

## RESEARCH LETTER

# Combining diffusive gradients in thin-films (DGT) and $^{31}\text{P}$ NMR spectroscopy to determine phosphorus species in soil

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## Abstract

The diffusive gradients in thin-films (DGT) technique shows in many publications a superior correlation to the amount of plant-available phosphorus (P) in soil. However, this technique cannot give information on the plant-available P species in soil. Therefore, we combined DGT with solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy. This was achieved by using a modified DGT device in which the diffusive layer had a larger pore size, the binding layer incorporated an adsorption material with a higher capacity, and the device had a larger exposure area. The spectroscopic investigation was undertaken after elution of the deployed DGT binding layer in a NaOH solution. Adsorption tests using solutions of known organic P compounds showed that a sufficient amount of these compounds could be adsorbed on the binding layer in order for them to be analyzed by solution  $^{31}\text{P}$  NMR spectroscopy. Furthermore, various intermediates of the hydrolysis of trimetaphosphate in soil could be also analyzed over time.

## 1 | INTRODUCTION

The amount of plant-available phosphorus (P) in soil strongly influences the yield of plants in agriculture. Therefore, various simple chemical extraction methods have been developed to estimate the plant-available P pools in soil (Kratz et al., 2019). More recently, several experiments using the diffusive gradients in thin-films (DGT) technique showed that it has a much better correlation to plant-available P in soils than standard chemical extraction methods (e.g., calcium–acetate–

lactate [CAL], Colwell, Olsen, water) when soils with different characteristics are considered (Arias et al., 2021; Mason et al., 2010; Mason et al., 2013; Menzies et al., 2005; Six et al., 2012; Vogel et al., 2017). Furthermore, various researchers discovered through the use of  $\text{P}^{33}/\text{P}^{32}$ -labeled P sources that maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) plants accessed the same pool of labile soil P as the DGT method (Mason et al., 2013; Six et al., 2012). Therefore, the soil P species that were responsible for high yields of maize and wheat diffused and bound to the DGT binding layer.

Previously, we showed that the spectroscopical investigation of DGT binding layers with P K- and  $\text{L}_{2,3}$ -edge X-ray adsorption near-edge structure (XANES) and infrared

**Abbreviations:** DGT, diffusive gradients in thin-films; NMR, nuclear magnetic resonance; TMP, trimetaphosphate.

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spectroscopy could determine the identification of single P compounds (Vogel et al., 2019). However, this approach was limited to only a few P species on the DGT binding layer and the analysis of a mixture (i.e., various inorganic poly-P species) was very challenging. In particular, various organic P could not be analyzed in parallel with these method combinations.

Therefore, the objective of this paper was to identify the plant-available P species of soil by combining the DGT method with solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy, a spectroscopic technique that is also sensitive for the analysis of organic P compounds (Doolette & Smernik, 2011; McLaren et al., 2020; Turner et al., 2005). However, due to the very low concentration of P on the DGT binding layer, the direct application of solid-state  $^{31}\text{P}$  NMR spectroscopy is challenging (Doolette & Smernik, 2011; He et al., 2007). But solution  $^{31}\text{P}$  NMR spectroscopy can be used after the extraction of the DGT binding layers with NaOH solution and a subsequent concentration step.

To obtain a sufficient amount of P species on the DGT binding layer to be subsequently analyzed with solution  $^{31}\text{P}$  NMR spectroscopy, the DGT device has to be modified. Therefore, we used a DGT device that has a diffusive layer with a larger pore size, a binding layer incorporated an adsorption material with a higher capacity for P, and a device with a larger exposure area (see comparison to a conventional P DGT device in Table 1).

This device was used to test the adsorption capacity of organic P species on the DGT binding layer and the hydrolysis of trimetaphosphate (TMP) in soil over time, where the absorbed P on the DGT binding layer was analyzed after extraction by solution  $^{31}\text{P}$  NMR spectroscopy.

## 2 | MATERIALS AND METHODS

For the experiments, agarose-based DGT diffusive gels were prepared as previously described in Huang et al. (2016). The agarose diffusive gels were washed at least three times with deionized water and afterward stored in 0.01 mol/L  $\text{NaNO}_3$  solution before use. The zirconium (Zr) oxide DGT binding layers were prepared according to Guan et al. (2015) with some modifications. The prepared agarose-based diffusive

### Core Ideas

- We show a promising combination of DGT technique and solution  $^{31}\text{P}$  NMR spectroscopy.
- This technique combination can identify organic phosphorus species in solutions.
- Modified DGT device can analyze the hydrolysis of trimetaphosphate in soil.

gel layers were first immersed in a solution of 32.2 g/L of  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  for 2 h. Afterward, immersion in MES monohydrate (10.66 g/L) for 40 min at pH 6.7 (pH adjusted using 0.1 mol/L NaOH) was applied. Finally, we received precipitated Zr oxide binding gel layers with 0.2-mm thickness. The Zr oxide binding gel layers were washed several times with deionized water and finally stored in deionized water before use. Commercially available DGT devices with a rectangular sampling window (1.8 cm  $\times$  15 cm = 27 cm<sup>2</sup>) were used (DGT Research Ltd.; see comparison to conventional DGT in Figure 1a).

For the organic P adsorption tests, the DGT devices were deployed in 4 L of 50 mg P/L solutions (see Figure 1b) of adenosine-5'-monophosphate (AMP) Na-salt and a glycerol 3-phosphate/glycerol 2-phosphate isomer mixture, respectively (both Sigma-Aldrich). The DGT devices were deployed for 24 h at 22 °C in constantly agitated solutions. Finally, the DGT binding layers were removed from the DGT devices and eluted in 0.5 mol/L NaOH for 24 h.

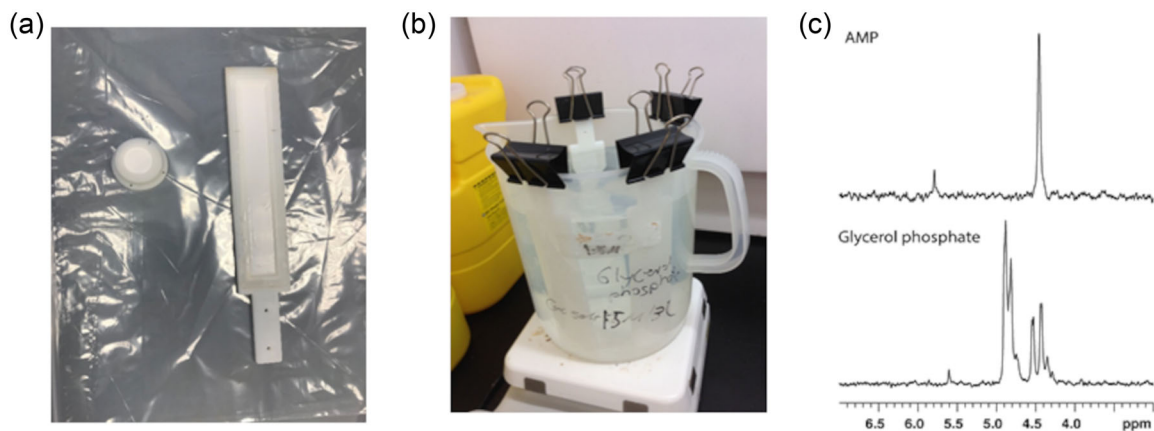
Time-resolved DGT measurements were performed in soil with TMP applied as sodium salt, ( $\text{NaPO}_3$ )<sub>3</sub> (Alfa Aesar). The used subsoil was from a loess-derived brown soil, which was mixed with quartz sand at 1:1 mass ratio to decrease the P amount ( $P_{\text{Total}} = 180$  mg/kg,  $P_{\text{Calcium-Acetate-Lactate}} = 10$  mg/kg, pH in 0.01 mol/L  $\text{CaCl}_2 = 6.7$  of mixture). Two hundred grams of this soil-sand mixture was subsequently mixed with 20 mg P of TMP (= 100 mg P/kg of soil, as in previous pot experiments [Vogel et al., 2017; Vogel et al., 2019]) in 500-ml plastic containers. The soil-TMP mixtures were maintained daily at 60% of the water-holding capacity at 22 °C. Afterward, DGT devices were deployed for 24 h (see Figure 2a) at three time points:

TABLE 1 Type of conventional and modified diffusive gradients in thin-films (DGT) devices used for phosphorus

DGT	Diffusion layer	Binding layer	Exposure area	Elution
Conventional	Polyacrylamide cross-linked with an agarose derivative (APA)	Ferrihydrite (max. binding capacity: 7 $\mu\text{g}/\text{cm}^2$ ) <sup>a</sup>	2.54 cm <sup>2</sup>	Acid
Modified	Agarose (larger pore size)	Zirconium oxide (max. binding capacity: 18.2 $\mu\text{g}/\text{cm}^2$ ) <sup>b</sup>	27 cm <sup>2</sup>	NaOH

<sup>a</sup>Santner et al., 2010.

<sup>b</sup>Arias et al., 2021.



**FIGURE 1** (a) Conventional and modified large diffusive gradients in thin-films (DGT) device; (b) adsorption test with organic P compounds in solution; (c) Solution <sup>31</sup>P nuclear magnetic resonance spectra of the organic P compounds adenosine monophosphate (AMP) and glycerol phosphate isomer mixture after extraction from the DGT binding layer

at the start of the incubation experiment (DGT-1), after 2 d (DGT-2), and after 7 d (DGT-3) of incubation. The water content of the soil–TMP mixtures was increased to 100% water-holding capacity before DGT deployment. The binding layers from these DGT experiments with the soil–TMP mixture were eluted in 0.5 mol/L NaOH for 24 h for P analysis.

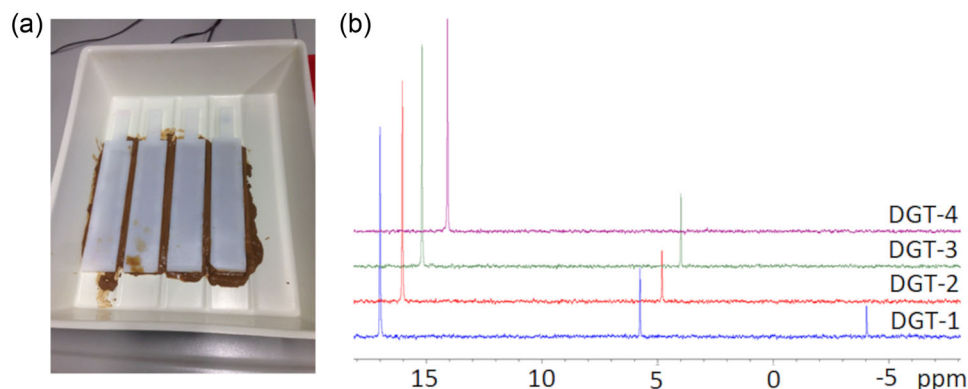
For solution <sup>31</sup>P NMR analysis, replicate NaOH extracts, following the rinsing of each DGT sample, were combined and freeze-dried. A 800-mg subsample of each freeze-dried extract was ground and redissolved in 2 ml of deionized water. The supernatant solution (0.5 ml) and D<sub>2</sub>O (0.1 ml) were placed in a 5-mm NMR tube. For quantification, methylenediphosphonic acid (MDP; 3 g/L) was added as an internal reference standard to the D<sub>2</sub>O. The resultant pH of the samples was >13. Solution <sup>31</sup>P NMR spectra were collected using a Bruker Avance NEO 400 MHz spectrometer equipped with a BBO Broad Band Probe operating at a <sup>31</sup>P frequency of 161.9 MHz. Spectra were acquired with the Bruker pulse sequence *zpgp30* using a 30° pulse and power-gated <sup>1</sup>H decoupling over the <sup>31</sup>P range of 30 to –20 ppm. Acquisition parameters were as follows: recycle delay (d1) of 60 s, time domain points (td) 64 K, and acquisition time (aq) of 4.0 s. A total of 352 scans and 4 dummy scans were acquired for each sample, representing acquisition times of 6 h 20 mins. The chemical shift is reported in parts per million (ppm) relative to an external standard of 85% (w/w) phosphoric acid.

### 3 | RESULTS AND DISCUSSION

Figure 1c shows the solution <sup>31</sup>P NMR spectra from the adsorption test with the organic P compounds AMP and glycerol phosphate isomer mixture on the DGT binding layer. Both spectra show a clear <sup>31</sup>P NMR signal of the respec-

tive compound (Doolette et al., 2011). Thus, the modified DGT device setup is able to adsorb a sufficient amount of organic P compounds to analyze them by solution <sup>31</sup>P NMR spectroscopy.

Furthermore, Figure 2b shows the solution <sup>31</sup>P NMR spectra of the eluted DGT binding layers of the untreated soil (DGT-4) and the experiment that included the incubated soil–TMP mixtures (DGT-1 to DGT-3). At the start of the incubation experiment, peaks of orthophosphate (5.6 ppm) and pyrophosphates (approximately –4 ppm) were detected (DGT-1). Surprisingly, in contrast to previous analysis by Fourier transform infrared spectroscopy (Vogel et al., 2019) where polyphosphates were determined, in the spectral range of approximately –6 to –20 ppm polyphosphates (He et al., 2007) were not detected in this study using solution <sup>31</sup>P NMR spectroscopy. After 2 d of incubation, the pyrophosphate peak at approximately –4 ppm is no longer detectable (DGT-2). Finally, only orthophosphate was shown in the <sup>31</sup>P NMR spectra (DGT-3) after 7 d of incubation. Thus, the time-resolved hydrolysis of inorganic TMP in soil could also be analyzed with the combination of DGT and solution <sup>31</sup>P NMR spectroscopy. However, a faster degradation of polyphosphates to orthophosphate could be detected. Unlike the previous study with Fourier transform infrared spectroscopy, where the DGT binding layers were directly investigated, for solution <sup>31</sup>P NMR spectroscopy, the P compounds must be eluted from the DGT binding layer using NaOH solution. The alkaline treatment with the NaOH solution might increase the hydrolysis of the polyphosphates. Previous work showed an increased polyphosphate hydrolysis rate with higher concentrations of NaOH in solutions (Shen, 1966). Furthermore, the effects of alkaline hydrolysis for a wide range of organic phosphorus species in soils have been reported (Doolette et al., 2009; Turner et al., 2005) and cannot be excluded for polyphosphates because Dick and Tabatabai (1987) showed that the



**FIGURE 2** (a) Application of large diffusive gradients in thin-films (DGT) devices to soil samples; (b) Solution  $^{31}\text{P}$  nuclear magnetic resonance spectra of the eluted DGT binding layers from the incubated experiment with the soil–trimetaphosphate mixtures (DGT-1: start; DGT-2: after 2 d; DGT-3: after 7 d) and the untreated soil (DGT-4); for ease of comparison each spectrum is off set from the lowest trace (DGT-1) by increasing the chemical shift in 1 ppm increments; peak at  $\sim 17$  ppm is the internal standard methylenediphosphonic acid (MDP), 5.6 ppm is ortho-P, and approximately  $-4$  ppm is pyro-P

hydrolysis of polyphosphates was higher in soils with a higher pH-value.

Additionally, the untreated soil was analyzed by solution  $^{31}\text{P}$  NMR spectroscopy (see DGT-4 in Figure 2b). However, because it is a very P-poor soil, no organic and inorganic P compounds could be detected in this soil.

## 4 | CONCLUSION

Our experiments showed that the combination of the DGT technique with solution  $^{31}\text{P}$  NMR spectroscopy has great potential to analyze P species in soil. The modified DGT device setup, where the diffusive layer had a larger pore size, the binding layer incorporated an adsorption material with a higher capacity for P, and the device had a larger exposure area, was able to adsorb the necessary amount of organic and inorganic P compounds to analyze them by solution  $^{31}\text{P}$  NMR spectroscopy. Therefore, the organic P compounds from the adsorption test and the inorganic P compounds from the TMP hydrolysis could be identified. In the untreated soil, no P compounds could be detected; however, this was a sandy P poor soil and other soils with a higher amount of plant-available P have the potential to be analyzed using these combined methods.

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## AUTHOR CONTRIBUTIONS

All authors contributed equally to this work. Christian Vogel: Conceptualization; Formal analysis; Methodology; Writing – original draft. Ashlea Doolette: Conceptualization; Formal analysis; Methodology; Writing – review & editing. Jianyin Huang: Conceptualization; Formal analysis; Methodology; Writing – review & editing.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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## REFERENCES

- Arias, D. M., Teasdale, P. R., Doolette, C. L., Lombi, E., Farquhar, S., & Huang, J. (2021). Development and evaluation of a new colorimetric DGT technique for the 2D visualisation of labile phosphate in soils. *Chemosphere*, 269, 128704. <https://doi.org/10.1016/j.chemosphere.2020.128704>
- Dick, R. P., & Tabatabai, M. A. (1987). Factors affecting hydrolysis of polyphosphates in soils. *Soil Science*, 143, 97–104. <https://doi.org/10.1097/00010694-198702000-00003>
- Doolette, A. L., & Smernik, R. J. (2011). Soil organic phosphorus speciation using spectroscopic techniques. In E. Bünemann, A. Oberson, & E. Frossard (Eds.), *Phosphorus in action: Biological processes in soil phosphorus cycling* (pp. 3–36). Springer.
- Doolette, A. L., Smernik, R. J., & Dougherty, W. J. (2009). Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Science Society of America Journal*, 73, 919–927. <https://doi.org/10.2136/sssaj2008.0192>



- Doolette, A. L., Smernik, R. J., & Dougherty, W. J. (2011). A quantitative assessment of phosphorus forms in some Australian soils. *Soil Research*, 49, 152–165.
- Guan, D.-X., Williams, P. N., Luo, J., Zheng, J.-L., Xu, H.-C., Cai, C., & Ma, L. Q. (2015). Novel precipitated zirconia-based DGT technique for high-resolution imaging of oxyanions in waters and sediments. *Environmental Science & Technology*, 49, 3653–3661. <https://doi.org/10.1021/es505424m>
- He, Z., Honeycutt, C. W., Xing, B., McDowell, R. W., Pellechia, P. J., & Zhang, T. (2007). Solid-state Fourier transform infrared and  $^{31}\text{P}$  nuclear magnetic resonance spectral features of phosphate compounds. *Soil Science*, 172, 501–515. <https://doi.org/10.1097/SS.0b013e318053dba0>
- Huang, J., Bennett, W. W., Welsh, D. T., Li, T., & Teasdale, P. R. (2016). Development and evaluation of a diffusive gradients in a thin film technique for measuring ammonium in freshwaters. *Analytica Chimica Acta*, 904, 83–91. <https://doi.org/10.1016/j.aca.2015.11.022>
- Kratz, S., Vogel, C., & Adam, C. (2019). Agronomic performance of P recycling fertilizers and methods to predict it: A review. *Nutrient Cycling in Agroecosystems*, 115(1), 1–39. <https://doi.org/10.1007/s10705-019-10010-7>
- Mason, S. D., McLaughlin, M. J., Johnston, C., & McNeill, A. (2013). Soil test measures of available P (Colwell, resin and DGT) compared with plant P uptake using isotope dilution. *Plant and Soil*, 373, 711–722. <https://doi.org/10.1007/s11104-013-1833-7>
- Mason, S., McNeill, A., McLaughlin, M. J., & Zhang, H. (2010). Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. *Plant and Soil*, 337(1), 243–258. <https://doi.org/10.1007/s11104-010-0521-0>
- McLaren, T. I., Smernik, R. J., McLaughlin, M. J., Doolette, A. L., Richardson, A. E., & Frossard, E. (2020). Chapter two: The chemical nature of soil organic phosphorus—A critical review and global compilation of quantitative data. *Advances in Agronomy*, 160, pp. 51–124.
- Menzies, N. W., Kusumo, B., & Moody, P. W. (2005). Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. *Plant and Soil*, 269, 1–9. <https://doi.org/10.1007/s11104-004-1725-y>
- Santner, J., Prohaska, T., Luo, J., & Zhang, H. (2010). Ferrihydrite containing gel for chemical imaging of labile phosphate species in sediments and soils using diffusive gradients in thin films. *Analytical Chemistry*, 82, 7668–7674. <https://doi.org/10.1021/ac101450j>
- Shen, C. Y. (1966). Alkaline hydrolysis of sodium trimetaphosphate in concentrated solutions and its role in built detergents. *I&EC Product Research and Development*, 5, 272–276. <https://doi.org/10.1021/i360019a015>
- Six, L., Pypers, P., Degryse, F., Smolders, E., & Merckx, R. (2012). The performance of DGT versus conventional soil phosphorus tests in tropical soils: An isotope dilution study. *Plant and Soil*, 359, 267–279. <https://doi.org/10.1007/s11104-012-1192-9>
- Turner, B. L., Cade-Menun, B. J., Condron, L. M., & Newman, S. (2005). Extraction of soil organic phosphorus. *Talanta*, 66, 294–306. <https://doi.org/10.1016/j.talanta.2004.11.012>
- Vogel, C., Sekine, R., Steckenmesser, D., Lombi, E., Herzel, H., Zuin, L., Wang, D., Félix, R., & Adam, C. (2019). Combining diffusive gradients in thin films (DGT) and spectroscopic techniques for the determination of phosphorus species in soils. *Analytica Chimica Acta*, 1057, 80–87. <https://doi.org/10.1016/j.aca.2019.01.037>
- Vogel, C., Sekine, R., Steckenmesser, D., Lombi, E., Steffens, D., & Adam, C. (2017). Phosphorus availability of sewage sludge-based fertilizers determined by the diffusive gradients in thin films (DGT) technique. *Journal of Plant Nutrition and Soil Science*, 180, 594–601. <https://doi.org/10.1002/jpln.201600531>

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