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

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ABSTRACT

We applied a novel approach-the diffusive gradients in thin films technique (DGT)-for investigating the *in situ* distributions of labile phosphorus (P) and iron (Fe) in close proximity to Asian rice (*Oryza sativa L.*) roots at submillimeter to millimeter spatial resolutions at the seedling and booting stages in this study. The experiment was conducted in a seven-year field trial plot with four 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 rice P concentration ($R^2 = 0.62$, $P < 0.05$). Furthermore, the results on one- and two-dimensional changes of DGT-labile P revealed that the fertilization only in the wheat season had sufficient labile P pools in the flooded soil, similar to those in the fertilization only in the rice season treatment, despite a lower dissolved P concentration. The co-occurrence and significantly positive correlation ($P < 0.01$) between DGT-labile P and Fe indicates the Fe-coupled mobilization of P in flooded soils. These results collectively show that the DGT technique provides the firsthand information on the *in situ* distribution of labile P and its variability in close proximity to the roots of rice plants. It implies that the DGT technique can improve our understanding of *in situ* and high-resolution of labile P processes in paddy soils and can provide useful information on optimal application of P fertilizers.

Keywords Fertilization reduction, Labile P, Rice root, Zr-oxide DGT, Fe-P coupling

INTRODUCTION

Owing to dual concerns on the risk of water quality and finite phosphorus (P) reserves on the earth, it is imperative that more efficient use of P in broadacre agriculture is required and has been highlighted recently (Cordell and White, 2014). Our previous work in the Taihu Lake region in China identified from chemical extraction method indicated that P fertilization during the wheat season only in a rice-wheat crop rotation could sustain rice crop yield due to a sufficient supply of available P for crop growth (Wang et al., 2016a,b). However, due to the operationally defined nature of chemical extraction methods, there are limitations both on methodological and physico-chemical interpretation.

Rice makes up a large proportion of people's diet and is a major crop in the region in Southeast Asia. During the rice-growth season, the physiochemical condition of flooded paddy soils and the release of P may fluctuate under anoxic condition (Upreti et al., 2015). Furthermore, Fe(III)-oxyhydroxides are reductively dissolved, and due to the O_2 release by the rice roots, Fe(II) in turn is oxidized in the vicinity of the roots, which leads to a formation of iron plaque on submerged rice roots (Williams et al., 2014). Thus elevated levels of Fe(II) exist at the boundary of the aerobic rhizosphere area submerged, and affect the rate of P uptake by rice plants (Reddy and Patrick, 1976). Considering localized P, such as around crop roots, may be capable of resupplying P at a much higher rate than the surrounding soil. Therefore, detailed information on the spatial pattern of labile P distribution along the root axis and with depth could provide an assessment of plant P needs and provide a valuable contribution to the efforts towards P fertilizer reduction program. However, assessing the spatial distribution of root-induced changes of P lability in the root zone is experimentally challenging.

The diffusive gradients in thin films (DGT) technique has been shown to be an optimal indicator of P bioavailability in soil relative to chemical extractions because it mimics the uptake of solutes by plant roots by providing a sink for the free orthophosphate and other ions (Mason et al., 2013), despite it falls short of mimicking processes at the root surface (Kruse et al., 2015). Using a specially designed passive sampler, DGT techniques can detect labile P fractions released from soil solids, then diffused through diffusive layer and assimilated by the binding layer (Li et al., 2018). This technique provides a reasonably effective way to assess P availability for crops and could provide an improved recommendation for P fertilizer application in nutrient management (Zhang et al., 2013, 2014) than reagent based extraction techniques. Since first conceptualized, the DGT technique has been applied to predict plant response (specifically for wheat, tomato, and maize) to P fertilizer inputs (Mason et al., 2010; Mcbeath et al., 2007; Menzies et al., 2005; Six et al., 2012, 2013; Tandy et al., 2011). However, most of these studies are done on dry-farming crops and soils sampled at crop maturation, and application of the DGT technique to predict P availability for plants in water-logged and anoxic soils has been tested less. Only recently, a few pioneering studies have used for specifically mapping the distribution and changes of labile and plant-available P around single, soil-grown plant roots at sub-mm scale (Kreuzeder et al., 2018; Santner et al., 2010, 2012; Stockdale et al., 2008).

As we know, in flooded paddy soils, under anoxic condition, the retention of P by iron (oxyhy)droxides could be reduced, which led to the release of P and Fe(II) into the porewater. The simultaneous release and coupling between P and Fe(II) using DGT technology has been reported by Ding et al. (2016) and Xu et al. (2012) in sediment. For example, Ding et al. (2016) observed significantly positive correlations of DGT-labile P and Fe. Chen et al. (2018) also indicated 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 of note, that the Zr-oxide DGT has been developed for imaging the distribution of labile P in water-logged soils on sub-millimeter scale in combination with detection using computer-imaging densitometry (CID) (Ding et al., 2013). Moreover, the Zr-oxide DGT can provide a potentially powerful tool *in situ* and enable imaging of the heterogeneous distribution of labile P (Ding et al., 2015). Recently, Santner et al. (2012) compared soluble P change around the roots of two *Brassica napus* cultivars using two-dimensional DGT imaging. However, most of the DGT technology application is limited to laboratory trials, while it is seldom tested *in situ* on a field experiment scale.

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-growth season. The overall aim of this study was to generate 1) the potential applicability of the DGT technique in flooded soils, and 2) a visual assessment of P distribution to further compare and test of the sufficiency of P fertilization during the wheat season only in a rice-wheat rotation cropping system.

MATERIALS AND METHODS

Field experiment design

The paddy field experiment is located at the Yixing Agro-Environment Research Base (the northwest side of the Taihu Lake within one km of the shore) (31°16′N, 119°54′ E) and is managed by the Institute of Soil Science, Chinese Academy of Sciences. The experiment started from the rice-growth season in May 2010 and spanned seven consecutive integrated rice (*Oryza sativa L.*) and wheat (*Triticum aestivum L.*) rotation seasons, including the P fertilizer application in the rice season only (PR) and wheat season only (PW) treatments, and once in the rice season and once in the wheat season (PR+W) treatment. No P fertilizer was used in the control treatment in either of the crop-growth seasons (Pzero). Details of the agronomic management have been described in Wang et al. (2016b).

Measurement of labile P and Fe with DGT during rice-growth season

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

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

Analyses of soil basic properties

The basic chemical properties of the soil including temperature, pH, and redox potential were measured *in situ*. Soil Olsen P was calculated from 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 analyzed with a spectrometer (UV 2500 Japan) (Lu, 2000).

Calculations

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

$$C_{\mathrm{DGT}} = \frac{\mathrm{M}\Delta \mathrm{g}}{D_g A t} \quad (1),$$

where Δg is the thickness of the diffusive layer, D_g is the diffusion coefficient of phosphateor Fe in the diffusive layer, t is the deployment time, and M is the corresponding accumulated mass of P over the deployment time.

The M was calculated accroding to the eq. (2)

$$\mathrm{M} = C_e(V_g + V_e)/f_e \quad (2)$$

whereC_e is the concentration of the analyte in the elution solution, V_g is the volume of the gel, f_e is the elution efficiency. The values of f_e for P and Fe were 98 % and 88%, respectively (Xu et al., 2013).

The high-resolution imaging of labile P in soils is interpreted as the time-averaged flux (F_{DGT}, μg cm^{-2} s^{-1}). The grayscale intensity of the of the scanned gel surface (y) was transformed into the accumulation mass (M) according the eq. (3) (Ding et al., 2013):

$$y = -167.3e^{\frac{-M}{6.51}} + 214.63 \quad (3)$$

F_{DGT} was calculated according to eq. (4) (Ding et al., 2016a):

$$F_{DGT} = \frac{M}{t} \times 10^6 \quad (4)$$

The Duncan test at a significance level of $P < 0.05$ level and one-way ANOVA test were used to analyze the effect of different P fertilization treatments on the one-dimensional DGT-labile P and Fe in soils during the two rice-growth stages. Linear regression was used to analyze the relationship between DGT-labile P and Fe. All statistical analyses above were conducted using software SPSS 17.0 software.

RESULTS

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

The one-dimensional vertical distributions of labile P and Fe measured by DGT in the four different P fertilizer treatments under two stages of rice growth are shown in Fig. 1. The concentration of averaged DGT-labile P at the rice seedling stage was higher than that at the booting stage, and the rice and wheat treatment showed the highest concentration of labile P compared to the wheat only, rice only, and control treatments at both stages of the rice-growth season. The positive relationship between DGT-labile P and Fe can be well fitted using a line for each P fertilization treatment both at the rice seedling and booting stages (Fig. 2), all with significance levels of $P < 0.001$, except the wheat only treatment at the seedling stage ($P < 0.01$).

Fig. 1

Fig. 1 One-dimensional distribution of labile P and Fe measured by DGT in paddy soil around rice roots during two rice-growing stages (seedling and booting) under four P fertilizer treatments *in situ*. PW indicates P fertilization during the wheat-growing season only, PR indicates P fertilization during the rice-growing season only, PR+W indicates P fertilization during both the rice- and wheat-growing seasons, and Pzero indicates no P fertilization during either season. Error bars indicate ± standard deviation of the mean ($n = 3$).

Fig. 2

Fig. 2 Correlation analysis between labile Fe and P measured by DGT during two rice-growth stages (seedling and booting) under four P fertilizer treatments *in situ.* Each treatment includes three replicates.

The DGT-labile Fe and P were enriched in a similar depth and exhibited similar variations in both the vertical and horizontal directions, reflecting that the enhanced flux of P to the DGT probe is closely related to Fe redox cycling. The positive relationship between DGT-labile P and Fe can be well fitted using a line for each P fertilization treatment both at the rice seedling and booting stages, all with significance levels of $P<0.01$. The slopes in the linear equations (the values of the Fe/P ratio) ranged from 0.16 to 0.707 in the booting stage and from 0.023 to 0.077 in the seedling stage, and these values were smaller in the rice only, rice and wheat treatment compared to the wheat only and control treatments.

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

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

Fig. 3

Fig. 3 Correlation between the labile P measured by DGT and soil Olsen P (left axis) and total P concentration (right axis) in rice plants. The value of labile P measured by DGT was calculated by averaging the concentration for each profile.

Distribution of two-dimensional DGT-labile P in soils during the rice-growth season

The two-dimensional distribution of DGT-labile P was also obtained at a fine resolution based on color development (Fig. 4). The results (interpreted as F_{DGT}, Fig. 4) showed higher F_{DGT} values in the wheat only, rice only, and wheat and rice treatments in both rice-growing stages, compared with that of control treatment. The wheat and rice treatment showed the highest values of DGT-labile P. In addition, spatial variation in labile P was apparent both along vertical and lateral dimensions. Particularly, a higher concentration of labile P was mostly present between 0 and 60 mm soil depth in the rice seedling stage and below 60 mm in the rice booting stage. This result is consistent with the one-dimensional distributions of labile P (Fig. 1).

Fig. 4

Fig. 4 Two-dimensional distribution of labile P measured by DGT and coloration in paddy soil around rice roots during two rice-growth stages (seedling and booting) under four P fertilizer treatments *in situ*. Numbers 0 to 100 indicate an increase in the value of the flux (pg cm^{-2} s^{-1}). Each treatment includes three replicates.

DISCUSSION

Given that plant uptake of P from soil is a complex process that involves a dynamic interaction among the solid phase, soil solution, and root system. Results showed that the average value of DGT-labile P for each profile had a significant and positive correlation with soil Olsen P and rice total P concentration. This is the first time the DGT technique is used to determine the labile P distribution and variations in close proximity to the roots of rice plants during the two rice-growth stages *in situ*, and our data reveal that the P measured by the technique is bioavailable P to rice plants. A previous study on the application of DGT to predict P uptake was on winter barley in a greenhouse experiment (Tandy et al., 2011). However, until now, very little other work using DGT to study crop uptake of P has been published, but the technique has been used to measure the yield response to P fertilizer addition, especially for dry-farming crops (Mcbeath et al., 2007; Six et al., 2013, 2014).

Importantly, the assessment of P bioavailability in flooded soils has neglected the heterogeneity in biogeochemical properties before. Visual information on the spatial variability of P in this study provided easy-to-judge results that are convincing farmers and thus could be used as a metrics for optimal P fertilizer application and any adjustment or reduction needed on P application. The capability of DGT to grasp this variability at different times asserts it as promising tool in the assessment of P bioavailability and could account heterogeneous feature of the flooded soils into consideration, analogous to a study on the bioavailability of heavy metals in sediments (Amato et al., 2015), and provided information on the spatial distribution and release kinetics of P in soils at the sub-mm scale (Kruse et al., 2015) that DGT can offer attest its applicability in flooded soils *in situ*. Moreover, two-dimensional distributions of DGT-labile P reflected the heterogeneous distribution character of soil P and the concentration translocated from topsoil in the seedling stage to deeper soil depth in the booting stage. This spatial heterogeneity was more influenced by the growth of the rice root tips and changes in redox condition in flooded soils. Santner et al. (2012) also observed elevated P concentrations at the root tips and along the root axis of *Brassica napus* cv.

Furthermore, our previous work on four-year results identified that P fertilization during the wheat season only in a rice-wheat crop rotation could supply a sufficient soil available P for rice and wheat growth by chemical extraction method. In fact, in the seven-year, there were still no significant differences in rice biomass for the four P fertilization treatments (0.356-0.469 t ha^{-1} in rice seedling stage, and 9.071-11.10 t ha^{-1} in rice booting stage). Moreover, the values of DGT-labile P in the two-dimensional distributions here also suggested that the wheat only treatment was not significantly different from the rice only and rice and wheat treatments. Additionally, compared with the wheat only treatments, lower Fe/P ratios for the rice treatments indicated a relatively higher risk and strength of P release from soils (Ding et al., 2016a), especially in the rice seedling stage, due to the external application of P fertilizer was used as base fertilizer. Hence this also provides evidence for reducing environmental risk and the feasibility of P fertilizer reduction in the rice season.

CONCLUSION

The results presented in this study indicate that DGT appear to be a suitable tool for assessing P bioavailability in paddy soils, based on the strong positive relationships with soil Olsen P and rice P. Furthermore, the results provided a visual perspective for understanding the mobilization of soil P and an estimate of sufficiency of P for crop. DGT is a new and direct technique for evaluating the feasibility of P fertilization during the wheat season only in a rice-wheat crop rotation, as this P reduction regime potentially supplies sufficient labile P pools in the flooded soils similar to P fertilization in both the rice and wheat seasons. This knowledge base helps optimize the application of P fertilizers and minimize the subsequent nutrient enrichment in open waters and eutrophication risks.

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Figure captions:

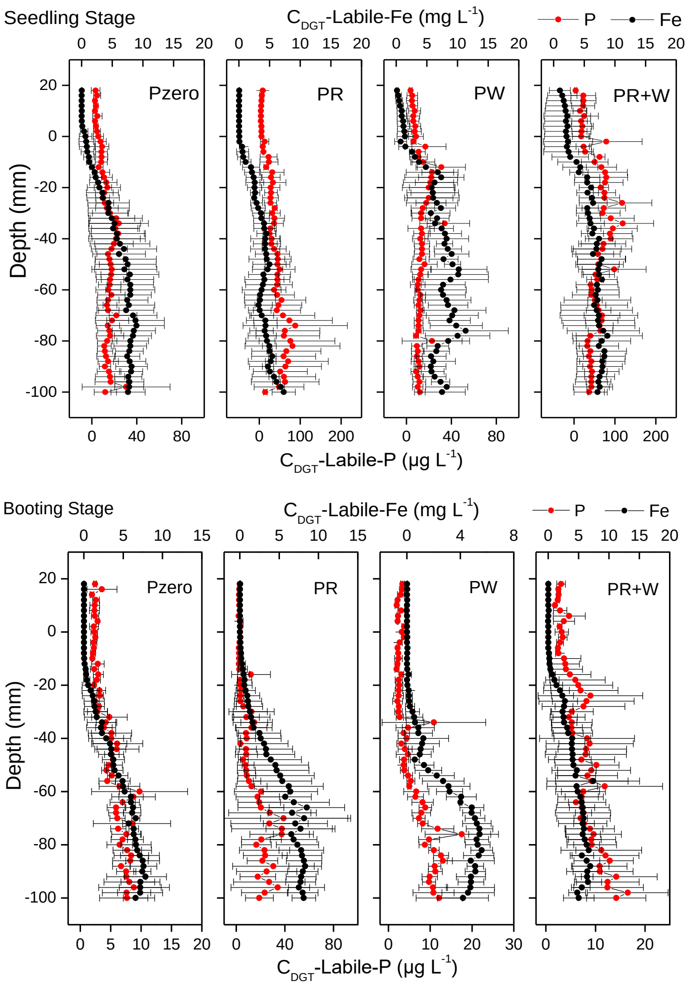
Fig. 1 One-dimensional distribution of labile P and Fe measured by DGT in paddy soil around rice roots during two rice-growing stages (seedling and booting) under four P fertilizer treatments *in situ*. PW indicates P fertilization during the wheat-growing season only, PR indicates P fertilization during the rice-growing season only, PR+W indicates P fertilization during both the rice- and wheat-growing seasons, and Pzero indicates no P fertilization during either season. Error bars indicate ± standard deviation of the mean (n = 3).

Fig. 2 Correlation analysis between labile Fe and P measured by DGT during two rice-growth stages (seedling and booting) under four P fertilizer treatments *in situ.* Each treatment includes three replicates.

Fig. 3 Correlation between the labile P measured by DGT and soil Olsen P (left axis) and total P concentration (right axis) in rice plants. The value of labile P measured by DGT was calculated by averaging the concentration for each profile.

Fig. 4 Two-dimensional distribution of labile P measured by DGT and coloration in paddy soil around rice roots during two rice-growth stages (seedling and booting) under four P fertilizer treatments *in situ*. Numbers 0 to 100 indicate an increase in the value of the flux (pg cm^{-2} s^{-1}). Each treatment includes three replicates.

Fig. 1



Seedling Stage
C$_{DGT}$-Labile-Fe (mg L^{-1})
P
Fe
Pzero
PR
PW
PR+W
Depth (mm)
C$_{DGT}$-Labile-P (μg L^{-1})
Booting Stage
C$_{DGT}$-Labile-Fe (mg L^{-1})
P
Fe
Pzero
PR
PW
PR+W
Depth (mm)
C$_{DGT}$-Labile-P (μg L^{-1})

Fig. 2

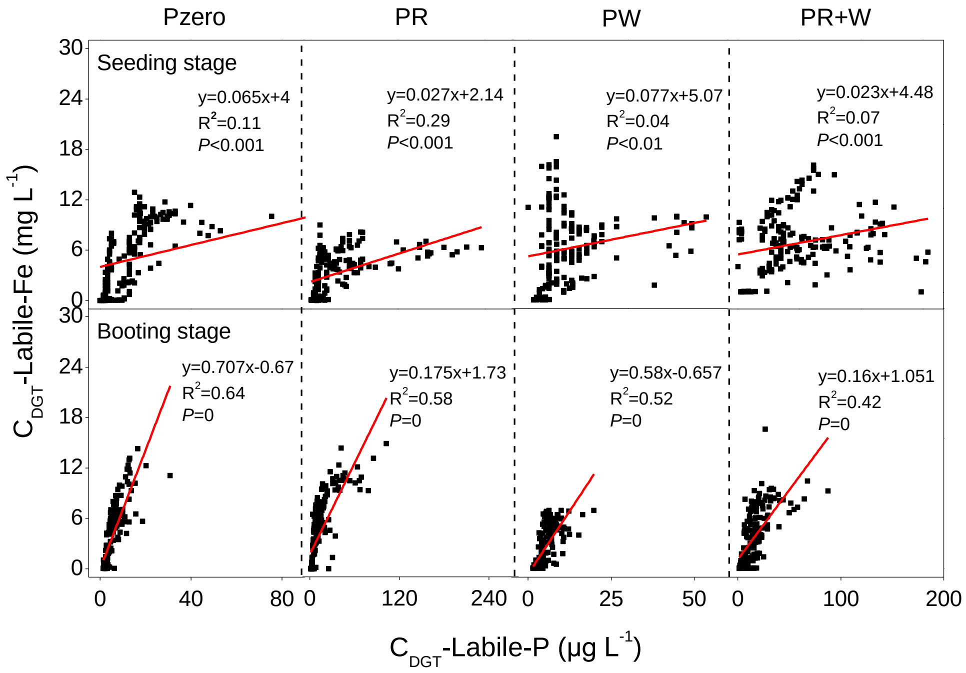
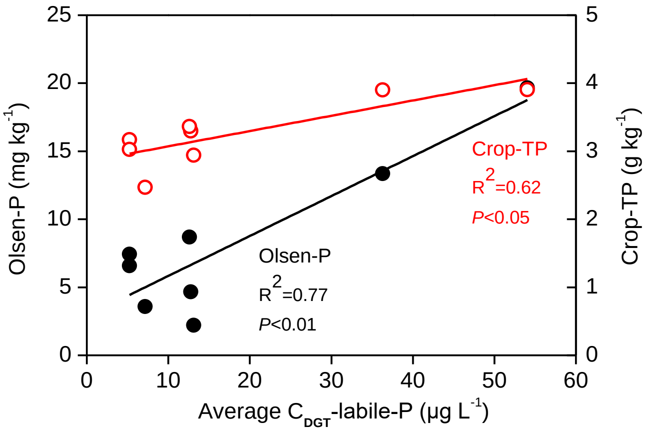
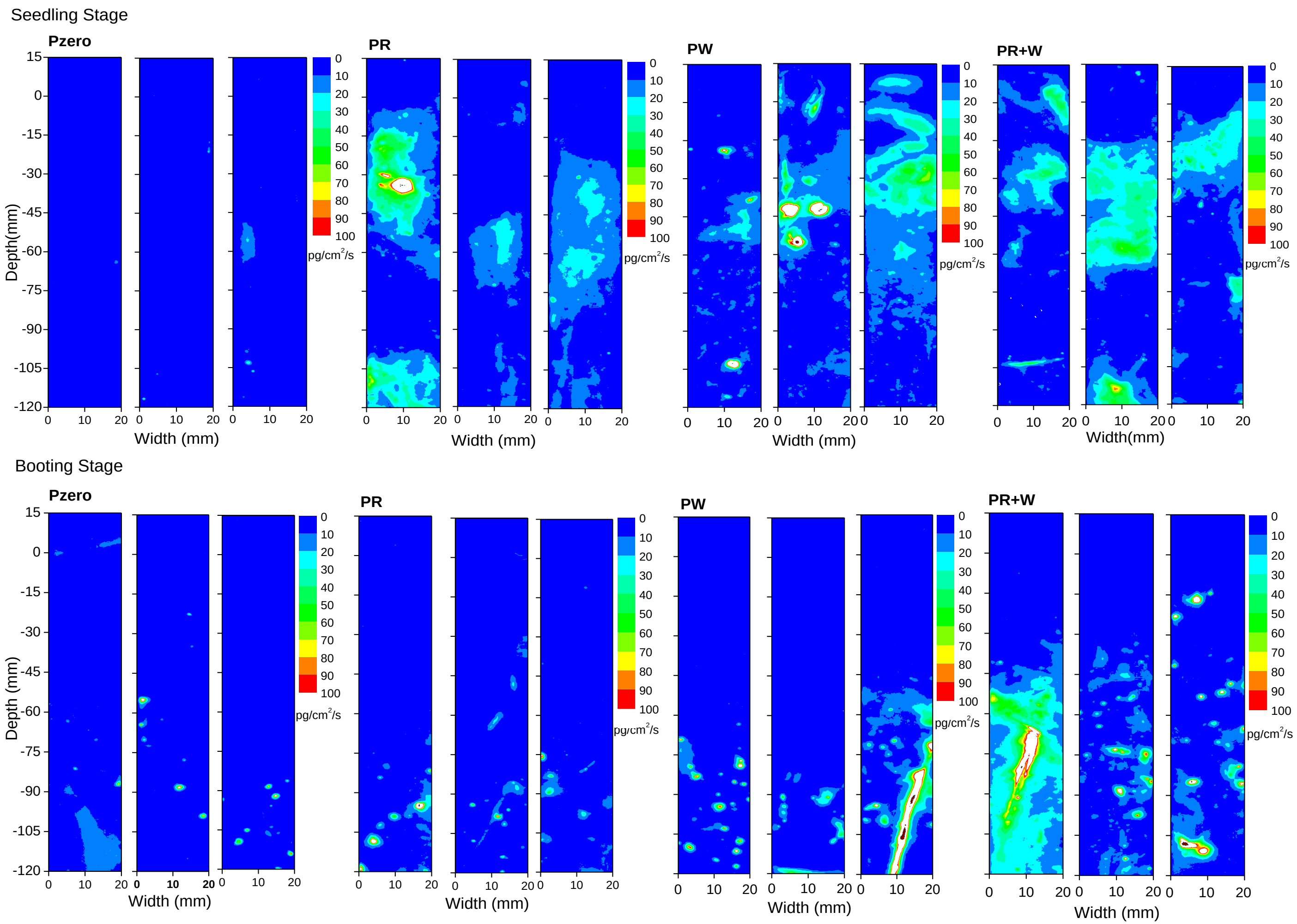

Pzero
PR
PW
PR+W
Seeding stage
y=0.065x+4
R2=0.11
P<0.001
y=0.027x+2.14
R2=0.29
P<0.001
y=0.077x+5.07
R2=0.04
P<0.01
y=0.023x+4.48
R2=0.07
P<0.001
Booting stage
y=0.707x-0.67
R2=0.64
P=0
y=0.175x+1.73
R2=0.58
P=0
y=0.58x-0.657
R2=0.52
P=0
y=0.16x+1.051
R2=0.42
P=0
CDGT-Labile-Fe (mg L-1)
CDGT-Labile-P (μg L-1)

Fig. 3



25
20
15
10
5
0
0
10
20
30
40
50
60
5
4
3
2
1
0
Olsen-P (mg kg-1)
Crop-TP (g kg-1)
Average CDGT-labile-P (μg L-1)
Crop-TP
R2=0.62
P<0.05
Olsen-P
R2=0.77
P<0.01

Fig. 4


Seedling Stage
Pzero
PR
PW
PR+W
Depth(mm)
Width (mm)
Width(mm)
pg/cm^2/s
0
10
20
30
40
50
60
70
80
90
100
15
-15
-30
-45
-60
-75
-90
-105
-120
Booting Stage
Depth (mm)