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Research article

Investigation and insights into the technical strategies for reducing agrochemical inputs in rice farming to enhance agroecosystem resilience and food safety, A case study in China

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ABSTRACT

The growing demand for cereal production has led to increasing agrochemical inputs; therefore, evaluation and adjustment of current practices are required to maintain and improve sustainable cropping systems. A Four-years study of multiple practices with reduced agrochemical application for rice farming was conducted and investigated in southern China to assess impacts on food safety and ecological resilience. A 30 % reduction in total pesticide use resulted in a 20 % decrease in ecological risk to earthworms, primarily due to reduced application of key pesticides: pymetrozine, pretilachlor, difenoconazole, propiconazole, thifluzamide, tricyclazole, and hexaconazole. A 22 % reduction in total mineral fertilizer use had a slight impact on soil fertility; however, certain practices involving partial replacement of chemical fertilizers with organic manure enhanced soil enzyme activity. This improvement was also linked to changes in the soil bacterial community, particularly the enrichment of *Gemmatimonadetes*, *Actinobacteria*, and *Cyanobacteria*, which contributed to enhanced soil fertility. Additionally, reduction in agrochemical application was accompanied by a declining trend in heavy metal accumulation; however, exposure risks of arsenic and Cd still require consideration. Our study demonstrates that progressive reduction of agrochemical inputs can mitigate pollutant risks and reactivate soil self-restoration processes, thereby enabling the design of adaptable sustainable cropping systems with optimized ecological trade-offs.

1. Introduction

Over the past half-century, the global arable land area has decreased by 48 %, while cereal production has increased by 238 %(Ritchie, 2017). Elevated crop yields are largely dependent on the application of agrochemicals, such as chemical pesticides and mineral fertilizers(Gagic et al., 2017). Global chemical pesticide usage is estimated to be 3.5 million tons by 2020(Sharma et al., 2019). The annual pesticide application in China is approximately 1.8 million tons (most of which is used for rice production), which is the highest in Asian countries(FAO, 2017a). High levels of pesticide residues in the agricultural environment and crop products could negatively impact human health and ecosystems(Sharma et al., 2019). High crop yields also consume large amounts of mineral fertilizers. The global input of total fertilizers (nitrogen, N; phosphate, P; potassium, K) was estimated to be 201.66 million tonnes in 2020(FAO, 2017b). Applying large amounts of fertilizers not only causes low fertilizer use efficiency(Yu et al., 2021) but also leads to heavy metal contamination, water eutrophication, and the greenhouse effect(Q. F. Li et al., 2021), thereby reducing agricultural sustainability (Savci, 2012).

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Global efforts have been made to reduce the use of agrochemicals. France has limited pesticide usage since 2008 and set a target of a 50 % decrease in conventional pesticide use by 2025(Lamichhane et al., 2016). Similar actions have been implemented in North America, although with some reluctance from farmers(Chèze et al., 2020). Over 30 % of global pesticides and fertilizers were applied in China with only 9 % of global cropland. The continuously increasing intensity of agrichemical use is associated with the existence of numerous small farms in China. Since 2005(Wu et al., 2018), China has planned to reduce chemical pesticide and fertilizer applications, named the Double Reduction Plan (DRP). From 2005 to 2015, DRP-guided Chinese smallholder farmers accomplished average increase in crop yield of 11 % with reduced fertilizer use by 15 %(Cui et al., 2018). Starting in 2016, China launched "Zero Growth of Chemical Fertilizer and Pesticide Use Active" to balance the sustainability and productivity of agriculture(Cui et al., 2018). Multiple agrochemical-reducing management practices have been implemented in China, but a comprehensive evaluation of these practices is still lacking. French researchers did not detect any conflict between low pesticide use and high productivity in 77 % of 946 tested non-organic arable commercial farms(Lechenet et al., 2017), suggesting that reducing the inputs of agrochemicals may not counteract crop productivity. One possible explanation is that reducing agrochemicals may benefit the recovery of agricultural ecosystems. However, knowledge is scarce regarding the effects of reducing agrochemicals on soil fertility recovery, biodiversity, and food safety risks. What is the key technical path to reduce or adjust the use of agrochemicals? Which pesticide would be the key component that should be minimized or limited for use, and at which crop growth stage?

In this study, we investigate the risks of environmental pollution, food safety as well as soil fertility before and after adopting DRP-guided management practices through a four-year evaluation in the main rice production area of southern China, located in the middle and low reaches of the Yangtze River. The effect of agrichemicals on soil microbes was investigated as well. We aim to evaluate and provide insights into different practices for reducing agrochemical inputs in rice farming, focusing on agroecosystem impacts and food safety. Furthermore, we seek to understand and propose a potential running mechanism for rebuilding sustainable cropping systems and to identify key technical pathways for rational agrochemical reduction and adjustment. The results will contribute to optimizing DRP in rice production and promoting sustainable development of agriculture.

2. Materials and methods

2.1. Field trial setup

JiangSu and JiangXi are two of the major rice production provinces along the middle-lower Yangtze River in China, crop production is mainly dependent on small farms (Cui et al., 2018). The field experiments were conducted in JiangSu and JiangXi Province, four sites (JinTan, HaiAn, JiangYan, and HongZe) and three sites (JiAn, FengCheng, and ShangGao) were selected from JiangSu and JiangXi, respectively. Three sties (JiAn, FengCheng, and ShangGao) in JiangXi have more rainfall and relatively higher temperatures than the other four sites (JinTan, HaiAn, JiangYan, and HongZe) in JiangSu (Table S1). All the selected sites are small conventional rice farms, which are typical Chinese croplands, including single-season rice and multiple-season rice cultivation patterns. Given the complexity and diversity of DRP practices in China, we designed specific improved management practices by reducing the usage of both pesticides and yield-related chemical fertilizers based on traditional agrochemical usage at each site (Tables S2 and S3), named double-reduction area (DRA) and control group, respectively. Individual experimental plots with reduced-input management covered 1.4 ha, and adjacent plots with the same size were used as controls. Three replicates were performed for each treatment. The experiment was initiated in 2016 until 2019. All the soil and rice samples and relevant parameters, such as pesticide residue and microbes, were collected and measured seasonally and annually. Then, we evaluated the pesticide residue and heavy metal risks as well as the soil quality at three rice growth stages (tillering, heading, and harvesting). Unfortunately, for the short-term of study period, it was not possible to discern statistically significant directional trends in DRP efficacy. Therefore, after four years of continuing management, the data collected from soil and rice samples at each experimental plot in 2019 were used for the final analysis in this study compared with 2016.

2.2. Sampling

Soil samples (0–20 cm depth) were collected from paddy fields during the tillering, heading, and harvesting stages of rice. Six soil samples were randomly collected from each 66.7 m^2 experimental plot and homogenized to form a composite sample. The collected soil was frozen in liquid nitrogen immediately followed by storage at −80 °C. Rice grains were randomly sampled from each plot, air-dried, and dehulled prior to analysis.

2.3. Determination of pesticide residue content

Pesticide residues were extracted from soil and rice samples using a QuEChERS kit (Thermo Fisher Scientific)(Perestrelo et al., 2019). Pesticides quantification was performed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6470 triple quadrupole mass spectrometer with an electrospray ionization (ESI) source (Agilent Technologies, USA). Chromatographic separation was achieved on a Poroshell 120 EC-C_{18} column (2.1 mm × 150 mm, 2.7 μm) maintained at 30 °C. The gradient elution system consisted of two mobile phases: (A) 0.1 % (v/v) formic acid in water and (B) acetonitrile. The elution gradient was programmed as follows: 0–8 min, 20 %–90 % A; 8–12 min, 90 %–100 % A; 12–14 min 100 % A; 14–15 min, 100 %–20 % A. The flow rate and injection volume were set to 0.2 mL/min and 5 μL, respectively. Table S4 summarizes the mass spectrometry parameters for detecting 51 commonly applied pesticides in rice fields.

2.4. Assessment of ecological risk of soil pesticide residues

The concentration addition-based mixture risk of soil pesticides was evaluated by calculating the risk quotient (RQ) using the ecological relative risk (EcoRR) method(Vašíčková, 2019). The RQ for individual pesticide was calculated using Equation (1). MEC_{soil} represents the measured environmental concentration of the pesticide. $PNEC_{mss}$ (predicted no-effect concentration of pesticide) was derived from the most sensitive toxicological endpoints (e.g., no observed effect concentration (NOEC), median lethal concentration (LC_{50}), and median effective concentration (EC_{50}) based on data for soil-dwelling organisms(EC, 2002). Earthworms were selected as model organisms for ecological risk assessment in this study. RQ values were categorized into four risk levels: negligible ($RQ < 0.01$), low risk ($0.01 \le RQ < 0.1$), medium risk ($0.1 \le RQ < 1$), and high risk ($RQ \ge 1$). Total RQ for a specific location (considering multiple pesticides) or a specific pesticide (across multiple locations) was calculated as the sum of individual RQ values (RQs).

$$RQ = \frac{MEC_{soil}}{PNEC_{mss}} \quad (1)$$

2.5. Assessment of dietary exposure to pesticide residues

Chronic and acute dietary exposure of pesticide residues in rice grains was evaluated based on the percentage of acceptable daily intake (ADI) and acute reference dose (ARfD), respectively(GEMS/Food, 1997). For chronic exposure assessment, estimated daily intake (EDI) firstly calculated via Equation (2) as micrograms of pesticide per kilogram of body weight per day (μg/kg bw/day). Supervised trial median residue (STMR) values represent the average pesticide concentration in rice grains. K denotes the average daily rice consumption (g bw/day) for different age groups(CNS, 2016; GAS, 2015). Population characteristics and dietary intake data are provided in Table S5. Then, EDI values were expressed as percentage of ADI (%ADI) using Equation (3). Maximum ADI values for critical pesticides were adopted from the Joint FAO/WHO Meeting on Pesticide Residues (JMPR)(JMPR, 2014). For example, the ADI for pymetrozine is 0.3 mg/kg bw. Chronic dietary risk is considered acceptable if %ADI <100.

$$EDI = STMR \times K \tag{2}$$

$$\%ADI = \frac{EDI}{ADI} \times 100 \tag{3}$$

For acute exposure assessment, the estimated short-term intake (ESTI) was firstly calculated via Equation (4), expressed in micrograms of pesticide per kilogram of body weight per day (μg/kg bw/day). LP represents the maximum daily food consumption (kg). HR (highest residue) refers to the maximum residue level (mg/kg) in edible portions from supervised trials. A variability factor (v) of 3 was applied, as recommended by JMPR(JMPR, 2014). bw denotes the average body weight (kg) of specific population age group. ARfD values for pesticides were adopted from JMPR(JMPR, 2014). For example, the ARfD for pymetrozine is 0.1 mg/kg bw. Acute dietary risk is deemed acceptable if % ARfD <100 Equation (5).

$$ESTI = \frac{LP \times HR \times v}{bw} \tag{4}$$

$$\%ARfD = \frac{ESTI}{ARfD} \tag{5}$$

2.6. Determination of heavy metal content

Dry soil or rice grains, straws, fertilizers, animal wastes, etc. were ground into powder. Then, the samples were digested with concentrated HNO_3 with hydrogen peroxide (10 % w/v) in a microwave digestion system (MARS6, CEM Corporation, USA). The digested solution was filtrated and diluted for measurement of Cd, Pb, As, and Hg using ICP–MS (inductively coupled plasma–mass spectrometry) (iCAP Q, ThermoFisher, USA).

2.7. Assessment of ecological risk of soil heavy metals

The ecological risk of soil heavy metals was assessed using the Hakanson potential ecological risk index method(Hakanson, 1980). The risk index (RI) for individual heavy metals was calculated using Equation (6). C represents the measured concentration of heavy metals (mg/kg) in soil. C_n denotes the background concentration of heavy metals (mg/kg) in soils with no anthropogenic input. C_n values for heavy metals in paddy soil with varying pH levels are listed in Table S6, based on the Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (China)(MEE, 2018). C_n values were selected according to measured soil pH values (Table S6). T is the toxic coefficient of soil heavy metal (As, 10; Cd, 30; Hg, 40; Pb, 5)(Hakanson, 1980). RI values were categorized into five risk levels: low potential ecological risk (RI < 40), moderate potential ecological risk (40 ≤ RI < 80), considerable potential ecological risk (80 ≤ RI < 160), high potential ecological risk (160 ≤ RI < 320), and very high potential ecological risk (RI ≥ 320)(Hakanson, 1980).

$$RI = \frac{C}{C_n} \times T \tag{6}$$

The composite risk index (CRI), calculated as the sum of RI values for all heavy metals Equation (7), represents the integrated ecological risk of multi-metal contamination. The CRI was classified into four risk levels: low ecological risk (CRI <150), moderate ecological risk (150 ≤ CRI <300), considerable ecological risk (300 ≤ CRI <600), and very high potential ecological risk (CRI ≥600)(Hakanson, 1980).

$$CRI = \sum_{i}^{m} RI^{i} \tag{7}$$

2.8. Assessment of dietary exposure to heavy metals

Health risks associated with heavy metal exposure via rice ingestion were assessed for non-carcinogenic and carcinogenic effects using the US Environmental Protection Agency (USEPA) methodology(USEPA, 2004). Chronic daily intake (CDI, mg/kg/day) was calculated via Equation (8). C_{rice} represents the heavy metal concentration (μg/kg) in rice grains. IR (daily ingestion rate) for Chinese adults (218.7 g/day) was adopted from (MEE-PRC, 2013). Rice intake values for other age groups are provided in Table S5. EF (exposure frequency) was set to 365 days/year. ED (exposure duration for adults) was defined as 70 years. BW (average body weight of Chinese adults: 62 kg)(MEE-PRC, 2013). AT (average time) was calculated as 70 years × 365 days/year. Hazard quotient (HQ) - defined as the ratio of CDI to reference dose (RfD)-was used to evaluate non-carcinogenic risk via Equation (9). RfD values for heavy metals were adopted from USEPA(USEPA, 2021a). For example, the RfD for As is 0.0003 mg/kg/day. Non-carcinogenic risk is considered acceptable if HQ < 1.

$$CDI = \frac{C_{rice} \times IR \times EF \times ED}{BW \times AT} \tag{8}$$

$$HQ = \frac{CDI}{RfD} \tag{9}$$

Cancer risk (CR) was used to assess the carcinogenic potential of heavy metals in rice ingestion. CR was calculated using Equation (10). CSF (carcinogenic slope factor) quantifies the carcinogenic potency of heavy metals. CSF values were obtained from the Integrated Risk Information System (IRIS)(USEPA, 2021b). For example, the CSF of As is 1.5 kg/mg/day. Carcinogenic risk is deemed acceptable if CR < 1×10^{-4}(USEPA, 2004).

$$CR = CDI \times CSF \tag{10}$$

2.9. Determination of soil chemical properties

Soil chemical properties were analyzed following the methods of Pansu and Gautheyrou(Pansu and Gautheyrou, 2003). Total N was determined via the Kjeldahl method. Available P was extracted with $NaHCO_3$ and quantified by molybdenum-antimony spectrophotometric. Available K was extracted with CH_3COONH_4 and analyzed by flame emission spectroscopy. Soil organic matter (SOM) content was determined using the mass loss on ignition method. Soil pH was determined with pH meter in a soil suspension.

2.10. Assessment of soil fertility

Soil fertility was evaluated using the Nemerow index method(Zhang et al., 2018). The single fertilizer index (F_i) for each soil chemical property was calculated using Equation (11). C_i represents the measured values of the target soil property, while x_a, x_p, and x_c denote the Nemorow grading indices for specific soil properties (Table S7).

The composite fertility index (F) was derived from the individual F_i via Equation (12). $\overline{F_i}$ denotes the mean of all Fi values, F_{min} indicates the minimum Fi, and n represents the total number of indices. Soil fertility levels were categorized as: barren (F < 0.9), general (0.9 ≤ F < 1.8), fertile (1.8 ≤ F < 2.7), and very fertile (F ≥ 2.7).

$$\left\{\begin{array}{c} F_i = \frac{C_i}{x_a}, C_i \leq x_a, (F_i \leq 1) \\ F_i = 1 + \frac{(C_i - x_a)}{(x_c - x_a)}, x_a < C_i \leq x_c, (1 < F_i \leq 2) \\ F_i = 2 + \frac{(C_i - x_c)}{(x_p - x_c)}, x_c < C_i \leq x_p, (2 < F_i \leq 3) \\ F_i = 3, C_i > x_p \end{array}\right\} \tag{11}$$

$$F = \sqrt{\frac{(\overline{F}_i)^2 + (F_{min})^2}{2}} \times \left(\frac{n-1}{n}\right) \tag{12}$$

2.11. Determination of soil enzyme activity

Soil samples were air-dried and ground to analyze soil enzyme activities. Urease activity was measured via the indophenol colorimetric method using urea as the substrate(Xue et al., 2017). Saccharase activity was quantified by the 3,5-dinitrosalicylic acid(DNS) colorimetric method with sucrose as the substrate(Xue et al., 2017). Dehydrogenase activity was assessed using the 2,3,5-triphenyltetrazolium(TTC) chloride colorimetric method(Małachowska-Jutsz and Matyja, 2019).

2.12. Analysis of the soil bacterial community

Soil bacterial community analysis was conducted by Majorbio Bio-Pharm Technology Co., Ltd., (Shanghai, China). Total DNA was extracted from soil samples using the Power Soil DNA Isolation Kit (MoBio Laboratories Inc., USA). The V4-V5 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5′-ATCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). TransStart Fastpfu DNA Polymerase (TransGen Biotech Co., LTD, China) was selected to perform polymerase chain reaction (PCR) by using an ABI GeneAmp PCR system 9700 (Thermo Fisher Scientific, USA). The library was constructed using a TruSeq™ DNA Sample Prep Kit, followed by sequencing using an Illumina MiSeq Benchtop Sequencer (Illumina, CA, USA). Sequences were *de novo* clustered into operational taxonomic units (OTUs) at a 97 % similarity. Taxonomic information of the sequence was obtained through BLAST (Basic Local Alignment Search Tool) based on SILVA database (https://www.arb-silva.de/)(Quast et al., 2013).

2.13. Data analysis

The results of the statistical analysis are presented as the mean of three replicates with standard deviation (SD). One-way analysis of variance (ANOVA) was performed, followed by F test for evaluating the significant differences at $P < 0.05$. Pearson correlation analysis among different parameters was conducted using the “psych” package in *R*(Wei and Simko, 2017). For heatmap analysis, the data were first transformed into $\log_2$-fold changes relative to the control group. The heatmap was subsequently generated using TBtools(Chen et al., 2020) and the “heatmap” package in *R*(R-Core-Team, 2020).

3. Results and discussion

3.1. Reduced agrochemical management

The total pesticide used in DRA decreased by 13.2 %–60.5 % compared to the control, with an average reduction of 29.5 % (Fig. S1). The usage of different kinds of fertilizers was reduced in DRA at different sites (Table S3). The total chemical fertilizer usage in DRA decreased by 3.33 %–54.6 % compared to the control, with an average reduction of 22.3 % (Fig. S2A). The average reductions in the calculated usage of total N, P, and K were 19.2 % (2.50 %–46.5 %), 21.8 % (15.6 %–73.8 %), and 22.5 % (1.89 %–68.4 %), respectively (Fig. S2B–D). The improved management practices were implemented over four years (2016–2019).

3.2. Risk evaluation of pesticide input

For the pesticide residues investigation in the soil, among the 51 pesticides, tricyclazole, hexaconazole, and propiconazole had a detectable rate of 100 % at all three stages throughout the entire area (Fig. S3). Tricyclazole was used to control rice blast disease at all sampling sites. Hexaconazole and propiconazole were only applied in some sampling areas (Table S2), but these pesticides with relatively long half-lives may represent residues from previous growing seasons(Kim et al., 2002; Zhang et al., 2016). Pymetrozine was detected in all the soil samples at the heading stage, with a slight decrease at the harvesting stage (Fig. S3). This was because pymetrozine was generally applied to control aphids and planthoppers at the heading stage at all sampling sites (Table S2). Then, some of them might be degraded at the harvesting stage due to the relatively short half-life of pymetrozine(Li et al., 2011).

For the ecosystem impact of pesticide residues in soil, the total soil pesticide RQ of DRA practice in JiangSu and JiangXi decreased by 29.4 % and 12.0 %, respectively, compared to the control groups of traditional practice (Fig. 1A). Approximately 38 % of the DRA sites showed decreased soil RQ (Fig. 1B). Most DRA sites showed lower soil RQ at the tillering stage (Fig. 1B), which was mainly attributed to the decrease in pymetrozine, propiconazole, and pretilachlor (Fig. 1C). The RQs of pymetrozine, difenoconazole and thifluzamide contributed greatly to the total RQ at the heading stage (Fig. 1D). The RQs of pymetrozine, propiconazole, hexaconazole and tricyclazole contributed greatly to the total RQ at the harvesting stage (Fig. 1E). Most of the single RQs of these pesticide residues at each site were less than 0.1 (Fig. S4), indicating that they posed low risk to the soil environment(Vašíčková, 2019). However, higher risks were observed during the heading stage in traditional practice plots at Hongze and JiAn (>0.2; Fig. S4), indicating that the absence of Pymetrozine in DRA practices significantly altered ecosystem impact. A similar impact was found in the heading stage at Hongze in the DRA practice plots (>0.2; Fig. S4), where difenoconazole was adopted. In the harvesting stage, the RQs of DRA increased compared to the control in ShangGao and JiAn (Fig. 1B), but most of the prominent pesticide residues were at low risk (RQ < 0.1) (Fig. S4).

The RQ of pymetrozine was dominant among all the detected soil pesticide residues (Fig. 1C–E). Reducing pymetrozine usage led to a remarkable decrease in soil pymetrozine RQ (Fig. 1C–E), which further contributed greatly to the decrease in total soil pesticide RQ, especially in the heading stage (Fig. S4). Difenoconazole is commonly used to control rice sheath blight and brown spot disease occurring at the heading stage (K. Wang et al., 2012). As a result, soil difenoconazole residue was mainly detected in the heading stage (Figure. S3 and Fig. 1D). The RQ of soil difenoconazole decreased at most DRA sites (Fig. S4). However, the total difenoconazole RQ increased in DRA (Fig. 1D). This was mainly due to the high difenoconazole RQ (>0.1; medium risk) detected in the HongZe DRA (Fig. S4). The reason was finally confirmed that farmers in HongZe DRA did not strictly follow the guidelines of improved pesticide usage and applied more difenoconazole in DRA plots than in the control in the heading stage because of severe sheath blight disease.

Pymetrozine was the most frequently detected pesticide residue in polished rice grains, with a detection rate of 93 % across all samples (Fig. S5). We evaluated the risk of acute dietary exposure and chronic dietary exposure upon detectable pesticide residues. All the rice grain samples from both the control area and DRA had low acute dietary risk. A %ARfD value under 100 % indicated an acceptable risk of acute dietary exposure. For pymetrozine, the %ARfD was 0.13 %–0.75 %, although the children had a higher %ARfD than the elderly individuals (upper panel in Fig. 1F). In JiAn and JinTan, the %ARfD decreased dramatically in the DRA group compared with the control group. This may result from the 40 % and 20 % reductions in pymetrozine use in DRA practices at JiAn and JinTan, respectively (Table S2). In HaiAn,

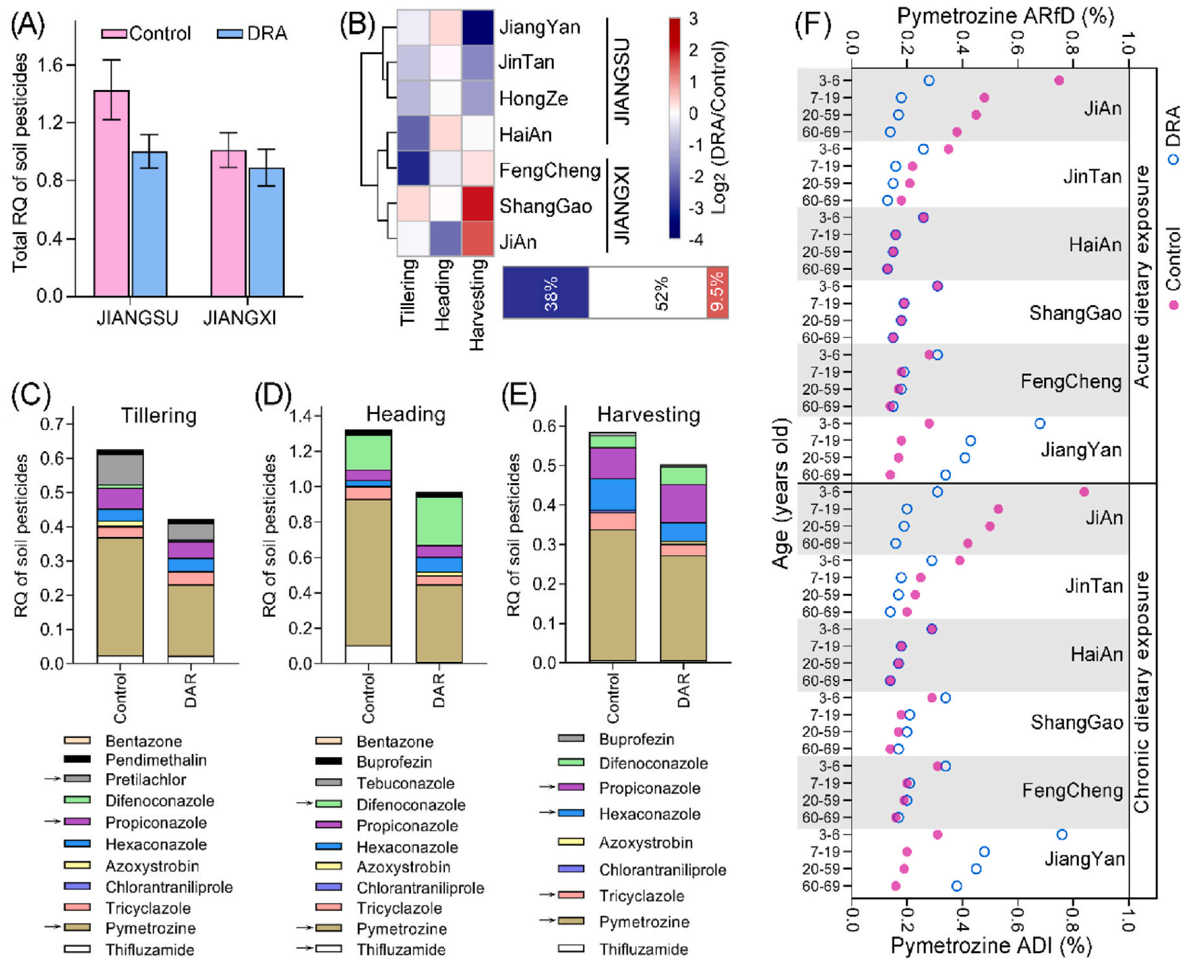

Fig. 1. Evaluation of pesticide residue risk. (A) Total RQ of soil pesticide residues in JiangSu and JiangXi. (B) Relative fold change of total RQ of soil pesticide residues between DRA and control in different sites. (C–E) Distribution of the soil RQ for different pesticide residues at different rice growth stages. (F) The ARfD and ADI of pymetrozine in rice grains, indicating acute dietary exposure and chronic dietary exposure, respectively.

ShangGao, and FengCheng, the %ARfD did not show a significant difference between DRA and the control (upper panel in Fig. 1F) because the similar key pesticides mentioned above were adopted or excluded in both practices. The chronic dietary exposure of detectable pesticide residue was indicated by %ADI. All the rice grain samples from both the control area and DRA had low chronic dietary risks. For pymetrozine, the %ADI value showed similar changes with %ARfD at different sampling sites (lower panel in Fig. 1F). The pymetrozine usage in JiangYan DRA decreased by 67 % (Table S2), but both %ARfD and %ADI increased (Fig. 1F). We found that the farm holders in this plot may have failed to strictly follow improved practices. More pymetrozine was applied in JiangYan DRA to control pests to maintain yields. It has been reported that the risk of yield losses due to pests strongly limits farmers' willingness to change their conversional practices(Chèze et al., 2020). Therefore, it is important to understand farmers' reluctance to perform improved practices with lower pesticide usage.

Collectively, these results suggested that the risk of pesticide residues in both soil and rice grains was low in the sampled rice farms located in JiangSu and JiangXi. Efforts to reduce pesticide usage tended to decrease the environmental risk and health risk of pesticide residues in rice fields. We need to reiterate that pymetrozine, pretilachlor, propiconazole, tricyclazole, difenoconazole, and hexaconazole are the key points and critical chemicals contributing to the control of the ecological risk of pesticide residues. Reducing or adjusting the application of key chemicals during the appropriate rice growth stage can significantly mitigate the impact on soil ecosystems. Across all study sites, the average 30 % reduction in total pesticide use did not strictly correlate with the 20 % average decrease in total soil pesticide RQ. Apart from the varying impacts of different pesticides, such as critical chemicals that contribute major risks, they may also be affected by the different dissipation dynamics among different pesticides used previously(Kaur et al., 2015; Li et al., 2011; K. Wang et al., 2012). Taking into account the crop planting patterns and environmental fates of corresponding pesticides would help further evaluate the management of pesticide application to further decrease the ecosystem impact of pesticide residues in rice fields.

3.3. Risk evaluation of heavy metal/metalloid input

The ecological risk of heavy metals in agricultural environments has been linked to agricultural management practices, including fertilizer and pesticide application(Gimeno-García et al., 1996). We evaluated the RI of four heavy metals (arsenic, As; cadmium, Cd; mercury, Hg; lead, Pb) in soils using the method proposed by Hakanson(Hakanson, 1980). All the single RI values of soil samples were below 40 (Fig. 2A), indicating low potential ecological risk. In China, elevated levels of heavy metals in soils have been documented in JiangXi Province, an area rich in polymetallic mineral resources(Liu et al., 2014). Here, we also found higher average RI values of soil heavy metals in JiangXi than in JiangSu (Fig. 2A). Compared to the control, DRA-managed soils in JiangXi, but not in JiangSu, showed a slightly decrease in average RI (Fig. 2A). Then, we found that Cd and Hg contributed the most to the total RI, while Pb contributed the least (Fig. 2B). This is consistent with reports that Cd

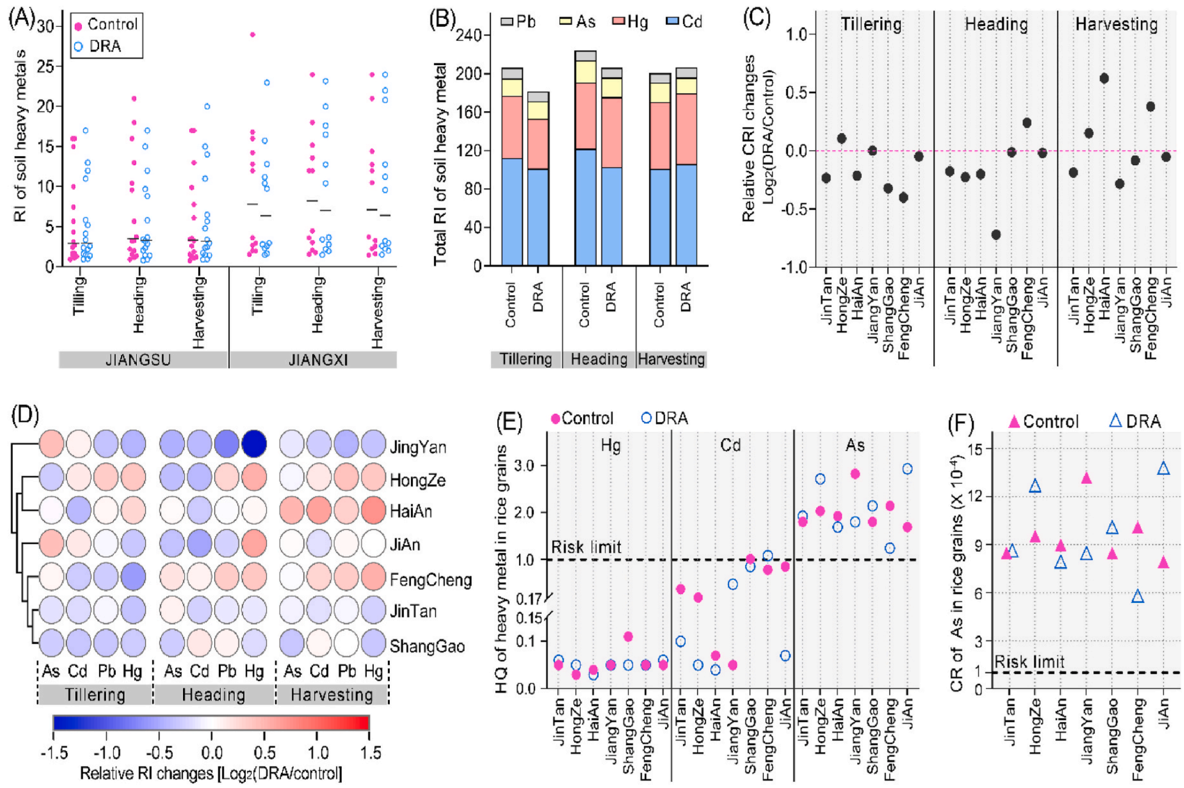

Fig. 2. Evaluation of heavy metal/metalloid risk. (A) The distribution of RI values for single heavy metal/metalloid in soil. (B) Distribution of the total soil RI for four different heavy metals/metalloid (Pb, As, Hg, and Cd). (C) The relative fold change of CRI (sum of the RI values of four metals/metalloid, Pb, As, Hg, and Cd) in DRA. (D) Relative fold change of soil RI for each heavy metal/metalloid between DRA and control. (E) The HQ values of heavy metals/metalloid in rice grains, indicating non-carcinogenic health risk. (F) The CR values of As in rice grains, indicating carcinogenic health risk.

and Hg were the two main metals in agricultural soils in China(Song et al., 2021; Wang et al., 2019). At the tillering and heading stages, the decrease in the total RI of soil heavy metals was mainly attributed to the decrease in Cd (Fig. 2B).

CRI indicates the total ecological risk of multiple metal-like substances. All the CRI values for each site were below 50 (Fig. S6), indicating a low potential ecological risk of heavy metals/metalloid for both the control and DRA(Hakanson, 1980). Compared to the control, most DRA sites showed unchanged or decreased CRI values at the tillering and heading stages, while some DRA sites showed increased CRI values at the harvesting stage (Fig. 2C). Similar changing patterns were also observed for the single RI distribution for each metal/metalloid at each experimental site (Fig. 2D). The DRA in JinTan and JiangYan had a decreased RI throughout all three stages (Fig. 2C and D). A possible explanation was the reduction in fertilizer usage. Intensive fertilizer application could increase the concentration of active heavy metals/metalloid in cultivated soil by either introducing additional metals or enhancing the distribution of metals(Atafar et al., 2010; Wei et al., 2020). Applying P fertilizer increased the concentrations of Cd and Hg in soil(Dharma-Wardana, 2018; Tang et al., 2018). The considerable reduction in the usage of fertilizers in the DRA of JinTan and JiangYan may have contributed to decreasing the ecological risk of soil heavy metals/metalloid (Figure. S2A, Fig. 2C). The DRA in FengCheng showed increased risks of heavy metals/metalloid at the heading and harvesting stages (Fig. 2C and D). This may be explained by the application of straw return in this area (Table S3), as straw return can enhance the risks of metal release in soil(Su et al., 2021). These results suggested that fertilizer reduction may help decrease the ecological risks of heavy metals in rice fields. However, the integration of straw return with fertilizer reduction should be carefully managed, which requires a more comprehensive assessment.

To assess health exposure risks from heavy metals in rice grains, the heavy metal concentrations in rice grains were used to calculate non-carcinogenic (HQ) and carcinogenic (CR) risks. Pb had the lowest ecological risk, and there is an absence of the RfD of Pb in the database (EPA). Therefore, we calculated the HQ and CR for the other three metals (Cd, Hg, and As) (USEPA, 2001). The results revealed that the younger generation faces a relatively low noncarcinogenic and carcinogenic health risk, while adults face a higher risk associated with arsenic As exposure. All CR values for arsenic As in rice exceeded the risk threshold (CR > 1), indicating a high carcinogenic risk.

For noncarcinogenic risk to adults, most of the HQ values of Hg and Cd were below the acceptable limit (HQ < 1), suggesting no noncarcinogenic health risk (Fig. 2E). Only the HQ of Cd in the DRA of FengCheng exceeded the acceptable limit (Fig. 2E), which may have resulted from straw return (Table S3). Unmodified straw return may pose a potential Cd risk in rice paddies because rice straw accumulates high concentrations of Cd(Shan et al., 2021). For Hg, the DRA in ShangGao showed decreased HQ, while the HQ values of other sites remained unchanged. For Cd, most DRAs showed decreased HQ values compared to the control groups, except for JiangYan (Fig. 2E). Urea application promotes Cd uptake and accumulation in crops(Ji et al., 2020). The high HQ of Cd in the DRA of JiangYan might have resulted from the application of much more urea than that of the control (Table S3). The health risk of Hg and Cd was below the risk limit in all tested spots, reducing agrochemicals in DRA tended to further decrease the health risk of these metals. Arsenic (As), even at low levels, is highly toxic to humans and crops. Rice plants are more efficient in assimilating As into their grains than other cereal crops(Islam et al., 2016). Here, we found that all the HQ values of rice as were above the risk limit (HQ > 1), indicating a high noncarcinogenic health risk (Fig. 2E). The DRA of three sites (HaiAn, JiangYan, and FengCheng) showed decreased HQ of As, but the HQ values of As were enhanced in the DRA of the other three sites (HongZe, ShangGao, and JiAn) compared to the control (Fig. 2E). The results showed that As poses a high noncarcinogenic health risk, further carcinogenic health risk of As was analyzed as follows.

The results revealed that all the CR values of As in rice exceeded the risk limit (CR > 1), indicating a high carcinogenic risk. The changes in the CR of As showed a similar pattern to the HQ of As at different experimental sites (Fig. 2F). The elevated arsenic risk observed in this study may stem from high baseline As levels in the Yangtze River Basin environment. Both natural and anthropogenic sources contribute to As distribution in rice paddy soil. Arsenic pollution in China mainly comes from the release of industrial waste/waste water into the environment (Kumarathilaka et al., 2018). A national investigation of As contamination demonstrates that 64.4 % of As comes from human activities in China, among which the Yangtze River basin has a high level of As due to industrialization(Han et al., 2019). The multiple changes in the health risks of As may result from the variable transformations of As. The toxicity and bioavailability of As depend on its variable chemical forms. The inorganic forms of As are more toxic than the organic forms. As(III) is the predominant form in paddy soils. As(III) is readily taken up by rice plants and is more toxic than As(V)(Dominguez-Gonzalez et al., 2020). Different chemical forms of As might pose different risks to human health. In this study, we only detected the total As content in rice grains. Further risk profiling of different chemical forms of As may help clarify the detailed role of reducing agrochemicals in lowering the health risks of As in rice grains.

3.4. Evaluation of soil fertility

Continuous overuse of chemical fertilizers may impair soil fertility, which is vital for crop productivity and sustainable agriculture(Ning et al., 2017). In this study, we measured soil nutrient content (N, P, and K), SOM, and soil pH (Fig. S7). Soil fertility indices (N, P, and K fertility) and the composite fertility index were calculated using the Nemerow index method (F. Q. Li et al., 2021). More obvious changes in soil fertility were observed along with the rice growth stages (from tillering to harvesting) (Fig. 3A). Decreased soil fertility was found in the DRA of JiangYan and JiAn. Soil fertility was rarely affected in the DRA of JianTan and HaiAn compared to the control. Intriguingly, soil fertility was enhanced in the DRA of ShangGao and FengCheng, especially for P-fertility and K-fertility at the heading and harvesting stages (Fig. 3A and B). Most of the soil total fertility was at a general level (0.9–1.8) based on the Nemerow Index classification (Fig. 3B). Chemical fertilizer reduction tends to impair soil fertility(Ning et al., 2017), as evidenced by observations in JiangYan and JiAn in this study (Fig. 3A). This finding aligns with previous results reported by Wang et al. (2012a,b) and Ning et al. (2017). This phenomenon can be attributed to the competition for available soil nutrients between proliferating microbial communities and crop requirements under reduced chemical fertilizer input (Geisseler et al., 2010; Wang et al., 2012a). Soil fertility in the DRA of HaiAn remained almost unchanged (Fig. 3A), which may have resulted from the slight reduction in fertilizer usage (only −3.33 %) (Fig. S2A). Generally, chemical fertilizer reduction combined with organic fertilizer supplementation can help compensate for the loss of soil fertility to a certain degree(Han et al., 2021; Ning et al., 2017). Under this condition, the average total fertility in the DRA of ShangGao and FengCheng increased by 23.7 % and 13.5 %, respectively (Fig. 3A), while fertilizer reductions of 13.5 % and 12.1 % occurred in these two areas, respectively (Fig. S2A). Thus, reducing chemical fertilizers properly might be potentially beneficial for the recovery of soil fertility.

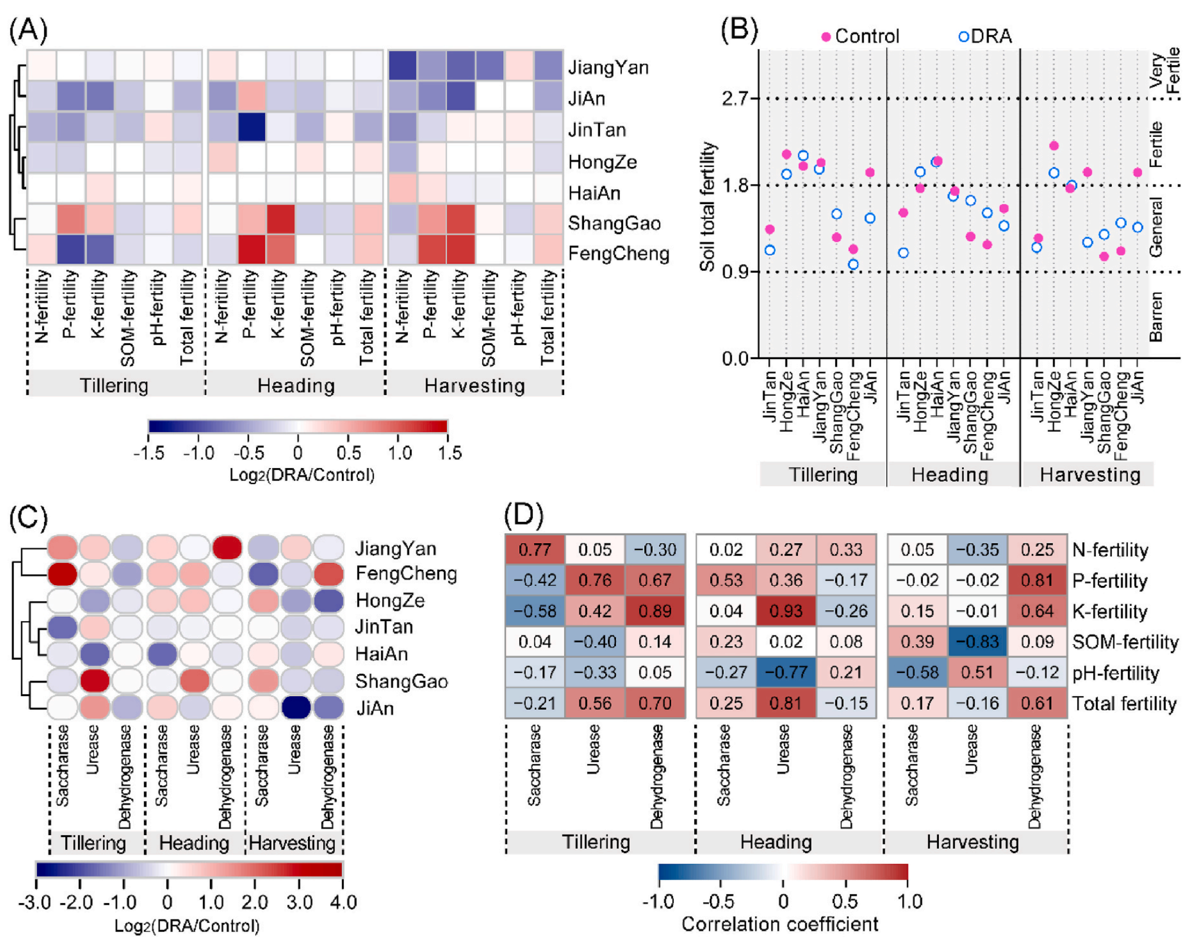

Fig. 3. Evaluation of soil fertility and soil enzyme. (A) Relative fold change of soil fertility indices. (B) Soil total fertility index. (C) Relative fold change of soil enzyme activities. (D) Pearson correlation analysis between soil fertility and soil enzyme activity.

Long-term overuse of chemical fertilizers diminishes fertilizer efficiency by enhancing SOM decomposition, which can further damages soil structure and lead to nutrient loss from soils(Roba, 2018). We found a slight increase in SOM content in the DRA of ShangGao and FengCheng compared to the control groups (Fig. S7E), which may help recover soil fertility in these two areas. Soil fertility depends on the chemical, physical, and biological properties of the soil. In addition to chemical fertilizer reduction, several alternative fertilizer source practice patterns have been investigated for comparison, such as straw return, organic manure of animal feces, and reduced chemical fertilizer plus organic manure compensation. Soil enzymes activity and microbial communities are critical drivers of nutrient cycling, forming the cornerstone of soil health and ecosystem sustainability, directly influencing soil fertility and crop productivity.

Soil enzymes are suitable bioindicators of soil fertility because they play important roles in soil nutrient cycling and distribution(Gunjal et al., 2019). Here, we determined the activity of three soil enzymes (saccharase, urease, and dehydrogenase). Compared to the control, DRA showed variable changes in soil enzyme activity across rice growth stages. Approximately one-third of soil enzyme activities were enhanced in DRA, while others remained unchanged or declined (Fig. 3C). We first observed changes in enzyme activities in ShangGao and FengCheng, two DRA areas with enhanced soil fertility. Urease activity was enhanced at the tillering and heading stages in the DRA of ShangGao (Fig. 3C). Urease hydrolyzes urea into NH_3, which can be assimilated by rice plants(Gunjal et al., 2019). The same quantity of urea was applied in both the control and DRA in ShangGao (Table S3), and the enhanced urease activity in DRA may improve urea utilization efficiency, thereby providing N for rice plants. In the DRA of FengCheng, the activities of saccharase, urease, and dehydrogenase increased at the tillering, heading, and harvesting stages, respectively (Fig. 3C). Soil saccharase and dehydrogenase are hydrolytic enzymes involved in the transformation and cycling of soil organic carbon(Gunjal et al., 2019; Xu et al., 2021). Straw return replaced partial complex fertilizers in the DRA of FengCheng (Table S3), potentially enhancing saccharase and dehydrogenase activity by supplying additional organic carbon as substrate(Liu et al., 2010). Among all three growth stages in DRA, enzymatic activities were most pronounced at the heading stage but declined at the harvesting stage (Fig. 3C). There trend was consistent with observed change in soil fertility (Fig. 3C). A possible explanation is that rice plants require additional nutrients during the heading stage, which may stimulate both soil fertility and enzyme activity under reduced fertilizer conditions in DRA.

To better understand the relationship between soil fertility and enzyme activity under reduced chemical fertilizer conditions, we performed correlation analyses. The enzyme activity showed positive correlations with soil fertility. The comprehensive soil fertility index was positively correlated with urease activity at the heading stage (Fig. 3D). This result confirms the importance of supplying N nutrients during the heading stage of rice growth(Tang et al., 2019). Additionally, dehydrogenase activity was positively correlated with K-fertility at the tillering stage and P-fertility at the harvesting stage. It has been indicated that dehydrogenase plays a key role in P nutrient cycling by mediating oxidative phosphorylation, a critical process in microbial energy production that drives nutrient transformations in soil (Trevors, 1984). Reducing fertilizer application decreased dehydrogenase activity at most sites, paralleled by reduced in P-fertility at the harvesting stage (Fig. 3A and 3C), suggesting improved phosphorus management is required during this period.

Overall, our results demonstrate that fertilizer reduction variably affects soil fertility. However, reducing chemical fertilizers or substituting them with alternatives may enhance soil enzyme activity. Improved soil fertility observed in certain DRA areas further indicates that appropriate chemical fertilizer reduction could aid soil recovery in regions degraded by long-term chemical fertilization. The maintenance or restoration of soil fertility under reduced fertilizer input may be linked to soil microbial community adaptation, which was further investigated and discussed below.

3.5. Analysis of the soil bacterial community

The soil microbiome is a ubiquitous and indispensable agroecosystem component that sustains soil fertility, nutrient cycling, and crop productivity(Falkowski et al., 2008). Both fertilization and pesticide residues influence soil microbial diversity(Wang et al., 2020; Zhao et al., 2019). As previously found in this study, the DRA areas of HaiAn/FengCheng, JiAn, and JiangYan exhibited remarkable reduction in pesticide-related risks, significantly mitigating their impact on local ecosystems Log_2 (fold change) > 2, at the tillering, heading, and harvesting stages, respectively (Fig. 1A). Therefore, to understand and investigate shifts in microbial composition and elucidate the functional roles of the changed soil microbiome, these areas were selected for soil bacterial community analysis using 16S rRNA gene sequencing. Sequencing yielded an average of 4248 OTUs with 94.8 % coverage (Figure. S 8D and 8E). Approximately 50 % of OTUs were shared between control and DRA groups across all site (Fig. S8A–C). No significant differences for bacterial richness indices (Chao and ACE) were observed between control and DRA groups (Fig. S8D and 8E), suggesting that reduced agrochemical practices may require extended periods to induce bacterial enrichment or exert minimal effects. Bacterial community alpha diversity was assessed using the Shannon and Simpson indices. While the Shannon index showed no significant differences between groups (Fig. S8I), the Simpson index (weighted toward community evenness) decreased in the DRA areas of JiAn and JiangYan (Fig. S8J). Thus, short-term agrochemical reduction may not alter overall bacterial diversity but could reduce community evenness in specific DRA areas (e.g., JiAn and JiangYan).

We further analyzed shifts in relative bacterial community abundance. Approximately 90 % of bacterial were classified into seven dominant phyla: *Proteobacteria* (27.3 %), *Chloroflexi* (20.5 %), *Actinobacteria* (16.1 %), *Acidobacteria* (13.0 %), *Nitrospirae* (5.18 %), *Bacteroidetes* (4.16 %), and *Firmicutes* (3.73 %). Similar phyla dominance patterns have been reported in paddy soils(Zhang et al., 2019). A secondary group comprised *Gemmatimonadetes* (1.90 %), *Cyanobacteria* (1.48 %), *Planctomycetes* (1.10 %), *Patescibacteria* (1.08 %), and *Rokubacteria* (0.89 %) (Fig. 4A).

DRA exhibited distinct phylum-level bacterial compositions compared to controls, highlighting significant restructuring of soil microbiomes. The phylum abundance between the DRA and control was compared, and a log2 fold change of DRA/control greater than 0.5 was considered a significant change (Fig. S9). In the DRA of FengCheng, the proportion of *Acidobacteria* increased, while *Actinobacteria* and *Firmicutes* showed a decreased proportion. In the DRA of HaiAn, the proportion of three phyla (*Nitrospirae*, *Bacteroidetes*, and *Patescibacteria*) decreased. In the DRA of JiAn, *Firmicutes* abundance decreased, whereas *Gemmatimonadetes* increased. In the DRA of JiangYan, an increased abundance was observed in *Acidobacteria*, while *Bacteroidetes* showed a decreased proportion (Fig. 4A; Fig. S9). Integrated deep sequencing and machine learning-based co-occurrence network analysis revealed that soil keystone functions are mediated by specific bacterial taxa(Xun et al., 2021). For example, phosphorate metabolism is suggested to be closely related to *Gemmatimonadetes* and *Actinobacteria*(Xun et al., 2021). In the DRA of JiAn at the heading stage, the increased abundance of *Gemmatimonadetes* and *Actinobacteria* may have contributed to increased P-fertility (Fig. S9; Fig. 3A). In the DRA of FengCheng at the tillering stage, the decreased P-fertility may be associated with the decreased abundance of *Actinobacteria* (Fig. S9; Fig. 3A). Keystone bacterial taxa play important roles in sustaining the stability of the soil microbiome and soil nutrient metabolism(Xun et al., 2021). In this study, a much greater reduction in the usage of P fertilizers was conducted in FengCheng (−73.8 %) compared to JiAn (−28.6 %) (Fig. S2C). The observation suggested that P metabolism-related bacteria may survive and

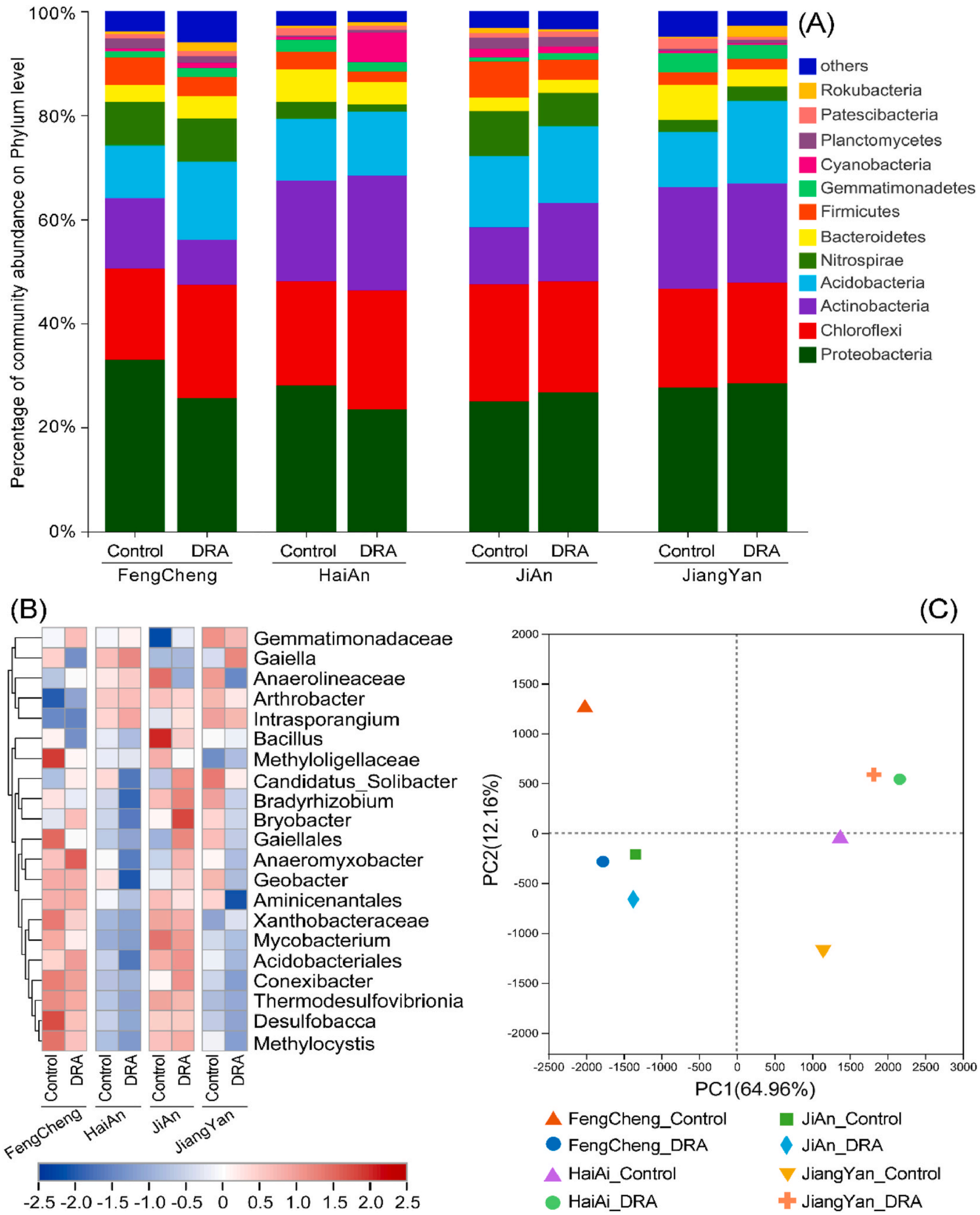

Fig. 4. Analysis of soil bacterial community. (A) Relative abundance of different bacterial communities at phylum level. (B) Relative abundance of different bacterial communities at genus level. (C) PCA analysis of soil bacterial communities at genus level across the soil samples.

play a role in sustaining soil P-fertility under conditions of mild P deficiency. However, it is likely that these bacteria may not be sufficient to cope with severe P deficiency. In addition, it has been suggested that soil *Cyanobacteria* can improve soil fertility and stability(Chamizo et al., 2018). The soil fertility in the DRA of HaiAn was sustained or even slightly increased compared to the control after the slight reduction in fertilizer usage (Fig. 3A; Fig. S2), suggesting that the resilience of the soil bacterial community could be favored by the stress release of chemical fertilizers. Another possible reason for the observation could be the significant increase in the abundance of *Cyanobacteria* (3.25-fold enhancement) observed in the DRA soil of HaiAn (Fig. S9). Thus, the appropriate reduction of fertilizers may positively modulate the beneficial bacteria in soil, which at least in part further activates the ability of soil self-restoration.

To elucidate soil bacterial community adaptation in DRA, we analyzed genus-level abundance changes, identifying 21 genera with significant differential abundance between DRA and control groups. In general, the bacterial abundance was relatively lower in HaiAn and JiangYan compared to FengCheng and JiAn (Fig. 4B). PCA (principal component analysis) of samples at genus level was performed to determine the impact of DRA and location (Fig. 4C). Soil bacteria were well separated between DRA and control, indicating shifted bacterial genera in response to practices of reducing agrochemicals. The genus abundance showed considerable correlations with P-fertility, pH and total fertility (Fig. S10A). A remarkable increase in *Intrasporangium* (a kind of *Actinobacteria*) abundance was detected in the DRA of JiAn (Fig. 4B), consistent with an increase in *Actinobacteria* (Fig. 4A). The changes in *Intrasporangium* abundance were positively correlated with soil P-fertility as well (Fig. S10A). This result suggested that *Intrasporangium* may contribute to P metabolism upon proper reduction of P fertilizer. *Anaerolineaceae* frequently exists in anoxic ecosystems such as rice paddy soil. Members of *Anaerolineaceae* are able to survive by utilizing organic matter (e.g., sugars and proteinaceous compounds)(Yamada and Sekiguchi, 2018). Here, a decreased abundance of *Anaerolineaceae* was observed in the DRA of JiAn (heading stage) and JiangYan (harvesting stage) (Fig. 4B), coinciding with the decrease in SOM fertility (Fig. 3A). *Anaerolineaceae* was positively correlated with SOM fertility (Fig. S10A). Thus, reducing chemical fertilizers in JiAn ad JiangYan decreased the abundance of *Anaerolineaceae*, which may have further limited the utilization of SOM. In addition, an increased abundance of *Arthrobacter*, a plant growth-promoting bacterium, was found in the DRA of FengCheng and HaiAn (Fig. 4B). *Arthrobacter* may be recruited by root-secreted organic acids to enhance N use efficiency, resulting in the adaptation of crops to changing environments(Chen et al., 2019).

Compared to soil fertility, we did not find a remarkable correlation between soil enzymes and bacterial genera upon reducing fertilizer usage (Fig. S10B). Soil enzymes were closely related to the soil microbiome, but plant roots and soil properties were also key factors determining the activity of soil enzymes(Maurya et al., 2020). Therefore, further studies are needed to investigate the relationship between soil enzymes and the soil microbiome by integrating rice root exudates and soil properties under the condition of reducing agrochemical usage in paddy soil.

3.6. A proposed practice of agrichemical application for sustainable agriculture

Based on the investigation and assessments of ecosystem impacts, soil fertility, and health risk, a potential agrichemical application strategy for sustainable agriculture is proposed as follows. For pesticide reduction, as indicated in Fig. 1B, the following practices are recommended: FengCheng DRA (pre-tillering), Jian DRA (tillering to heading), and JiangYan DRA (heading to harvesting). Consequently, a pesticide optimization strategy for rice growth is proposed (Table S8), though further validation and refinement are required. For fertilizer reduction, as illustrated in Fig. 2C and D, S2A, 3, and S7E, the following practices may serve as references: ShangGao DRA or FengCheng DRA (pre-tillering), JiangYan DRA or ShangGao DRA (tillering to heading), and ShangGao DRA (heading to harvesting). Future studies should focus on long-term, large-scale field trials combined with continuous monitoring and validation to optimize precision and sustainability of DRA practices.

4. Conclusion

Meeting the food requirements of the burgeoning global population necessitates the development of sustainable agriculture practices. The over-extended and excessive use of agrochemicals presents threats to both soil and public health. In this study, we investigated agrochemical reduction strategies in two Chinese rice-producing regions through four-year field trials. Reduced pesticide/fertilizer application decreased ecological and dietary risks from pesticide residues and heavy metals. While two experimental sites showed decreased soil fertility, most maintained or relatively improved fertility levels, potentially mediated by keystone soil bacterial taxa. Strategic urea and P fertilizers reductions effectively lowered heavy metal risks without compromising soil fertility. Targeted restriction of pesticides, such as pymetrozine and difenoconazole during specific growth phases, or coupled with substitution of less-toxic alternatives, proved critical. These empirically validated patterns provide critical insights for optimizing scientific farming practices in rice cropping systems. While our investigation may not fully elucidate the optimal mechanism framework for agricultural system profiling, our findings represent a significant advancement in enabling science-driven transitions toward sustainable cropping systems. It provides actionable strategies for optimizing agrochemical use to minimize ecological risks and dietary exposure risks throughout rice cultivation cycles.

CRediT authorship contribution statement

Yaodong Zhang: Writing – original draft, Formal analysis, Investigation, Methodology. **Haoran Zhang:** Formal analysis, Methodology, Investigation. **Tingting Tao:** Investigation, Formal analysis. **Jian Chen:** Writing – original draft, Investigation, Formal analysis. **Pan Li:** Investigation, Formal analysis. **Yulong Wang:** Investigation, Formal analysis. **Pengyan Liu:** Formal analysis. **Yiyong Zhu:** Writing – review & editing. **Michael N. Routledge:** Writing – review & editing. **Cuifeng Yang:** Funding acquisition, Writing – review & editing. **Cunzheng Zhang:** Writing – review & editing, Conceptualization, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.126619.

Data availability

Data will be made available on request.

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