



# Variations of soil phosphatase activity and phosphorus fractions in ginger fields exposed to different years of chloropicrin fumigation

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## Abstract

**Purpose** Although soil fumigation efficiently controls soil-borne diseases, the effects of repeated soil fumigation on soil phosphorus (P) cycling are still largely unknown.

**Methods** In this study we conducted a field experiment to explore soil phosphorus availability in ginger fields with 0, 3, and 7 years (F0, F3, and F7) of annual chloropicrin (CP) fumigation history in Shandong Province of China. Soil samples (0–20 cm) were collected at four different times in 2019. Ginger yield, soil phosphatase (acid and alkaline) activities, and soil P fractions were measured.

**Results** Results showed that ginger rhizome yield was similar in F0 and F3 (70.0 t ha<sup>-1</sup>), but significantly lower in F7 (37.5 t ha<sup>-1</sup>). The acid phosphatase (AiP) activity was significantly higher in F0, while alkaline phosphatase (AlP) activity was the highest in F3. There was no significant difference in the available P (resin-P + NaHCO<sub>3</sub>-P + NaOH-P) between F0 and F7, with 33.6 to 57.5% of total P (TP), while the available P was significantly lower in F3, being less than 30% of TP. Redundancy analysis (RDA) showed that the highest pH values in F3 contributed to the lowest soil P availability there. AiP activities showed highly positive effects on the soil labile P contents.

**Conclusion** Results suggested that more P fertilizers are needed after 3 years of CP fumigation to avoid soil P deficiency for ginger growth. The death of ginger became the main limitation for ginger production after 7 years of CP fumigation, at which time, comprehensive agricultural practices should be considered to control ginger soil-borne diseases.

**Keywords** Fumigation · Chloropicrin · Ginger · Phosphatase activity · Soil P fractions

## 1 Introduction

Ginger (*Zingiber officinale* Rosc.) is an important commercial crop in China with high agricultural and medical value (Zhang et al. 2017a, b). During cultivation, ginger is prone

to soft rot, bacterial wilt, leaf spot, and yellowing caused by soil-borne pathogens such as *Pythium*, *Pseudomonas solanacearum*, *Ralstonia solanacearum*, *Phylosticta*, and *Fusarium* (Jiang et al. 2018). Under appropriate environmental conditions, soil-borne pathogens can invade ginger roots and stem bases, causing ginger diseases and reducing production (Mansfield et al. 2012).

To prevent soil-borne diseases in ginger crops, soil fumigation has become a key step prior to ginger cultivation in China (Rokunuzzaman et al. 2016). Among the various fumigants, chloropicrin (trichloronitromethane, CP) is one of the most widely used compounds owing to its advantageously high inhibitory efficiency on soil-borne pathogens and low environmental residues (Sun et al. 2018). Within several days after application, CP is broken down into nitrogenous compounds via both chemical and microbial processes (Neilson et al. 2020). The degradation rates of CP can be influenced by the application rate, soil moisture,

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organic matter content, and other soil properties (Ashworth et al. 2018; Ashworth and Yates 2019). Soil microorganisms that can metabolize and use CP as a source of energy and nutrients are main players in CP biodegradation (Qin et al. 2016). CP has received increasing attention over the last few years due to its broad antimicrobial activities, environmental behaviors, and ecological effects on the soil microbiome, soil enzymes, and ultimately on nutrient cycling and soil health (Pecina et al. 2016; Li et al. 2017a, b; Huang et al. 2020a).

Previous studies have shown that soil microbial communities recovered relatively rapidly after soil fumigation (Dangi et al. 2014, 2015; Liu et al. 2015; Rokunuzzaman et al. 2016; Fang et al. 2018; Zhang et al. 2019). Zhang et al. (2017a, b) found that after repeated application of soil fumigants, susceptible soil microbes could disappear while the proportion of those tolerant microbes could increase gradually and even eventually dominate the soil microbiome. They concluded that after 3 years of continuous chloropicrin fumigation, seven genera of *Actinobacteria* and one genus in *Bacteroidetes* disappeared while *Saccharibacteria* increased significantly. Dangi et al. (2017) also found that there were significant differences in soil microbial communities between untreated sites and sites exposed to 15 and 30 years of annual fumigation with methyl bromide. All of these long-term studies revealed that the changes in the soil microbiome after long-term repeated soil fumigation were irreversible. Scientists do not yet know how long it would take for the soil microbial community to recover from repeated fumigation if it can recover at all.

With the potential for the reduced activity of soil organisms after soil fumigation, one major question is how nutrient cycles in soil could be affected. Among the diverse nutrient cycling processes in soil, many researchers have explored the detrimental effects of CP on nitrogen (N) cycling and concluded that soil nitrification processes could be inhibited by soil fumigation in various soil types (Li et al. 2017a, b; Yan et al. 2017; Fang et al. 2018; Sun et al. 2018).

Soil phosphorus (P) is the second most important plant growth-limiting nutrient after nitrogen. Although the soil system is rich in P, 95–99% of the soil total P is present in an insoluble form (Wan et al. 2020) due to binding to the inorganic minerals or organic matter (Schaller et al. 2019), causing about 43% of the soils to be deficient in phosphorus available for plant growth (Zhao et al. 2019). On the other hand, phosphate rock as a raw material for phosphate fertilizer is a limited resource, which also emphasizes the urgent need to improve the availability of soil phosphorus for plant growth (Lemming et al. 2019). The soil P availability for plant uptake depends on the composition of different forms of P existing in the soil. Hedley's sequential extraction method (Hedley et al. 1982) for P, based on the P availability for plants, classifies soil

P fractions as (1) resin-P (easily available P), a soluble inorganic orthophosphate ( $\text{H}_2\text{PO}_4^{2-}$  or  $\text{H}_2\text{PO}_4^-$ ) in the soil solution and the only form that can be taken up by plants directly (Wang et al. 2012); (2)  $\text{NaHCO}_3$ -P ( $\text{NaHCO}_3$ -Pi and  $\text{NaHCO}_3$ -Po, labile P), inorganic P fractions slightly complexed with Fe/Al minerals and labile organic P fractions complex with organic matter surfaces; (3)  $\text{NaOH}$ -P ( $\text{NaOH}$ -Pi and  $\text{NaOH}$ -Po, moderately labile P), P fractions strongly complexed with Fe/Al minerals and calcic compounds, as well as organic P fractions more strongly associated with soil organic matter; and (4) occluded P (unavailable P), which is occluded by soil aggregate and cannot be used by plants (Motavalli and Miles 2002; Koch et al. 2018; Fan et al. 2019). Among these soil P fractions, inorganic P fractions ( $\text{NaHCO}_3$ -Pi and  $\text{NaOH}$ -Pi) are available for plants after solubilization, while organic P fractions ( $\text{NaHCO}_3$ -Po and  $\text{NaOH}$ -Po) need to be mineralized for plant uptake (Spohn and Kuzyakov 2013). Inorganic P solubilization and organic P mineralization are mainly controlled by phosphorus solubilizing microorganisms (PSMs) that secrete organic acid and synthesize various phosphatase enzymes (Richardson et al. 2009). Acid phosphatases (AiP; EC3.1.3.2) and alkaline phosphatases (AlP; EC3.1.3.1) are two non-specific phosphohydrolases able to hydrolyze simple phosphate monoesters to orthophosphate. The AiP can be excreted by plant roots, nodules, and soil microbes, while the production of AlP is carried out by soil bacteria and some fungi (Fraser et al. 2017).

Many studies have found that PSM species showed various responses to fumigant composition (Pecina et al. 2016; Zhang et al. 2019; Huang et al. 2020a) and application rates (Huang et al. 2020b). For example, Pecina et al. (2016) proved that the population of *Pseudomonas* ssp. and *Bacillus* ssp. increased immediately after CP fumigation in intensive tomato production fields with alkaline soil. Dangi et al. (2017) concluded that the proportion of arbuscular mycorrhizal fungi (AMF) was lower in sites with 15, 33, and 39 years of annual methyl bromide fumigation as compared to non-fumigated sites. The variation of PSMs leads to a change in P solubilizing genes and phosphatase enzyme activities, which essentially determine soil P availability. Recently, researchers like Huang et al. (2020a, b) found that soil fumigation using chloropicrin and dazomet significantly increased the plant available P content in soil and the amount of leached soil P during the first 2 weeks of incubation. No significant difference was observed for the amounts of available P and leached P after 30 days, regardless of the concentrations of chloropicrin and dazomet applied.

However, the response patterns of soil P cycling to long-term repeated soil fumigation in agricultural systems are still far from clear. Understanding the soil P transformation in fields exposed to long-term soil fumigation is crucial in providing deeper insight into the interaction between soil

fumigation and soil phosphorus availability. Therefore, in this study, we aimed to understand the effects of repeated chloropicrin fumigation on ginger yield, P uptake, soil phosphatase activity, and soil P fractions in a real-world field. We hypothesized that the repeated chloropicrin fumigation could increase ginger yield, but decrease the soil available P contents by increasing ginger P uptake.

## 2 Material and methods

### 2.1 Experiment design

The sampling sites were located in Anqiu, in the Shandong Province of China (36°21'42"N, 119°10'38"E; Fig. 1), a region with a temperate continental monsoon climate. The mean temperature is 12.2 °C and the mean annual precipitation is 646.3 mm.

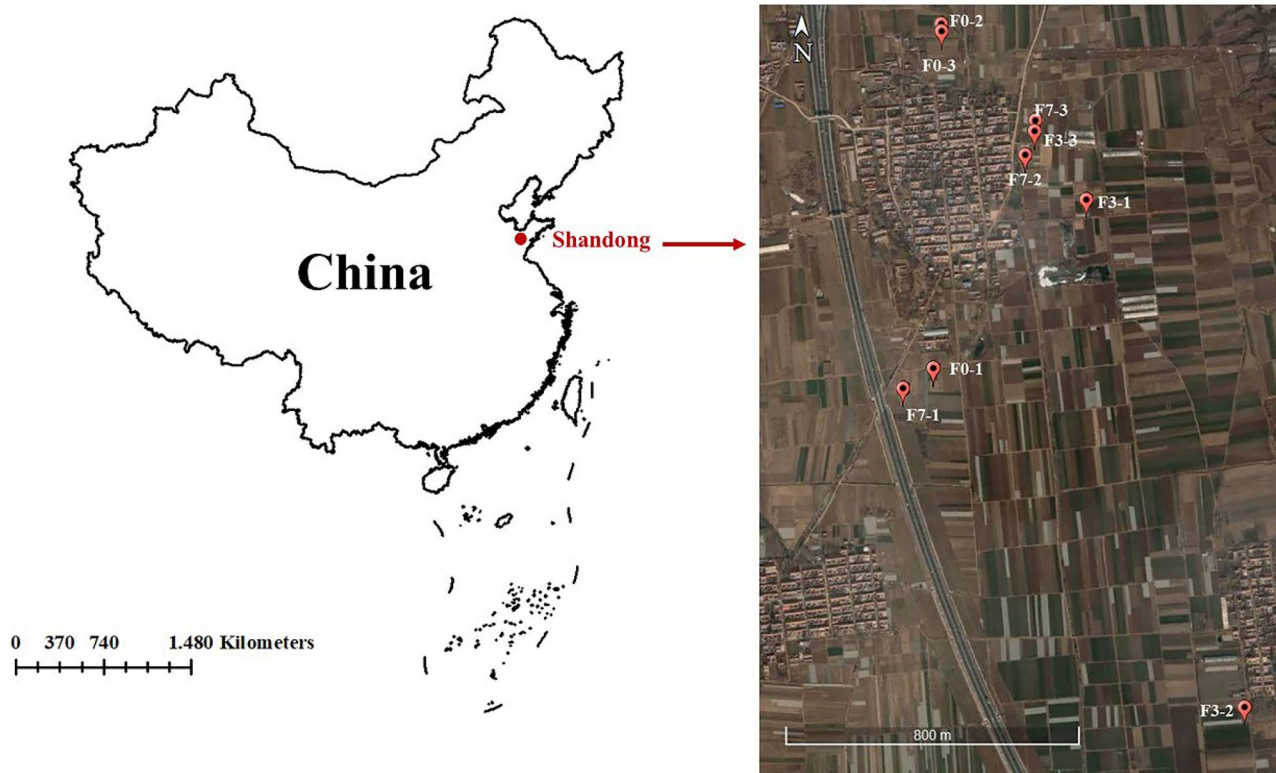
Ginger production fields belonging to the local small-holder farmers in a plain region were selected (Fig. 1). The historical cropping systems in all farms were wheat-corn rotations. These systems were changed at different years to monoculture ginger production in 2012, 2016, and 2019. The application of the soil fumigant chloropicrin to prevent

soil-borne diseases has started with the cultivation of ginger production. We selected fields with 0, 3, and 7 years of annual chloropicrin fumigation (F0, F3, and F7; 3 replicates each). The size of the replicate field ranges from 530 to 1300 m<sup>2</sup>. Five soil samples were taken from each replicate field to take soil heterogeneity into consideration and were analyzed separately.

Agricultural management practices, such as tillage, then fumigation, rides and furrows, fertilization with biofertilizers (microbial biocontrol agent) before plantation, fertilization with compound mineral (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O:18–15–22) and organic fertilizers (soybean meal), and open ditch irrigation during a vegetation period of 8 months, were similar in all fields. During the entire ginger growth period, the total P input was about 48 kg ha<sup>-1</sup>.

The soils are classified as sandy loam (sand: 52.4~69.8%, silt: 16.1~37.4%, clay: 10.3~20.7%; International soil classification system). The initial soil properties are presented in Table 1.

Chloropicrin (Dalian Lv Feng Chemical Co. Ltd. (Dalian, China)) was applied in all selected fields before ginger planting in March 2019. Chloropicrin was injected 15 to 30 cm into the soil using a specialized machine and the average application rate was 375 kg ha<sup>-1</sup>. The fields were then



**Fig. 1** The locations of sampling sites. The red dot names with Shandong indicate the location of the sampling region in China. The picture on the right is a screenshot of the sampling fields from Google

Earth. F0, F3, and F7 represent fields with 0, 3, and 7 years of chloropicrin fumigation history before 2019, with three replicated fields for each of them (F0-1, F0-2, F0-3; F3-1, F3-2, F3-3; F7-1, F7-2, F7-3)

**Table 1** Soil physiochemical properties in the selected fields at the beginning of the experiment in 2019

Field	pH (H <sub>2</sub> O) <sup>a</sup>	SOM (g kg <sup>-1</sup> ) <sup>b</sup>	TP (g kg <sup>-1</sup> ) <sup>c</sup>	TN (g kg <sup>-1</sup> ) <sup>d</sup>
F0-1	5.1 ± 0.1	18.3 ± 2.4	1.5 ± 0.3	0.7 ± 0.0
F0-2	4.9 ± 0.2	25.3 ± 3.4	1.6 ± 0.2	0.6 ± 0.1
F0-3	6.3 ± 0.3	20.8 ± 1.1	1.2 ± 0.1	0.3 ± 0.2
F3-1	6.6 ± 0.2	21.4 ± 5.8	2.2 ± 0.3	0.7 ± 0.1
F3-2	7.7 ± 0.2	24.9 ± 3.0	2.4 ± 0.3	0.8 ± 0.1
F3-3	7.1 ± 0.1	23.2 ± 3.0	2.3 ± 0.2	0.6 ± 0.1
F7-1	5.7 ± 0.3	17.2 ± 1.5	1.4 ± 0.2	0.8 ± 0.1
F7-2	6.1 ± 0.2	24.4 ± 2.4	2.4 ± 0.1	1.2 ± 0.1
F7-3	5.5 ± 0.3	27.0 ± 2.5	1.7 ± 1.5	1.0 ± 0.1

F0, F3, and F7 represent fields with 0-, 3-, and 7-year chloropicrin fumigation history before 2019, with 3 replicated fields for each of them. Data represents the means of five replicates in each field with standard deviation

<sup>a</sup>pH (H<sub>2</sub>O) soil:ultra-pure water with a ratio of 1:2.5

<sup>b</sup>SOM soil organic matter (g kg<sup>-1</sup>), colorimetric method after H<sub>2</sub>SO<sub>4</sub>-K<sub>2</sub>CrO<sub>7</sub> oxidation (Sun et al. 2019)

<sup>c</sup>TP total phosphorus (g kg<sup>-1</sup>), analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) after HNO<sub>3</sub>-HF-H<sub>2</sub>O<sub>2</sub> digestion at 180 °C using a MARS 5 Xpress microwave system (CEM, USA)

<sup>d</sup>TN total nitrogen (g kg<sup>-1</sup>), measured using an elemental analyzer

covered by plastic films for 2 weeks. The plastic films were removed to release built-up gas 1 week before planting. A previous study showed that the half-life of CP was about 6 days under the following conditions: an application rate of 392 kg ha<sup>-1</sup> under 10 °C, 8% soil moisture content, and 1% soil organic matter content (Ashworth and Yates 2019), which was similar to conditions in our sampling region.

Topsoil samples from 0–20-cm depth were collected at four stages: before fumigation (05/03/2019), after fumigation (28/03/2019), middle growth period (24/08/2019), and harvest (23/10/2019). Each sample was divided into two sub-samples: one was air-dried for soil chemical properties and the other was stored at 4 °C for analysis of soil phosphatase enzyme activities.

## 2.2 The ginger yield and P uptake

Ginger rhizome yields were estimated at the harvesting stage. Three ginger plants were selected randomly in every field except for the plot F7-3 in which all ginger plants died due to a soil-borne disease before sampling could be carried out. For ginger plant samples, the biomass weight of fresh shoots and rhizomes were measured by gravimetric analysis. The yield of ginger rhizome was estimated by local farmers, while the yield of ginger shoot was calculated based on the ratio of shoot biomass and rhizome biomass measured from ginger plant samples. The shoot and rhizome samples

were separated, and the total P content was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES) after HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> digestion (Rosa et al. 2020). The total P uptake was calculated by multiplying the P content per unit biomass by the total biomass in each field (Zhao et al. 2019).

## 2.3 Soil phosphatase activity

Soil AIP was measured according to the protocol described by Tabatabai and Bremner (1969). Briefly, 1.0 g of fresh soil was added (< 2 mm) to a 50-mL flask, along with 0.2 mL methylbenzene, 1.0 mL 0.05 M p-nitrophenyl phosphate, and 4.0 mL modified universal buffer of pH 6.5, mixed and incubated at 37 °C for 1 h. Then, 1.0 mL 0.5 M CaCl<sub>2</sub> and 4.0 mL 0.5 M NaOH were added to terminate the reaction and then the mixture was filtrated. Absorbance of the filtrate was measured using a spectrophotometer at 410 nm. To measure AIP activity, the universal buffer of pH 6.5 was replaced by a universal buffer of pH 11 (Zhao et al. 2019).

## 2.4 P fractions in soil

Soil Olsen-P proposed by Olsen et al. (1954) was analyzed colorimetrically using the molybdate blue method after extraction with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> at pH 8.5 for 30 min (Hu et al. 2012). Soil P fractions were sequentially extracted using the methods slightly modified according to Tiessen (2006). Briefly, 0.5 g of air-dried soil (< 0.25 mm) was put into a 50-mL centrifuge tube and sequentially extracted with (1) ultrapure water with two anion exchange resin membrane strips (1 cm × 2 cm) converted to the bicarbonate form, (2) 0.5 M NaHCO<sub>3</sub>, and (3) 0.1 M NaOH and 1 M HCl at 25 °C for 16 h (180 rpm). The soil suspension was centrifuged at 10,000 g for 10 min at 0 °C and decanted. After adjusting pH, inorganic P (Pi) fractions in each extract were measured using the molybdate ion colorimetry method at 700 nm (Costa et al. 2016; Zhou et al. 2018). Total P in the extracts was determined using ICP-OES while the organic P fractions (Po) were calculated as the difference between TP and Pi. The P fractions were interpreted as resin-P, NaHCO<sub>3</sub>-P (NaHCO<sub>3</sub>-Pi and NaHCO<sub>3</sub>-Po), NaOH-P (NaOH-Pi and NaOH-Po), and occluded P (HCl-Pi and residual P) (Hedley et al. 1982). The concentration of occluded P was estimated by subtracting the sum of the total other P fractions from the TP concentrations of samples (Koch et al. 2018).

## 2.5 Statistical analysis

The statistical analysis was performed by IBM SPSS Statistic 20. Normality of the measured data and homogeneity of variance were tested using the Kolmogorov–Smirnov and Levene tests ( $p > 0.05$ ). Due to the non-normal distribution



of Olsen-P, P fraction composition, and AiP and AIP activity values, the non-parametric Kruskal–Wallis analysis with Wilcoxon test ( $p < 0.05$ ) was used to facilitate the comparisons between the different treatments. One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) at  $p < 0.05$  was applied to normally distributed data (ginger P uptake). Redundancy analysis (RDA) was performed to establish the relationship among P fractions (response variables) and soil properties (explanatory variables) using Origin 2020. All of the figures were created using Origin 2020.

### 3 Results

#### 3.1 The ginger yield and P uptake

The average ginger rhizome yields in F0 and F3 were almost the same at  $70.0 \text{ t ha}^{-1}$ , while the rhizome yield of F7 was the lowest at  $37.5 \text{ t ha}^{-1}$  (Fig. 2a).

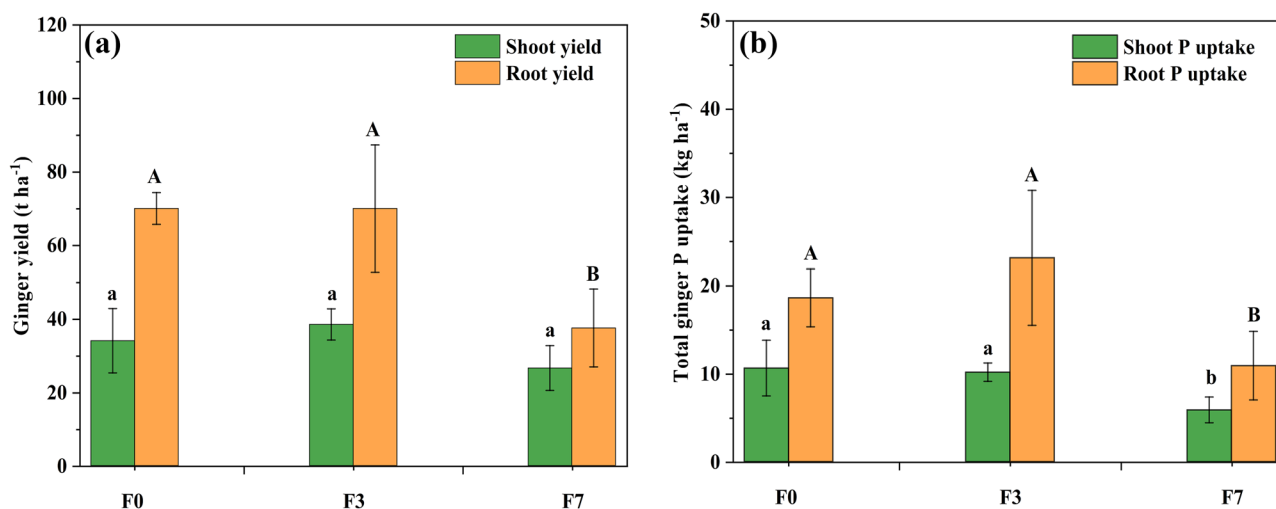
The total ginger P uptake showed similar trends as ginger biomass (Fig. 2b). There was no significant difference in shoot P uptakes between F0 and F3 with  $11.0$  and  $10.1 \text{ kg ha}^{-1}$ , respectively, which were significantly higher than shoot P uptake in F7 ( $5.9 \text{ kg ha}^{-1}$ ). No significant difference was observed for root P uptake between F0 and F3

(F0:  $19.3 \text{ kg ha}^{-1}$ ; F3:  $23.1 \text{ kg ha}^{-1}$ ), while the root P uptake was significantly lower in F7 ( $10.9 \text{ kg ha}^{-1}$ ).

#### 3.2 Phosphatase activity

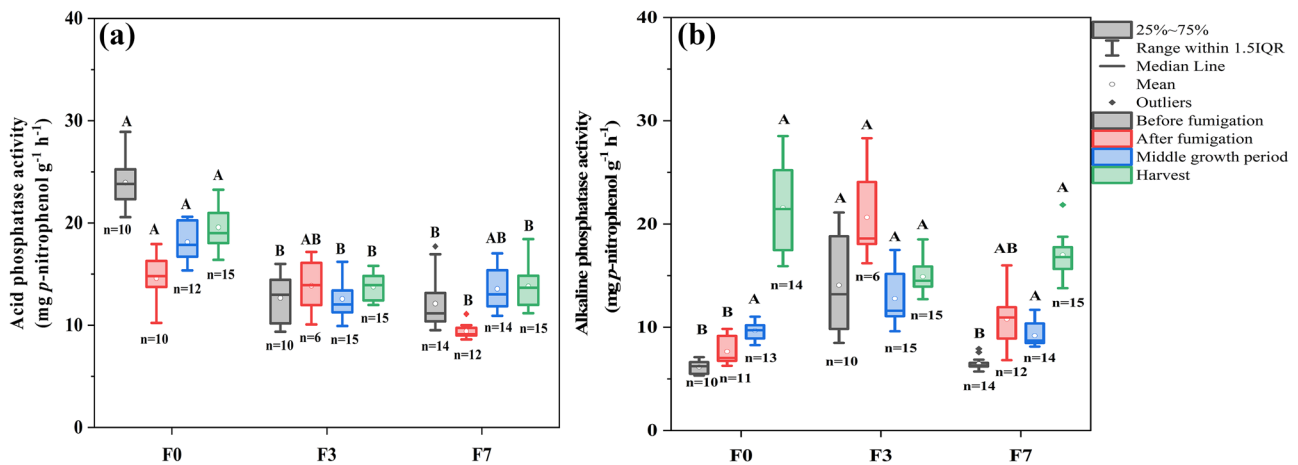
The average AiP activity was significantly higher in F0 than F3 and F7 during the entire ginger growth period (Fig. 3a). In F0, the average AiP activity changed from  $24.0 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$  before fumigation to  $14.6 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$  after fumigation, and recovered to  $19.6 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$  after harvest. There were no significant differences between F3 and F7 ( $12.1$  to  $13.8 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ) except for the samples taken after fumigation. For samples collected after fumigation, the average AiP activity was significantly higher in F3 ( $13.9 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ) than in F7 ( $9.4 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ).

For samples collected before fumigation, the average AIP activity was significantly higher in F3 ( $14.1 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ) than in F0 ( $6.1 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ) and F7 ( $6.5 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ), while there was no significant difference between F0 and F7 (Fig. 3b). For samples collected after fumigation, the average AIP activity in F3 ( $20.6 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ) was still significantly higher than in F0 ( $7.7 \text{ mg p-nitrophenol g}^{-1} \text{ h}^{-1}$ ). For samples collected during the middle growth period and harvest, no significant difference in the average AIP activity ( $14.9$  to



**Fig. 2** The average of ginger yield (a) and total ginger P uptake (b) after harvest. F0, F3, and F7 represent fields with 0, 3, and 7 years of chloropicrin fumigation history before 2019, with 3 replicated fields for each of them. Legends indicate the average ginger shoot (light green) and root (light orange) yield per hectare in Fig. 2(a) and the total P content in ginger shoots (light green) and roots (light orange) per hectare in Fig. 2(b). Column represents the average of replicates

with standard deviation. In Fig. 2(a), the uppercase letters (A and B) and lowercase letters (a and b) indicate the significant difference in ginger root and shoot yield among three treatments, respectively. In Fig. 2(b), the uppercase letters (A and B) and lowercase letters (a and b) indicate the significant difference in ginger root and shoot P uptake among three treatments, respectively. Treatments with the same letter had no significant difference (ANOVA with LSD test,  $p < 0.05$ )



**Fig. 3** Soil acid (a) and alkaline (b) phosphatase activity in the three treatments and sampling times. F0, F3, and F7 represent fields with 0, 3, and 7 years of chloropicrin fumigation history before 2019, with 3 replicated fields for each of them. Legend indicates the sampling time, including before fumigation (gray), after fumigation (red), mid-

dle growth period (blue), and harvest (green). *n* is the number of actual samples in the corresponding box. The uppercase letters (A and B) indicate the significant difference among the three treatments during each sampling time (Wilcoxon test,  $p < 0.05$ )

21.6 mg *p*-nitrophenol g<sup>-1</sup> h<sup>-1</sup>) was observed among three treatments.

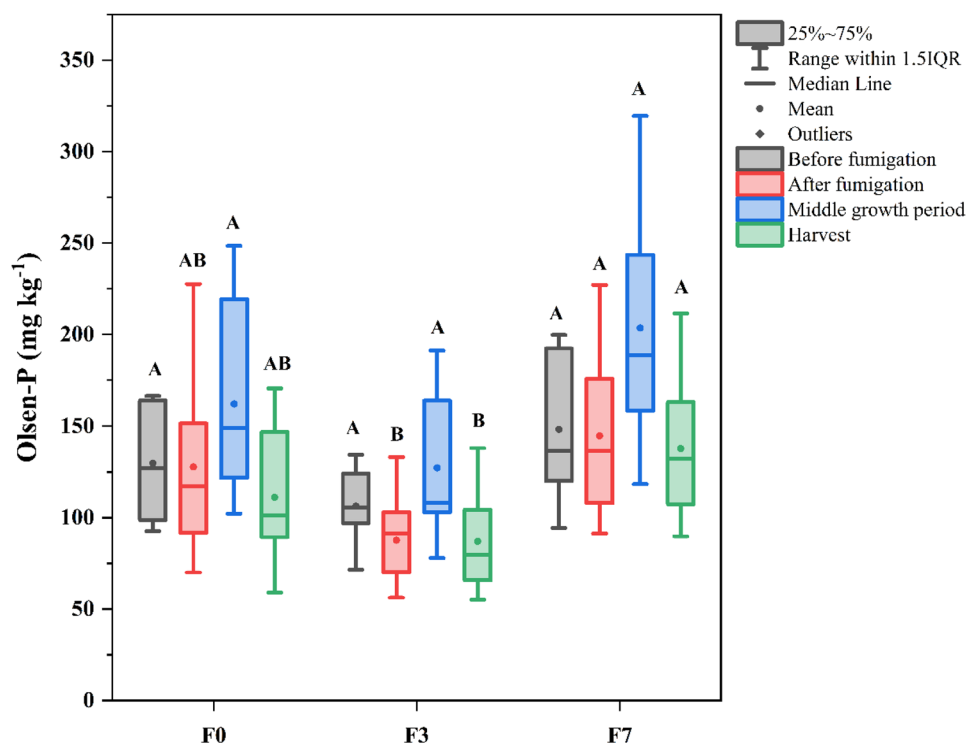
### 3.3 Variation of soil P in the different treatments

For samples collected before fumigation, the content of soil Olsen-P were 129.8, 106.2, and 148.3 mg kg<sup>-1</sup> in F0, F3, and F7, respectively, and the differences among the three

fumigated fields were not statistically significant (Fig. 4). During the whole ginger growth period, the application of chemical fertilizers by local farmers and the P uptake by ginger caused the variation of soil Olsen-P content to be 111.1 ~ 162.1, 87.1 ~ 127.2, and 137.8 ~ 203.6 mg kg<sup>-1</sup> in F0, F3, and F7, respectively.

The average TP contents were significantly lower in F0 (1179 to 1523 mg kg<sup>-1</sup>) than that in F3 (1994 to

**Fig. 4** Soil Olsen-P in different fields and sampling times. F0, F3, and F7 represent fields with 0, 3, and 7 years of chloropicrin fumigation history before 2019, with 3 replicated fields for each of them. Legend indicates the sampling time, including before fumigation (gray), after fumigation (red), middle growth period (blue), and harvest (green). The number of samples for every box were 15 ( $n = 15$ ) except for samples of F0 and F3 before fumigation ( $n = 10$ ). The uppercase letters (A and B) indicate the significant difference among the three treatments during each sampling time (Wilcoxon test,  $p < 0.05$ )



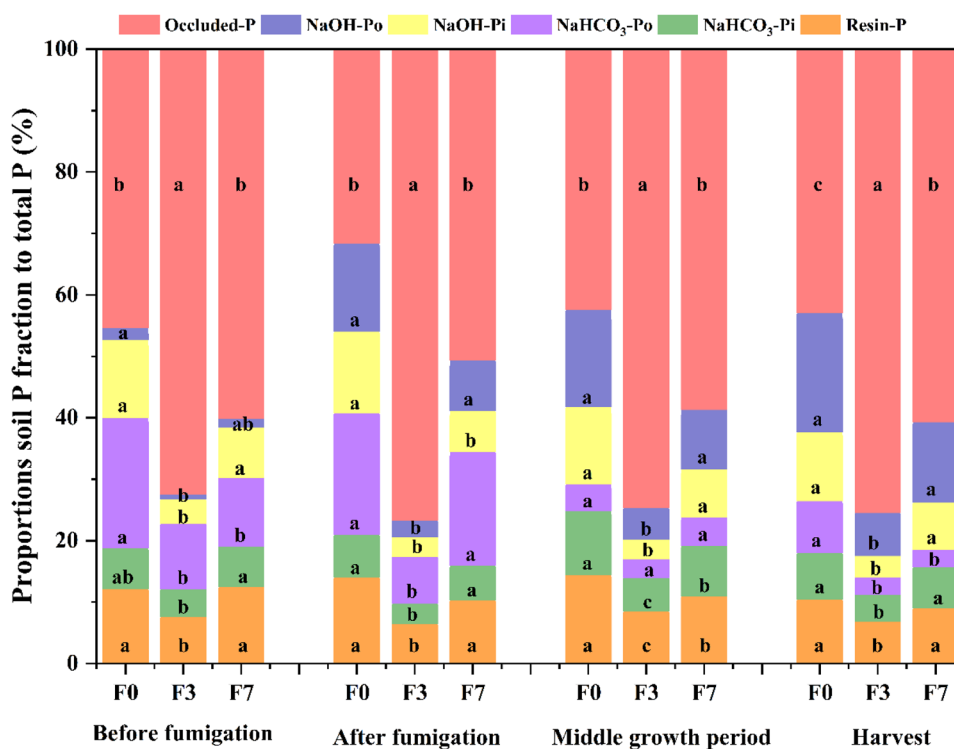
2487 mg kg<sup>-1</sup>) and F7 (1967 to 2257 mg kg<sup>-1</sup>) during the whole growing period. After measuring the total phosphorus (TP) contents, different soil P fractions were extracted using modified Hedley's sequential extraction method (Fig. S1).

For each of the P fractions, unavailable Occluded-P showed similar variation as TP with significantly lower contents in F0 (461 to 738 mg kg<sup>-1</sup>) than in F3 (1485 to 2019 mg kg<sup>-1</sup>) and F7 (1237 to 1522 mg kg<sup>-1</sup>). Significantly lower contents of NaOH-Po and NaOH-Pi were found in F3 (NaOH-Po: 20 to 153 mg kg<sup>-1</sup>; NaOH-Pi: 65 to 89 mg kg<sup>-1</sup>) as compared to F0 (NaOH-Po: 32 to 273 mg kg<sup>-1</sup>; NaOH-Pi: 159 to 204 mg kg<sup>-1</sup>) and F7 (NaOH-Po: 28 to 275 mg kg<sup>-1</sup>; NaOH-Pi: 133 to 159 mg kg<sup>-1</sup>). There were no significant differences in the contents of NaHCO<sub>3</sub>-Po among the three treatments, while the contents of NaHCO<sub>3</sub>-Pi and resin-P were significantly higher in F7 than in F0 and F3 (Fig. S1).

The proportions of soil P fraction to total P were also calculated to eliminate the basic differences among fields by dividing the soil total P contents by every P fraction content (Fig. 5). For samples collected before fumigation, the proportion of resin-P was significantly lower in F3 (7.6%) than in F0 (12.1%) and F7 (12.4%). The NaHCO<sub>3</sub>-Pi proportion appeared to have the same variation as resin-P with 6.7%, 4.5%, and 6.6% in F0, F3, and F7, respectively. The proportion of resin-P and NaHCO<sub>3</sub>-Pi remained significantly lower in F3 than F0 and F7 during the whole growth period. The proportion of NaHCO<sub>3</sub>-Po was significantly

higher in F0 with 21.2% as compared to the proportions measured in F3 (10.6%) and F7 (11.2%) before fumigation. After fumigation, the proportions of NaHCO<sub>3</sub>-Po became 19.7%, 7.7%, and 18.1% in F0, F3, and F7, respectively. During the middle growth period, there was no significant difference in the proportions of NaHCO<sub>3</sub>-Po among three treatments. However, the proportions of NaHCO<sub>3</sub>-Po were 8.4% in F0 but still remained at 2.8% and 2.9% in F3 and F7 after harvest. The proportion of NaOH-Pi was significantly lower in F3 with 3.9% and there were no significant differences between F0 (12.7%) and F7 (8.2%) before fumigation. The proportion of NaOH-Pi remained almost constant through the whole sampling time in all three treatments. However, NaOH-Po showed similar changes as NaHCO<sub>3</sub>-Po among different CP fumigation fields and sampling times. The proportions of NaOH-Po were 2.0%, 0.9%, and 1.4% before fumigation which increased to 7.5%, 2.5%, and 5.0% in F0, F3, and F7 fields, respectively, after fumigation. The NaOH-Po proportion increased during the whole ginger growth period and reached up to 19.4%, 6.9%, and 13.0% in F0, F3, and F7 fields, respectively, after harvest. The proportion of occluded-P showed opposite trends to available P fractions with significantly higher values in F3 with 72.5% and there were no significant differences between F0 (45.3%) and F7 (60.1%) for samples before fumigation. The sampling time did not have a significant influence on the proportion of occluded-P.

**Fig. 5** Proportions of various soil P fraction to total P in the three treatments and four sampling times. F0, F3, and F7 represent fields with 0-, 3-, and 7-year chloropicrin fumigation history before 2019, with 3 replicated fields for each of them. Sampling times were (1) before fumigation, (2) after fumigation, (3) middle growth period, and (4) harvest. Legend indicates the soil P fractions, including resin-P (orange), NaHCO<sub>3</sub>-Pi (green), NaHCO<sub>3</sub>-Po (purple), NaOH-Pi (yellow), NaOH-Po (blue), and occluded-P (red). The lowercase letters (a, b, and c) indicate the significant difference of each P fraction among the three treatments for every sampling time (Wilcoxon test,  $p < 0.05$ )



### 3.4 Correlation analysis among soil properties, P fractions, and phosphatase enzymes

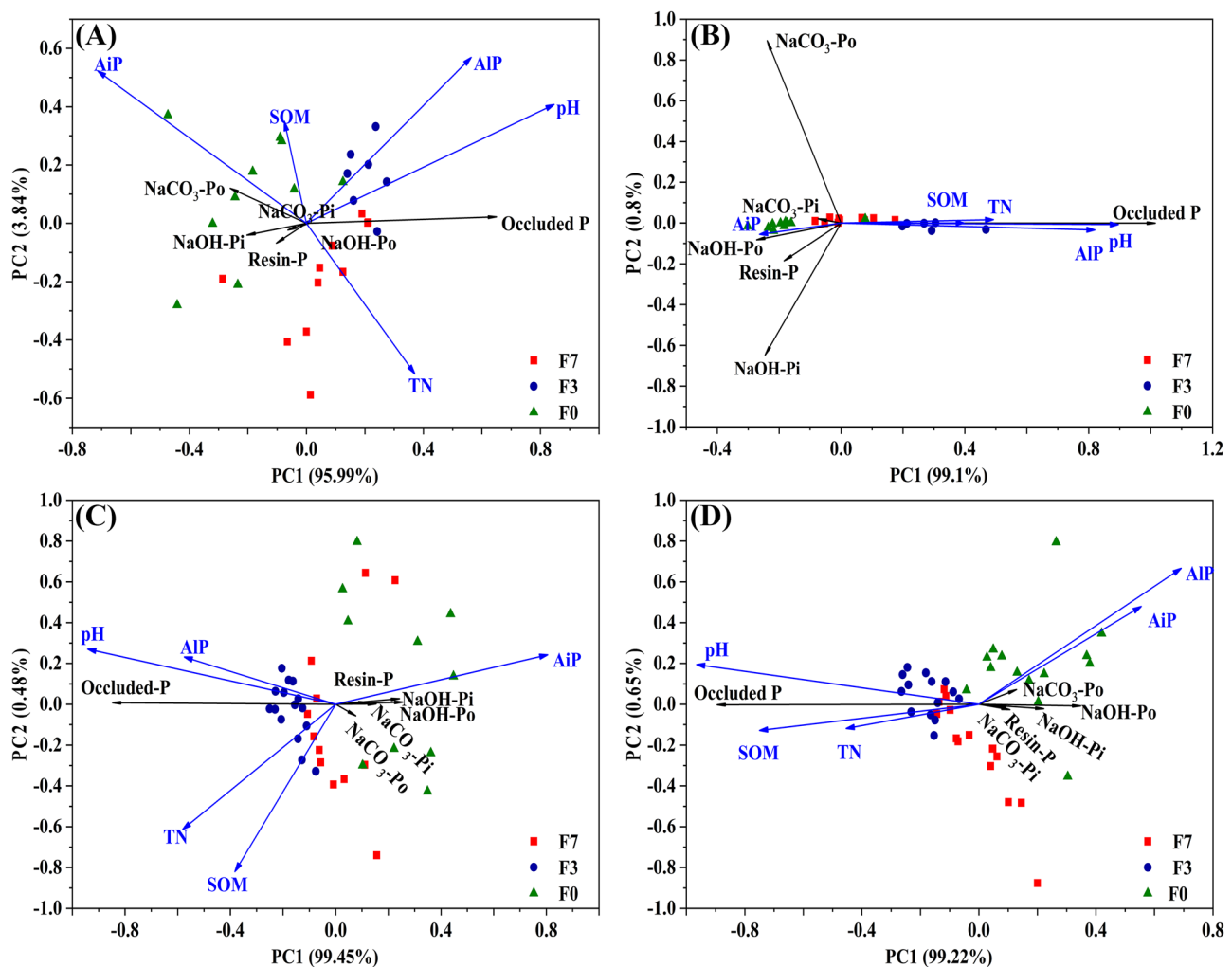
The effects of selected soil properties on the composition of soil P fractions were further analyzed using redundancy analysis (Fig. 6). The first two axes explained over 99% of the total variation. The results showed that occluded-P fractions were the main forms in F3, while other labile P fractions took the main place in F0 and F7 and no significant differences were observed among these fractions.

According to the indicating arrows, pH, AIP activity, SOM, and TN were highly positively correlated with the proportion of occluded-P to TP. Labile P fractions (especially  $\text{NaHCO}_3\text{-Po}$ ) were highly associated with AiP activity.

## 4 Discussion

### 4.1 The effects of different years of CP fumigation on ginger yield and P uptake

In this study, we found no significant differences in ginger yields among F0 and F3, while the yields decreased significantly in F7 due to the high level of soil-borne diseases. Similar results were also found in a study by Yao et al. (2006), in which they concluded that apple tree growth and yield were not affected by pre-plant fumigation with a mixture of 78% dichloropropene + 17% chloropicrin as compared to untreated controls after 2 years. They suggested that the results might have been related to the decreased inhibitory



**Fig. 6** Redundancy analysis (RDA) of P fractions and soil properties. Every figure refers to the RDA analysis for different sampling times including (A) before fumigation, (B) after fumigation, (C) middle growth period, and (D) harvest. Legend indicates the samples from F0 (green triangle), F3 (blue dot), and F7 (red square) treatments. The

blue arrows are soil properties including pH, SOM, AIP, AiP, and TN. Black arrows are soil P fractions including resin-P,  $\text{NaHCO}_3\text{-Pi}$ ,  $\text{NaHCO}_3\text{-Po}$ ,  $\text{NaOH-Pi}$ ,  $\text{NaOH-Po}$ , and occluded-P. The position and length of arrows indicate the direction and strengths of the effects of soil properties on P fractions



effects of CP on soil-borne diseases. Our study is also in line with a study conducted by Zhang et al. (2020), who found that the strawberry yield increment varied with different consecutive years of CP fumigation with a maximum of  $2.0 \text{ kg m}^{-2}$  after 2 years, then decreased to  $0.9 \text{ kg m}^{-2}$  after 5 years. In the current study, all of the ginger plants in F7-3 field died gradually during the later ginger growth period because of the severe soil-borne diseases occurring there. It was also confirmed by the local ginger growers that the inhibitory effects of CP fumigation on soil-borne diseases decreased greatly after several times of CP applications. This finding underlines the need for other comprehensive agricultural management practices, rather than sole CP fumigation, to control soil-borne diseases in ginger crops.

The calculated ginger P uptake showed the same variation as ginger yields with no significant difference among F0 and F3, while F7 was significantly decreased in this study, which also perhaps contributed to the decrease of arbuscular mycorrhizal fungi (AMF) in CP fumigated soil (Smith and Smith 2011). There is another long-term study based on chronosequence sites which showed that proportions of AMF were significantly lower for sites exposed to 15, 33, and 39 years of fumigation as compared to their non-fumigated counterparts. AMF has been proved to contribute to the mycorrhizal uptake pathway in delivering soil phosphorus to plants (Smith and Smith 2011), so the decrease of AMF could have detrimental effects on plant P uptake and soil P availability.

## 4.2 The effects of different CP fumigation years on phosphatase activities

Our study showed that soil AiP activities were significantly lower in F3 and F7 than in F0 except for samples taken after fumigation. The first time of CP fumigation in F0 put the most evident inhibitory effects on AiP. Even so, AiP activities recovered more quickly in F0 during the ginger growth. AiP activities in F3 and F7 remained almost constant among the different sampling times, meaning that soil CP fumigation did not have an obvious influence on AiP activities in F3 and F7.

On the other hand, the highest values for soil AIP activities were observed in F3 except for the samples gathered after harvest. Similar AIP activity values were observed for F0 and F7. It has been proven that AIP increases with the increase of soil pH (Dick et al. 2000). In this study, AIP activity was also significantly positively correlated with soil pH (Fig. S2). Therefore, we assume that the highest pH values in F3 are one of the most important reasons for the highest AIP activity there. On the other hand, AIP mainly is originated from soil microorganisms (Acosta-Martínez and Tabatabai 2015). A study by Huang et al. (2020b) found that the application of dazomet fumigant at  $50 \text{ mg kg}^{-1}$  showed a transient inhibitory effect on AIP activity before 14 days,

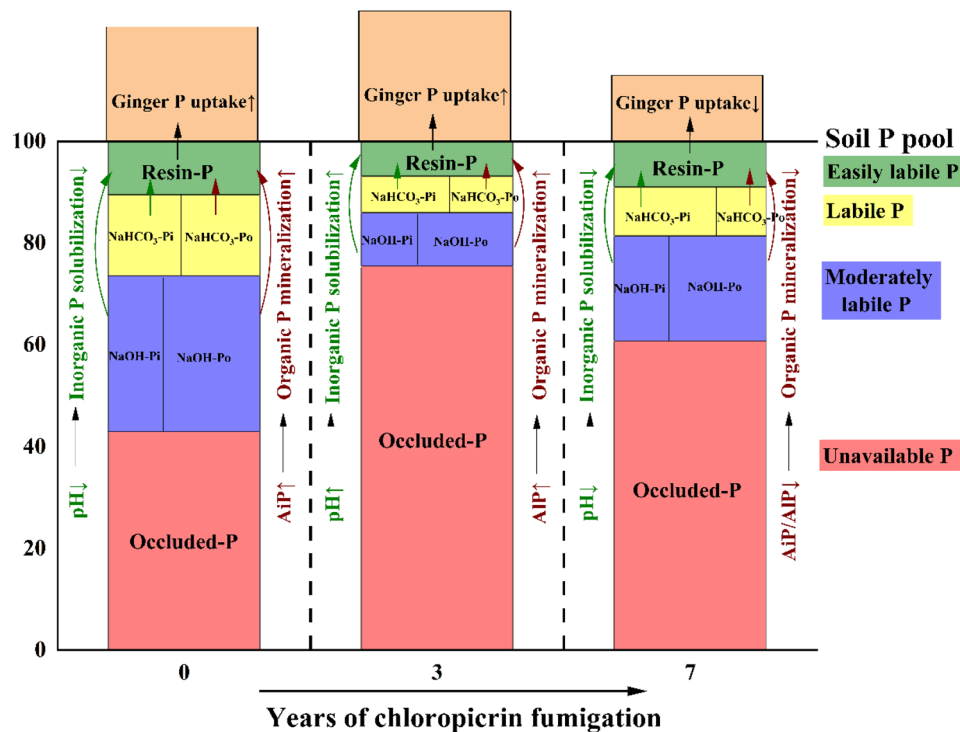
which may be due to the decrease of soil *phoD* gene abundance which is response for the production of AIP. However, in our research, the microbial mechanism of AIP activity changes in different fields needs further study.

## 4.3 The effects of different years of CP fumigation on P fractions

Soil P fractions extracted using sequential methods with different extracting agents are indicators of their solubility in soil solution and their availability for plants (Milić et al. 2019). In our experimental fields, the historical cropping system was maize-wheat rotation before the ginger planting. As a cash crop, ginger planting requires more fertilizers input than cereal crops (maize and wheat), causing significantly higher total P levels in F3 and F7 than in F0 (Table 1). Fertilization increased the concentration of P in the soil solution, thereby enhancing the adsorption of phosphorus by soil particles (Weihrauch and Opp 2018), making soil P unavailable for ginger plants in F3 and F7 (Fig. S1). Therefore, we did not refer to the total content in this study but we focused on the proportion of each P fraction to total P to compare the composition of soil phosphorus (Fig. 5).

The proportion of plant available phosphorus (resin-P +  $\text{NaHCO}_3$ -P +  $\text{NaOH}$ -P) was the lowest in F3, which may be due to the higher pH value in F3 (pH: 6.6 to 7.7) than F0 (pH: 4.9 to 6.3) and F7 (pH: 5.5 to 6.1). The optimum pH for the highest soil P availability is 5.5 to 6.0, because when the soil pH is lower than 5.0, soil P can form insoluble Fe/Al-P-minerals (Zhou et al. 2018), while in alkaline soil ( $\text{pH} > 7$ ), soil P combined with  $\text{Ca}^{2+}$  and precipitates in the form of Ca-P-minerals (Weihrauch and Opp 2018). The RDA analysis in our study also showed that the proportions of soil occluded-P are highly positively associated with soil pH. Compared with F7, the significantly higher ginger yield and ginger P uptake in F3 may also attribute to the lower proportions of soil available P. The lowest proportion of soil available P in F3 indicates that, although the yields of ginger rhizomes were similar between F0 and F3, the significantly lower proportion of soil available P in F3 may be a key limiting factor for higher ginger yields (Fig. 7).

On the other hand, the significant decrease in ginger yield and P uptake, and the lack of reduction in the application of phosphate fertilizer in F7, may further cause more available P fractions to be delayed on the soil surface, because plant uptake is one of the most important outputs of P in the soil system. Therefore, the low P use efficiency and high proportions of easily labile P (resin-P), labile P ( $\text{NaHCO}_3$ -P), and moderately labile P ( $\text{NaOH}$ -P) in F7 may cause severe environmental problems such as water eutrophication washed by surface runoff or P leaching into the groundwater system (Zhang et al. 2021).



**Fig. 7** Schematic diagram of the composition of soil P pool, the role of soil pH and phosphatase activity in soil P transformation, and the ginger P uptake. Data used in this figure is the data measured for the samples collected at harvest. Numbers 0, 3, and 7 represent fields with 0, 3, and 7 years of chloropicrin fumigation before 2019. The numbers at Y axis means the proportions of each P fraction to total P.

P fractions are resin-P,  $\text{NaHCO}_3\text{-Pi}$ ,  $\text{NaHCO}_3\text{-Po}$ ,  $\text{NaOH-Pi}$ ,  $\text{NaOH-Po}$ , and occluded-P. The distribution of  $\text{NaOH-Pi}$  and  $\text{NaOH-Po}$  as well as the distribution of  $\text{NaHCO}_3\text{-Pi}$  and  $\text{NaHCO}_3\text{-Po}$  in the figure were decided according to their own proportions to TP. The soil P pool was divided into easily labile P, labile P, moderately labile P, and unavailable P according to their solubility and availability for plant

Among different soil P fractions, the proportions of organic P (especially  $\text{NaOH-Po}$ ) increased significantly after CP fumigation again in 2019, which may be caused by the release of microbial organic P from the lysis of soil microorganisms (Fang et al. 2018). The released microbial organic P fractions are then mineralized into available inorganic P by various soil phosphatases (Acosta-Martínez and Tabatabai 2015). However, CP fumigation also reduced soil phosphatase activity (Huang et al. 2020a), especially  $\text{AiP}$  in our study, which may then retard the mineralization of organic P and eventually lead to the accumulation of organic P in the soil (Fig. 5). However, the detail microbial mechanisms also need to be studied further.

A previous lab incubation study conducted by Huang et al. (2020a) found that CP fumigation significantly increased the proportion of  $\text{NaHCO}_3\text{-Po}$  for up to 14 days in acidic soil, and significantly decreased the proportion of  $\text{NaHCO}_3\text{-Po}$  up to 49 days in alkaline soil. All of the soil P fractions recovered to a similar level after 49 days among all CP treatments in two kinds of soil. They suggested that farmers could reduce the P fertilizer application in the early stage (<30 days) due to the increase of soil available P. However, our study found that the release of available P by

the lysis of dead microbes may not satisfy the increasing demand for soil phosphorus by ginger, at which time, P deficiency may become a major limitation for getting a higher yield in F3. With the increasing of CP fumigation years, soil-borne pathogens may become resistant to CP (Qiao et al. 2010), leading to a serious ginger death in F7. At this time, we should first consider finding another effective method to prevent soil-borne diseases.

## 5 Conclusion

In this field study, we tested the effects of different years of chloropicrin fumigation on ginger yield, phosphatase activities, and soil P availability. The results showed that, compared with the ginger yield in fields with the first CP fumigation, no significant difference was observed on the ginger yields in the fields with 3 years of CP fumigation. However, the contents of soil available P decreased significantly after 3 years of CP fumigation, which may become an important limiting factor for the ginger yields. The ginger yield decreased significantly after 7 years of repeated CP fumigation, at which time, ginger death became the main

limiting factor for ginger production, and controlling soil-borne diseases using a better method rather than sole CP fumigation should be the first consideration. The lower phosphatase activities after repeated CP fumigation were also important influencing factors for the reduced soil P availability, which highlighted the fact that microbial mechanisms, especially P solubilizing microbes, need to be studied further to understand the variations in ginger yield and soil P availability after long-term repeated CP fumigation.

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## Declarations

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

**Conflict of interest** The authors declare no competing interests.

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