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Response of soil phosphatase activity and soil phosphorus fractions to the application of chloropicrin and azoxystrobin in ginger cultivation

Yan Wang,[a,b] Xiaomei Yang,[b,c] Minggang Xu[a,d*] and Violette Geissen[b]

Abstract

BACKGROUND: Soil fumigation can change soil nutrient cycling processes by affecting soil beneficial microorganisms, which is a key issue for soil fertility. However, the effect of combined application of fumigant and fungicide on soil phosphorus (P) availability remains largely unclear. We investigated the effects of the fumigant chloropicrin (CP) and the fungicide azoxystrobin (AZO) on soil phosphatase activity and soil P fractions in ginger production using a 28-week pot experiment with six treatments: control (CK), a single application of AZO (AZO1), double applications of AZO (AZO2), CP-fumigated soil without AZO (CP), CP combined with AZO1 (CP + AZO1) and CP combined with AZO2 (CP + AZO2).

RESULTS: AZO application alone significantly increased the soil labile P fractions (Resin-P + $NaHCO_3$-Pi + NaOH-Pi) at 9 weeks after planting (WAP) but decreased the soil phosphatase activity at 28 WAP. CP fumigation significantly reduced the soil phosphatase activity but increased the proportions of soil labile P fractions (Resin-P + $NaHCO_3$-Pi + $NaHCO_3$-Po) to total P (TP) by 9.0–15.5% throughout the experiment. The combined application of CP and AZO had a synergistic effect on soil phosphatase activity and soil P fractions compared with a single application.

CONCLUSION: Although AZO application and CP fumigation can increase soil available P in the short term, they might negatively affect soil fertility in the long run by inhibiting soil phosphatase activity. Soil microbial activities, especially microorganisms related to P cycling, may be responsible for the variations in soil P availability, but further research is needed.

Supporting information may be found in the online version of this article.

Keywords: azoxystrobin; chloropicrin; phosphatase activity; soil P fractions; ginger

INTRODUCTION

Soil fumigation has increased steadily over the last decades, driven by the demand for control of crop diseases caused by soil-borne pathogens[1] to achieve a high crop yield. For example, Ślusarski and Spotti found that chloropicrin (CP), applied using drip irrigation at 40 g m^{-2}, reduced the inoculum density of *Verticillium dahlia* in the soil by 86% and improved the average pepper marketable yields by 10.9–90.0% in different trials.[2] In addition to soil fumigation before planting, other fungicides such as azoxystrobin (AZO) are also applied during crop growth to reduce any soil-borne diseases (such as ginger wilt and rot diseases) caused by the interaction of crop and the surrounding environment.[3-5]

However, owing to its broad antimicrobial activity, CP fumigant is indiscriminately poisonous to all organisms – not only targeted harmful pests, pathogens and nematodes, but also beneficial soil microorganisms[6] – which may negatively affect the associated soil nutrient cycling processes. Previous studies have detected that CP fumigation could significantly inhibit the nitrification processes in various soils[6-8] but significantly increase the amount of soil available phosphorus (P) and leached P by altering the structure of the microbial community encoding the alkaline phosphatase *phoD* gene.[9] AZO fungicide also has detrimental effects on non-target functional microorganisms and their governed

* *Correspondence to: Minggang Xu, Key Laboratory of Arable Land Quality Monitoring and Evaluation, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China. E-mail: xuminggang@caas.cn*

a *Key Laboratory of Arable Land Quality Monitoring and Evaluation, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China*

b *Soil Physics and Land Management Group, Wageningen University, Wageningen, The Netherlands*

c *College of Natural Resources and Environment, Northwest A&F University, Yangling, China*

d *Shanxi Province Key Laboratory of Soil Environment and Nutrient Resources, Institute of Eco-environment and Industrial Technology, Shanxi Agricultural University, Taiyuan, China*

enzyme activities such as soil urease, invertase and phosphatase.[10,11] However, up to now, the combined effects of CP fumigation and AZO application on soil phosphatase activity and the governed soil P cycling process still remain largely unclear due to the great diversity and non-specific forms of soil P.

Soil P exists in different chemical forms, including inorganic P (Pi) and organic P (Po), which determine the soil P availability for plant uptake.[12] According to the solubility in the soil solution and the availability for plant uptake, soil P is interpreted by Hedley's sequential extraction methods as Resin-P (easily available P), $NaHCO_3$-P ($NaHCO_3$-Pi and $NaHCO_3$-Po: labile P), NaOH-P (NaOH-Pi and NaOH-Po: moderately labile P) and Occluded-P (HCl-Pi and residual P: unavailable P).[13] Only dissolved inorganic P fractions in the soil solution such as Resin-P can be taken up directly by plants. Other insoluble inorganic P fractions such as $NaHCO_3$-Pi and NaOH-Pi need to be solubilized by organic acids, while insoluble organic P factions such $NaHCO_3$-Po and NaOH-Po need to be mineralized by phosphatases before being taken up by plants.[14,15] Acid phosphatase (AiP, EC 3.1.3.2) and alkaline phosphatase (AlP, EC3.1.3.1) are two important extracellular phosphomonoesterases produced by soil microorganisms (or plants) that can hydrolyze simple phosphate esters ($NaHCO_3$-Po and NaOH-Po) into orthophosphate (PO_4^{3-}), which can be taken up by the plants.[16,17] Accordingly, CP fumigation and AZO application may potentially alter the soil P fractions, either by killing soil microbes and releasing the phosphorus inside microorganisms,[18] or by changing the composition of soil P solubilizing microorganisms (PSMs) such as *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter*, and fungi like *Penicillium* and *Aspergillus*[9,19-21] which can secrete organic acids to dissolve Pi and produce phosphatase to mineralize Po.[22,23] Therefore, understanding the composition of different soil P fractions and phosphatase activity is a key part of understanding microbial functions in soils contaminated by fumigants and fungicides.

Nonetheless, most previous studies focused only on the individual fumigant or fungicide application in the soil, ignoring the fact that fumigants and other fungicides coexist in real agricultural systems.[24] Some studies have observed that CP fumigation could decrease the adsorption of AZO fungicide on soil particles and extend the degradation time of AZO.[4] Soil microbiome recovery after initial soil fumigation may be affected by AZO application during crop growth, resulting in different effects on soil P cycling. In addition, our previous study discovered that only CP fumigation combined with double application of AZO increased the ginger height and root P uptake, while a single application of AZO did not affect the ginger growth regardless of whether CP fumigation was applied.[25] Therefore, in this study, we want to go further in exploring the variations in soil phosphatase activity and soil P fractions under the combined application of CP fumigant and AZO fungicide, and try to answer whether CP and AZO application could change the soil P availability. The study was conducted using the same greenhouse experiment as our previous study,[25] in which CP and AZO were applied individually and in combination. The AZO was also applied once and twice to study the effects of recurrent application of AZO on the soil P availability. We hypothesize that CP and AZO can increase the soil available P content, and the dual applications of AZO and the combined application of CP and AZO can enhance this effect.

MATERIALS AND METHODS

Experiment materials and design

From March to October of 2019 a greenhouse experiment was conducted at the Chinese Academy of Agricultural Sciences, Beijing, China. Details of the experiment are summarized in Table 1. Briefly, before the experiment, a field (1.2 × 2.2 m, in Anqiu, Shandong Province of China) that had never been planted with ginger and had never been fumigated before was selected. Chloropicrin (CP)[CCl_3NO_2] (Dalian Lvfeng Chemical Co. Ltd, China) was injected at 37.1 g m^{-2} (161.3 mg kg^{-1}) into a depth of roughly 15 cm. After the injection, the field was immediately covered by plastic film for 1 week to prevent the escape of CP gas. The plastic film was then removed, and the field was ventilated for another week to release the residual CP gas. After CP fumigation, the topsoil (0–20 cm) of the CP-fumigated field (for CP treatments) and an adjacent field without CP fumigation (for non-CP treatments) were passed through a 4 mm sieve and taken into the greenhouse for the next step.

In the greenhouse, ginger (*Zingiber officinale*) was used as the model plant. Before the experiment, 6 kg collected soil and 100 g germinated ginger rhizome were put into each pot (diameter 30 cm, height 25 cm), making 90 pots in total including six treatments with five replicates of each treatment made for every sampling time (Table 1). Treatments were as follows: control (CK); a single application of AZO (AZO1); double applications of AZO (AZO2); CP fumigated soil without AZO (CP); CP combined with

Table 1. Chloropicrin, azoxystrobin and fertilizer application

Treatment	Chloropicrin (g m^{-2}) (28 March)	Azoxystrobin (mg m^{-2}) 8 WAP (8 June)	Azoxystrobin (mg m^{-2}) 16 WAP (29 July)	Compound chemical fertilizer (N ≥ 16%, P ≥ 5.2%, K ≥ 16.6%, g) 17 WAP (11 August)
CK	—	—	—	5.0
AZO1	—	47.1	—	5.0
AZO2	—	47.1	47.1	5.0
CP	37.1	—	—	5.0
CP + AZO1	37.1	47.1	—	5.0
CP + AZO2	37.1	47.1	47.1	5.0

Abbreviations: AZO1, single application of AZO; AZO2, double applications of AZO; CK, control; CP, CP fumigated soil without AZO; CP + AZO1, CP combined with AZO1; CP + AZO2, CP combined with AZO2; WAP, weeks after planting.

AZO1 (CP + AZO1); and CP combined with AZO2 (CP + AZO2). Azoxystrobin (AZO; Hebei Zhongbao Green Crop Technology Company, China) was applied by suspension spray method. For AZO1 and CP + AZO1 treatments, 0.55 mg kg^{-1} AZO was applied at 8 weeks after planting (WAP), while for AZO2 and CP + AZO2 treatments the same amount of AZO was applied again at 16 WAP.

The soil was destructively sampled four times during the different growth periods of ginger, including BP: before planting (12 April); 9 WAP (seedling stage, 17 June); 17 WAP (flourishing growing stage, 10 August) and 28 WAP (harvest stage, 15 October). After the 17 WAP sampling, 5.0 g (about 70.8 g m^{-2}) compound chemical fertilizer (N ≥ 16%, P ≥ 5.2%, K ≥ 16.6%) was applied to each pot to supply the nutrients for ginger growth.

For the soil samples, one part was air dried and sieved to 2 mm for analysis of soil pH, soil organic matter (SOM) and Olsen-P, and to 0.25 mm for the analysis of total N (TN), total P (TP) and different soil P fractions using methods described in Wang et al.[25] Briefly, pH was determined using a pH meter with a soil:water ratio of 1:2.5. Soil organic matter (SOM) was analyzed using a colorimetric method after H_2SO_4–K_2CrO_7 oxidation, while TP was determined using inductively coupled plasma–optical emission spectroscopy (ICP-OES) after HNO_3–HF–H_2O_2 digestion. Soil Olsen-P was determined using spectrophotometry after extraction with 0.5 mol L^{-1} $NaHCO_3$. Total nitrogen (TN) was measured using an elemental analyzer. Another part was directly sieved to 2 mm and stored at 4 °C for phosphatase activity analysis.

Soil phosphatase activity

Soil acid (AiP) and alkaline (AlP) phosphatase activity was measured using a modified method of Tabatabai and Bremner.[26] 1.0 g of a fresh soil sample (<2 mm) was incubated with 0.2 mL toluene, 1.0 mL of 0.05 mol L^{-1} *p*-nitrophenyl phosphate and 4.0 mL modified universal buffer (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase) in a 50 mL centrifuge tube at 37 °C for 1 h. After 1 h, the reaction was ended by adding 1.0 mL of 0.5 mol L^{-1} $CaCl_2$ and 4.0 mL of 0.5 mol L^{-1} NaOH solution. The mixture was then filtrated and the absorbance of the filtrate at 410 nm was measured using a spectrophotometer. The concentration of *p*-nitrophenol in soil extract was calculated according to the *p*-nitrophenol standard curve and the phosphatase activity was quantified by the amount of *p*-nitrophenol produced per gram soil per hour (mg *p*-nitrophenol g^{-1} h^{-1}).

Soil phosphorus fractions

A modified method described by Tiessen[27] and Hedley *et al.*[13] was used to fractionate the soil P.[28] Briefly, 0.5 g air-dried soil (<0.25 mm) was added to a 50 mL centrifuge tube and extracted in the following sequential order: (1) 30 mL ultra-pure water with two anion-exchange resin membrane strips (1 × 2 cm) converted to the bicarbonate form (Resin-P, easily available P); (2) 30 mL of 0.5 mol L^{-1} $NaHCO_3$ solution ($NaHCO_3$-Pi + $NaHCO_3$-Po, labile P); and (3) 30 mL of 0.1 mol L^{-1} NaOH (NaOH-Pi and NaOH-Po, moderately labile P). For each extraction step, the mixture of soil and extractant was shaken at 25 °C for 16 h (180 rpm). The soil suspension was then centrifuged at 10 000 × *g* at 0 °C for 10 min and decanted. Inorganic P fractions (Pi) in each extract were measured at 700 nm using the molybdate ion colorimetry method.[29,30] TP in the extracts was determined using ICP-OES, while Po was calculated as the difference between TP and Pi. The concentration of Occluded-P was estimated by subtracting the sum of the total of other P fractions from the total P concentrations of samples.[15]

Statistical analysis

The ratio of the sum of Resin-P, $NaHCO_3$-Pi and NaOH-Pi to the sum of $NaHCO_3$-Po and NaOH-Po (Pi/Po = (Resin-P + $NaHCO_3$-Pi +NaOH-Pi)/($NaHCO_3$-Po + NaOH-Po)) was calculated to evaluate the transformation between these plant available inorganic and organic P fractions.

The statistical analysis was performed using IBM SPSS Statistic 20. Normality and homogeneity of variance of the measured data were tested using the Kolmogorov–Smirnov and Levene test ($P > 0.05$). Non-parametric Kruskal–Wallis analysis was used for the variance of Pi/Po and the Wilcoxon test was applied to compare the differences between each pair of treatments. For the normally distributed values (soil P fraction contents and proportions of soil P fractions to total P, soil phosphatase activity), one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) at $P < 0.05$ were used for significance comparison among treatments.

Random forest (RF) analyses were conducted to predict the importance of different soil properties (explanatory variables, mainly as TP (total P), Olsen-P, SOM (soil organic matter), pH, AiP and AlP) on each of the soil P fractions (response variables) using *R4.0.5*[31] and '*randomForest*' (v4.6–14)[32] package. Redundancy analysis (RDA) was also performed to establish the relationship among P fractions (response variables) and soil properties (explanatory variables) using Origin 2020 (http://www.originlab.com). All the figures were made using Origin 2020.

RESULTS

Soil phosphatase activity

The variations in soil AiP and AlP activity among different treatments and sampling times are shown in Fig. 1. AiP activity was significantly lower in CP, CP + AZO1 and CP + AZO2 (27.1–35.5 mg *p*-nitrophenol g^{-1} h^{-1}) as compared to the activity in the CK (38.3–44.2 mg *p*-nitrophenol g^{-1} h^{-1}) during the whole experiment. There was no significant difference in the average AiP activity among CK, AZO1 and AZO2 until 28 WAP. At 28 WAP, the average AiP activity was significantly lower in AZO1 and AZO2 (35.3–36.7 mg *p*-nitrophenol g^{-1} h^{-1}) as compared to that in CK (39.8 mg *p*-nitrophenol g^{-1} h^{-1}). In CP-fumigated treatments, no significant difference in the average AiP activity was observed between CP and CP + AZO1, while the average AiP activity in CP + AZO2 treatment (32.3–34.3 mg *p*-nitrophenol g^{-1} h^{-1}) was significantly higher than that in the CP treatment (28.4–29.4 mg *p*-nitrophenol g^{-1} h^{-1}) from 17 WAP (Fig. 1(A)).

The average AlP activity showed similar variations to the average AiP activity, with significantly lower values in CP, CP + AZO1 and CP + AZO2 (17.1–26.8 mg *p*-nitrophenol g^{-1} h^{-1}) than that in CK (22.7–33.2 mg *p*-nitrophenol g^{-1} h^{-1}) during the whole experiment (Fig. 1(B)). No significant difference was observed in the average AlP activity among CK, AZO1 and AZO2 until 28 WAP, when the average AlP activity in the AZO2 (21.4 mg *p*-nitrophenol g^{-1} h^{-1}) was significantly lower than that in CK (22.7 mg *p*-nitrophenol g^{-1} h^{-1}). However, in CP-fumigated treatments, the average AlP activity in CP + AZO2 (18.7–26.8 mg *p*-nitrophenol g^{-1} h^{-1}) was significantly higher than that in CP treatment (17.1–22.1 mg *p*-nitrophenol g^{-1} h^{-1}) from 17 WAP. There was still no significant difference between CP and CP + AZO1 treatments.

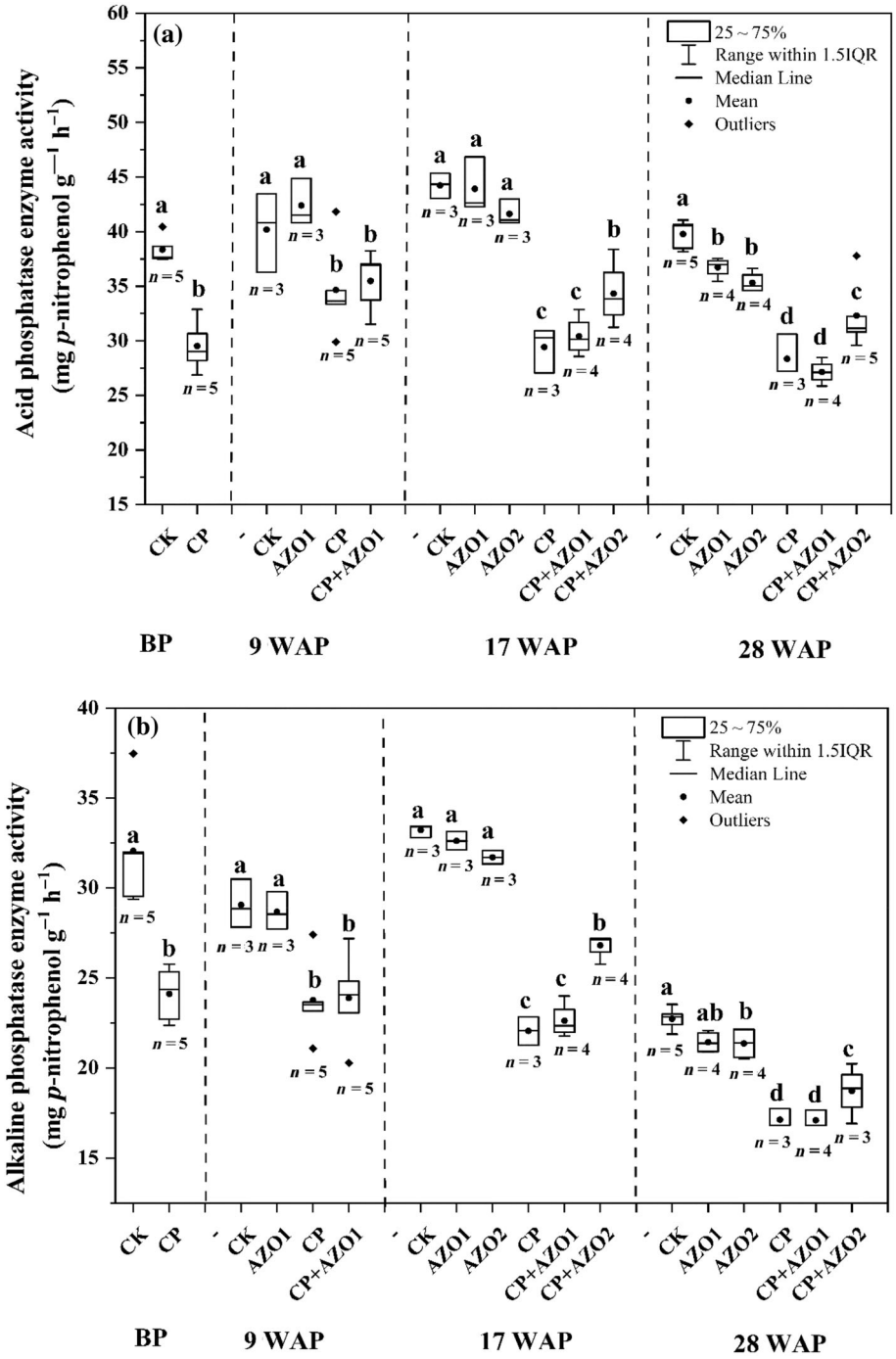

Figure 1. Soil acid (A) and alkaline (B) phosphatase activity in different treatments and at different sampling times. Treatments include control (CK), a single application of AZO (AZO1), double applications of AZO (AZO2), CP (CP fumigated soil without AZO), CP combined with AZO1 (CP + AZO1), and CP combined with AZO2 (CP + AZO2). Sample times were: before planting (BP); 9, 17 and 28 weeks after planting (9 WAP, 17 WAP and 28 WAP). *n* is the actual number of samples from the corresponding box. Lower-case letters indicate a significant difference between treatments during each sampling time (ANOVA with least significant difference (LSD) test; $P < 0.05$).

Soil P fractions

The values of soil TP were from 1.1 to 2.0 g kg^{-1} during the whole experiment, and no significant difference was observed between different treatments (Supporting Information, Table S1). For samples collected at 9 WAP, the content of Occluded-P was significantly higher in CK (927.0 mg kg^{-1}) treatment than that on other treatments (409.3–476.1 mg kg^{-1}). For samples collected at 17 WAP, the content of $NaHCO_3$-Po was significantly higher in CP-fumigated treatments (93.9–161.0 mg kg^{-1}) than that in CK (27.0 mg kg^{-1}). For samples collected at 28 WAP, the content of $NaHCO_3$-Po was significantly higher in AZO2 (46.1 mg kg^{-1}) and CP-fumigated treatments (66.2–89.0 mg kg^{-1}) than that in CK (6.1 mg kg^{-1}) (Fig. 2(A)). In CP-fumigated treatments, the content of $NaHCO_3$-Po was significantly higher in CP + AZO1 (136.6 mg kg^{-1}) and CP + AZO2 (161.0 mg kg^{-1}) as compared to that in CP treatment (93.9 mg kg^{-1}) at 17 WAP, while only

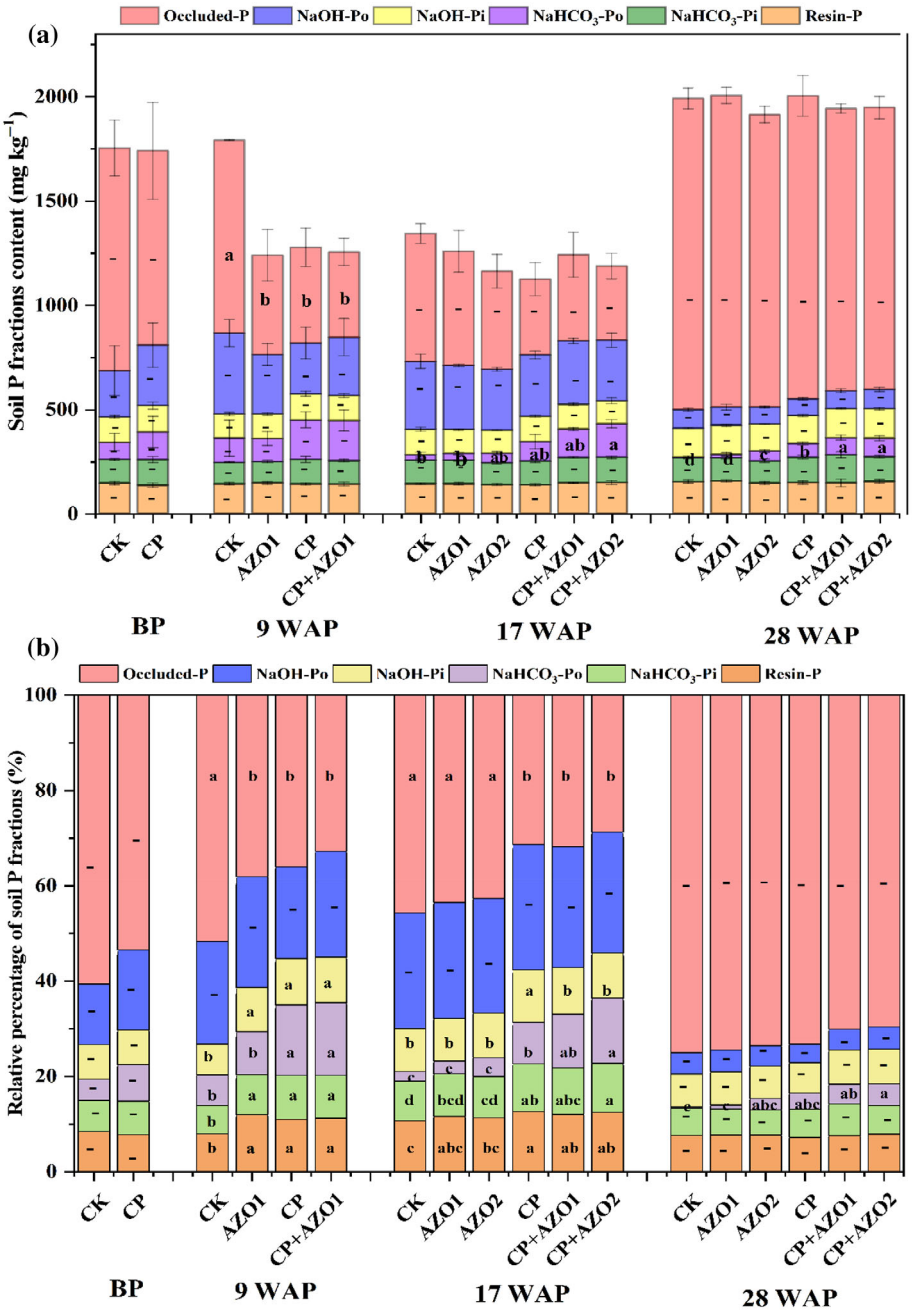

Figure 2. Absolute content of soil P fractions (A) and relative percentages of soil P fractions (B) in different treatments and with different sampling times. Treatments include control (CK), a single application of AZO (AZO1), double applications of AZO (AZO2), CP fumigated soil without AZO (CP), CP combined with AZO1 (CP + AZO1) and CP combined with AZO2 (CP + AZO2). Sample times were: before planting (BP); 9, 17 and 28 weeks after planting (9 WAP, 17 WAP and 28 WAP). Resin-P (orange, inorganic P fractions extracted by water), $NaHCO_3$-Pi (green, inorganic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), $NaHCO_3$-Po (purple, organic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), NaOH-Pi (yellow, inorganic P fractions extracted by 0.5 mol L^{-1} NaOH solution), NaOH-Po (blue, organic P fractions extracted by 0.5 mol L^{-1} NaOH solution) and Occluded-P (red, unavailable P). Columns represent the mean values of replicates with standard deviation (SD). Lower-case letters (a–d) indicate a significant difference in each P fraction between treatments during each sampling time. '-' means no significant differences (ANOVA with least significant difference, LSD test, $P < 0.05$).

CP + AZO2 (89.0 mg kg^{-1}) had a significantly higher content of $NaHCO_3$-Po than CP treatment (66.2 mg kg^{-1}) at 28 WAP.

The proportions of each soil P fraction to TP are shown in Fig. 2 (B), and there was no significant difference between CK and CP for samples collected before planting (BP). For the samples collected at 9 WAP, the proportions of Resin-P, $NaHCO_3$-Pi and NaOH-Pi to TP were significantly higher in AZO1, CP and CP + AZO1 (Resin-P: 11.0–12.0% of TP; $NaHCO_3$-Pi: 8.3–9.2% of TP; NaOH-Pi: 9.3–9.7% of TP) as compared to that in CK (Resin-P: 8.0% of TP; $NaHCO_3$-Pi: 6.0% of TP; NaOH-Pi: 6.5% of TP). The proportions of $NaHCO_3$-Po to TP were significantly higher in CP (14.7% of TP) and CP + AZO1 (15.2% of TP) than that in CK (6.5% of TP). No significant difference in the proportions of NaOH-Po to TP was observed between different treatments (19.2–23.2% of TP).

At 17 WAP, no significant difference in the proportions of all soil P fractions to TP was measured between CK, AZO1 and AZO2.

The proportions of Resin-P, $NaHCO_3$-Pi and $NaHCO_3$-Po to TP were significantly higher in CP, CP + AZO1 and CP + AZO2 (Resin-P: 12.0–12.7% of TP; $NaHCO_3$-Pi: 9.8–10.2% of TP; $NaHCO_3$-Po: 8.7–13.8% of TP) than that in CK (Resin-P: 10.7% of TP; $NaHCO_3$-Pi: 8.3% of TP; $NaHCO_3$-Po: 2.0% of TP). In CP-fumigated soils, the proportion of $NaHCO_3$-Po was significantly higher in CP + AZO2 (13.8% of TP) than that in CP treatment (8.7% of TP). No significant difference in the proportions of NaOH-Pi to TP was observed between CK (9.0% of TP) and all of the other treatments, except for that in the CP treatment (11.0% of TP). There was no significant difference in the proportions of NaOH-Po to TP between all of the treatments (24.0–26.3% of TP).

The soil P fraction composition changed dramatically from 17 WAP to 28 WAP due to the application of chemical fertilizer. However, only the proportions of $NaHCO_3$-Po to TP were significantly higher in CP + AZO1 (4.1% of TP) and CP + AZO2 (4.6% of TP) than that in the CK (0.3% of TP). Until 17 WAP, the Pi/Po ratio was about 1.0 in all treatments. At 28 WAP, the Pi/Po values increased to 4.5 in the CK which was significantly higher than that in AZO2 (3.0), CP (2.7), CP + AZO1(2.5) and CP + AZO2 (2.3) (Fig. 3).

Soil P fractions in relation to phosphatase activity and soil properties

Based on the data collected during the whole experiment, random forest analysis was used to calculate the importance of selected soil chemical properties (TP, Olsen-P, pH, SOM; Supporting Information, Table S1) and soil AiP/AlP activity on the proportions of different soil P fractions (Fig. 4). The larger values of increase of mean square error (MSE) indicate the greater importance. For the proportions of different soil P fractions, soil TP was ranked as the most important influential factor. Olsen-P was the second most important factor affecting the composition of different soil P fractions (excluding $NaHCO_3$-Po). For the soil organic P fractions, SOM (16.0–29.3 mg kg^{-1}) was the most important influencing factor for $NaHCO_3$-Po (19.8% of increase of MSE), followed by AiP, AlP and pH (6.5–6.9), which were of similar importance (11.9–13.8% of increased of MSE). AlP was of the highest importance for NaOH-Po (14.3% of increase of MSE), while AiP was the least important (0.8% of increase of MSE).

Nonetheless, selected soil properties showed less influence on soil inorganic P fractions than soil organic P fractions. pH and AlP were considered to be the most important influencing factors for NaOH-Pi (6.1% and 7.9% of increase in MSE) and Occluded-P (11.4% and 12.1% of increase in MSE), while SOM and pH were the most important influencing factors for $NaHCO_3$-Pi (7.5% of increase in MSE). All selected soil properties had much less important impacts on Resin-P (<7% of increase in MSE).

The results of redundancy analysis (RDA) showed that the first two principal components explained more than 95% of the variations of soil P fraction across treatments (Fig. 5). Along RDA1, soil samples were clearly separated between no-CP fumigation treatments (CK, AZO1 and AZO2) and CP fumigation treatments (CP, CP + AZO1 and CP + AZO2) according to the soil P composition. Among different soil P fractions, $NaHCO_3$-Po mainly appeared in CP-fumigated soils (CP, CP + AZO1, and CP + AZO2), while Occluded-P mainly appeared in no-CP fumigated soils (CK, AZO1 and AZO2). Except for samples collected before planting (BP), soil pH, AiP and AlP activities were significantly negatively correlated with $NHCO_3$-Pi and $NaHCO_3$-Po, but significantly positively correlated with Occluded-P (Supporting Information, Table S2).

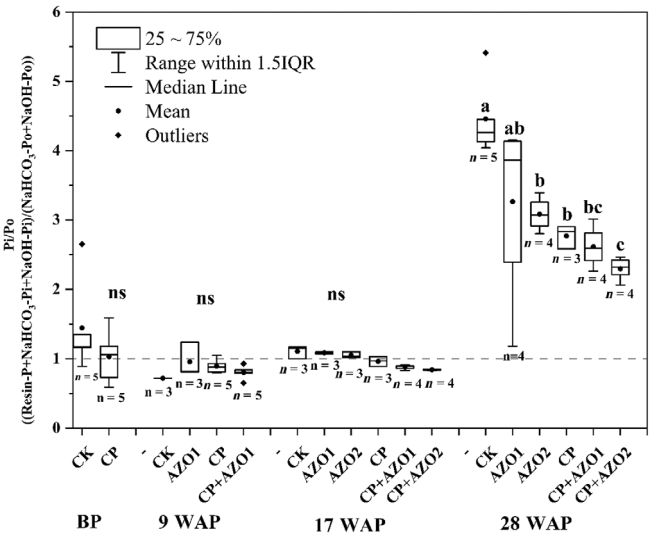

Figure 3. The ratio of available Pi (Resin-P + $NaHCO_3$-Pi + NaOH-Pi) to Po ($NaHCO_3$-Po + NaOH-Po) among different treatments for each sampling time. Pi, inorganic P; Po, organic P; Resin-P, $NaHCO_3$-P and NaOH-P are P fractions extracted sequentially using water, 0.5 mol L^{-1} $NaHCO_3$ solution and 0.5 mol L^{-1} NaOH solution. Treatments include control (CK), a single application of AZO (AZO1), double applications of AZO (AZO2), CP fumigated soil without AZO (CP), CP combined with AZO1 (CP + AZO1) and CP combined with AZO2 (CP + AZO2). Sample times were: before planting (BP); 9, 17 and 28 weeks after planting (9 WAP, 17 WAP and 28 WAP). *n* is the actual number of samples from the corresponding boxes. Lower-case letters (a–c) indicate significant differences between CK and other treatments for each sampling; ns, means no significant differences (Wilcoxon test, $P < 0.05$).

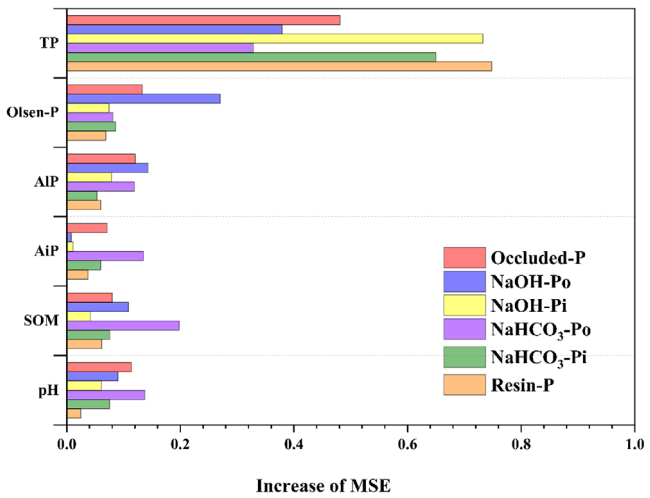

Figure 4. Random forest mean predictor importance (percentage of increase of mean square error, MSE) of major soil properties as drivers of the soil P fractions. The variables are shown in importance of descending order. An increase in MSE indicates the contribution to RF prediction accuracy for the variable. Soil properties include total phosphorus (TP), Olsen-P, alkaline phosphatase (AlP), acid phosphatase (AiP), soil organic matter (SOM) and pH. The legend indicates soil P fractions, including Resin-P (orange, inorganic P fractions extracted by water), $NaHCO_3$-Pi (green, inorganic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), $NaHCO_3$-Po (purple, organic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), NaOH-Pi (yellow, inorganic P fractions extracted by 0.5 mol L^{-1} NaOH solution), NaOH-Po (blue, organic P fractions extracted by 0.5 mol L^{-1} NaOH solution) and Occluded-P (red, unavailable P).

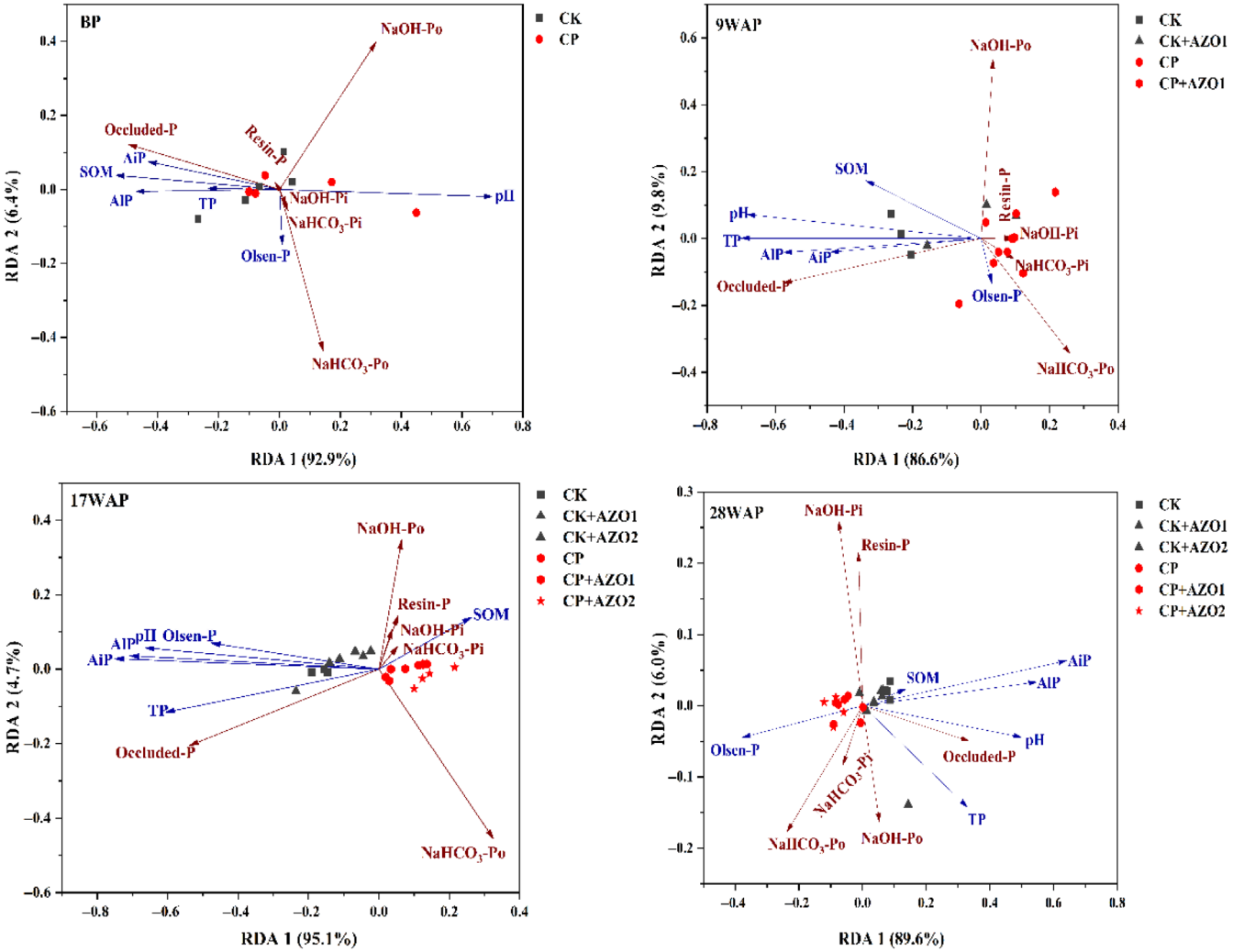

Figure 5. Redundancy analysis (RDA) of P fractions and soil basic properties. Symbols with the same shape were samples of the same treatment, including CK (control, square), a single application of AZO (AZO1), up triangle, double applications of AZO, down triangle (AZO2), CP fumigated soil without AZO (CP), circle, CP + AZO1 (CP combined with AZO1, hexagon) and CP + AZO2 (CP combined with AZO2, star). Red arrows indicate different soil P fractions extracted using Hedley's sequential extraction methods, including Resin-P (inorganic P fractions extracted by water), $NaHCO_3$-Pi (inorganic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), $NaHCO_3$-Po (organic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution), NaOH-Pi (inorganic P fractions extracted by 0.5 mol L^{-1} NaOH solution), NaOH-Po (organic P fractions extracted by 0.5 mol L^{-1} NaOH solution) and Occluded-P (unavailable P). Blue arrows indicate soil properties, including pH, soil organic matter (SOM), acid phosphatase (AiP), alkaline phosphatase (AlP), Olsen-P and total phosphorus (TP). The position and length of arrows indicate the direction and strength of the effects of soil properties on P fractions. Sample times were: before planting (BP); 9, 17 and 28 weeks after planting (9 WAP, 17 WAP and 28 WAP).

DISCUSSION

Soil phosphatase activity

This study found that, in soils without CP fumigation, AZO application did not affect soil phosphatase activity until 28 WAP, which may be caused by the low application rate of AZO (0.55 mg kg^{-1}) and rapid degradation rate.[25] This result was also in line with a previous study conducted by Wang et al.,[33] who discovered that the application of AZO at a rate of 2.0 mg kg^{-1} had no significant effect on the phosphatase activity on the 7th day of incubation in Spodosols. Similarly, in our study, soil samples were collected 1 week after the AZO application. The short period between AZO application and soil sampling could be another reason why no significant difference in soil phosphatase activity was observed between CK, AZO1 and AZO2 treatments. In addition, for samples collected at 28 WAP, the AiP activity of AZO1 and AZO2 treatments was significantly lower than that of CK treatment, confirming that AZO may take a longer time to show its effect on soil phosphatase activity.

Unlike AZO application, AiP and AlP activity in the treatments with CP fumigation (CP, CP + AZO1 and CP + AZO2) was significantly lower than that in the treatments without CP fumigation (CK, AZO1 and AZO2), which may be due to the stronger inhibitory effect of CP fumigant than AZO fungicide. Another study, conducted by Huang *et al.*, also found a 32.2% reduction in AlP activity in CP-fumigated soil (application amount was 53 mg kg^{-1}).[9] However, in soils with CP fumigation, there was no significant difference in the AiP and AlP activity between CP and CP + AZO1 treatments, while the AiP and AlP activity in the CP + AZO2 treatment was significantly higher than that in the CP treatment. The unexpectedly higher soil phosphatase activity in the CP + AZO2 treatment was probably due to the higher restoration of associated soil phosphorus solubilizing

bacteria (PSBs) responsible for AiP and AlP secretion after AZO application in CP-fumigated soils.[3] In CP-fumigated soils, soil fungi and bacteria recovered simultaneously.[34] In the CP + AZO2 treatment, those soil fungi that recovered might be killed again by the AZO fungicide,[33] leading to a faster recovery of soil PSBs due to the lack of competitors. However, further studies are needed for the detail microbial mechanisms.

Soil P fractions

In this study, the proportion of soil labile P fractions (especially soil labile organic P ($NaHCO_3$-Po)) increased with the application of AZO and CP, with the highest value for the CP + AZO2 treatment. The ratio of inorganic P (Resin-P + $NaHCO_3$-Pi + NaOH-Pi) to organic P ($NaHCO_3$-Po + NaOH-Po) content also confirmed that more soil organic P rather than soil inorganic P fractions were accumulated in treatments with AZO and CP application. The increased soil organic P fractions may come from dead microbial cells killed by AZO fungicide and CP fumigant.[35] Among soil microbes, 50–75% of microbial P exists in the form of nucleic acid, 20% exists in P monoesters and 5% exists in phospholipids.[12] AZO application and CP fumigation could kill and break down the microbial cells, releasing the organic P inside microbial cells, which then could be subsequently extracted using 0.5 mol L^{-1} $NaHCO_3$ ($NaHCO_3$-Po) and 0.1 mol L^{-1} NaOH (NaOH-Po) solution.[35] Those released soil organic P ($NaHCO_3$-Po and NaOH-Po) can be then hydrolyzed by AiP and AlP to orthophosphate for plant uptake.[36] However, the decreased activity of AiP and AlP after AZO and CP application might slow down the mineralization process of soil organic P and lead to the accumulation of organic P fractions (particularly $NaHCO_3$-Po)[12,36] (Fig. 6). The hypothesis was supported by the RF and RDA results that the proportions of $NaHCO_3$-Po to TP were highly negatively correlated with the activity of AiP and AlP (Fig. 5).

However, in CP-fumigated soils, the CP + AZO2 treatment had higher phosphatase activity as well as higher proportions of soil labile P fractions compared with the CP treatment. The reason may be that the combination of CP and AZO had a stronger killing effect on the soil microorganisms than that of a single application, releasing more microbial P into the soil. However, the addition of AZO to CP-fumigated soils may promote the recovery of some microbes that can produce phosphatase, resulting in higher phosphatase activity in CP + AZO2 than in CP. The released amount of $NaHCO_3$-Po may be greater than the amount being mineralized, leading to the accumulation of $NaHCO_3$-Po in CP + AZO2. However, further studies are needed to explain the underlying mechanism.

In our study, RF analysis showed that pH was the most important factor affecting the proportions of inorganic P fractions ($NaHCO_3$-Pi and NaOH-Pi) (Fig. 4). We found that soil pH was significantly lower in CP-fumigated soils (6.6) than in CK (6.9) 9 weeks after planting (Supporting Information, Table S1), which might be due to the release of organic acids from those dead and lysed microbial cells after CP fumigation (Fig. 6). The released organic acids then promote the dissolution of Ca-P-minerals and Fe/Al-P-minerals and improve the availability of soil P.[12]

From 17 WAP to 28 WAP, the ginger plants were in the flourishing growth and mature stages. At this time, ginger plants absorbed a large amount of soil available P, as the P in the ginger seeds cannot meet the needs of ginger growth. Therefore, in addition to soil chemical properties, ginger plants could also change the response of soil P fractions to the application of AZO and CP fumigation by absorbing soil P into plants. In this study, there was no significant difference in the soil available P content between treatments with and without CP fumigation (Fig. 2), while some previous studies that observed fumigants such as CP,[9] dazomet[19] and ethylicin[37] significantly increased the soil available P content. The inconsistent results might be due to the higher ginger P uptake in CP-fumigated soils (Supporting Information, Table S3)[38] in our study. However, Rodriguez-Morelos *et al.* found that, after 30 days of growth, AZO (applied at 1.17 mg g^{-1}) decreased the uptake of inorganic P by potato plants with and without AM fungus.[39] Our previous study also

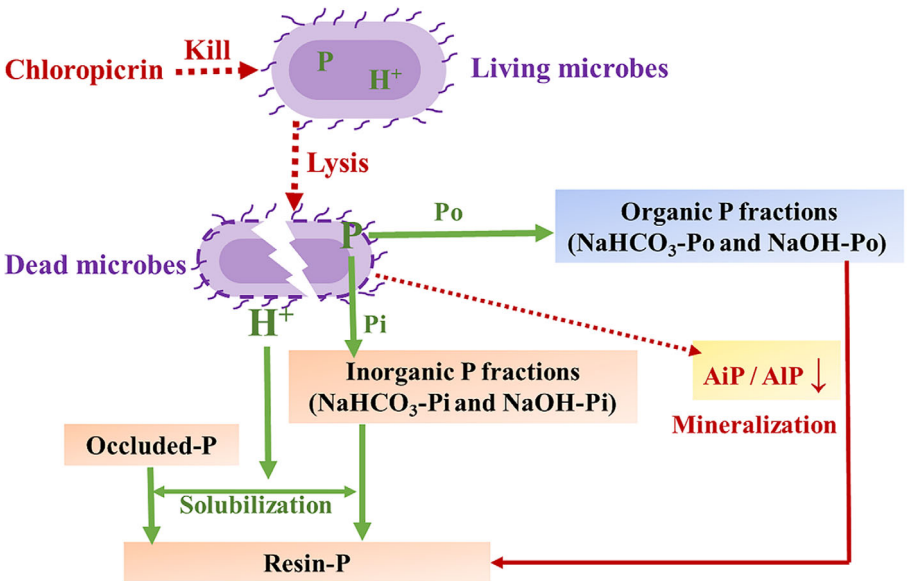

Figure 6. Schematic diagram of soil phosphorus (P) transformation process in CP fumigated soil. AiP, acid phosphatase; AlP, alkaline phosphatase; $NaHCO_3$-Pi, inorganic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution; $NaHCO_3$-Po, organic P fractions extracted by 0.5 mol L^{-1} $NaHCO_3$ solution; NaOH-Pi, inorganic P fractions extracted by 0.5 mol L^{-1} NaOH solution; NaOH-Po, organic P fractions extracted by 0.5 mol L^{-1} NaOH solution and Occluded-P (unavailable P); Pi, inorganic P; Po, organic P; Resin-P, inorganic P fractions extracted by water.

found that the average values of physiological P use efficiency (PPUE) of ginger showed a decreasing trend in CP, CP + AZO1 and CP + AZO2 treatments (589.9–723.0 g^2 dry matter g^{-1} P) as compared to the values in CK (1799.8 g^2 dry matter g^{-1}P) (Supporting Information, Table S4).[25] The inconsistent results between the increased P uptake and the decreased PPUE suggested that, after CP fumigation, the available P released at the early stage was mainly used for the ginger shoot growth rather than later ginger rhizome production.[25] A previous study suggested a reduction in the amount of P fertilizers used during the early growing stages after CP fumigation (<30 days).[9] However, the reduction of P fertilizers at the ginger early seedling stage may further aggravate the soil P deficiency during the later growth stage of the ginger rhizome.[38]

Apart from the soil properties and plant uptake, agricultural management practices during ginger growth could also lead to a different response of the soil P cycling process to the CP fumigation.[38] First, irrigation during ginger cultivation may reduce the soil available P content through P leaching, which has been proved by a previous study conducted by Huang *et al*., who found that CP fumigation increased P leaching from the soil column.[9] In our experiment, the P present in rhizome seeds was sufficient to meet the needs of the ginger plants at the early growth stage. Therefore, the increased soil available P after AZO application and CP fumigation would be washed out of the pot along with the irrigation water, leading to significantly lower soil total P and Occluded-P concentrations in AZO1, CP and CP + AZO1 treatments compared with the CK treatment at 9 WAP. In our field observation, significant reductions in ginger yield and P uptake in fields with 7 years of continuous CP fumigation resulted in more available P fractions being stored in the soil,[28] which could lead to severe environmental problems such as water eutrophication caused by P leaching into the groundwater.[34]

In addition to irrigation, fertilization during ginger growth significantly changed the soil P cycling process. In this study, chemical fertilizers (70.8 g m^{-2} of compound chemical fertilizer; $P \geq 5.2\%$) was applied after 17 WAP sampling to provide nutrients for ginger growth, resulting in a significant increase in soil TP at 28 WAP (Supporting Information Table S1). P-containing fertilizers could quickly increase soil available P content in a short period of time, but the increased soil soluble P fractions could promote the adsorption of P by soil particles, making it impossible to extract. Finally, the proportion of soil Occluded-P at 28 WAP was significantly higher than that at 17 WAP due to the increased uptake of soil available P by ginger plants and the increased soil P adsorption by soil particles (Fig. 2). At 28 WAP, the proportion of $NaHCO_3$-Po in CP-fumigated soils was still significantly higher than that in untreated soils.

Overall, AZO application and CP fumigation increased soil available P content under ginger cultivation, and combined application of AZO and CP released more soil available P than the single application. However, application of AZO and CP suppressed soil phosphatase activity, which might negatively affect later soil P availability. Soil P fractions are determined by the combined effects of soil properties, microorganism activities, plant uptake and agricultural management practices, leading to different issues at the different ginger growth stages. In the early growth stage of ginger, CP fumigation increased the content of soil available P, but that soil available P cannot be absorbed by ginger seed plants and might be washed away by irrigation water, causing serious environmental problems such as eutrophication. At this time, the amount of P fertilizer can be appropriately reduced. In the later stage of ginger growth, soil might become P deficient due to the increases in ginger P uptake and the decreases in soil phosphatase activity. At this time, more P fertilizer should be applied to meet the phosphorus demand of ginger plants to ensure the high yield of ginger.

CONCLUSIONS

AZO application and CP fumigation could increase the content of soil labile P fractions, making soil P become more available for plant uptake. Combined application of AZO and CP has more pronounced effects on soil P availability compared to the individual application of either AZO or CP alone. However, CP fumigation and AZO application significantly inhibit soil phosphatase activity, which might slow down the mineralization process of soil organic P and negatively affect soil fertility in the long run. The transformation of soil P after AZO and CP application may be due to the changes in soil microbial community, especially P-solubilizing microorganisms. However, further studies are needed to explain the microbial mechanisms underlying changes in soil P availability and ginger P uptake.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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