

Impact of agricultural activities on pesticide residues in soil of edible bamboo shoot plantations

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Abstract

Edible bamboo shoot is one of the most important vegetables in Asian countries. Intensive agricultural management measures can cause many negative influences, such as soil acidification and excessive pesticide residues. In the present study, more than 300 soil samples were collected from edible bamboo shoot plantations in six areas throughout Zhejiang province, China, to investigate the soil pesticide pollution and its change after different agricultural activities. Thirteen organic chemicals were detected; nine less than that detected during a similar study executed in 2003–2004. All the detected residues were far below the Chinese national environmental standards for agricultural soils. The pesticide residues in bamboo plantations showed a decline over the past decade. Organic materials used for mulching and plantation's background of being formerly a paddy field are two important factors increasing the pesticide residues. Conversely, lime application to acidified soil and mulching with uncontaminated new mountain soil could decrease the residues significantly. Our results indicated that the current agricultural activities are efficient in reducing pesticide residues in the soil of bamboo shoot plantations and should be further promoted.

Keywords: agricultural activity, bamboo plantation, remediation of soil, soil pollution

1 Introduction

Bamboo shoots are new culms that come out of the ground of many bamboo species, such as *Bambusa vulgaris* Schrad. and *Phyllostachys edulis* (Carrière) J. Houz. Due to its rich nutrients, crisp texture and delicious taste, bamboo shoots have been used as vegetables for thousands of years in numerous Asian countries. In China, *Phyllostachys praecox* f. *prevernalis* Chen et Yao (Lei bamboo) is one of the most popular species for bamboo shoot production (Jiang *et al.*, 2002). Since the 1990s, Lei bamboo has been transplanted and cultivated in large areas and many paddy fields have been transformed to bamboo shoot cultivation in several provinces, and particularly in Zhejiang because of the

increase in market demand (Fang *et al.* 1994; Zhou *et al.*, 1998).

To gain higher profits, intensive agricultural management measures have been adopted for bamboo shoot production, like weeding, chemical fertiliser application, as well as pesticide usage for pest control. In addition, bamboo plantations are mulched with a thick layer of straw, bran or some other organic material before the shoot harvest in winter time (Huang *et al.*, 2007). Through mulching, the bamboo shoot yield could be brought forward with two months, in order to coincide with the Chinese Spring Festival and obtain highest prices. On the other hand, however, these intensive management measures have caused many problems, including soil acidification, declining shoot yield, pest infestation, organic pollution of the soil, and excessive pesticide residues in the bamboo shoots (Guo *et al.*, 2011; Gui *et al.*, 2013).

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In order to achieve a sustainable production and to guarantee the quality of bamboo shoots, several farmer cooperative societies were established, and professional experts were hired to provide technical support to the farmers. By these means, several novel agricultural activities have been extensively carried out in the past decade, such as using low-toxicity and easily degradable pesticides, applying lime to increase pH in acidified soil, and mulching the bamboo plantation with new mountain soil (Zhuang *et al.*, 2014).

In the present paper, a field survey on organic pollutants, including organochlorine pesticides (OCPs), organic phosphorus pesticides (OPPs) and pyrethroids (PYs) was conducted during 2015–2016 in bamboo plantations from 6 major Lei bamboo shoot production areas in Zhejiang province. The pollution was analysed with respect to agricultural activities and also compared to historical data collected in the same areas during 2003–2004. Pesticide residues in bamboo shoots were not further analysed since these were all lower than the detection lines and not detectable from the samples collected in 2015–2016. The aims of this work were to examine the current state of organic pollutants in bamboo plantation soils, to elucidate their spatiotemporal dynamic characteristics, and to assess the efficacy of different agricultural activities for decreasing soil pesticide residues.

2 Materials and methods

2.1 Soil sampling

A total of 349 surface soil samples were collected from the six major bamboo shoot production counties in Zhejiang province (Fig. 1). All the bamboo plantations selected for soil sampling were once investigated in 2003–2004. Bamboo plots located in hilly uplands are usually sloping fields. In contrast, those fields that were originally paddy farmlands are relatively small, flat fields and are distributed separately on terraces or platforms as a result of land fragmentation.

The sampling plots were defined according to their former use [former paddy field (FPF) or hilly upland (HU)] and the different soil management measures previously conducted: non-mulched (NOMM) or organic material mulched (OMM), lime applied (LA), non-lime applied (NLA), new mountain soil mulched (NSM) plots and non-NSM. Soil samples taken from the surface (0–20 cm in depth) at five random points in each plot were thoroughly mixed to form a composite sample. One such sample was collected from each plot. The number of soil samples collected from the different types of plots is listed in Table 1.



Fig. 1: Location of the six sampling areas in Zhejiang Province (shown divided into prefectures).

2.2 Sample preparation, extraction and clean-up

The soil moisture content was determined by weighing subsamples of the soils before and after drying for ca. 20 h at 105 °C. The samples were air-dried at 30 °C and sieved through a 1 mm polyethylene sieve. The prepared soil samples were sealed in polyethylene bags and stored at –20 °C for further analysis.

Pesticide residues in all samples were extracted, cleaned up and analysed using the national standard methods (AQSIQ, 2003a, b), which were also used in 2003–2004 for data collection. For OPPs, the extraction and clean-up procedures were as follows: 20 g of the air-dried soil sample was extracted for 8 h with 100 ml acetone. After 4 h oscillation, the mixture was passed through a Buchner funnel with vacuum suction filtration packed with one layer of filter aid agent (celite 545) and several layers of filter paper. The eluate was added with 10 ml coagulation liquid (20 g NH₄Cl + 40 ml 85 % H₃PO₄ at constant volume of 2000 ml with pH 4.5–5.0) and 1 g filter aid agent (celite 545), oscillated 1 min, stood for 3 min and filtered. The eluate was then extracted 3 times with 50 ml dichloromethane and passed through a funnel packed with 1 g anhydrous sodium sulphate and 1 g celite 545. The solvent extracts were collected in a 250 ml flat bottom flask, added with 0.5 ml ethyl acetate and concentrated to ca. 3 ml with a rotating evaporator. The

Table 1: Number of soil samples collected from different types of plots.

Site	Location	OMM Plantation				NOMM Plantation		
		FPF			HU		FPF	HU
		LA	NLA		LA	NLA		
			NSM	non-NSM				
1	Deqing	20	4	6	7	8	10	6
2	Linan	20	5	7	7	6	12	7
3	Fuyang	20	3	8	8	6	8	6
4	Longyou	20	3	3	6	9	9	6
5	Jinyun	21	6	7	2	9	7	6
6	Qingyuan	17	5	6	2	8	7	6
Total		118	26	37	32	46	53	37

Note: OMM, organic material mulched; NOMM, non-mulched; FPF, former paddy field; HU, hilly upland; LA, lime applied; NLA, non-lime applied; NSM, new mountain soil mulched.

residue was dried with a nitrogen flux and then dissolved in 5 ml acetone for further chromatographic analysis.

For OCPs and PYs, a 20 g soil sample was mixed with 4 g diatomite and 100 ml acetone/petroleum ether (1 : 1, v/v) for 12 h. Then residues were extracted for 4 h at 80 °C in a Soxhlet extractor. After being cooled down, the solvent was added with 100 ml sodium sulfate solution, oscillated for 1 min and stood for several min until getting different layers. The acetone solution in the lower layer was discarded and the supernatant solution was eluted through a Florisil column with 100 ml petroleum ether/ethyl acetate (95 : 5, v/v). The eluate was collected, condensed with a rotating evaporator, dissolved in petroleum ether and finally reduced to 1 ml with a nitrogen flux.

2.3 Chromatographic analysis

All standard chemicals, including HCHs, DDX, quin-tozene, chlorothalonil and dicofol were supplied by J&K Chemica, Beijing, China and used as references. For OPPs, the samples were analysed using an Agilent Gas Chromatograph 7890A equipped with a flame photometric detector (FPD) and a DB-1701 column (30 m × 0.32 mm i.d. × 0.25 μm film thickness). The samples were injected by auto-sampling in the splitless mode with a venting time of 1 min. The oven temperature was programmed to increase from 100 to 200 °C at a rate of 10 °C min⁻¹, then to 250 °C at 25 °C min⁻¹ and maintained for 10 min. Nitrogen was used as the carrier gas (2 ml min⁻¹) and make-up gas (25 ml min⁻¹). The injector and detector temperatures were 200 and 220 °C, respectively. For OCPs and PYs, samples were analysed using

an Agilent Gas Chromatograph 6890N and 5973-MSD. A HP-5 column 30 m × 0.32 mm i.d. × 0.25 μm film thickness was used. The samples were injected by auto-sampling in the splitless mode with a venting time of 2 min. The temperature program was as follows: initial temperature held at 50 °C for 2 min, increased to 150 °C at a rate of 10 °C min⁻¹, then to 240 °C at 3 °C min⁻¹ and maintained for 28 min. Nitrogen was used as the carrier gas (1 ml min⁻¹) and make-up gas (37.25 ml min⁻¹). The injector and detector temperatures were 240 and 280 °C, respectively.

2.4 Quality control

The recovery rates of pollutants with samples fortified by pesticides (0.032–4.000 μg in weight for different ones) ranged from 88.95 % (methamidophos) to more than 100 % (γ-HCH, chlorothalonil, chlorpyrifos, parathion-methyl and parathion). The detection limits for the soil samples ranged from 0.05 (α-HCH) to 3.0 (malathion) ng g⁻¹ (Table 2). A procedural blank was run with every set of twenty samples to check for contamination. Three replicates for every soil sample were performed to reduce error levels in the analysis.

2.5 Statistical analysis

Average concentrations of pesticide residues were calculated by geometric mean. Concentrations from the same type of plantation were tested by normal distribution. All data were in line with normal distribution and homogeneity of variance, and were analysed using single-factor analysis of variance (ONE-WAY ANOVA) (SAS OnlineDoc®, Version 8.01, Statistical Analysis System Institute, Cary, NC).

Table 2: Organic pollutants in soils collected from bamboo shoot plantations.

	Concentration (ng g ⁻¹)			Occurrence rate (%)	Detecting limit (ng g ⁻¹)	Recovery (%)	
	Lowest	Highest	Average [†]				
<i>α</i> -HCH	0.17 (0.31)	0.24 (0.61)	0.20 (0.38)	16.62 (33.03)	0.05	96.01	
<i>β</i> -HCH	0.10 (0.19)	9.66 (24.79)	1.49 (2.44)	35.24 (77.46)	0.08	99.40	
<i>γ</i> -HCH	0.55 (0.59)	14.22 (37.61)	2.35 (5.01)	32.38 (93.51)	0.07	100.40	
<i>δ</i> -HCH	1.17 (3.11)	2.19 (4.22)	1.27 (3.35)	6.88 (11.46)	0.18	96.22	
∑ HCH	0.22 (0.58)	17.13 (35.57)	2.43 (6.03)	46.70 (100)			
OCPs [‡]	<i>p, p'</i> -DDT	0.52 (0.78)	1.82 (3.73)	0.91 (1.43)	11.75 (37.81)	0.49	93.71
	<i>p, p'</i> -DDE	0.36 (0.73)	3.13 (5.66)	1.85 (2.46)	25.47 (36.55)	0.17	98.20
	<i>p, p'</i> -DDD	N.D. (0.51)	N.D. (2.31)	N.D. (0.92)	N.D. (43.68)	0.48	99.65
	<i>o, p'</i> -DDT	N.D. (0.24)	N.D. (6.12)	N.D. (1.91)	N.D. (37.81)	0.19	92.87
	∑ DDT	0.24 (0.36)	4.33 (8.82)	1.88 (2.55)	31.23 (83.10)		
Quintozene	0.30 (0.30)	0.48 (0.66)	0.29 (0.53)	16.33 (79.58)	0.20	99.60	
Chlorothalonil	0.62 (1.55)	27.67 (72.25)	8.83 (20.20)	24.93 (67.23)	0.30	103.41	
Dicofol	0.96 (1.62)	32.62 (118.32)	2.35 (17.16)	17.19 (72.54)	0.80	101.00	
∑ OCP	1.01 (2.48)	61.25 (151.35)	11.16 (33.23)				
OPPs [§]	Chlorpyrifos	2.8 (2.8)	200.00 (421.22)	23.13 (74.14)	47.85 (83.32)	2.00	103.41
	Methamidophos	N.D. (13.22)	N.D. (37.57)	N.D. (23.23)	N.D. (64.78)	1.00	88.95
	Dichlorvos	N.D. (4.12)	N.D. (68.90)	N.D. (36.77)	N.D. (4.67)	1.00	94.88
	Malathion	N.D. (3.61)	N.D. (37.22)	N.D. (4.66)	N.D. (7.12)	3.00	98.79
	Methidathion	N.D. (2.33)	N.D. (11.15)	N.D. (3.24)	N.D. (4.56)	2.00	97.89
	Parathion-methyl	N.D. (1.21)	N.D. (26.65)	N.D. (4.38)	N.D. (97.12)	1.00	108.43
	Parathion	N.D. (1.53)	N.D. (3.69)	N.D. (2.32)	N.D. (36.61)	1.00	102.41
	Dimethoate	1.46 (3.17)	9.85 (26.62)	4.31 (14.45)	4.87 (9.12)	1.00	97.39
	∑ OPP	1.52 (2.11)	203.65 (518.12)	27.29 (82.13)			
PYs [¶]	Cypermethrin	1.27 (1.33)	5.22 (4.21)	1.76 (2.32)	16.33 (20.13)	1.00	98.89
	Fenvalerate	2.46 (5.84)	132.36 (312.25)	16.61 (123.38)	18.91 (71.16)	2.00	97.39
	Deltamethrin	N.D. (2.01)	N.D. (5.17)	N.D. (2.41)	N.D. (3.73)	2.00	95.88
	∑ PY	2.26 (2.96)	134.56 (312.25)	16.92 (147.55)			

Note: data in parentheses are historical data collected during 2003–2004 (unpublished). These original data were obtained from Forestry Bureau of Zhejiang Province. N.D. means not detectable. Detecting limit and recovery refer to data collected during 2015–2016.

[†] Average means the geometric mean. [‡] OCP represents organochlorine pesticides, [§] OPP organic phosphorus pesticides and [¶] PY pyrethroid.

3 Results

3.1 Concentration of pesticide residues

The results are summarized in Table 2. A total of 22 compounds were investigated. Residues of nine OCPs, including four HCHs, two DDX and three others; two OPPs and two PYs were detected in the surface soil samples. Both the concentration and occurrence rate of all these residues were obviously lower than those found during 2003–2004. Moreover, nine residues (*p, p'*-DDD, *o, p'*-DDT, deltamethrin, dichlorvos, malathion, methamidophos, methidathion, parathion and parathion-methyl), which had been detected 10 years earlier, were not detectable in our study. Our data showed a dramatic decrease in the pesticide residues during the last 10 years.

From Table 2, it can be seen that DDX and HCHs were still detectable although these pesticides have been banned for more than 30 years in China (since 1983), indicating their persistent-polluting characteristics in soil. The detected values are all below the Chinese national environmental standards for agricultural soil: <0.05 mg kg⁻¹ for ∑ DDT and <0.01 mg kg⁻¹ for ∑ HCH (Environmental Quality Standard for Soils of China, GB15618-2008).

3.2 Effects of mulching with organic material

The samples collected on plantations mulched with organic material (OMM) were compared with those from non-mulched (NOMM) plantations. However, plantations which were seriously deteriorated due to long-term intensive management and mulched with new mountain soil were not

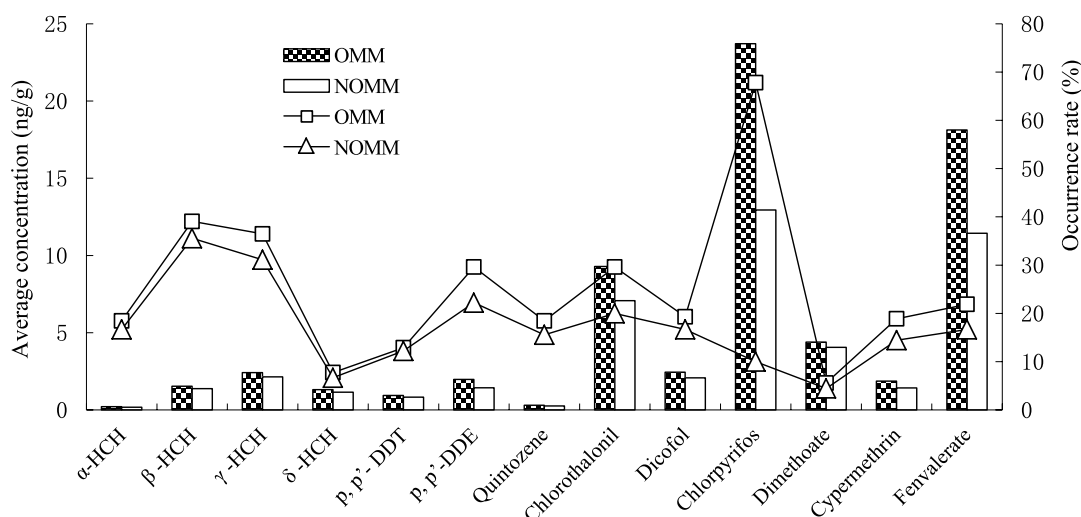


Fig. 2: The mean concentrations and occurrence rates of residues from different bamboo shoot production plots classified according to mulching. (OMM: organic material mulched plots; NOMM: non-mulched plots).

included in the analysis because we used the surface soil for residue analysis and the effect of new mulched soil on residues was significant. So the samples were divided into two groups based on organic material mulching, one for the OMM ($n = 233$) and the other for the NOMM ($n = 90$) (see Table 1). The results of the residue analysis are presented in Fig. 2.

From Fig. 2, it can be seen that the concentration of pesticide residues found on OMM plantations were nearly all higher than those from NOMM plantations; the differences in chlorothalonil ($p = 0.042$), chlorpyrifos ($p = 0.0001$), and fenvalerate ($p = 0.006$) were significant. A similar picture was found for the occurrence rates of pesticide residues.

3.3 Effects of lime application

In order to control soil acidification, lime is extensively used by farmers in their plantations, which have been mulched with straw, bran or other organic materials for several years. We divided the samples from OMM plantations into two groups based on lime application, one for the lime applied (LA) ($n = 150$) and the other for non-lime applied (NLA) ($n = 83$) (see Table 1). Again, the samples from plantations which were mulched with new mountain soil were not included. The results are presented in Fig. 3.

From Fig. 3, we can see that the concentrations of all types of residues in LA plantations were significantly lower than those from NLA plantations ($p = 0.037$ for \sum OCP, 0.006 for \sum OPP and 0.028 for \sum PY). The soil pH values were 3.89 ± 0.17 and 6.73 ± 0.14 in NLA and LA plantations respectively and significant differences were detected ($F = 27.62$, $df = 1$, $p = 0.0186$). Our results show that lime

can increase the soil pH value and accelerate the decomposition of residues.

3.4 Effects of land-use history

Before 1990s, nearly all bamboo fields were planted in hilly upland and most lowland areas were used to grow rice in Zhejiang province. From the beginning of 1990s, some of the paddy farmlands have been changed and used for bamboo cultivation because of the better economic benefits. Currently, more than 60% of all Lei bamboo shoot plantations have been planted on former paddy fields (FPF). The soil samples collected from FPF plantations ($n = 208$) were compared with those from hilly uplands (HU) ($n = 115$). Again, the samples from plantations which were mulched with new mountain soil were not included. The results are presented in Fig. 4.

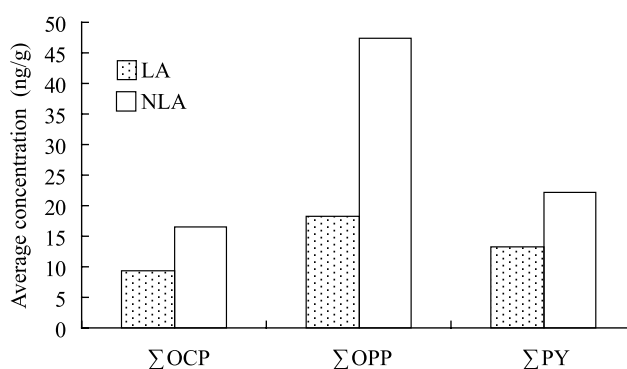


Fig. 3: The average concentrations of different types of residues from bamboo shoot production plots classified by lime application (LA: residues from lime applied plots; NLA: residues from non-lime applied plots; OCP: organochlorine pesticides; OPP: organic phosphorus pesticides; PY: pyrethroid).

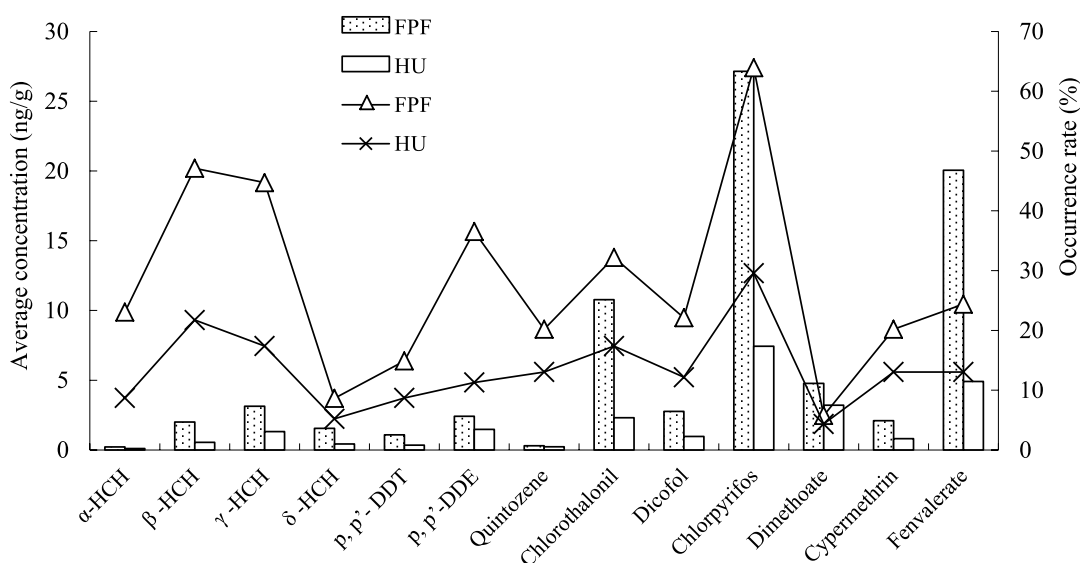


Fig. 4: The mean concentrations and occurrence rates of residues from bamboo shoot production plots classified by different land-use history (FPF: former paddy field; HU: hilly upland).

From Fig. 4, it was clear that both the average concentration and occurrence rate of pesticide residues were higher in FPF plantations than those in HU plantations. For eleven residues, significant differences were observed between the plantations with different land use histories (α -HCH: $p = 0.048$; β -HCH: $p = 0.016$; γ -HCH: $p = 0.035$; δ -HCH: $p = 0.022$; p,p' -DDT: $p = 0.030$; p,p' -DDE: $p = 0.043$; chlorothalonil: $p = 0.0001$; chlorpyrifos: $p = 0.0006$; cypermethrin: $p = 0.036$; dicofol: $p = 0.028$; fenvalerate: $p = 0.0003$).

3.5 Effects of mulching with new mountain soil

After several years of mulching with organic materials, the yield of bamboo shoot declined seriously because the soil properties were changed with excessive organic-matter input (Huang *et al.*, 2007; Gui *et al.*, 2013). In such cases, uncontaminated soils from mountain areas were used to mulch the seriously deteriorated plantations with a layer in thickness of about 15 cm. After the reestablishment of uncontaminated mountain soil system, yield of bamboo shoots can return to the normal level. In this study, no pesticide residue was detected for the samples collected from plantations mulched with new mountain soil. Although the pesticide residues in this soil are not really degraded and just buried in the underlying soil, the bamboo root tends to grow upward into the newly added surface soil, reducing the final concentration of residues in the edible bamboo shoot.

4 Discussion

Thirteen types of pesticide residues were detected in the surface soil from bamboo plantations in six major bamboo shoot producing areas of Zhejiang province. During the 2003–2004 study (unpublished data), nine further residues were detected. Both the concentrations and occurrence rates of the detected residues were reduced. These results indicate that pesticide residues in bamboo plantations have decreased over the past 10 years. Feng *et al.* (2003) reported that HCH would decrease faster than DDT in soil-water system of lake, but our results showed that both the concentrations and occurrence rates of HCH residues were higher than those of DDT in bamboo plantations, despite the fact they were banned at the same time in 1983 in China. This seems to indicate that HCH was not completely stopped in the first few years after the ban in Zhejiang province. For DDX, p,p' -DDT and p,p' -DDE were detected, but p,p' -DDD and o,p' -DDT were not detectable in our samples. The possible reason might be that under the conditions found in bamboo plantations, p,p' -DDT and p,p' -DDE are more difficult to decompose and more persistent than p,p' -DDD and o,p' -DDT, a case which has been found by others (Aguilar, 1984; Gong *et al.*, 2004). In China, methamidophos, parathion-methyl, and parathion have been banned since 2008. This might be the reason why the residues of these three pesticides were not detected in our study. In contrast to DDX and HCHs, other pesticides are still widely used in China as these decompose easier. For example, chlorpyrifos is intensively used for controlling the rice borer pest, *Tryporyza incertulas*. Straw and bran with residues of

chlorpyrifos are used as organic materials to mulch bamboo plantations and this might have led to chlorpyrifos being the highest residue in our study.

The kind of organic material used for mulching the bamboo plantation was an important factor in increasing the residues in the soil. For all of the detected residues, both the concentration and occurrence rate were higher in the samples from OMM plantations than those from NOMM plantations. Significant differences were found with chlorothalonil, chlorpyrifos and fenvalerate. The possible reason might be that mulching with organic materials helped subterranean pest insects to overwinter, causing infestation in the next year, like *Melanotus cribricollis* (Shu et al., 2012). The concomitant pest control measures increased the usage of pesticides, leading to a higher level of residues. In addition, organic materials used for mulching might contain some pesticides per se and so increase the residues in soil as shown above for chlorpyrifos. Furthermore, long-term mulching with organic materials could change the soil properties and decrease its pH value (Naramabuye & Haynes, 2006; Gui et al., 2013). An acidic environment might slow down the degradation rate of some pesticides, such as fenamiphos, chlorpyrifos, DDT and its metabolites (Singh et al., 2003a, b; Gong et al., 2004).

Compared to hilly-upland bamboo plantations, residues were significantly increased in soil samples from plantations that had formerly been paddy fields, implying that the historical background of a plantation is an important factor affecting the level of residues. The residues left from the original paddy field might contribute to the differences found in our investigation, especially for persistent organic pollutants like HCHs, DDT and its metabolites. In another study, it was found that the population of subterranean pest insects in FPF plantations is much higher than that in HU plantations (Shu et al., 2012). This causes the application of more chemicals for pest control, increasing the difference in residue concentrations. Also the higher runoff in sloping HU plantations could have contributed to the residue differences found. In contrast, lime application in the acidified soil was found to reduce the residues efficiently because organic pesticides tend to decompose faster with an increase in pH. For example, the half-life of imazapyr has been negatively correlated with the pH of soil: with an increase of alkaline, the degradation rate of imazapyr is faster (Wang et al., 2004). Similar results have also been found in other studies (Bobé et al., 1998; Singh et al., 2003a, b; Gunasekara et al., 2007; Gianelli et al., 2014; Martin-Reina et al., 2017). On the other hand, however, exogenous lime might also affect the balance of soil microbial flora, which could adversely reduce the function of microbes in decomposing pesticides. Therefore, further research is necessary

to be conducted on the reasonable application of lime in Lei bamboo shoot plantations. Moreover, no residues were detected in surface soil samples collected from plantations mulched with uncontaminated mountain soil, implying that such an application on plantations deteriorated through excessive mulching is an efficient measure, although pesticide residues are just buried in the deeper soil layers.

Our findings inferred that the present-day agricultural activities suggested by professional experts, such as application of lime and mulching with uncontaminated mountain soil, as well as using chlorpyrifos, chlorothalonil and malathion instead of methamidophos, parathion-methyl and parathion as pesticides, were efficient in reducing the pesticide residues in soil of bamboo plantations. These agricultural activities could be applied extensively or used as reference for other bamboo shoot producing areas.

Competing interests

The authors declare that they have no conflict of interest.

Author contributions

JH designed the study, and drafted the manuscript. YW carried out the soil sampling, sample preparation and chromatographic analysis. HY performed in the statistical analysis. WG participated in the sample collection, preparation and chromatographic analysis. LB participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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