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Effect of fly ash amendment on sandy soil properties and peanut yields

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ABSTRACT: Fly ash is a solid residual by-product of coal combustion in thermal power plants and considered a problematic solid waste. To justify the application of fly ash in agriculture, the Pha Lai (Vietnam) thermal power plant-derived fly ash (FA) was amended, along with farmyard manure (FYM) and NPK chemical fertilizer (NPK), to sandy soil in Quang Binh (Vietnam) for peanut (*Arachis hypogaea* L.) cultivation. The effect of FA amendment was investigated, based on the changes in soil properties and peanut yields. The results revealed that the FA amendment had positive benefits to soil properties and peanut yields. Especially, the amendment of 5% FA in combination with FYM and NPK increased notably the proportion of silt-sized particles, surface charges, pH, electrical conductivity, cation exchange capacity, contents of Ca^{2+} and Mg^{2+}, contents of total and available P and K, and hydraulic conductivity. Microbial populations, enzyme activities, and bacterial community diversity were considerably improved. More dominant species, viz. *Paraburkholderia sacchari*, *Rheinheimera tangshanensis*, and *Betaproteobacteria bacterium*, were observed. Moreover, the FA increased dry peanut grain yield from 0.19 to 2.3 t/ha (12.1-fold). It is recommended to utilize 5% FA along with FYM and NPK in peanut production for economically valuable and environmentally friendly disposal.

KEYWORDS: fly ash, soil amendment, sandy soil properties, soil microbial activities, peanut

INTRODUCTION

Fly ash is a coal combustion residue of thermal power plants and considered a problematic solid waste. The utilization of coal to meet progressively energy needs results in an exponential increase in the production of massive amounts of fly ash to approximately 750 Mt in 2015. Contrarily, the global average utilization of fly ash is only 25%; the rest is landfilled and surface impounded, with potential risks of air pollution and water contamination due to leaching. Meanwhile, when compared with conventional P fertilizers, the cost of fly ash (1.5% P) (2926 $AUS) is more economical than that of single superphosphate fertilizers (9% P) (8800 $AUS) in supplying P to a 100 ha farm in Australia. Also, an economic analysis similar to that of P can be used to determine the value of fly ash as a liming agent for ameliorating soil acidity. It showed that Class C fly ash (43% calcium carbonate equivalent (CCE)) may be an economical resource for raising the soil pH when compared with agricultural limes (80–100% CCE) [1].

Quang Binh is a province in the North Central Coast of Vietnam with at least 6000 ha of unutilized sandy soil. However, the coastal sandy soil has very specific properties, such as low natural fertility, acidity, low content of organic matters, and poor in humus, due to the intensive mineralization process, high proportion of coarse particles, discrete structure, and low absorption capacity, resulting in the limited water and fertilizer holding capacities. Meanwhile, it proves that: (i) the peanut grows best in a well-drained, slightly acidic soils with a pH of 6.0 to 6.5, and can fix atmospheric N_2 with the aid of root nodule bacteria [2]; (ii) the fly ash may act as a liming agent to neutralize soil acidity and provide plant-available nutrients [3]; (iii) the amendment with fly ash can improve sandy soil properties [4]; (iv) the co-application of fly ash with inorganic and organic amendments has many advantages, i.e. improving environments for water, air and nutrient interactions in soil, enhancing nutrient availability, decrease in bioavailability of toxic metals, pH buffering, organic matter (OM) addition, microbial stimulation, overall improvement in the

general health of the soil, and increase in peanut yields and quality [5]; and (v) the potential release of trace elements from fly ash into soil environment is dependent on the composition of the coal used in combustion, combustion conditions, methods of disposal, and climatic conditions [6].

This study aimed to investigate the effect of FA on the physical, chemical and biological properties of sandy soil and yields of peanut (*Arachis hypogaea* L.) in Quang Binh Province. The successful value-added application would help to reduce cost and environmental concern of FA disposal and create a possible solution to improve sandy soil properties and increase crop yields.

MATERIALS AND METHODS

Collection and properties of FA

FA was collected from a 3-year old covered dumping site (weathered) in Pha Lai thermal power plant. The parental coal source of FA was Quang Ninh (Vietnam) anthracite. FA was derived from pulverized fuel coal combustion and captured by the emission control device. Properties of FA were previously described [7]. Briefly, the proportion of silt-sized particles was 75.88%. FA had low bulk density and high specific surface area. pH in 1:5 (v/w) 1 N KCl was 9.7. Extractable P, K, Ca^{2+}, and Mg^{2+} were 112.4, 397.9, 5.26, and 0.88 mg/kg, respectively. The major oxide components were SiO_2, Al_2O_3, K_2O, and Fe_2O_3. The effective specific activities of ^{226}Ra, ^{238}U, ^{232}Th, and ^{40}K were lower than the limit for building materials. The concentrations of heavy metals in the toxicity characteristic leaching procedure (TCLP) extracts from FA were lower than the soluble threshold limit concentrations (STLC) set by the USEPA (1992) [8].

Experimental treatments

The soil is sandy soil, cleared of native vegetation 2 years before the plots being planted. The treatments were: (i) control (100% sandy soil); (ii) 5% FA; (iii) 10% FA; (iv) FYM; (v) 5% FA + FYM; (vi) 10% FA + FYM; (vii) NPK; (viii) 5% FA + NPK, (ix) 10% FA + NPK; (x) FYM + NPK; (xi) 5% FA + FYM + NPK; and (xii) 10% FA + FYM + NPK. The experimental dose of FA was 80 t/ha (5% w/w) or 160 t/ha (10% w/w), and FYM (0.5% N, 0.4% P_2O_5, 0.5% K_2O, 0.2% CaO, and 0.1% MgO) was 8 t/ha. The recommended dose of NPK fertilizer (40 N + 90 P_2O_5 + 60 K_2O kg/ha) was added to the treatments through urea $CO(NH_2)_2$ (46.3% N), Lam Thao superphosphate $Ca(H_2PO_4)_2$ (16.5% P_2O_5) and KCl (61% K_2O). FA was incorporated into the top 15 cm of a series of 18 m^2 individual plots arranged in a completely randomized design. Three replicates of each treatment were established and planted with peanut for 3 months. Composite surface soil samples of 5 sub-samples were collected from the rhizosphere layer (0–20 cm) of each plot at the end of the planting period. The samples were placed in plastic bags and brought to the laboratory where they were sieved (2-mm mesh size), homogenized, and stored at 4 °C.

Analysis of soil physical and chemical properties

Particle size distribution was determined by PIPET method [9]. Bulk density was measured as suggested [10]. Surface charges were measured with a particle charge detector (Mütek PCD-05, Germany). Hydraulic conductivity was measured by the constant head test method [11]. The pH of 1 N KCl after being mixed with sandy soil (1:5 w/v) and EC of the extract in deionized water (1:5 w/v) were measured with a pH meter and an EC meter, respectively. CEC was determined by the ammonium acetate method [12]. Contents of exchangeable Ca^{2+} and Mg^{2+} were determined with the titration of Ca^{2+}, Mg^{2+} by Trilon B titrant [13]. Organic carbon (OC) and total C were determined after dry combustion [14]. Total N was determined by the modified Kjeldahl method [14] and available N by distillation method [15]. Available P was determined by the Bray and Kurtz method [16]. Available K was measured with flame atomic absorption spectroscopy [16]. TCLP was used to determine the concentrations of heavy metals that could leach from soil [8].

Measurement of microbial populations and extracellular enzyme activity

Nutrient Agar, Potato Dextrose Agar, and Starch Casein Agar were selected for counting total aerobic bacteria, fungi, and actinomycetes, respectively. Modified Hans Agar and Ashby's Mannitol Agar were selected for cellulolytic microorganisms and nitrogen-fixing bacteria, respectively. Extracellular enzyme activity was assessed using API ZYM kit (bioMerieux) [17]. Soil extract was prepared by mixing 5 g soil with 7.5 ml distilled water. The mixture was blended for 10 min, allowed to settle for 10 min, and then centrifuged at 2000 g for 8 min. The colour reactions were read after 5 min, and a numerical value ranging from 0 to 5 was assigned [18].

Analysis of soil bacterial community

Total DNA was extracted from 0.25 g soil with Powersoil DNA Isolation Kit. Variable V3 regions of 16S rDNA gene were amplified by PCR using primers P2 and P3 [19] with AmpliTaq Gold, 10 × PCR buffer and dNTP AmpliTaq Gold kit. A touchdown protocol for PCR was employed [20]. Subsequently, the amplicons were analyzed with a Dcode™ DGGE system operated at 60 °C for 12 h at 100 V in a linear 25% to 65% denaturant agent gradient with 8% polyacrylamide gel and DGGE Marker I (Nippon Gene, Japan). DGGE gel was, then, soaked for 30 min in SYBR Gold Nucleic Acid Gel Stain. Band intensity was measured by a Gel Doc XR$^+$ and analyzed with the Image Lab software (Bio-Rad, Laboratories, CA, USA). Each band represents one population (species) within the community and its relative intensity represents the relative abundance of a particular species in the population [19]. Shannon-Weiner diversity index (H′), Simpson index of dominance (D), range-weighted richness (Rr), and species richness (S) were used to evaluate the bacterial diversity and calculated using the following equations: $H' = -\sum_{i=1}^{s} Pi \ln(Pi)$ [21]; $D = \sum_{i=1}^{s} Pi^2$ [21]; $Rr = S^2 \times D_g$ [22], where $Pi = ni/N$ is the relative intensity of each band; ni, the height of the peak; N, the sum of all peak heights in the curve; S, the total number of bands in each lane; and D_g, denaturing gradient of the gel (in %) comprised between the first and last bands of the lane. H′ is based on measuring uncertainty. The uncertainty degree of predicting the species of a random sample is related to the diversity of a community. If a community has low diversity (dominated by one species), the uncertainty of prediction is low; a randomly sampled species is most likely going to be the dominant species. However, if diversity is high, uncertainty is high. Whereas, D is a weighted arithmetic mean of proportional abundance and measures the probability that two individuals randomly selected from a sample will belong to the same species. Since the mean of the proportional abundance of the species increases with decreasing number of species and increasing abundance of the most abundant species, D obtains small values in data sets with high diversity and large values in data sets with low diversity. The value of D ranges from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity; so the larger the value of D, the lower the diversity. Rr describes a carrying capacity of an environment containing wide species GC variability. The value of Rr < 10 is characterized by a low Rr, values intermediate (10–30) can be correlated with a medium Rr, while an Rr > 30 is characterized by a high microbial diversity with a high Rr [22]. S does not take into account the number of individuals of each species present.

Dominant DGGE bands were excised from the gel; then, their DNA samples were extracted with 100 µl TE buffer and purified by ethanol precipitation. The purified DNA was amplified using the primer pair 341f and 534r [19]. The 16S rDNA V3 region sequences were analyzed with an ABI PRISM 3100 xl Genetic Analyzer System using a BigDye Terminator v3.1 Cycle Sequencing Kit, DGGE band sequencing kit (TechnoSuruga Laboratory, Japan), and ChromasPro 1.7 software. The phylogenetic identity was determined by comparing the partial 16S rDNA gene sequences with sequences in GenBank using the BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The statistical significance differences between replicated samples were determined by Duncan's test (SPSS 17.0). Differences between means were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Soil physical properties

Soil amendment has been a topic of interest in agriculture using various treatments including addition of inorganic [23] and organic materials from industrial or agricultural wastes [24]. In this study, the amendment of FA alone into sandy soil increased significantly the proportion of silt-sized particles from 6.92% to 16.15% in 10% FA. Surface charge increased from 0.004 mol/kg in the control to 0.13 mol/kg in 10% FA + FYM and 5% FA + FYM + NPK and 0.14 mol/kg in 10% FA + FYM + NPK. However, there was no significant difference in bulk density between the control and other treatments. Hydraulic conductivity decreased from 158.8 mm/h in the control to 7.8 mm/h in 5% FA + FYM + NPK and 5.6 mm/h in 10% FA + FYM + NPK. FA amended at sufficient rates increased water holding capacity, which in turn improved hydraulic conductivity.

Soil chemical properties

As shown in Table 1, soil pH increased slightly in treatments amended with FA alone compared with the control. The highest pH was recorded in 10%

Table 1 Effects of different treatments on some soil chemical properties.

Treatment	pH 1:5 KCl	EC (dS/m)	CEC (cmol/kg)	Ca^{2+} (mg/kg)	Mg^{2+} (mg/kg)
Control	4.8	0.04	3.75	0.15	0.10
5% FA	4.9	0.08	4.31	0.40	0.15
10% FA	5.0	0.10	4.85	0.68	0.19
FYM	4.9	0.05	4.05	0.22	0.12
5% FA + FYM	5.1	0.11	5.28	0.48	0.16
10% FA + FYM	5.3	0.13	5.82	0.75	0.21
NPK	4.7	0.06	4.05	0.66	0.14
5% FA + NPK	4.8	0.12	4.78	0.92	0.18
10% FA + NPK	4.9	0.14	4.98	1.19	0.23
FYM + NPK	4.9	0.11	4.12	1.12	0.18
5% FA + FYM + NPK	4.9	0.12	5.32	1.38	0.41
10% FA + FYM + NPK	5.0	0.14	5.88	1.58	0.55

Table 2 Effect of different treatments on some soil chemical properties.

Treatment	OC (%)	Total N (%)	Total P (%)	Total K (%)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Control	0.650	0.12	0.018	0.174	1.13	11.10	12.80
5% FA	0.673	0.13	0.020	0.192	1.17	16.81	32.70
10% FA	0.684	0.13	0.022	0.206	0.85	22.36	50.59
FYM	0.742	0.15	0.021	0.219	1.16	20.08	13.21
5% FA + FYM	0.766	0.16	0.025	0.228	1.28	39.98	33.11
10% FA + FYM	0.789	0.17	0.026	0.248	1.02	59.87	52.89
NPK	0.603	0.09	0.022	0.382	1.27	25.106	16.92
5% FA + NPK	0.621	0.10	0.025	0.421	1.41	45.001	36.82
10% FA + NPK	0.609	0.11	0.052	0.456	1.11	64.896	55.71
FYM + NPK	0.911	0.17	0.035	0.651	1.97	47.810	17.12
5% FA + FYM + NPK	0.951	0.18	0.038	0.721	1.69	67.705	37.02
10% FA + FYM + NPK	1.021	0.18	0.046	0.803	1.26	87.600	56.89

Table 3 Effect of treatments on heavy metal concentrations in TCLP extracts (mg/l).

Treatment	As	Cd	Cu	Pb	Zn
Control	0.080	0.001	0.055	<0.002	1.84
5% FA	0.245	0.003	0.618	0.035	2.09
10% FA	0.320	0.004	0.700	0.049	2.32
FYM	0.145	0.002	0.186	0.032	1.92
5% FA + FYM	0.245	0.013	0.473	0.035	2.88
10% FA + FYM	0.245	0.015	0.538	0.044	2.89
NPK	0.163	0.003	0.453	0.012	1.73
5% FA + NPK	0.250	0.010	0.443	0.023	2.09
10% FA + NPK	0.320	0.008	0.430	0.027	2.37
FYM + NPK	0.238	0.001	0.370	0.012	2.07
5% FA + FYM + NPK	0.318	0.002	0.515	0.027	2.55
10% FA + FYM + NPK	0.320	0.003	0.535	0.042	2.63
STLC levels	5	1	25	5	250

FA + FYM. The increase in pH can be attributed to the alkaline nature of FA and the significant contents of alkaline metals in FA. Besides, the supplement of FYM along with FA may result in additional pH buffering capacity of Ca^{2+} [5]. Compared with the control, EC values in 5% FA and 10% FA treatments increased 2.0 and 2.5-fold, respectively. However, EC increased significantly in the combination of FA with NPK or with FYM and NPK. The highest EC was observed in 10% FA + NPK and 10% FA + FYM + NPK. It may be due to the high content of soluble salts in FA and deposition of additional salts in surface soil by FYM incorporation. Moreover, CEC increased in 5% and 10% FA treatments, and the highest CEC was found in 10% FA + FYM + NPK. The application of FYM increased the CEC by increasing soil OM and OC contents. Furthermore, the lowest contents of Ca^{2+} and Mg^{2+} were observed in the control, while the highest contents were observed in 10% FA + FYM + NPK.

The addition of FA in combination with FYM and NPK has significantly increased the OC (Table 2). Total N content was not significantly affected by the addition of FA. The lower contents of total and available N, P, and K were found in the control, while their higher contents were obtained in FA + FYM +

NPK. The increased contents of available N, P, and K may be connected to the considerable contents of P and K in FA and high contents of N, P, and K in FYM and NPK.

The concentrations of heavy metals increased in all treatments in comparison to the control (Table 3). It could be mainly attributed to their presence in FA. However, these concentrations in TCLP extracts from treatments were lower than the STLC level set by the USEPA (1992) [8].

Soil microbial populations

The microbial numbers were affected by different treatments (Table 4). The number of aerobic bacteria increased by 5% FA and decreased by 10% FA as compared with the control. The low levels of FA may have increased bacterial numbers because of the addition of nutrients. However, the numbers of fungi in 5% FA and 10% FA were lower than those in the control. This was probably due to alkaline FA containing a high content of CaO which raises soil pH and, thereby, inhibits alkali-intolerant fungal growth. Compared with the control, the actinomycetes numbers increased by 5% FA, though not significantly, and did not change in 10% FA. The numbers of cellulolytic bacteria in 5% and 10% FA were lower than those in the control. Besides, the addition of FYM resulted in a significant increase in microbial numbers. The FYM itself may have contained substantial numbers of microorganisms. Also, FYM may supply the macronutrients deficient in FA, improve soil conditions and serve to increase microbial numbers. In comparison to the control, heterotrophic aerobic bacterial, actinomycetes, fungal, and cellulolytic bacterial numbers in 5% FA + FYM + NPK increased about 5-, 8-, 7-, and 5-fold, respectively. However, the number of nitrogen-fixing bacteria decreased notably in 5% FA + FYM + NPK. This might be due to free-living N-fixing bacteria can potentially suppress N fixation when N is relatively abundant.

Soil extracellular enzyme activities

API ZYM strip with 19 enzymatic activities was used for evaluating enzyme activity in soil [25]. As shown in Table 5, phosphatase and protease activities were observed in all treatments. Despite higher levels of fly ash limited enzyme production [26], phosphatase activity in 10% FA alone treatment and the control was not different. FYM addition increased remarkably phosphatase activity, presumably due to the increases in microbial biomass and organic P. Thereby, it enhances the hydrolysis of organic P esters to orthophosphate, which can be metabolized by plant. The activity of proteases, that act on proteins and polypeptides, increased considerably with FYM application. In general, the highest enzyme activities were found in 5% and 10% FA + FYM + NPK.

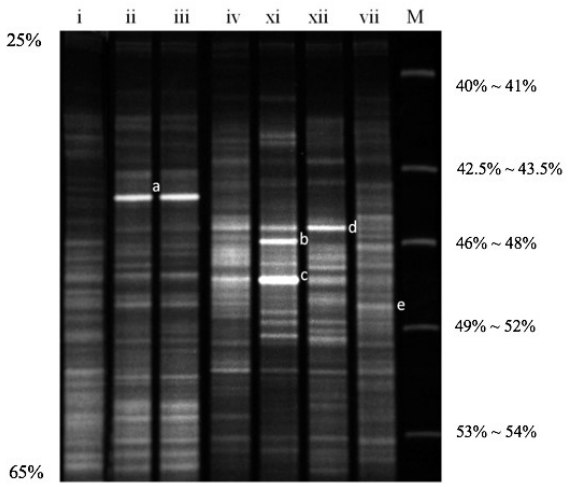

Fig. 1 DGGE band pattern of 16S rDNA gene fragments from treatments (i) control, (ii) 5% FA, (iii) 10% FA, (iv) FYM, (vii) NPK, (xi) 5% FA + FYM + NPK, and (xii) 10% FA + FYM + NPK. The bands represented by letters were excised and sequenced. M is DGGE Marker I (5 fragments).

Soil bacterial community diversity

The DGGE profile showed significant differences in the banding pattern of 12 treatments (Fig. 1). The lane with the lowest number of bands was found in the FA alone treatment, whereas the lane with the highest number and intensity of bands was found in the 5% FA + FYM + NPK. The presence of OM has an additive effect as it promotes microbial proliferation and diversity [27]. As shown in Table 6, H′ decreased by 5% and 10% FA treatments and exceeded in 5% FA + FYM + NPK compared with the control. D and H′ exhibited opposite trends, reflecting the fact that the two results were consistent. Also, Rr and S agreed with those of H′. Obviously, the most diverse bacterial community was obtained with 5% FA + FYM + NPK.

Soil dominant bacterial populations

Five dominant bands that could represent the DGGE profile of the treatments (Fig. 1) were excised from the lanes, reamplified, sequenced, and identified by

Table 4 Effect of different treatments on soil microbial populations.

Treatment	Aerobic bacteria ($\times 10^7$ CFU/g)	Fungi ($\times 10^6$ CFU/g)	Actinomycetes ($\times 10^4$ CFU/g)	Cellulolytic bacteria ($\times 10^2$ CFU/g)	Nitrogen-fixing bacteria ($\times 10^6$ CFU/g)
Control	0.59	0.50	0.43	0.68	1.21
5% FA	0.65	0.40	0.45	0.51	1.31
10% FA	0.45	0.35	0.43	0.46	1.25
FYM	2.61	2.65	2.27	3.01	1.60
5% FA + FYM	2.73	2.82	2.52	3.15	1.77
10% FA + FYM	2.47	2.94	2.62	2.85	1.85
NPK	1.27	1.21	0.45	1.46	0.78
5% FA + NPK	1.53	1.05	0.39	0.61	0.83
10% FA + NPK	1.45	0.85	0.32	0.58	0.67
FYM + NPK	2.85	3.86	2.72	3.29	0.52
5% FA + FYM + NPK	2.97	4.02	2.97	3.43	0.57
10% FA + FYM + NPK	2.59	3.95	3.07	2.99	0.58

Table 5 The relative activities of extracellular enzymes extracted from treatments.

Enzyme	Treatment											
	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Phosphatases												
Alkaline phosphatase	1	1	1	4	3	3	1	1	1	4	5	4
Acid phosphatase	1	1	1	3	3	3	1	1	1	4	3	3
Phosphohydrolase	1	1	1	4	3	2	1	1	1	3	3	3
Esterases												
Lipase	1	1	1	0	1	1	0	0	0	0	1	0
Esterase-lipase	1	1	1	1	1	1	0	0	1	1	1	0
Esterase	0	1	1	1	1	1	0	0	0	0	0	1
Aminopeptidases												
Leucine aminopeptidase	1	0	1	2	1	1	1	0	0	1	1	1
Valine aminopeptidase	0	0	0	1	1	1	0	0	2	1	1	1
Cystine aminopeptidase	0	0	0	1	1	1	0	0	0	1	1	1
Proteases												
Chymotrypsin	1	1	1	5	4	4	1	1	1	4	5	3
Trypsin	1	1	1	4	3	3	1	3	2	3	4	3
Glycosyl hydrolases												
α-galactosidase	1	0	1	1	0	1	0	0	0	0	0	0
β-glucosidase	1	1	1	0	0	1	1	0	0	0	1	0
β-galactosidase	0	0	0	0	1	0	0	0	0	0	0	1
β-glucuronidase	0	0	0	0	1	0	1	0	0	0	0	0
α-mannosidase, α-fucosidase	0	0	0	0	0	0	0	0	0	0	1	0

(i) control; (ii) 5% FA; (iii) 10% FA; (iv) FYM; (v) 5% FA + FYM; (vi) 10% FA + FYM; (vii) NPK; (viii) 5% FA + NPK, (ix) 10% FA + NPK; (x) FYM + NPK; (xi) 5% FA + FYM + NPK; and (xii) 10% FA + FYM + NPK. The results were reported as colour intensity of enzymatic reactions (0: no intensity; 1: low intensity; 2–3: moderate intensity; 4–5: high intensity). N-acetyl β-glucosaminidase and α-glucosidase are nil in all treatments.

BLAST with their 16S rDNA V3 region sequences. The 16S rDNA sequence corresponding to band "a" was 100% identical to the sequence of *Bacillus koreensis* TC4 (MF062974.1). A spore-forming bacterium of this species is isolated from the rhizosphere [28]. Band "b" was 99% identical to the sequence of *Rheinheimera tangshanensis* HMF2735 (KP099960.1). A gram-negative, aerobic, rod-shaped bacterium of this species is isolated from the roots of fresh rice plants (*Oryza sativa*) [29]. The plant growth-promoting rhizobacteria (PGPR) affect growth by increasing nutrient cycling and suppressing pathogens by producing bacterial and fungal antagonistic substances or biologically active

Table 6 Effects of different treatments on the diversity of soil bacterial communities.

Treatment	Diversity index			
	H′	D	Rr	S
Control	2.3254	0.1175	56.5	14
5% FA	2.0746	0.1715	42.0	13
10% FA	2.1080	0.1601	32.2	12
FYM	2.5885	0.1037	127.6	20
5% FA + FYM	2.4249	0.1189	93.1	18
10% FA + FYM	2.4153	0.1142	73.2	17
NPK	2.2515	0.1366	59.1	16
5% FA + NPK	2.3116	0.1246	58.6	15
10% FA + NPK	2.2710	0.1316	50.6	14
FYM + NPK	2.4806	0.1144	116.8	20
5% FA+FYM+NPK	2.6104	0.0978	140.0	21
10% FA+FYM+NPK	2.3273	0.1410	120.9	19

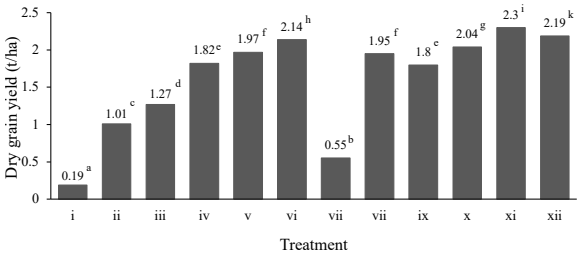

Fig. 2 Effects of different treatments (i) control; (ii) 5% FA; (iii) 10% FA; (iv) FYM; (v) 5% FA + FYM; (vi) 10% FA + FYM; (vii) NPK; (viii) 5% FA + NPK, (ix) 10% FA + NPK; (x) FYM + NPK; (xi) 5% FA + FYM + NPK; and (xii) 10% FA + FYM + NPK on peanut yields. Different letters next to the values indicate significantly different means (Duncan's test, $p < 0.05$).

substances [30]. Band "c" had a 100% sequence identical to that from *Betaproteobacteria bacterium* IMCC25669 (KY053193.1). *Betaproteobacteria* occurs in diverse environments with roles in maintaining soil pH and in elementary cycling [31]. The sequence from band "d" shared a low degree of identity (92%) to that from *Paraburkholderia sacchari* AQ5-11 (KX792232.1). *Burkholderia sacchari* LMG 19450, recently reclassified as *P. sacchari*, accumulates polyhydroxyalkanoates from carbohydrates under unbalanced growth conditions as a mechanism to store excess C and energy [32]. Analysis of 16S rDNA sequence of a N_2-fixing plant-associated bacterium showed 97.2âĂŁ% similarity to *B. sacchari* [33]. *Burkholderia* can effectively control soil diseases and improve solubilization of fixed soil P [34]. Finally, the sequence from band "e" shared a low degree of identity (93%) to that from *Sorangium cellulosum* 38 (KX572696.2). *S. cellulosum* is a saprophyte bacterial species deriving its nutrition from cellulose aerobically [35]. *Sorangium* produces 50% of all known metabolites including antifungal and antibacterial compounds [36]. Obviously, *B. koreensis* was dominant in treatments amended with 5% and 10% FA alone, while *P. sacchari* was dominant in treatments amended with 5% and 10% FA in combination with FYM. Besides, *R. tangshanensis* and *B. bacterium* were dominant in treatments amended with 5% FA + FYM + NPK. *S. cellulosum* was dominant only in treatment amended with NPK alone.

Yields of peanut

To produce 1.5–2.0 t/ha of grain yield, the peanut cultivated on coastal sandy soil in Vietnam requires 30–40 kg N, 60–90 kg P_2O_5, and 30–60 kg K_2O per ha. The Ca, K, P, and S are involved in the seed filling and oil synthesis and required in higher quantity (100 mg/kg Ca). Besides, FYM (5–15 t/ha) is also combined with NPK fertilizer to increase peanut grain yields [37]. In this study, a significant difference on the dry peanut grain yield ($p < 0.05$) was observed among all treatments and the control (Fig. 2). The highest grain yield with 5% FA + FYM + NPK showed an increase of 12.1-fold compared with the control. It might be due to the soil physicochemical properties in the 5% FA + FYM + NPK which provided an effective aeration for roots and nitrifying bacteria, suitable pH value for plant nutrient availability and *Bradyrhizobium* spp. growth, sufficient Ca^{2+} content for seed development, and proper OM content for soil water-holding capacity and plant nutrients. Besides, higher numbers of PGPR enhance nutrient cycling and activities of enzymes improve available N and P supply in peanut soil. Higher microbial diversity was favourable for positive plant-soil feedback. It is estimated that the average 100 grain mass was about 150 g, the 100 seed mass was 56 g, and the ratio of grain and seed was 72%. The seeds have pink coat, uniform sizes, and even spread. Fly ash amendment in sandy soil also increased wheat yield [38]. Thus, to get higher peanut yields, the amendment of FA along with FYM and NPK fertilizers is of prime significance.

CONCLUSION

This study has demonstrated positive benefits of amending FA on improving sandy soil properties and increasing peanut yields. Noteably, 5% FA can be used in combination with FYM and NPK to

get the best additional benefits. However, since there is a potential in harming the environment and human health, long-term studies of the impact of fly ash on soil health, heavy metal uptake, plant physiology and growth, crop quality, and continuous monitoring on the soil characteristics are essential before planning agriculture as a venue for fly ash utilization.

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REFERENCES

1. Yunusa IAM, Loganathan P, Nissanka SP, Manoharan V, Burchett MD, Skilbeck CG, Eamus D (2012) Application of coal fly ash in agriculture: A strategic perspective. *Crit Rev Environ Sci Technol* **42**, 559–600.
2. Weiss EA (1983) *Oilseed Crops*, Longman Inc., New York, USA.
3. Taylor EM, Schumann GE (1988) Flyash and lime amendment of acidic coal soil to aid revegetation. *J Environ Qual* **17**, 120–124.
4. Pandey VC, Singh N (2010) Impact of fly ash incorporation in soil systems. *Agric Ecosyst Environ* **136**, 16–27.
5. Ram LC, Masto RE (2014) Fly ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments. *Earth Sci Rev* **128**, 52–74.
6. Femández-Jiménez A, Palomo A (2005) Mid-infrared spectroscopic studies of alkali-activated fly ash structure. *Micropor Mesopor Mat* **86**, 207–214.
7. Le VT, Ngo TTC, Le TTH, Nguyen TT, Hiroyuki F (2019) Properties of fly ashes from thermal power stations in relation to use as soil amendments. *Sains Malays* **48**, 745–755.
8. USEPA (1992) *Method 1311: Toxicity Characteristic Leaching Procedure*, Publ. SW-846, EPA, Washington DC, USA.
9. Sheldrick BH, Wang C (1993) Particle size distribution. In: Carter MR (ed) *Soil Sampling and Methods of Analysis*, 1st edn, Canadian Society of Soil Science, Lewis Publishers, Boca Raton, FL, USA, pp 499–512.
10. Blake GR, Hartge KH (1986) Bulk density. In: Klute A (ed) *Methods of Soil Analysis, Part 1*, 2nd edn, Agron. Monogr. 9., ASA and SSSA, Madison, WI, pp 363–375.
11. Klute A, Dirksen C (1986) Hydraulic conductivity and diffusivity: Laboratory methods. In: Klute A (ed) *Methods of Soil Analysis, Part 1*, 2nd edn, Agron. Monogr. 9., ASA and SSSA, Madison, WI, pp 687–734.
12. MOSTE-Vietnam (2010) Standard 8568:2010 Soil Quality – Method for determination of cation exchange capacity (CEC) by ammonium acetate method.
13. MOSTE-Vietnam (1987) Standard 4406-87: Soil – Method for determination of total of exchange calcium and magnesium.
14. MOSTE-Vietnam (1995) Standard 6642:2000 (ISO 10694:1995)/6498:1999 (ISO 11261:1995): Soil Quality – Determination of organic and total carbon after dry combustion (elementary analysis)/Determination of total nitrogen- Modified Kjeldahl method.
15. MOSTE-Vietnam (2009) Standard 5255:2009: Soil Quality – Method for determination of bio-available nitrogen.
16. MOSTE-Vietnam (2011) Standard 8942:2011/ 8662:2011: Soil Quality – Method for determination of bio-available phosphorus/potassium.
17. Tiquia SM (2002) Evolution of extracellular enzyme activities during manure composting. *J Appl Microbiol* **92**, 764–775.
18. Martínez D, Molina MJ, Sánchez J, Moscatelli MC, Marinari S (2015) API ZYM assay to evaluate enzyme fingerprinting and microbial functional diversity in relation to soil processes. *Biol Fertil Soils* **52**, 77–89.
19. Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**, 695–700.
20. Nishijima M, Lindsay DJ, Hata J, Nakamura A, Kasai H, Ise Y, Fisher CR, Fujiwara Y, et al (2012) Association of thioautotrophic bacteria with deep-sea sponges. *Mar Biotechnol* **12**, 253–260.
21. Vivas A, Moreno B, Garcia-Rodriguez S, Benitez E (2009) Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. *Bioresour Technol* **100**, 1319–1326.
22. Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. *Environ Microbiol* **10**, 1571–1581.
23. Chaiyaraksa C, Tumtong M (2019) Acid soil amendment by zeolite, sepiolite and diatomite. *ScienceAsia* **45**, 253–259.
24. Chaiyaraksa C, Lokham N, Kuikrong R, Artsanapaiboon S (2020) Immobilization of cadmium in soil using magnetic biochar derived from *Eichhornia crassipes*. *ScienceAsia* **46**, 450–456.
25. Boluda R, Roca-Pérez L, Iranzo M, Gil C, Mormeneo S (2014) Determination of enzymatic activities using a miniaturized system as a rapid method to assess soil quality. *Eur J Soil Sci* **65**, 286–294.
26. Kirk J, Klironomos J, Lee H, Trevors JT (2005) The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum-contaminated soil. *Environ Pollut* **133**, 455–465.

27. Malik A, Thapliyal A (2009) Eco-friendly fly ash utilization: potential for land application. *Crit Rev Environ Sci Technol* **39**, 333–366.
28. Lim JM, Jeon CO, Lee JC, Ju YJ, Park DJ, Kim CJ (2006) *Bacillus koreensis* sp. nov., a spore-forming bacterium, isolated from the rhizosphere of willow roots in Korea. *Int J Syst Evol Microbiol* **56**, 59–63.
29. Zhang X, Sun L, Qiu F, McLean RJ, Jiang R, Song W (2008) *Rheinheimera tangshanensis* sp. nov., a rice root-associated bacterium. *Int J Syst Evol Microbiol* **58**, 2420–2424.
30. Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit Rev Microbiol* **30**, 205–240.
31. Slonczewski JL, Foster JW (2014) *Microbiology: An Evolving Science*, 3rd edn, W.W. Norton and Company, New York.
32. Oliveira-Filho ER, Silva JGP, de Macedo MA, Taciro MK, Gomez JGC, Silva LF (2020) Investigating nutrient limitation role on improvement of growth and poly (3-hydroxybutyrate) accumulation by *Burkholderia sacchari* LMG19450 from xylose as the sole carbon. *Front Bioeng Biotechnol* **7**, ID 416.
33. Reis VM, Estrada-de los Santos P, Tenorio-Salgado S, Vogel J, Stoffels M, Guyon S, Mavingui P, Baldani VL, et al (2004) *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *Int J Syst Evol Microbiol* **54**, 2155–2162.
34. Li Q, Chen J, Wu L, Luo X, Li N, Arafat Y, Lin S, Lin W (2018) Belowground interactions impact the soil bacterial community, soil fertility, and crop yield in maize/peanut intercropping systems. *Int J Mol Sci* **19**, ID 622.
35. Pradella S, Hans A, Spröer C, Reichenbach H, Gerth K, Beyer S (2002) Characterisation, genome size and genetic manipulation of the myxobacterium *Sorangium cellulosum* So ce56. *Arch Microbiol* **178**, 484–492.
36. Schneiker S, et al (2007) Complete genome sequence of the myxobacterium *Sorangium cellulosum*. *Nat Biotechnol* **25**, 1281–1289.
37. Ho KM (2007) Research on the technical solution to increase the yield and efficiency of peanut production (*Arachis hypogaea* L.) on the coastal sandy soil of Quang Binh province. PhD thesis, Univ of Agriculture and Forestry, Hue Univ, Vietnam.
38. Dhindsa HS, Sharma RD, Kumar R (2016) Role of fly ash in improving soil physical properties and yield of wheat (*Triticum aestivum*). *Agric Sci Digest* **36**, 97–101.