al. (2013) developed a colorimetric technique using DGT for measuring PO_4-P in sediment porewater. However, this technique is limited by its slow processing time as a 5-day heat pre-treatment (80°C) of binding layers is required and colour development requires gel incubation for 45 min at 35ºC.

The aim of our study was to develop and evaluate a new and fast colorimetric DGT technique with a thin diffusive layer and a high binding capacity to accurately visualise and quantify labile PO_4-P concentrations in soils using Computer Imaging Densitometry (CID). The visualisation of data can be a very effective way to engage and inform end users about the new fertiliser formulations and the optimum soil management practices. Also, it was important to develop a DGT that was easy to handle, could be manipulated without tearing, and could be prepared in various shapes and sizes. For these reasons and for the first time, we prepared a membrane-based binding layers for the 2D quantitative visualisation of phosphate diffusion from fertilisers in soil. Experiments were

carried out to: a) develop membrane-based zirconia (Zr)-oxide binding and diffusive gel layers (Zr-oxide was chosen due to its high binding capacity (Ding et al., 2010; Ding et al., 2013; Guan et al., 2015); b) determine the optimal reaction temperature and coloration time for the colorimetric technique; c) compare the efficacy of the new colorimetric DGT technique to elution DGT techniques; and, d) investigate the diffusion of phosphate away from granular P fertilisers in soils.

2. Methodology

2.1. Materials, reagents and solutions

AR grade chemicals reagents were used in all experiments and all solutions were prepared using ultrapure deionised water (18.2 MΩ.cm, Milli-Q Advantage 10, Millipore). The detail of materials, reagents and solutions used in this study are presented in Supporting Information (SI) 1.1.

2.2. Gel layer preparation

2.2.1. Preparation of membrane-based diffusive gel and Zr-oxide binding gel

Membrane-based diffusive and binding gel layers were used for their structural integrity; a requirement of these experiments was that the gel layers needed to be cut into different sizes and easily manipulated without tearing.

The bis-acrylamide gel stock solution was prepared according to previous studies (Robertson et al., 2008; Bennett et al., 2012). Membrane-based bis-acrylamide diffusive gels were then prepared by soaking PES filter membranes (25 mm and 76 mm) in the bis-acrylamide gel stock solution for 10-15 s. The membranes were then placed flat between two plastic sheets (making sure to remove air bubbles) and left to polymerise at room temperature for 40 min. The polymerised diffusive gels

were then rinsed three times with ultrapure deionised water within 24 h before storage. As a result, diffusive gel discs of 0.2 mm thickness were obtained, which was measured using an electronic stainless-steel micrometer (Mahr, TESA, Mitutoyo, Sylvac). The diffusive gel discs were stored in 0.01 mol/L NaCl before deployment.

The Zr-oxide binding layers were prepared according to Guan et al. (2015) with some modifications. Specifically, the membrane-based bis-acrylamide diffusive gel discs were immersed in a solution of 32.2 g/L of $ZrOCl_2 \cdot 8H_2O$ for 2 h, followed by immersion in MES monohydrate (10.66 g/L) for 40 min at pH 6.7 (pH adjusted using 0.1 mol/L NaOH). Precipitated Zr-oxide binding gel discs of 0.2 mm thickness were obtained.

2.2.2. Preparation of staining gel

The bis-acrylamide staining gels (15 cm×20 cm) – on which the binding gels were placed after DGT deployment – were prepared according to Bennett et al. (2012) and Pagès et al. (2011). The staining gels were soaked in a colour reagent, which was prepared based on the standard molybdite-ascorbic acid method (APHA P-E), for 1.5-2 h before use (Lin and Wu, 1996; Pagès et al., 2011).

2.3. Assembly and validation of DGT with membrane-based binding layer

2.3.1. Assembly of solution and soil standard DGT samplers

In this study, two types of standard DGT sampler were used for the method development, including solution DGT with an exposed surface area of 3.14 cm^2 and soil DGT with an exposed surface area of 2.54 cm^2. The DGT samplers were assembled using three different layers, from top (i.e. in

contact with soil) to bottom: a 0.45 μm PES membrane filter (0.1 mm), a bis-acrylamide gel (0.2 mm) and a Zr-oxide binding gel (0.2 mm). To ensure the gel assembly did not move within the DGT device, a 0.8 mm bis-acrylamide gel layer was placed under the Zr-oxide binding layer followed by a plastic cap placed on the top layer.

2.3.2. DGT calculations

The DGT-measured concentration (C_{DGT}: ng/mL=μg/L) of PO_4-P in solution and soil was calculated from the accumulated analyte mass using the DGT equation (Eq. 1) (Zhang and Davison, 1994),

$$c_{DGT} = \frac{M * \Delta g}{A * D * t} \quad (1)$$

where M is the accumulated mass of PO_4-P during the deployment time (ng), Δg is the thickness of the diffusive layer (cm), D is the diffusion coefficient for PO_4-P through the diffusive layer ($1.74 \times 10^{-6} cm^2/s$), t is the deployment time (s), and A is the area of the gel in contact with the solution or soil (cm^2).

2.3.3. Mass accumulation over time and diffusion coefficient

Solution DGT devices were deployed in 10 L of 1 mg PO_4-P/L solution with 0.01 mol/L $NaNO_3$. The solution was prepared 24 h before DGT deployment to allow the solution to equilibrate in the container. DGT devices were deployed for 4, 8, 12, 24 and 48 h (n=5). 10 mL aliquots of the phosphate solution were collected before the deployment and after the removal of DGT devices.

174 pH, electrical conductivity and temperature were also measured before the DGT devices were
175 deployed and after they are removed.
176
177 After removing the DGT probes from solutions, they were rinsed with deionised water and stored
178 at 4°C in sealed plastic bags until analysis. For the five DGT replicates deployed in 1 mg PO_4-P/L
179 solution, three were used for the colorimetric determination of PO_4-P concentrations (see section
180 2.4), and the other two were analysed using elution techniques. For the elution, DGT devices were
181 eluted with 0.5 mol/L NaOH using the reported elution efficiency of 96.2% (Guan et al., 2015),
182 then diluted with deionised water (1-100 times) before reactive phosphate analysis (molybdite-
183 ascorbic acid method) using a Skalar segmented flow analyser (Scan++, Skalar).
184
185 The diffusion coefficient, *D*, of PO_4-P from the membrane-based diffusive gel layer was based on
186 Eq. 2 (SI), which is determined from mass accumulation over time experiments as described
187 previously (Zhang and Davison, 1995).
188
189 2.4. Validation of the colorimetric method
190 2.4.1. Determining the optimum staining time and temperature
191 The membrane-based Zr-oxide binding layers, part of the DGT devices, were deployed in 10 mL
192 of 13 different PO_4-P concentrations (KH_2PO_4, Chem Supply), ranging from 0 to 50 mg/L for 24
193 h (n=6). After that, the membrane-based Zr-oxide binding layers were removed from solutions and
194 placed on staining gels to develop colour (see section 2.6). To determine the optimum staining
195 time, images were scanned after a 2 min reaction time, then every 3 min for up to 2 h. Two reaction
196 temperatures of 20ºC (room temperature) and 40ºC (using a laboratory oven, 5 ºC higher than Ding
197 et al. (2013)) were selected, in order to determine the optimum temperature.

198

199 2.4.2. Calibration curve and binding capacity

200 Solution DGT devices were deployed in nine different PO_4-P concentrations, ranging from 0 to
201 6.9 mg/L in 10 L of 0.01 mol/L $NaNO_3$ solutions. The solutions were prepared 24 h before DGT
202 deployment. Five DGT devices were deployed in each concentration for 24 h. 10 mL of the
203 phosphate solution were collected before the DGT devices were deployed and after they were
204 removed. Additionally, pH, electrical conductivity and temperature were measured before the
205 DGT devices were deployed and after they are removed. After removing the DGT probes from
206 solutions, they were rinsed with deionised water. Among the five DGT samplers, three of them
207 were used for colorimetric development to determine the grayscale intensity (GSI) values by
208 placing the membrane-based Zr-oxide binding layers on staining gels for scanning. The other two
209 gels were analysed using elution techniques to measure the PO_4-P mass on the binding layers and
210 the eluents were measured by Scan++. A calibration curve was determined by plotting the
211 grayscale intensity values (n=3) versus the different PO_4-P concentrations ($\mu g/cm^2$) measured
212 using the elution method (n=2). The calibration curve allowed us to determine the most suitable
213 PO_4-P concentrations for our subsequent experiments. This experiment also allowed us to
214 determine the binding capacity of Zr-oxide to be determined (see section 3.3).

215

216 2.5. DGT deployment in soils

217 2.5.1. Deployment in soils amended with P fertiliser

218 After validating the colorimetric technique, modified DGT devices were used to investigate the
219 diffusion of PO_4-P from a granular phosphate fertiliser in soil (Figure S1 in SI). Three different
220 Australian soils were used for this experiment: 1) a calcareous clay loam from South Australia
221 (SA), 2) a clay cropping soil from New South Wales (NSW) and 3) a clay ferrosol from

Queensland (QLD). The pH, EC, phosphate concentration, phosphorus buffering index (PBI), and cation concentrations varied between each soil (Table S1).

In this experiment, much larger (76 mm diameter) membrane-based bis-acrylamide diffusive gel layers and precipitated Zr-oxide binding layers were prepared. The experimental set-up was based on the method described previously by Lombi et al. (2004 and 2005). Where 75 g (dry weight) of each soil was weighed into a 76 mm diameter petri dish to give a soil density of 1.06 g/cm^3 for the SA soil, 1.02 g/cm^3 for the QLD soil, and 1.29 g/cm^3 for the NSW soil. Soils were brought to 80% MWHC, using deionised water, then incubated for 24 h. Six petri dishes were prepared for each soil, where two were used as a blank (no fertiliser added), and one P fertiliser granule added to each of the other four dishes. The monoammonium phosphate (MAP) granule (55 ± 3 mg) with 11 mg of P per dish was placed on top and in the middle of the soil, then pushed down (5 mm) to half the soil depth using a pin (Lombi et al., 2004; Lombi et al., 2005). Then the petri dishes were sealed with parafilm and incubated for 35 days at 25°C.

After the incubation, the membrane-based bis-acrylamide diffusive layers (which were the same size as the petri dish), were laid directly on top of the soil, followed by the Zr-oxide binding layer. To ensure that there was no air gap between the soil and membrane-based bis-acrylamide diffusive layers, and between the membrane-based bis-acrylamide diffusive layers and the Zr-oxide binding layer the upturned lid of the petri dish was placed on top of the gel layers. This facilitated complete contact between the soil and the gel layers and maintained the gel layer moisture for the 24 h deployment time (Figure S1). Nine of the DGT samplers were analysed using the novel colorimetric visualisation technique while the rest were measured using the elution and analysis

technique. The binding layers measured by the elution technique were sliced into four concentric rings (centred on the fertiliser granule) using stainless steel cylinders with different diameters to investigate the diffusion of PO_4-P away from the fertiliser granule in the different soil types. The ring diameters, from the innermost ring to the outermost, were 14, 30, 56, and 76 mm (Figures 2).

After removing the DGT from the petri dish, four concentric rings of soil – having the same diameters as the sliced DGTs – were collected from the petri dish. The same cylinders were pushed into the soil and the soil scooped out of each concentric ring using a plastic spatula. Soils were then dried at 60°C for 5 d and the Colwell P extraction technique (Colwell, 1963) was used to determine the PO_4-P concentration in each soil ring. Briefly, 0.5 g of soil was extracted with 50 mL of 0.5 mol/L bicarbonate ($NaHCO_3$) in a soil:solution ratio of 1:100, then shaken on an end-over-end shaker for 16 h. Phosphorus concentrations in the extractant were then measured using Scan++.

Finally, the results provided by the colorimetric technique were compared to those obtained from the standard elution technique and the Colwell P method. In addition, after DGT deployment, the fertiliser granules were removed from the soil using tweezers and dried at 60°C for 5 days. Adhered soil was gently removed using a brush and the granule weighed. The differences in PO_4-P diffusion from the fertiliser in the three soils were evaluated.

2.5.2. Deployment in unamended soils with low concentration of available P

Soil DGT devices were deployed in three contrasting soils collected from Western Australia; they were named as Kalannie (sandy loam), Condering (silt) and Jennacubine (sandy). The soils differed in their pH, phosphate buffering index (PBI), exchangeable iron and aluminium content,

and organic carbon content (Table S2). 50 g (dry weight) of each soil was weighed into a petri dish (76 mm diameter) with three replicates per soil. 24 h before DGT deployment, deionised water was added to each dish to bring the soil to its maximum water holding capacity (MWHC) (Mason et al., 2008). Two DGT probes were deployed in each petri dish i.e. a total of six DGT probes were deployed in each soil. After deployment for 24 h, the DGT devices were rinsed with deionised water to remove any residual soil, then disassembled for analysis. One DGT from each petri dish was analysed using the colorimetric technique, and the other analysed using the elution technique. Results from the colorimetric method (C_{DGT-C}) and elution method (C_{DGT-E}) were then compared.

2.6. Image scanning to determine PO_4-P concentration and distribution

Following 24 h deployment, the membrane-based Zr-oxide binding layers were placed on a 0.2 mm plastic spacer. The staining gels were laid on another 0.2 mm plastic spacer and excess reagent was wiped off to ensure that clear images were taken by the scanner. Then, the staining gel was placed on top of the membrane-based Zr-oxide binding layers to develop the blue colour. The intensity of the blue colour was measured using a CanoScan 9000F Mark II scanner at a resolution of 300 dpi, corresponding to a pixel size of 169 × 169 μm. The images were scanned for 26 minutes at 20ºC (room temperature). Then the scanned images of the coloured binding gels were formatted using GIMP 2.8.22, and ImageJ 1.51K1 software was used to produce/calculate the grayscale intensity in each Zr-oxide binding layer. All collected data were then analysed using Microsoft Excel 2016. For each PO_4-P concentration, the calibration curve was applied, the reaction time, temperature and average grayscale intensity for three replicate discs were calculated.

291 To quantify the two-dimensional (2D) diffusion of PO_4-P away from the fertiliser granule in each
292 soil (SA, NSW, and QLD), GIMP 2.8.22 and ImageJ 1.51K1 software were used following the
293 procedure described by Kankanamge et al. (2020) with some modification as described in the SI
294 1.4.
295

296 ## 3. Results and discussion

297 ### 3.1. Optimum staining time and temperature

298 The results indicate that the colour intensity was stable after approximately 38 minutes at 20ºC,
299 and 26 minutes at 40ºC (Figures S2 and S3). As expected, the higher temperature increased the
300 reaction rate meaning colour development was more rapid at 40ºC. According to Bennett *et al*.
301 (2012), the most appropriate staining time is when the grayscale intensity of each concentration
302 exceeds at least 80% of the highest intensity value during the colour development period, which
303 provides suitable colour development for scanning and also minimise diffusive relaxation.
304 Therefore, the optimal staining time was 26 minutes at 20ºC and 20 minutes at 40ºC, although we
305 note that if greater sensitivity is required these times can be extended. In the subsequent
306 experiments, 26 minutes at 20ºC was selected as the preferred staining time and temperature.
307 Additionally, Ding et al. (2013) reported a significant edge effects after staining without heat
308 treatment where there was a gradual change of the blue colour on the blank gel layer from the
309 sliced lateral edge to the center. Our results also show this edge effect, but the effect was not
310 significant (Figures S3 and S4). Our study shows that the differences between the center and the
311 lateral edge are less than 12 GSI while the difference was more than 50 GSI in Ding's study without
312 heat treatment. To avoid edge effects, the edge of the images should not be captured during image
313 and data processing.

314 3.2. Mass accumulation of labile P over time and determination of the diffusion coefficient

315 The mass of PO_4-P adsorbed to the membrane-based Zr-oxide binding layer increased linearly
316 over the 48 h deployment with an R^2 of 0.98 (Figure S5). Using Eq 2 from SI, the effective
317 diffusion coefficient of 1.74×10^{-6} cm^2/s for PO_4-P was obtained from the linear regression line
318 at 21ºC. The diffusion coefficient of PO_4-P from this study was only 32% of the diffusion
319 coefficient determined using an open diffusive gel layer (polyacrylamide with agarose cross-
320 linker) from DGT Research (5.42×10^{-6} cm^2/s at 21ºC) (Zhang et al., 1998; Price et al., 2013).
321 This was anticipated because our study used a 0.45 μm membrane-based bis-acrylamide diffusive
322 layer which further reduced the pore size of the diffusive layer. Shiva et al. (2015) showed that the
323 diffusion coefficients of oxyanions when using a bis-acrylamide diffusive gel layer was between
324 0.66 and 0.77 the diffusion coefficients of the open diffusive gel layer. This calculated DGT
325 diffusion coefficient, was corrected for temperature as appropriate using the Stokes-Einstein
326 equation (Zhang and Davison, 1995), and was used for all calculations in this study.

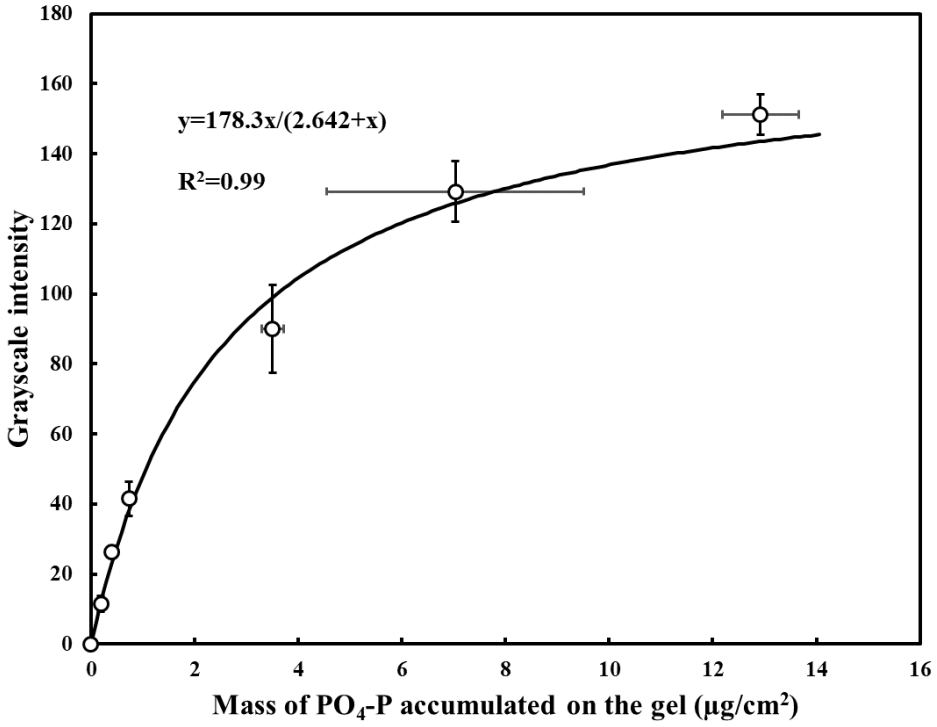
327

328 3.3. Calibration curve and determination of the binding capacity

329 In this study, a calibration curve was determined following 24 h DGT deployment in PO_4-P
330 solutions ranging in concentration from 0 to 6.9 mg/L. The average greyscale value for blank gels
331 (n=3) was 41.6 ± 1.10, which is equal to 3.2 μg/L PO_4-P. The grayscale intensity values at each
332 PO_4-P concentration were determined by the colorimetric method (n=3) with the blank value
333 subtracted. A calibration curve was determined by plotting the grayscale intensity values versus
334 the different PO_4-P mass densities ($\mu g/cm^2$) measured using the elution and analysis method (n=2).

335

A non-linear calibration curve (Hyperbola, Single Rectangular 2 Parameter) was achieved with an R^2 of 0.99 (Figure 1). This curve is comparable to calibration curves that are presented for other colorimetric DGT techniques (Teasdale et al., 1999; Robertson et al., 2008; Ding et al., 2013). This calibration curve was used to calculate all subsequent PO_4-P concentrations (section 3.3-3.6), and the limit of detection of PO_4-P (LOD = three times the standard deviation of the blank gel layers) which was 0.05 µg PO_4-P /cm^2 (n=3). This value corresponds to a LOD of 10.1 µg/L for the colorimetric technique following 24 h DGT deployment using a diffusive layer of 0.3 mm thickness at 21°C. The LOD for the colorimetric technique is approximately four times lower than that (40 µg/L) reported by Ding et al. (2013), and only slightly higher than that (6.8 µg/L) presented by Pagès et al. (2011). The LOD of our colorimetric method is higher than that for the elution technique (1.7 µg/L), while the LOD from the elution technique is similar to other LODs for PO_4-P (from 0.04 to 1.2 µg/L) determined for various binding materials, such as ferrihydrite, Metsorb, Zr-oxide, Magnesium Carbonate based on 24 h deployment (Zhang et al., 1998; Panther et al., 2010; Guan et al., 2015; Fa-Zhi et al., 2016). Most importantly, the colorimetric technique LOD of the current study is low enough to measure PO_4-P concentrations in most natural waters, sediment and soil. To further improve sensitivity, modifications can be made such as using shorter staining times or applying different colour channels during the scanning analysis stage (Bennett et al., 2012).



355

356 **Figure 1**. Plot of grayscale intensity (n=3) versus the mass of PO_4-P accumulated on the gel
357 (µg/cm^2) measured by the elution method (n=2) using a 26 min staining time and at 20ºC. The
358 error bars indicate the standard deviation of the mean (SD for the grayscale intensity ranged from
359 1.1 to 19 and SD for the mass of PO_4-P accumulated on the gel ranged from 0 to 3.3 µg/cm^2). The
360 calibration line was fitted using Sigma Plot 11.0 using the non-linear curve (Hyperbola, Single
361 Rectangular 2 Parameter) with the highest R^2.

362

363 The binding capacity of the membrane-based Zr-binding layer was also determined by the
364 colorimetric method using a 24 h DGT deployment across a wide range of PO_4-P equivalent
365 concentration solutions (0, 0.028, 0.052, 0.11, 0.60, 1.10, 2.20, 3.90 and 6.90 mg/L). Figure S6
366 shows that the accumulated mass in the binding layer, measured by the colorimetric method,
367 increased non-linearly and plateaued around 13 µg/cm^2 PO_4-P, whereas the value measured by the
368 elution technique continued to increase and reached 18.2 µg/cm^2 (Figure S7). The slightly lower

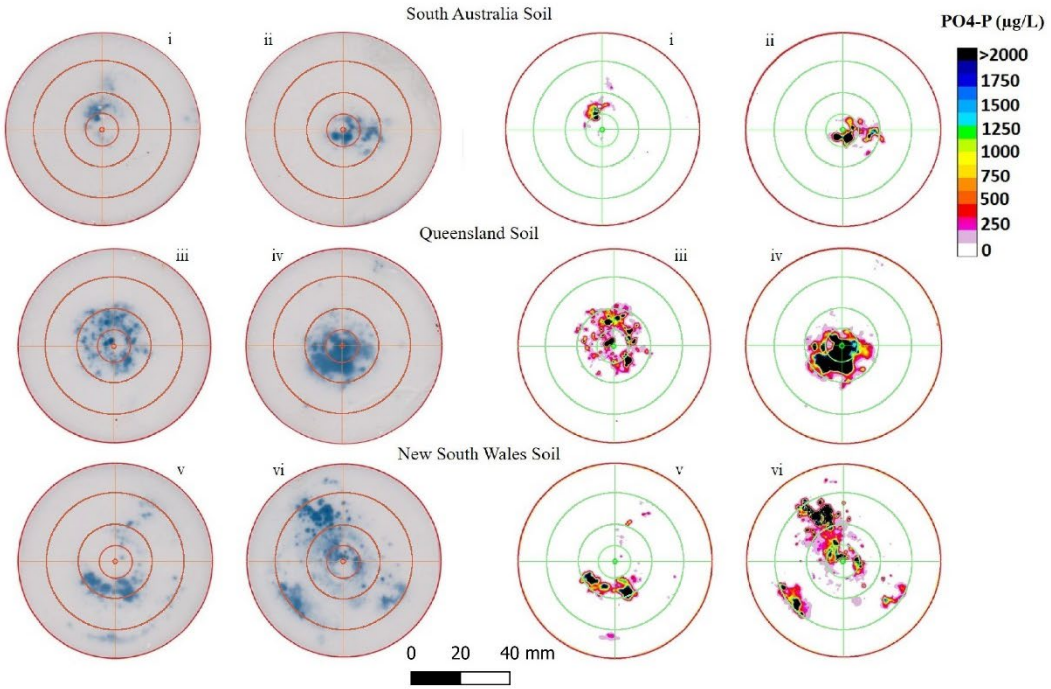
binding capacity determined by the colorimetric method is mainly due to the saturation effects in the colorimetric method. Additionally, the capacity of using the colorimetric method for the membrane-based Zr-oxide binding layer for PO_4-P at 13 μg/cm^2 corresponds to PO_4-P concentrations of 2.5 mg/L for 24 h deployment with the diffusion coefficient of 1.74×10^{-6} cm^2/s. For the elution method, an accumulation of 18.2 μg/cm^2 corresponds to PO_4-P concentrations of 3.5 mg/L based on the same deployment time and diffusion coefficient. This binding capacity is lower than the capacity obtained by both Guan et al. (2015) (65 μg/cm^2) and Ding et al. (2013) (23 μg/cm^2). One reason for the lower binding capacity in the current study is that our membrane-based binding gel was two times thinner than those used by Guan et al. (2015) and Ding et al. (2013). For thicker binding gel layers, the phosphate may not just be bound on the surface, but also retained to a greater extent inside the gel, leading to a higher capacity. Again, other options, such as using shorter staining times or applying different colour channels during the scanning analysis stage, can be applied to modify this calibration curve to increase the upper measurement limits as required (Bennett et al., 2012). Overall, the binding capacity of our current study is still higher than values previously reported for precipitated ferrihydrite gel (approximately 7.0 μg/cm^2) (Santner et al., 2010) and Metsorb gel layer (11.7 μg/cm^2) (Panther et al., 2010, 2011). In the study of Degryse and McLaughlin (2014), there was a negative relationship between the median color value and the P solution concentration, which was different from our technique. This is caused by the different procedures when processing the image; we used 256 (highest GSI value) to subtract the received GSI values after scanning (Kankanamge et al., 2020) while Degryse and McLaughlin (2014) did not use this approach. However, this does not affect the interpretation of the results. The visualization technique from Degryse and McLaughlin (2014) could determine PO_4-P

391 concentrations in $CaCl_2$ solution to approximately 100 mg/L based on a 30 min deployment time
392 while our technique was able to measure up to 120 mg/L PO_4-P for the same deployment time.
393
394 3.4. Validation of colorimetric technique for phosphate measurement in soils with low P
395 The colorimetric visualisation and elution Zr-oxide DGT techniques were used to obtain the PO_4-
396 P concentrations in three soil samples collected from Western Australia, named Kalannie (K),
397 Condering (C) and Jennacubine (J) (Table S3). Soils K and C had much lower PO_4-P
398 concentrations, as determined using both techniques, than soil J. The $C_{DGT\text{-}C}/C_{DGT\text{-}E}$ ratios were
399 found to be 0.66, 0.86 and 0.90 for soils K, C and J, respectively. There was close agreement
400 between two of the three soils, although the colorimetric visualisation did slightly underestimate
401 the DGT-labile P concentrations. The lower ratio for soil K is most likely due to the relatively low
402 PO_4-P concentration and some heterogeneity in the soil. Additionally, the PO_4-P concentrations in
403 K and C soils were close to the LOD (10.1 μg/L) of the colorimetric technique, and these levels
404 are still important for the interpretation of DGT for PO_4-P measurements as not all the soils have
405 high PO_4-P concentrations and increasing the deployment time can address this issue in future
406 studies. Although the ratio was lower for soil K, the PO_4-P concentrations measured by the two
407 techniques were not significantly different due to the variance in elution values (Independent T-
408 test, $p>0.05$). Overall, the colorimetric technique provided reliable measurements of PO_4-P in soils,
409 and further detailed investigations should be carried out to investigate the effect of soil
410 composition and phosphate concentrations on the colorimetric technique. However, the main
411 purpose of this study was to develop a two-dimensional visualisation study for soil fertiliser tests
412 and these results confirm that the colorimetric technique can provide accurate data, especially at
413 higher concentrations.

3.5. Two-dimensional measurement of phosphate diffusion in fertilised soils and comparison of $DGT_{-colorimetric}$, $DGT_{-elution}$ and Colwell P techniques

Colorimetric DGT devices were used to investigate the diffusion of PO_4-P away from a MAP fertiliser granule in three Australian soils (SA, QLD and NSW). After scanning the colorimetric gels from the three fertilised soils at the optimum staining time and temperature, the images were processed by GIMP and ImageJ software according to Kankanamge et al. (2020) and the two-dimensional measurement of PO_4-P concentration in the binding layers were obtained (Figure 2). Phosphate concentrations in the 2D-images ranged from 10.1 µg/L (white) to greater than or equal to 2000 µg/L (black) (Figure 2). Phosphate was relatively immobile in the calcareous SA soil, slightly more mobile in the QLD soil, and had the greatest mobility in the NSW soil. For both the SA and QLD soils, movement of potentially available P was limited to 15 mm from the fertiliser application point, with PO_4-P concentrations less than 50 µg/L at distances between 15 and 38 mm from the granule for both soils (Figure 2i-iv). For the NSW soil, potentially available P diffused further from the granule, with hotspots exceeding 2000 µg/L between 28 and 38 mm from the granule in more than one area (Figure 2 vi). Only one sample (Figure 2 iv), showed a relatively uniform high PO_4-P concentration within 7 mm from the granule which was most likely due to direct contact between the fertiliser granule and the DGT gel layers. In this study, we tried to avoid direct contact between the DGT gel layers and the fertiliser granule to prevent internal diffusion of P within the gel layer. Internal diffusion can occur when very high concentrations of PO_4-P bind

434 to the Zr-oxide binding layer (e.g. from direct contact with the P fertiliser granule) and result in an
435 overestimation of PO_4-P diffusion.
436
437 These data demonstrate how two-dimensional visualisation measurements contribute to a clearer
438 understanding of the effect of soil type on PO_4-P diffusion and can visually demonstrate the
439 heterogeneity of P diffusion. Any issues with DGT deployment also become readily apparent, such
440 as in Figure 2iv.

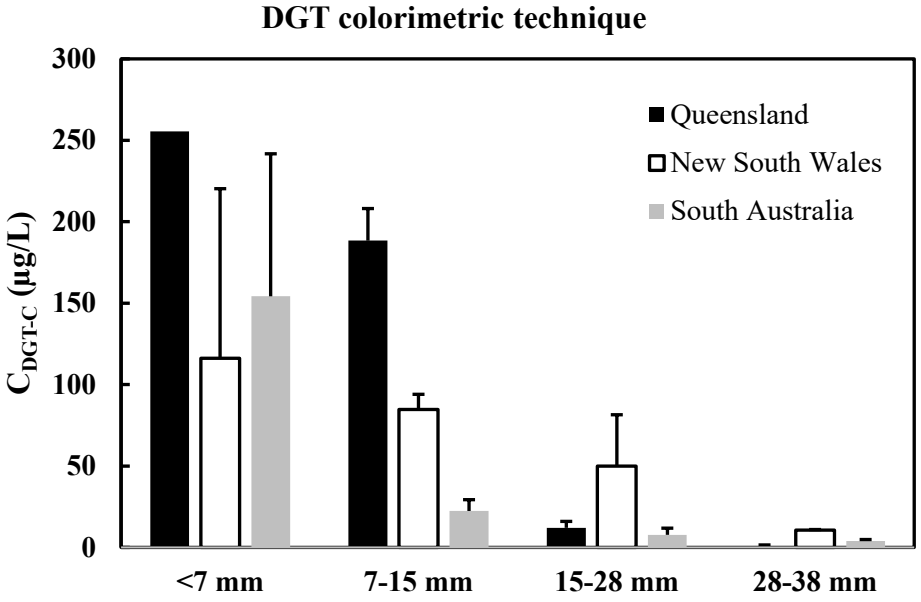


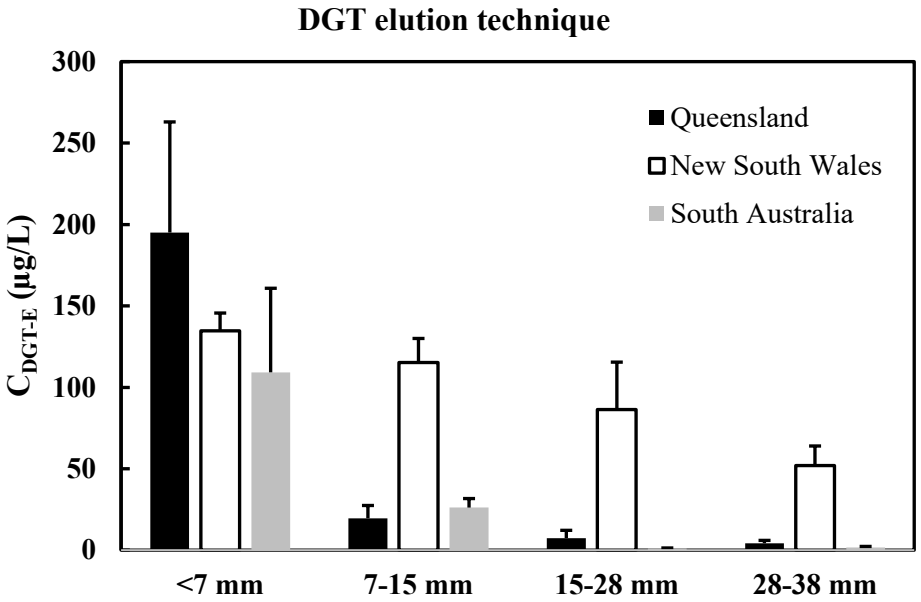
441

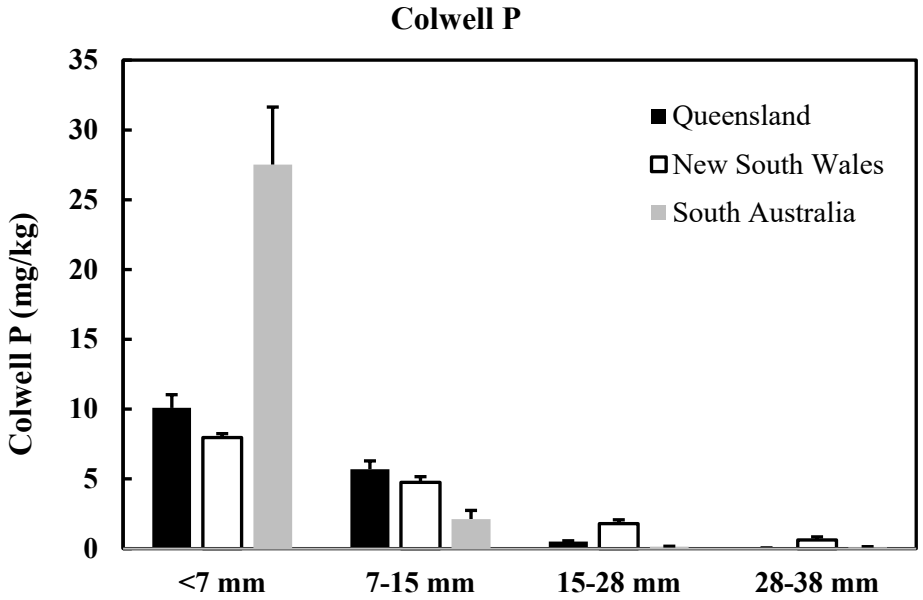
442 **Figure 2**. On the left, a picture of the binding layer after 26 minutes of staining time at 20ºC. Two
443 replicates of each of the three different soils were analysed. On the right, 2D-images obtained from
444 the scanned pictures on the left. The colour scale indicates the value for the phosphate
445 concentration in the binding layer (µg/L) determined by the colorimetric technique. The smallest
446 circles in the centre of binding layers and 2D images show the fertiliser application point. The

447 concentric circles surrounding this point (shown in red on the binding layers, and green on the 2D
448 images) indicate the distance from the fertiliser application point where the gels were sliced. These
449 circles have diameters of 14, 30, 56, and 76 mm, from the inner most ring to the outermost ring.
450
451 Regarding the PO_4-P diffusion within the soils and neglecting the PO_4-P concentration within 14
452 mm from the granule due to the high amount of variance between replicates, the three techniques
453 (DGT colorimetric, DGT elution, and Colwell P) gave very similar results. The qualitative trends
454 in PO_4-P distribution were the same among all methods for all treatments (Figures 2 and 3), i.e.
455 diffusion of PO_4-P away from the fertiliser granule increased as follows: SA < QLD < NSW.
456 Labile P concentrations measured colorimetrically (C_{DGT-C}) were compared to those measured
457 using the standard elution technique (C_{DGT-E}). Independent t-test results from Table S4 showed
458 that most PO_4-P values determined from the DGT colorimetric and elution measurements were not
459 significantly different ($p>0.05$) with only one exception which was the value between 7 and 15
460 mm from the granule of QLD soil ($P=0.015$). This was most likely caused by direct contact
461 between the fertiliser granule and the DGT gel layers, leading to the high PO_4-P concentration in
462 that area.
463
464 Overall, the DGT colorimetric method quantified analogous PO_4-P concentrations to the DGT
465 elution technique. Furthermore, both techniques demonstrated similar patterns of PO_4-P diffusion.
466 Conversely, the Colwell P method estimated much higher PO_4-P concentrations for the SA soil
467 compared to the two DGT techniques. Critical Colwell P concentrations are affected by the ability
468 of the soil to adsorb P as the Colwell P test measures less available P as well as readily available
469 P. This is because using bicarbonate as the extractant can remove large amounts of phosphate that
470 are fixed to aluminium and calcium particles contained in the soil (Mason et al., 2010).

Additionally, it has been shown that Colwell P is not a good predictor of available P in calcareous soils (Bertrand et al., 2003). Therefore, the Colwell P measurements may overestimate the true concentration of PO_4-P in the SA soil, which had a high $CaCO_3$ content.







476

477 **Figure 3**. Phosphate concentration (µg/L and mg/kg) determined by the DGT colorimetric, elution
478 technique and Colwell P method in three different soils within different distance from the granule
479 (Queensland, New South Wales and South Australia). The standard error was also plotted (n=2
480 for the DGT colorimetric and elution technique, n=4 for Colwell P method). For the DGT
481 colorimetric method, the value from <7 mm of QLD soil which is affected by the direct contact of
482 the fertiliser was excluded.

483

484 3.6 Fertiliser dissolution in different soil types

485 Following the 35-day incubation – and immediately after DGT collection – MAP granules were
486 extracted from the petri dishes and weighed. There was incomplete dissolution of MAP in all three
487 soils after the incubation; however, there were differences in the mass decrease of granules
488 between soils. In the QLD and NSW soils, MAP granules decreased by 74% (41 mg) and 76% (42
489 mg), respectively, whereas in the SA soil, MAP only decreased in mass by 57% (31 mg) (Table
490 S5). Therefore, as suggested by Lombi et al. (2004) who performed similar diffusion experiments,

491 the large amount of PO_4-P remaining within 14 mm of the QLD soil can be explained by a slower
492 rate of P diffusion compared to that in the NSW soil. In the SA soil, PO_4-P was also confined
493 within 14 mm, but in this case most likely due to the low dissolution rate of the MAP granule itself
494 and the low rate of P diffusion which can be attributed to soil properties. When the fertiliser granule
495 is initially added to the SA soil, the water drawn to the granule contains Ca^{2+}, which can precipitate
496 P (Hettiarachchi et al., 2006). Due to the high concentration of P in the fertosphere (the volume of
497 soil around the granule), precipitation reactions dominate initially (Hettiarachchi et al., 2006).
498 Then, as P moves away from the granule and the concentration of P in soil solution decreases,
499 adsorption becomes the dominant process.
500
501 3.7 Effect of soil properties on PO_4-P diffusion away from fertiliser granules
502 The advantage of the DGT method is that compared to other techniques such as Colwell P, it is
503 suitable for a range of soil types. In addition, DGT compares more favourably than Colwell P in
504 relation to plant available P (Mason et al., 2010; Mason et al., 2013). Phosphate diffusion is largely
505 controlled by soil properties, specifically soil pH, presence of $CaCO_3$, and the concentration of
506 Al/Fe oxides. These properties also influence the soil's PBI. The QLD and SA soils both had a
507 moderate PBI (210 and 180, respectively) while the NSW soil had a low PBI of 73. High PBI
508 values indicate that the soil can quickly bind P. In soils with a low PBI, applied P is generally more
509 labile in the short term as the soil has a limited ability to bind P. The soil properties that influence
510 PBI (pH, $CaCO_3$, Al/Fe oxides), can also explain the differences in P diffusion observed in the
511 current study. Our results correlate well with the PBI values in that PO_4-P was most labile and

512 diffused further in the NSW soil; the soil with the lowest PBI. While for the QLD and SA soils
513 with high PBI, the PO_4-P was less labile and the diffusion was lower.
514
515 When MAP dissolves in soil, the pH of the fertosphere soil solution becomes moderately acidic.
516 For this reason, MAP is advantageous in neutral and alkaline soils. However, in the presence of
517 Ca, P is rapidly removed from the soil solution. In calcareous alkaline soils, such as the SA soil
518 (pH 8.5, 36% $CaCO_3$ equivalent), orthophosphate ions can rapidly precipitate with Ca when the
519 soil solution concentration of PO_4^{3-} exceeds the solubility products for Ca-P solid phases (Freeman
520 and Rowell, 1981; Lindsay, 1979); effectively immobilising P. Therefore, this explains the limited
521 diffusion of P away from the fertiliser granule in the current study (Figures 2 and 3). In the acidic
522 QLD soil (pH=5.0), P fixation can also explain the limited diffusion of PO_4-P away from the
523 fertiliser granule. However, for this soil, precipitation of P with Fe and Al oxides is the most likely
524 fixation mechanism. In the fertosphere of non-calcareous soils, precipitation of Al-P minerals
525 dominates as shown by Meyer et al. (2020). Using synchrotron-based X-ray absorption near edge
526 structure (XANES) analysis the authors found that when granular MAP was applied to an acidic
527 Tenosol, Al-P minerals such as taranakite, and absorbed P were the dominant P species. Finally,
528 P diffusion was greatest in the relatively neutral NSW soil (pH=7.4) not only because soil pH was
529 favourable for MAP dissolution, but also because precipitation and absorption reactions that act to
530 limit PO_4-P diffusion were less prevalent. Specifically, Ca-P precipitation was less likely because
531 $CaCO_3$ was not detected in the soil, and, fixation by Al was reduced as exchangeable Al contributed
532 <0.1% to the soil's cation exchange capacity (CEC).
533
534

4. Conclusion

The novel colorimetric technique based on precipitated membrane-based Zr-oxide DGT was developed. The optimal staining temperature and time were 20ºC (room temperature) and 26 minutes. The new colorimetric technique was not only easier to perform without the need for any pre-treatment of the binding gel layers and heating during scanning, but was also able to provide faster results than the colorimetric technique developed by Ding et al. (2013). This makes the technique more suitable for in-field applications. The technique was able to measure phosphate concentration up to 2.5 mg/L for a 24-hour deployment with a detection limit of 10.1 µg/L. Due to the relatively high capacity of the precipitated Zr-oxide binding layer, it was possible to measure labile phosphate in a range of contrasting soils. The relatively low LOD will also allow for the measurement of phosphate in most waters, sediments and soils. The phosphate values measured by the colorimetric technique were similar to the values determined by the elution technique. Additionally, it has been shown that the new colorimetric technique can be used to visualise the diffusion of labile phosphate in soil types. This makes it possible to investigate the diffusion of potentially available phosphate from conventional and novel fertilisers, which can then be correlated with the plant uptake of P. Furthermore, the visualisation of data can be a very effective way to engage and inform end-users about novel fertiliser formulations and how to optimise soil management practices.

Acknowledgments

The authors would like to thank UniSA STEM for providing support to David Macias Arias's for his Master study. We also thank Nutrient Advantage for soil characterisation. The authors also like

to honour the contribution of Professor Peter Teasdale to this manuscript. Professor Peter Teasdale passed away during the revision of the manuscript, and this is a tough time for the team and his family. Peter was a loving family man, a talented researcher and a great mentor. This warm and kind man will be greatly missed by his family, friends, colleagues and students. His strong sense of integrity and desire for scientific truth will continue to inspire other researchers.

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