*Pedosphere* **31**(1): 76–82, 2021
doi:10.1016/S1002-0160(20)60009-1
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

# Using diffusive gradients in thin films technique for *in-situ* measurement of labile phosphorus around *Oryza sativa* L. roots in flooded paddy soils

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(Received November 9, 2018; revised February 25, 2019)

## ABSTRACT

Behavior of phosphorus (P) in flooded rice soil is controlled by iron (Fe) redox cycling in root-zone. In this study, we applied a novel approach—the diffusive gradients in thin films (DGT) technique—for investigating the *in-situ* distribution of labile phosphorus (P) and Fe in close proximity to Asian rice (*Oryza sativa* L.) roots at submillimeter to millimeter spatial resolutions during the seedling and booting stages. We conducted a seven-year field experiment under rice-wheat rotation with different P fertilizer treatments. The results showed a significant and strong positive relationship of the average DGT-labile P concentration with soil Olsen P ($R^2 = 0.77$, $P < 0.01$) and with rice total P concentration ($R^2 = 0.62$, $P < 0.05$). Furthermore, results on one- and two-dimensional changes of DGT-labile P indicated that fertilization only in the wheat season produced sufficient amounts of labile P in the flooded paddy soils, similar to when fertilizer was applied only in the rice season; dissolved P concentrations, however, were lower. A co-occurrence and significant positive correlation ($P < 0.01$) between DGT-labile P and Fe indicated Fe-coupled mobilization of P in flooded paddy soils. These results collectively indicated that the DGT technique provided information on *in-situ* distribution of labile P and its variability in close proximity to rice roots. This suggests that the DGT technique can improve our understanding of *in-situ* and high-resolution labile P processes in paddy soils and can provide useful information for optimizing P fertilization.

*Key Words*: fertilization reduction, labile P, rice root, Zr-oxide DGT, Fe-P coupling

**Citation**: Wang Y, Chen H, Wang L, Zhu W B, Yuan J H, Jaisi D P, Zhao X, Wang S Q. 2021 . Using diffusive gradients in thin films technique for *in-situ* measurement of labile phosphorus around *Oryza sativa* L. roots in flooded paddy soils. *Pedosphere*. **31**(1): 76–82.

## INTRODUCTION

Owing to dual concerns regarding water contamination risk and finite phosphorus (P) reserves, it is imperative for large-scale agriculture to adopt more efficient P usage strategies, as highlighted previously (Cordell and White, 2014). Our previous work on a rice-wheat crop rotation in the Taihu Lake region in China using chemical extraction methods indicated that P fertilization only during the wheat growing season may sustain the yield of the subsequent rice crop due to a sufficient supply of available P (Wang *et al*., 2016a, b). However, the operationally defined nature of chemical extraction methods limits both methodological and physico-chemical interpretation.

Rice is a major crop in Southeast Asia and represents a large proportion of the diet of the population. During the rice growing season, physico-chemical conditions of flooded paddy soils and release of P from the soil may fluctuate under anoxic conditions (Upreti *et al*., 2015). Furthermore, iron (Fe)(III)-oxyhydroxides are reductively dissolved, and due to the release of molecular oxygen ($O_2$) by rice roots, Fe(II) is oxidized in the vicinity of the roots, which leads to the formation of iron plaque on submerged rice roots (Williams *et al*., 2014). Therefore, elevated levels of Fe(II) exist at the boundary of the submerged aerobic rhizosphere area and affect the rate of P uptake by rice plants (Reddy and Patrick, 1976). Localized P, such as P around crop roots, may be able to re-supply P at substantially higher amounts than the surrounding soil. Therefore, detailed information on the spatial pattern of labile P distributions along root and vertical axes may provide insights regarding plant P requirements and may thus be valuable information to design P fertilizer reduction programs. However, assessing the spatial distribution of root-induced changes of P lability in the root zone is methodologically challenging.

The diffusive gradients in thin films (DGT) technique has been shown to be an optimal indicator of P bioavailability in soil and surpasses chemical extraction methods because

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it mimics the uptake of solutes by plant roots by providing a sink for free orthophosphate and other ions (Mason *et al*., 2013); however, processes at the root surface cannot be mimicked (Kruse *et al*., 2015). Using a specially designed passive sampling device, DGT techniques can detect labile P fractions that are released from soil solids and then diffused through a diffusive layer and are assimilated by the binding layer (Li *et al*., 2019). This technique is a comparably effective method for assessing P availability to crops and may help improve recommendations for P fertilizer application (Zhang *et al*., 2013), compared to reagent-based extraction techniques. The DGT technique has been applied to predict plant responses of crop plants (specifically wheat, tomato, and maize) to P fertilizer input (Menzies *et al*., 2005; Mcbeath *et al*., 2007; Mason *et al*., 2010; Tandy *et al*., 2011; Six *et al*., 2012, 2013). Most previous studies were performed on dry-farming crops and on soils sampled at crop maturation; however, few studies have applied the DGT technique to predict P availability to plants in water-logged and anoxic soils. Recently, several pioneering studies used this method to map the distribution and changes of labile and plant-available P in the proximity of single soil-grown plant roots at a sub-mm scale (Stockdale *et al*., 2008; Santner *et al*., 2010, 2012; Kreuzeder *et al*., 2018).

Retention of P by Fe (oxy)hydroxides may be reduced under anoxic conditions in flooded paddy soils, which leads to the release of P and Fe(II) into the porewater. Simultaneous release and coupling of P and Fe(II) in sediment was measured by Ding *et al*. (2016) and Xu *et al*. (2012) using DGT technology. For example, Ding *et al*. (2016) observed significant positive correlations of DGT-labile P and Fe. Li *et al*. (2019) suggested that Fe-P coupling mechanisms were responsible for the release of P from sediments. However, the release of P and Fe(II) is more complex in paddy soils than in sediments, because of the existence of rice roots. It is worth noting that zirconium (Zr)-oxide DGT in combination with computer-imaging densitometry (CID) has been developed for imaging the distribution of labile P in water-logged soils on sub-millimeter scale (Ding *et al*., 2013). Moreover, Zr-oxide DGT is potentially a powerful tool for imaging the heterogeneous *in-situ* distribution of labile P (Ding *et al*., 2015). Santner *et al*. (2012) used two-dimensional DGT imaging to compare soluble P changes near the roots of two *Brassica napus* cultivars. However, most applications of DGT technology have been limited to laboratory experiments so far, whereas few studies used this method under field conditions.

In this study, we applied the ZrO-Chelex DGT and Zr-oxide DGT to obtain one- or two-dimensional high-resolution profiles of the *in-situ* distribution of labile P in close proximity to the roots of Asian rice (*Oryza sativa* L.) plants in a rice growing season. The objectives of this study were i) to assess potential applicability of the DGT technique in flooded paddy soils and ii) to visualize soil P distribution to further compare and test whether in a rice-wheat rotation cropping system, P would suffice during P fertilization only in the wheat season.

## MATERIALS AND METHODS

### *Field experiment design*

The experimental field site is located at the Yixing Agro-Environment Research Base (about 1 km north-west of the Taihu Lake; 31°16′ N, 119°54′ E) and was managed by the Institute of Soil Science, Chinese Academy of Sciences. The experiment commenced with the rice growing season in May 2010 and spanned seven consecutive Asian rice and wheat (*Triticum aestivum* L.) rotation seasons. Three P fertilization treatments were applied: fertilization only in the rice growing season (PR), only in the wheat growing season (PW), or in both seasons (PR+W). The control treatment received no P fertilization (P0). Details of the agricultural management are described by Wang *et al*. (2016b).

### *Measurement of labile P and Fe using DGT*

*In-situ* field application of DGT was conducted twice during the 2016 rice growth season (the seventh year of the field experiment): at the seedling stage (June 30, 2016) and at the booting stage (August 30, 2016) when the soils were flooded at a depth of 3–5 cm. Two types of DGT probes were obtained from the Easysensor Ltd. (Nanjing, China): a ZrO-Chelex DGT probe previously used for simultaneous measurements of labile P and Fe (one-dimensional DGT) (Xu *et al*., 2013; Ding *et al*., 2016b), and a Zr-oxide DGT probe, which was previously used for two-dimensional DGT measurement of labile P (Ding *et al*., 2013; Kreuzeder *et al*., 2013). Both types of DGT (using an exposure window of 2 cm × 15 cm) were established in the field with three replicates at each rice growing stage. The probes were manually inserted in the rice root zone of soils with least possible disturbance to the root zone. The probes were retrieved after 24 h and transferred to the laboratory for analyses.

The ZrO-Chelex-binding gels were sectioned at 2.0 mm intervals. Analyses of P and Fe were performed by a miniaturized molybdenum blue and phenanthroline colorimetric method using an Epoch Microplate Spectrophotometer (BioTek, USA). The grayscale intensity of the Zr-oxide gel was measured by computer-imaging densitometry according to Ding *et al*. (2013). The Zr-oxide gel strip was first immersed in a reagent mix for surface coloration. The colored Zr-oxide gel was scanned using a flat-bed scanner (Canon 5600F, Canon Corporation, Japan) at a resolution of 600 dpi, corresponding to a pixel size of 42 μm × 42 μm, and the grayscale intensity of the scanned images was analyzed using ImageJ 1.46 software (http://rsb.info.nih.gov/ij).

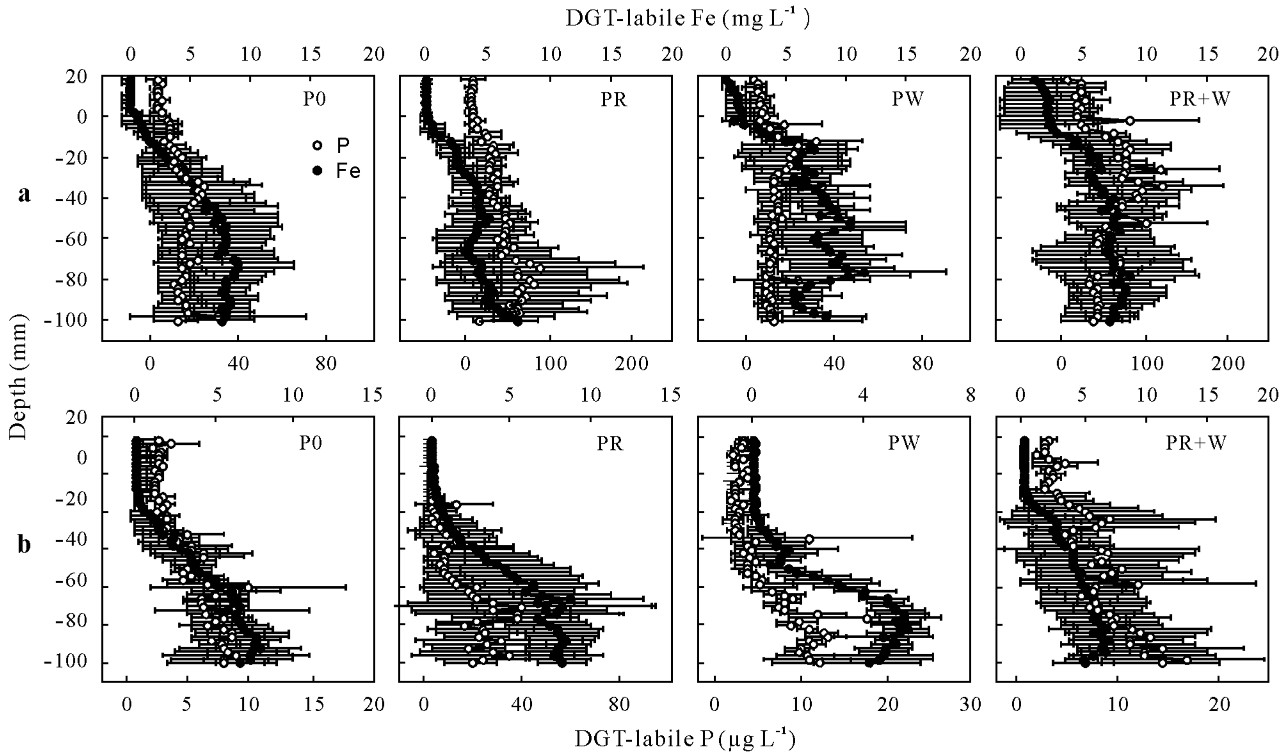


Fig. 1 One-dimensional distribution of labile P and Fe, measured *in-situ* using diffusive gradients in thin films (DGT), in flooded paddy soils near rice roots at two rice growth stages (seedling (a) and booting (b)) under four P fertilizer treatments. Error bars indicate standard deviations of means ($n = 3$). PW = P fertilization only in the wheat growing season; PR = P fertilization only in the rice growing season; PR+W = P fertilization in both seasons; P0 = no P fertilization.

*Analyses of basic soil properties*

The basic chemical properties of the soil including temperature, pH, and redox potential were measured *in-situ*. Soil Olsen P was calculated using the sodium bicarbonate ($NaHCO_3$, pH 8.5) extraction method. Crop total P was determined after digestion with sulfuric acid ($H_2SO_4$) and hydrogen peroxide ($H_2O_2$) oxidation and was analyzed using a spectrometer (UV 2500, SHIMADZU Corporation, Japan) (Lu, 2000).

*Calculations*

The concentrations of DGT-labile P and Fe were calculated using established equations for the DGT technique (Zhang *et al*., 1998; Davison and Zhang, 2012; Ding *et al*., 2013):

$$C_{\mathrm{DGT}} = \frac{M \Delta g}{D_{\mathrm{g}} A t} \tag{1}$$

where $\Delta g$ is the thickness of the diffusive layer, $D_{\mathrm{g}}$ is the diffusion coefficient of phosphate or Fe in the diffusive layer, $t$ is the deployment time, $M$ is the corresponding accumulated mass of P over the deployment time, and $A$ is the exposure area of the gel. The $M$ was calculated according to the following equation:

$$M = C_{\mathrm{e}}(V_{\mathrm{g}} + V_{\mathrm{e}})/f_{\mathrm{e}} \tag{2}$$

where $C_{\mathrm{e}}$ is the concentration of the analyte in the eluted solution, $V_{\mathrm{g}}$ is the volume of the gel, $V_{\mathrm{e}}$ is a known volume of 1 mol $L^{-1}$ NaOH, and $f_{\mathrm{e}}$ is the elution efficiency. The values of $f_{\mathrm{e}}$ for P and Fe were 98% and 88%, respectively (Xu *et al*., 2013).

High-resolution imaging of labile P in soils was interpreted as a time-averaged flux ($F_{\mathrm{DGT}}$, μg $cm^{-2}$ $s^{-1}$). The grayscale intensity of the scanned gel surface ($y$) was transformed into the accumulation mass ($M$) as per the following equation (Ding *et al*., 2013):

$$y = -167.3\mathrm{e}^{\frac{-M}{6.51}} + 214.63 \tag{3}$$

The $F_{\mathrm{DGT}}$ was calculated according to the following equation (Ding *et al*., 2016a):

$$F_{\mathrm{DGT}} = \frac{M}{t} \times 10^6 \tag{4}$$

*Statistical analysis*

The effects of P fertilization treatments on one-dimensional DGT-labile P and Fe in soil during the two rice growth stages were tested using a Duncan's test and a one-way analysis of variance. Statistical significance is reported at $P < 0.05$. A linear regression was used to analyze the relationship between DGT-labile P and Fe. All statistical analyses were performed using SPSS 17.0 software.

## RESULTS

### *Distribution of one-dimensional DGT-labile P and Fe in soils*

One-dimensional vertical distributions of labile P and Fe measured by DGT in four different P fertilizer treatments at two rice growth stages are shown in Fig. 1. The concentration of averaged DGT-labile P at the seedling stage was higher than that at the booting stage, and the PR+W treatment produced the highest concentrations of labile P at both growth stages. A linear positive correlation of DGT-labile P and Fe was observed under each P fertilization treatment at both growth stages (Fig. 2); all correlations were significant at $P < 0.001$, apart from that at the seedling stage in the PW treatment ($P < 0.01$).

The DGT-labile Fe and P were enriched at similar depths and exhibited similar variation in the vertical and horizontal directions, indicating that enhanced flux of P to the DGT probe is closely associated with Fe redox cycling. The positive linear correlation of DGT-labile P and Fe was significant in each P fertilization treatment at both seedling and booting stages ($P < 0.01$). The slopes in the linear equations (Fe/P ratio values) ranged from 0.16 to 0.707 at the booting stage and from 0.023 to 0.077 at the seedling stage; these values were lower in the PR and PR+W treatments, compared to the PW treatment and to the control.

### *Correlation between one-dimensional DGT-labile P and soil Olsen P and crop total P*

To better understand the correlation of labile P concentrations measured using DGT and soil Olsen P or P uptake by rice plants, the average concentration of P measured using the DGT technique for each depth profile and each treatment was calculated separately. A significant, strong positive correlation of DGT-labile P concentration with soil Olsen P was observed ($R^2 = 0.77$, $P < 0.01$) and with rice total P concentration ($R^2 = 0.62$, $P < 0.05$) (Fig. 3).

### *Distribution of two-dimensional DGT-labile P in soils*

The two-dimensional distribution of DGT-labile P was also assessed at a fine resolution based on color development (Fig. 4). The results (presented as $F_{\rm DGT}$, Fig. 4) showed higher $F_{\rm DGT}$ values at both rice growth stages in the PW, PR, and PR+W treatments, compared with that of the control. The PW+W treatment showed the highest values of DGT-labile P. In addition, spatial variation in labile P occurred along the vertical and lateral axes. A higher concentration of labile P was observed at depths of 0 and 60 mm at the seedling stage and below 60 mm at the booting stage. This result was consistent with the one-dimensional distributions of labile P (Fig. 1).

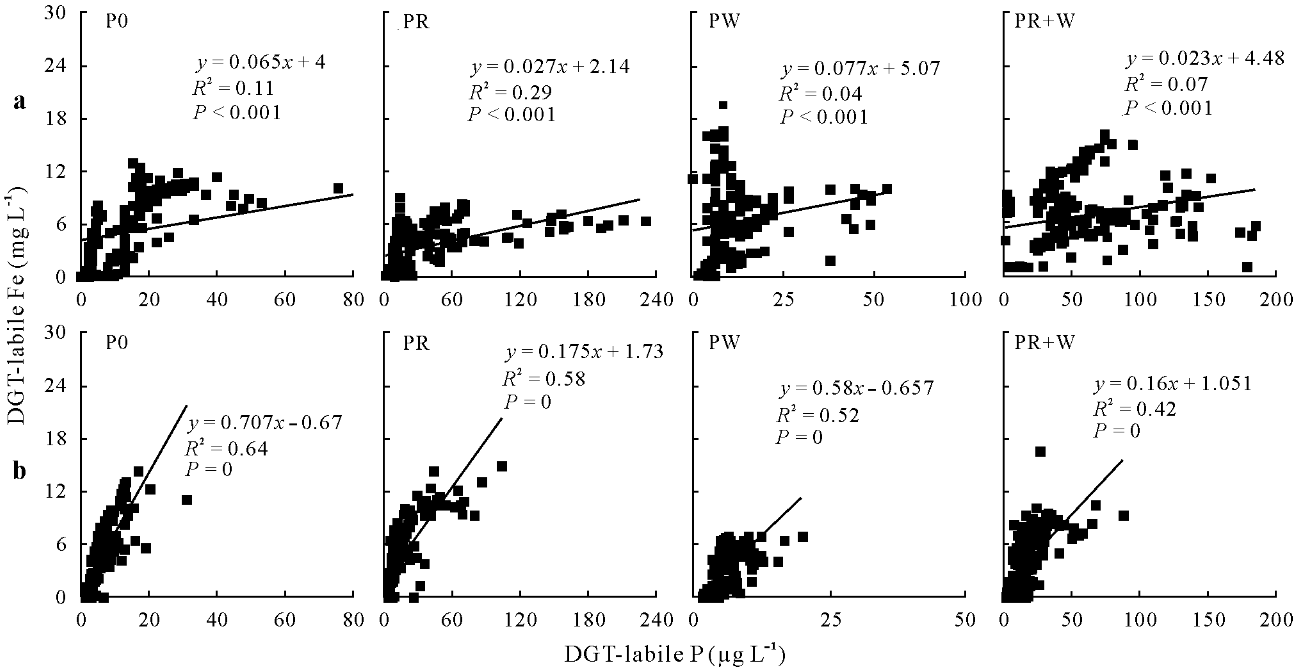


Fig. 2 Correlations between labile Fe and P, measured *in-situ* using diffusive gradients in thin films (DGT), in flooded paddy soils near rice roots at two rice growth stages (seedling (a) and booting (b)) under four P fertilizer treatments. Each treatment was performed in three replicates. PW = P fertilization only in the wheat growing season; PR = P fertilization only in the rice growing season; PR+W = P fertilization in both seasons; P0 = no P fertilization.

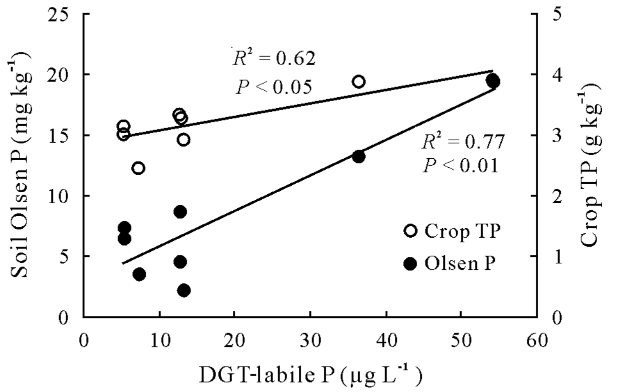


Fig. 3 Correlation of labile P (measured *in-situ* using diffusive gradients in thin films (DGT)) with soil Olsen P and crop total P (TP). The value of labile P was calculated by averaging the concentration of each profile.

## DISCUSSION

The uptake of soil P by plants is a complex process that involves dynamic interactions between the solid phase, soil solution, and the root system. Our results indicated a significant positive correlation of the average DGT-labile P value of each profile with soil Olsen P and rice total P concentration. Here, for the first time, the DGT technique was used to determine the distribution of labile P and to assess variations in close proximity of rice plant roots at two growth stages *in situ*, and our data revealed that the P measured using the DGT technique was bioavailable P to rice plants. A previous study using DGT to predict P uptake by winter barley was performed in a greenhouse experiment (Tandy *et al.*, 2011). So far, considerably few studies using DGT to assess P uptake by crop plants have been published; however, this technique has been used to measure the response of yield to P fertilization, particularly in dry-farming crops (McBeath *et al.*, 2007; Six *et al.*, 2013, 2014).

Importantly, previous studies on P bioavailability in flooded soils have mostly neglected the heterogeneity in biogeochemical properties. In this study, the spatial distribution of P was visualized to make our results more interpretable and evident to farmers; this method can potentially be used to assess optimal P fertilization and test the necessity for adjustments of the applied P fertilization scheme. The potential of DGT to determine this variability over time suggests its use for assessing P bioavailability by accounting for the heterogeneity of flooded soils, analogous to a study on bioavailability of heavy metals in sediments (Amato *et al.*, 2015). A previous study reported the spatial distribution and release kinetics of P in soils at a sub-mm scale (Kruse *et al.*, 2015), which emphasizes the advantages and potential application of DGT on flooded soils. Moreover, two-dimensional

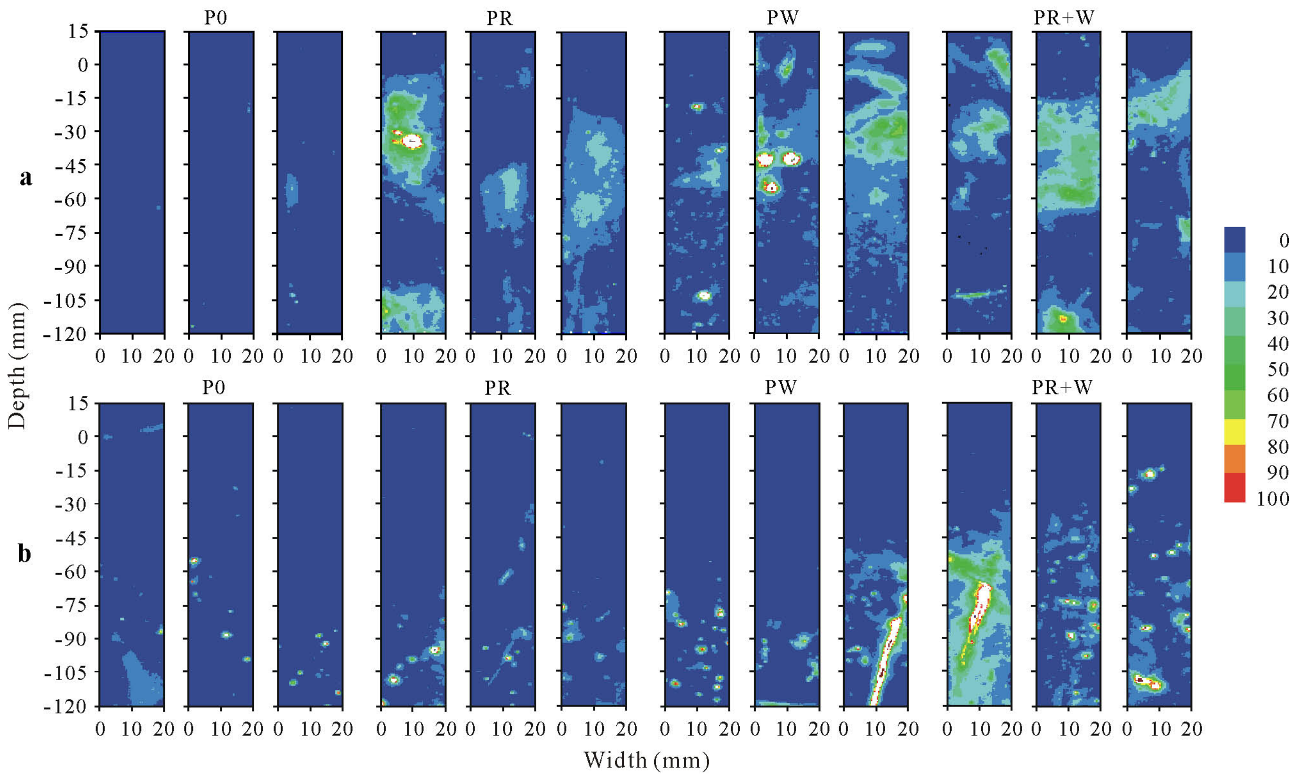


Fig. 4 Two-dimensional distribution of labile P in flooded paddy soils near rice roots at two rice growth stages (measured *in-situ* using diffusive gradients in thin films (DGT)) and coloration in flooded paddy soils near rice roots at two rice growth stages (seedling (a) and booting (b)) under four P fertilizer treatments. Numbers 0 to 100 indicate an increase in the flux of DGT-labile P (pg cm$^{-2}$ s$^{-1}$). Each treatment was performed in three replicates. PW = P fertilization only in the wheat growing season; PR = P fertilization only in the rice growing season; PR+W = P fertilization in both seasons; P0 = no P fertilization.

distributions of DGT-labile P indicated the heterogeneous distribution of soil P and a shift of high concentrations from the topsoil layer at the seedling stage to deeper soil layers at the booting stage. This spatial heterogeneity was influenced by rice root tip growth and by changes in the redox conditions in flooded soils. Santner *et al*. (2012) also observed elevated P concentrations near the root tips and along the root axes of *Brassica napus*.

Furthermore, we previously examined a rice-wheat rotation system over four years using a chemical extraction method and found that a PW treatment may provide sufficient soil available P for subsequent rice and wheat growth. In fact, even after seven years, no significant differences in rice biomass were observed between the four P fertilization treatments (0.356–0.469 t ha$^{-1}$ at the rice seedling stage, and 9.071–11.10 t ha$^{-1}$ at the rice booting stage). Moreover, the two-dimensional distribution of DGT-labile P in the PW treatment was not significantly different from that in the PR or PR+W treatment. Additionally, compared with the PW treatment, lower Fe/P ratios indicated a relatively higher risk and intensity of P release from soils (Ding *et al*., 2016a), especially at the seedling stage, as P fertilizer was applied as a base fertilization. This also provides evidence for reducing environmental contamination and the feasibility of avoiding P fertilization in the rice growing season.

## CONCLUSIONS

The results presented in this study indicated that DGT was a suitable tool for assessing P bioavailability in paddy soils, based on the strong positive relationships with soil Olsen P and rice total P. Furthermore, the results provided a visual perspective for understanding the mobilization of soil P and an estimate of sufficiency of P for crop. The new and direct DGT technique evaluation results also showed the feasibility of P fertilization only during the wheat season in a rice-wheat crop rotation, a reduced P application regime potentially supplying sufficient labile P in flooded soils like P fertilization in both the rice and wheat seasons. These insights may help optimize P fertilization and minimize the risk of environmental pollution.

## ACKNOWLEDGEMENT

This study was funded by the National Key Research and Development Program of China (No. 2017YFD0200206), the National Natural Science Foundation of China (No. 41671304), and the National Basic Research and Development Program of China (No. 2015CB150403).

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