# Contrasting Effect of Application of Chemical Fertilizers and/or Organic Manure on Soil Quality under a *Phyllostachys praecox* Stand in Subtropical China

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Abstract: Phyllostachys praecox (PP) is a favorite species for producing bamboo shoots that has been widely planted in southern China. A study was conducted under the cultivation of PP stand with extensive management treated with fertilizers at a bamboo forest site, Lin'an County, Zhejiang Province, China. The aim was to assess and compare the effect of chemical fertilizer (CF), organic manure (OM) and combination of chemical fertilizer and organic manure (CF+OM) on soil chemical properties, labile organic carbon and available heavy metals. Application of CF significantly tended to increase the contents of total P (TP), hydrolyzed N (HN) and available P (AP), but did not influence the contents of total organic carbon (TOC) and total N (TN) as compared with no fertilizer plots (CK). Application of CF+OM markedly increased the contents of soil TOC, TN and HN as compared with CF plots. Application of CF significantly decreased the contents of soil microbial biomass carbon (MBC) and soil water-soluble organic carbon (WSOC), but significantly increased mineralizable carbon (MC) as compared with CK. By contrast, CF+OM greatly increased the contents of TOC, WSOC, MBC and MC as well as the ratios of MBC/TOC, WSOC/TOC and MC/TOC as compared to CF plots. Application of CF alone significantly increased available contents of all the heavy metals studied, whereas CF+OM significantly decreased available contents of Pb, Cr and Cu as compared with CF plots. Significant positive correlations were observed between different soil carbon fractions. Total N or HN was highly related to soil carbon fractions except for between TN and WSOC. Available P was highly related to TOC and MC. Application of CF alone significantly increased the contents of heavy metals studied, whereas combined application of CF and OM significantly reduced the contents of available Pb, Cr and Cu in the soils as compared with CF. It was concluded that application of CF+OM fertilization exhibited a beneficial effect on promoting soil quality under Phyllostachys praecox stands and minimized the negative effects of over-application of CF on the environment.

**Key words:** Phyllostachys praecox • Soil quality • Organic manure • Chemical fertilizer

#### INTRODUCTION

Phyllostachys praecox (PP) is an important bamboo species producing mainly for producing edible bamboo shoots and has been planted in many parts of southern China, with a total of  $2.62 \times 106$  ha in throughout China [1]. Recent interest has focused on the introduction of intensive management, including heavy-application of chemical fertilizers (CF) and winter mulching in PP production to increase bamboo shoot yield and economic benefit. Intensive management has been adopted by majority farmers in PP production area. The rates of CF and organic manure (OM) application in

PP production are usually more than 3.0 and 100 t ha<sup>-1</sup> yr<sup>-1</sup>, respectively, which are close to those in protected vegetable soils.

Investigation of fertilization on protection vegetable soils in Shouguang county, Shandong province showed that the average annual rates of N,  $P_2O_5$  and  $K_2O$  applied as CF were 1331, 1287 and 480 kg ha<sup>-1</sup> yr<sup>-1</sup>, respectively [2]. Long-term application of CF can influence important soil properties such as soil structure and density, soil pH, the quantity, quality and distribution of soil organic matter and nutrient cycles within the soil profile [3,4]. Besides the impairment of environment quality, this practice also caused a

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decline in soil productivity through excessive soil erosion, nutrient runoff and deteriorated soil chemical properties.

Long-term over-application of CF in PP stands has resulted in over-accumulation of soil nutrients, especially soil P [5,6], decrease in soil enzymatic activities [7], the exceeded standard of nitrite contents in bamboo shoots [8] and increased available Zn, Cu and Pb [9]. Thus, the PP production area has become a potential pollution resource followed by protected vegetable soils. However, combined application organic manure and chemical fertilizers increased the yield and nitrogen use efficiency of rice, reduced the risk of environmental pollution and improved soil fertility greatly [10].

Understanding the effect of fertilization on soil quality and resulting soil sustainability would help in evaluating and choosing appropriate fertilization practices. The objectives of this study were to assess changes in the soil quality under PP stands induced by over-application of chemical fertilization and to compare the effects of application of CF alone and in combination of CF and OM on the soil quality under PP stand.

### MATERIALS AND METHODS

## Climate of Experimental Sites and Soil Properties:

Experimental site was located in Linan County (30°14′N and 119°4′E), Zhejiang Province, China. The area has central-subtropical climate with an average annual temperatures of 15.8 °C and, an average annual rainfalls of 1420 mm. The average annual sunshine hours and days free of frost in the region were 1774 h and 236 days, respectively. Red soils derived from siltstone are classified as Ferrisols under the U.S taxonomic classification system. The selected soil physical and chemical properties prior to the experiment were light loam, pH (soil:  $\rm H_2O=1:5)$  5.02; organic matter 44.4 g kg<sup>-1</sup>;

total N 1.71 g kg $^{-1}$ , total P 0.153 g kg $^{-1}$ ; hydrolyzed N 42.5 mg kg $^{-1}$ , available P 16.9 mg kg $^{-1}$ , available K 116 mg kg $^{-1}$ ; available Pb 0.62 mg kg $^{-1}$ , Cd 0.034 mg kg $^{-1}$ , Zn 4.44 mg kg $^{-1}$ , Cr 0.12 mg kg $^{-1}$  and Cu 1.42 mg kg $^{-1}$ .

Experimental Design: The experiment was al treatments were initiated in May 2008 on a 6-year-old bamboo stands. Treatments consisted of 6 fertilizer management practices treatments (Table 1). Treatments arranged in a randomized complete block design with three replications. The Experimental unit area was 15.0 m by 8.0 m. Fertilizer rate of the treatment of CF2.0 in this study were similar to one applied by the farmers.. Urea and compound fertilizer (N:  $P_2O_5$ :  $K_2O = 15:15:15$ ) were used as N, P and K resources, respectively. N content in 11.25 t of pig manure was the same as one in 1.88 t of rapeseed cake. Chemical fertilizers were applied in three splits of 35, 30 and 35% of designed amount chemical fertilizers applied on May 2, September 2 and December 4, 2008, respectively. Half of organic fertilizers were applied on May 2 and half on December 4, 2008, respectively. Winter mulching with 15 cm rice straw and 20 cm rice grain hulls was placed upon the soil surface on December 4, 2008.

Soil Sampling: Soil samples were collected from each treatment at 0-25 cm depth before the experiment commenced (May 1, 2008) and one year after fertilization (May 15, 2009). Twenty cores (4 cm diameter) per treatment were randomly taken and mixed together. Soil sample of each treatment was divided into two parts: one was passed through a 2 mm sieve and kept in the refrigerator at 4°C prior to analysis of water soluble organic carbon (WSOC), microbial organic carbon (MBC) and mineralizable carbon (MC) within 2 days and the left soil was air-dried at room temperature and crushed to pass a 2-mm sieve and mixed thoroughly for other analysis.

Table 1: Rates of chemical fertilizers and organic manures used in the different treatments

Treatment*	Chemical fertiliz	ter (kg ha <sup>-1</sup> )	Organic fertilizer (t ha <sup>-1</sup> )		
	 Urea	Compound fertilizer	Pig manure#	Rapeseed cake#	
CK (no fertilizer)	0	0	0	0	
CF1.5	1460	2000	0	0	
CF2.0	1952	3000	0	0	
CF0.5PM1.0	488	750	5.625	0	
CF1.0RC1.0	976	1500	0	1.88	
CF1.0PM2. 0	976	1500	11.25	0	

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake;

#Ingredients of pig manure: H<sub>2</sub>O 735 g kg<sup>-1</sup>, C 142 g kg<sup>-1</sup>, N 5.98 g kg<sup>-1</sup>, P 0.91 g kg<sup>-1</sup>, K 5.2 g kg<sup>-1</sup>;

Ingredients of rapeseed cake: C 441 g kg<sup>-1</sup>, N 35.9 g kg<sup>-1</sup>, P10.5 g kg<sup>-1</sup>, K 11.7 g kg<sup>-1</sup>.

Chemical and Biological Analyses: Soil MBC was estimated using the fumigation- extraction method [11]. The C concentrations of K<sub>2</sub>SO<sub>4</sub>-extracted solutions for the chloroform-treated and untreated soils were measured using an automated TOC Analyzer (Shimazu, TOC-Vcph, Japan). Soil MBC was calculated by subtracting K<sub>2</sub>SO<sub>4</sub>-extracted C of untreated soils from that of the chloroform-treated soil and calibrated using the extraction efficiency factor (Kc) of 0.38. Soil WSOC was extracted from 10 g moist soil with addition of 20 mL distilled water. The mixture was shaken for 30 min with 250 rpm and centrifuged for 10 min at 15,000 rpm. The supernatant liquid was filtered through 0.45 µm filterable membrane. The filtrate was stored at -18 °C until analysis.

Organic C in the extracted solutions was determined by an automated TOC Analyzer. Soil total organic C (TOC) was measured by means of wet combustion method with 133 mmol  $L^{-1}$   $K_2$ Cr $_2$ O $_7$  at 170–180 °C and total N was measured by the semi-micro- Kjeldahl method [12]. Soil HN was hydrolyzed using 0.1 mol  $L^{-1}$  NaOH; soil AP and AK were determined by Bray-1 method and the NH $_4$ OAc extract-flame photometric method, respectively; and soil pH (soil:  $H_2$ O = 1:5) was measured using a pH meter; soil available Cd, Pb, Cr, Cu and Zn were extracted with 0.1 mol  $L^{-1}$  HCl and determined by CP [12].

Data were statistically analyzed by using analysis of variance (ANOVA) and the treatment differences were adjudged by Duncan's Multiple Range Test.

#### RESULTS AND DISCUSSION

Soil Total N and P, Hhydrolyzed N and Available P: Total nitrogen (TN) and total phosphorus (TP), hydrolyzed N (HN) and available P (AP) in the soil were measured to assess treatment effect on the accumulation and availability of N and P in the soil (Table 2). Combined application of chemical fertilizer (CF) and organic manure (OM) significantly increased TN and TP contents as compared with CK (P<0.05), while application of CF alone significantly increased TP contents, but the insignificant variation in soil TN contents between CF and CK (no fertilizers) plots was found.

Marked higher contents of TN and TP, HN and AP in the soil were found in the CF1.0PFM2.0 and CF1.0RC1.0 plots than in the CF1.5 plots although the total input amounts of N and P in the fertilizers were less in the formers than in the latter (Table 3). This was attributed to the retention of N and P by the organic matter produced from pig manure and rapeseed. The addition of certain organic fertilizers causes a net immobilization of soil

nitrogen [13], which would maintain soil quality and productivity and minimize the negative effects of overapplication of CF on the environment.

Application of CF alone significantly increased the contents of HN and AP in the soil as compared with CK (P<0.05). The AP contents in the soil were much higher (p<0.05) in the CF2.0 plots than in the CF1.5 plots, but the insignificant variation in HN was observed, which suggesting that loss amount of N from the soil was much greater than that of P.

Application of CF or CF + OM increased HN and AP contents by 2.09-10.56 and 16.46-18.58 times, respectively, as compared with CK. The reason for this is that the ratios of surplus amounts/output amount of N and P for the PP stand were 1.21 –2.70 and 3.55–13.44, respectively [14]. Such high HN and AP contents have approached or exceeded those in protected vegetable land applied by over-application CF [15].

Rapid accumulation of the nutrients in the soils has resulted in the pollution of water system around PP stands [16]. The determination results showed that the concentrations of TN, NO<sub>3</sub>-N and TP in the river water around PP stands with intensive management were 3.13, 5.07 and 2.39 times greater than those around natural forests, respectively [16]. When the concentrations of TN and NH<sub>4</sub>\*-N were used as the index of water quality, the polluted water is classed as ? kind water [17].

Soil Organic Carbon: Total organic carbon (TOC) in the soils ranged from 19.34 g kg<sup>-1</sup> in the CF1.5 to 24.21 g kg<sup>-1</sup> in the CF1.0PFM2.0 and insignificant variation within the treatments of CF and CK was observed (Table 2). Combined application of CF and OM significantly increased TOC contents as compared with CK and CF (P<0.05). Although the amount of organic C applied in the CF1.0RC1.0 was almost double than in the CF1.0PFM2.0 (Table 3), TOC content in the former were slightly greater than that in the latter, which might be due to faster decomposition rate of organic C in rapeseed cake. The highest TOC in the treatments applied with OM could be due to the supplies of large amounts of available C, resulting in a more diverse and dynamic microbial system than inorganically fertilized soil (Peacock et al. 2001). In contrast, application of CF alone was deleterious to soil quality because of depletion of organic matter, the reservoir of plant available N and P in weathered, tropical soils [18]. The nutrient turnover and long-term productivity of the soil were enhanced by using organic amendments along with inorganic fertilizers [19]). This finding was supported by the results of Meng et al. [14]

Table 2: Contents of total N and P and available N and P in the soil under the bamboo stands one year after fertilization

Treatment*	Total N (g kg <sup>-1</sup> )	Total P (g kg <sup>-1</sup> )	Hydrolyzed N (mg $kg^{-1}$ )	Available P (mg kg <sup>-1</sup> )
CK (no fertilizer)	1.28b	0.150c	21.4d	13.2c
CF1.5	1.35b	0.33 <i>6</i> b	66.1c	230.4b
CF2.0	1.49ab	0.353a	73.5c	285.0a
CF0.5PM1.0	1.55a	0.330b	202.4b	237.7b
CF1.0RC1.0	1.64a	0.362a	234.3a	258.3a
CF1.0PM2. 0	1.60a	0.356a	247.4a	256.0a

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake.

Table 3: Input amounts of C, N, P and K in different treatments through chemical fertilizer and organic manure

	Chemica	al fertilizer (	kg ha <sup>-1</sup> )		Organic fertilizer (kg ha <sup>-1</sup> )			Total (kg ha <sup>-1</sup> )			
Treatment*	N	P	K	С	N	P	K	C	N	P	K
CK (no fertilizer)	0	0	0	0	0	0	0	0	0	0	0
CF1.5	898	131	249	0	0	0	0	0	898	131	249
CF2.0	1350	198	395	0	0	0	0	0	1350	198	395
CF0.5PM1.0	338	49	94	2110	9	1	8	2110	347	50	102
CF1.0RC1.0	675	99	188	4220	18	2	16	4220	693	101	204
CF1.0PM2. 0	675	99	188	8290	30	8	10	8290	705	107	198

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake.

Table 4: Effects of fertilization on total organic carbon (TOC), microbial organic carbon (MBC), water-soluble organic carbon (WSOC) and mineralizable carbon (MC) in the soil

Treatment*	TOC (g kg <sup>-1</sup> )	WSOC (mg kg <sup>-1</sup> )	MBC (mg kg <sup>-1</sup> )	MC (mg kg <sup>-1</sup> )
CK (no fertilizer)	20.85c#	38.5b	306.1ab	12.1c
CF1.5	19.34c	33.7c	222.9c	18.0b
CF2.0	20.45c	29.7c	189.9c	18.8b
CF0.5PM1.0	22.93 ab	51.6ab	321.4ab	27.3a
CF1.0RC1.0	24.21a	58.7a	465.3a	31.3a
CF1.0PM2. 0	23.81a	58.8a	478.8a	28.17a

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake;

who found that combined application CF and PM (CF1.0+PM1.0) increased internal nutrient use efficiency of N, P and K by 30.2, 31.2 and 95.0%, respectively and improved bamboo shoot yield by 2.16 times as compared with CF1.0.

Labile Organic Carbon: Soil MBC generally comprises 1-4% of TOC [20]. The results in Table 4 showed that fertilization had a great effect on TOC, MBC, WSOC and MC in the soils under PP stand. Compared with the CK, soil MBC contents significantly increased with combined application of CF and OM except for CF0. 5PM1.0 plots and significantly decreased with CF application alone. Our results are in agreement with other researchers who

reported that manure fertilization usually increased soluble organic C or increased carbon input to the soil [21,22], while inorganic fertilizer reduced soil MBC [23,24]. The decrease in soil MBC induced by application of CF might be due to high level of mineral N availability, high mineralization rate of soil organic C and changes in substrate quality and root growth [25], whereas the increase in soil MBC induced by applying CF+OM was attributed to more energy for soil microbes and higher level microbial biomass. A higher MBC content was found in the high OM rate plots than in low OM rate plots, whereas a lower MBC content was found in the high CF rate plots than in low CF rate plots. However, the differences in soil MBC contents resulting from fertilizer

<sup>#</sup> Means followed by the same letter are not significantly different from each other at p<0.05.

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rates did not approach significance at 5 % level of probability. Significant higher contents of soil MBC were found in the treatments of CF +OM than in the treatments of CF alone, which indicating that the formers could improve soil microbial activity. Under the condition of the same CF rate, the soil MBC in the CF1.0PM2.0 plots was significantly greater than in CF1.0RC1.0 plots, indicating PM had a higher capacity to increase soil microbial activity.

As shown in Table 3, the soil WSOC contents in the OM plots were higher (P < 0.05) than those in CF plots, which was in agreement with the results of McGill et al. [26]. Soil WSOC contents in the CF1.0RC1.0 and CF1.0PM2.0 plots were higher (P < 0.05) than in the CF0.5PM1.0 plots, which were attributed to greater amounts of organic carbon. Application of CF resulted in a significant decline in soil WSOC content, but there was no difference in soil WSOC contents between CF treatments. Decreases in soil WSOC induced by application of CF might be due to the increase of substrate obtained from CF The results of this study corroborated the findings of Liang et al. [22] and McGill et al. [26]. Long term-application of CF could result in a great decline in soil WSOC contents [27,28]. Chantigny et al. [29] also reported that soil WSOC contents decreased with the increasing CF rates.

The contents of MC in the soils with OM was markedly higher than with CF. No significant differences in soil MC contents were observed between different fertilizer rates and kinds. The results of this study indicated that fertilization greatly influenced the size and activity of labile C pool in soil organic matter, which are consistent with other studies [30,27,8].

Ratios of soil labile carbon to soil total organic carbon: The MBC/TOC ratios are useful measures to monitor soil organic matter and provide a more sensitive index than TOC measured alone [20]. The MBC/TOC ratios has been used as an indicator for C availability [31]. Compared to CK, combined application of CF and OM

increased WBC/TOC ratios by 30.9-37.0% except for CF0.5FM1.0, while application of CF decreased WBC/TOC ratios by 21.5-36.6% (Table 5). The results obtained here were in agreement with those observed by Jiang and Xu [8], Jiang *et al.* [27] and Liu *et al.*[32], The ratio of WBC/TOC in the CF1.0FM2.0 plots was 42.1% greater than that in the CF0.5PM1.0 plots, which was due to higher OM rate in the former than in the latter.

Mean ratios of WBC/TOC in paddy field, woodland, upland and orchard were 3.01, 1.57, 1.29 and 1.14%, respectively [32]. The results of this study showed that mean ratios of WBC/TOC in the treatments of CF and CF+OM were 1.041% and 1.778%, respectively, which were close to the finding obtained from woodland [32]. Shen and Cao [33] reported that the MBC/TOC ratios in the rice field and red soil were 3.36-4.92% and 2.51-4.28%, respectively, which were much greater than those obtained from the present study.

Combined application of CF and OM significantly increased ratios of WSOC/TOC compared to the CK. However, no significant differences were observed between the treatments of CF and CK. Application of CF or/ and OM significantly increased ratios of MC/TOC compared to the CK, but the increase in the treatments applied OM was greater than those applied CF alone (Table 5).

Relationships between soil carbon fractions as well as between soil Carbon fractions and soil nutrients: It can be seen from Table 6 that significant positive correlations were observed between soil carbon fractions. Total N or hydrolyzed N was highly correlated with soil carbon fractions except for between total N and WSOC. Available P was highly correlated with TOC and MC, but was not correlated with WSOC and MBC. The above results are consistent with earlier data (Jiang *et al.*, 2002), which suggesting that TOC, WSOC, MBC and MC in the soil are important indexes for evaluating soil quality because they plays a role in maintaining soil nutrients and biological fertility [20].

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Treatment*	MBC/TOC (%)	WSOC/TOC (%)	MC/TOC (%)
CK (no fertilizer)	1.468b#	0.1846b	0.0580c
CF1.5	1.153c	0.1742b	0.0931b
CF2.0	0.930c	0.1423b	0.0919b
CF0.5PM1.0	1.402b	0.2250a	0.1190a
CF1.0RC1.0	1.922a	0.2425a	0.1293a
CF1.0PM2. 0	2.011a	0.2470a	0.1183a

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake;

<sup>#</sup> Means followed by the same letter are not significantly different from each other at p<0.05.

Table 6: Relationships between different soil carbon fractions and between soil carbon fractions and soil nutrients

	TOC	WSOC	MBC	MC	Total N	HN#	AP#	AK#
TOC	1	0.598**	0.473*	0.667**	0.789**	0.734**	0.470*	0.238
WSOC		1	0.522*	0.471*	0.315	0.548*	0.237	-0.015
MBC			1	0.701**	0.667**	0.490*	0.313	0.218
MC				1	0.519*	0.818**	0.495*	0.038

<sup>\*</sup> p< 0.05 and \*\* p< 0.01

Table 7: Effects of fertilization on the contents of available heavy metal in the soil

Treatment*	Pb (mg kg <sup>-1</sup> )	Cd(mg kg <sup>-1</sup> )	Zn(mg kg <sup>-1</sup> )	Cr(mg kg <sup>-1</sup> )	Cu(mg kg <sup>-1</sup> )
CK (no fertilizer)	0.62b#	0.031c	4.22c	0.10b	1.39b
CF1.5	1.03a	0.044ab	5.14b	0.15a	1.66a
CF2.0	1.18a	0.045ab	6.41a	0.16a	1.80a
CF0.5PM1.0	0.68b	0.046a	6.75a	0.10b	1.20b
CF1.0RC1.0	0.86b	0.056a	7. <b>54a</b>	0.13b	1.36b
CF1.0PM2. 0	0.77b	0.048a	6.88a	0.13b	1.31b

<sup>\*</sup>CF= chemical fertilizers; PM=pig manure; RC=rapeseed cake;

The good relationships between soil nitrogen and soil labile carbon indicated that organic carbon level and microbial activity dominated soil nitrogen level, but less related to available P and K supply.

Available heavy metals: Application of CF alone significantly increased the contents of Pb, Cd, Zn, Cr and Cu by 66.1-87.1, 41.9-45.2, 21.8-52.0, 50.0-60.0 and 19.4-29.5%, respectively, as compared with CK (Table 7). This is because of fact that marked decline in soil pH induced by over-application CF [15] caused significant increase in availability of heavy metals [34].

Combined application of CF significantly increased the contents of available Cd and Zn because significant increase in soil WSOC contents (Table 3), but had little effect on the contents of available Pb, Cr and Cu as compared with CK (Table 7). However, the contents of available Pb, Cr and Cu were much higher in the CF+OM plots than in the CF plots. These results were similar to that of Walker et al. [35] who found that manure application greatly reduced available contents of Cu, Zn and Mn in the soil. This effect appeared to be related to a decline of soil pH. For the soil with high CF rate, liable to acidification, manure application appears to be able to inhibit soil acidification [35] and to enhance complication of humus formed from OM with heavy metal.

There was clear difference in available contents of heavy metals between different rates of CF or OM except for Zn in the F1.5 and CF2.0 plots (Table 7).

#### CONCLUSIONS

Degradation in soil quality resulting from overapplication of CF was characterized by the decreases in the contents of SOC, MBC, WSOC and MBC/TOC ratio, by rapid and excessive accumulation of available N, P and K in the soils and by the increases in available contents of Pb, Cd, Zn, Cr and Cu in the soil. Based on our study results, the following conclusions were made:

- Application of CF alone could resulted in a greater decrease in soil quality, while combined application of CF and OM could counteracted or eliminated this adverse effect.
- Application of CF alone also resulted in too high contents of HN and AP, which may induce the pollution of water system around bamboo stands.
- The study of correlation studies between soil nutrients and soil labile carbon that TOC, MBC and MC in the soil were better indexes for evaluating soil quality.
- Application of CF alone significantly increased the contents of heavy metals studied, whereas combined application of CF and OM significantly reduced the contents of available Pb, Cr and Cu in the soils as compared with CF.
- Based on this study results, CF rate should be reduced and combined application of CF and OM should be advocated in fertilization management of PP stands.

<sup>#</sup> HN = hydrolyzed N; AP = available P; AK = available K

<sup>#</sup> Means followed by the same letter are not significantly different from each other at p<0.05.

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