Effect of fulvic acid on the phosphorus availability in acid soil

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Abstract

The incubation experiment was conducted to evaluate the influence of fulvic acid (FA) on phosphorus (P) movement and transformation in acid soil. FA at the rate of 0, 0.1, 0.2, 0.3 g and in combination with 0.19 g KH_2PO_4 was sprinkled uniformly on the surface of the filter paper on the soil cylinder for 21 days incubation, respectively. The result showed that the pH of soil treated with FA increased from the original value of 5.83 to the highest value of 6.27 in the surface soil layer after 21 days incubation. Due to the application of FA, exchangeable Al concentration evidently decreased in comparison to the only P treatment, which in turn significantly increased available P concentration. FA also increased the movement length of P that reached 4.5, 5.5 and 6.5 cm depth for treatments reinforced with 0.1, 0.2 and 0.3 g FA per column, respectively. As FA was added into soil, microbial population, organic matter content and cation exchange capacity (CEC) all clearly elevated. It is suggested that P fertilizer and FA are fertilized together may be considered as an optimum choice for the improvement of P availability and soil physicochemical conditions.

Keywords: Aluminium, microbial population, phosphorus availability, organic matter

1. Introduction

Phosphorus fertilizer efficiency in acid soil is less than 20% (Lindsay, 1988) unfavorable for agricultural production. Therefore, we must completely comprehend the relationship between P availability and soil acidity as well as pH buffering capacity. Soil acidity refers to a variety of possible chemical process, which are known to occur in acid soil (Rengel, 2003). The combined interaction of these process influences the amount and speciation of nutrient ions in soil solution and thus their availability for plant growth. Normally, acid soil is associated with a decrease in the availability of nutrients (particularly phosphorus, calcium and magnesium) and an increase in the presence acidic cations (hydrogen H^+), aluminum and iron (Robson and Abbott, 1989). The decrease in availability of phosphorus in acid soil is mainly because of its strong affinity for soil surface and reaction with Al and Fe (Eghball *et al.*, 1990), which results in P adsorption and precipitation. Soil pH buffering capacity is a key factor determining the rate of pH change during soil acidification (Moody and Aitken, 1997). The great contribution of change in

CEC to soil pH has been confirmed by recent study (Nelson and Su, 2010), suggesting the soil properties of organic carbon, clay content and effective CEC were highly correlated with the change in CEC with pH and thus affected soil buffering capacity. A significant component of pH buffering capacity in soil arises from dissolution/precipitation reactions and protonation/deprotonation reactions on variable charge minerals and organic matter (Aitken and Moody, 1994).

In the south of China, acid soil is abundant. Lime is often used to neutralize the acidity of acid soil but there is necessary in order to avoid over-liming which can have a negative impact on plant growth (Kamprath, 1971). Due to insufficient understanding of relationship between acidity present and pH, liming acid soil has not been completely accepted by most of farmers. For the present situation, researchers have brought up a new thinking, that is, granular organic amendments banding P fertilizer is applied.

FA is a macromolecular complex mixture comprised of aromatic hydroxy carboxylic acid. Although sources of FA are different, it is a vital constitute and an ultimate part of soil organic structure that has been considered by many scientists as an essential constitute of organic matter and highly effective in improving soil condition and plant growth (Lee and Bartlette, 1976). FA contains many nutritional elements beneficial for improving crop yields together with improvement of physicochemical and biological environment of soil (Brannon and Sommers, 1985). Previous report demonstrated FA significantly improved aggregate content in soil and increased tomato yield by 27.4% (Gao *et al.*, 2011) due to its chelating property to hold and release nutrient ions as and when required by plants (Linchen, 1978). And the same time, one of the most important properties of FA is the production of negative charge that arises essentially from the dissociation of acidic functional groups (Campitelli *et al*., 2003; Ceppi *et al*., 1999). Thereby, the addition of FA could contribute to the soil buffering capacity, or exert a marked control on the physicochemical behavior.

While FA has so many advantages for soil, little research is available on the effect of FA on the movement of P from fertilizer into soil and consequent P transformation. The objective of this experiment was to study the effect of KH_2PO_4 alone and in combination with different levels of FA on P availability.

2. Materials and Methods

The experiment was conducted in Yantai Institute, China Agricultural University, Yantai city, Shandong Province (119°34′~121°57′ E, 36°16′~38°23′ N). The soil used in this study was laterite obtained from the top 15 cm of a cultivated layer in Dongchuan city, Yunnan province (108°08′ E, 26°04′ N). Its main physical and chemical properties included available P (2.4 mg kg^{-1}), pH value (5.83), bulk density (1.34 g cm^{-3}), moisture content (280 g kg^{-1}), cation exchange capacity (11 cmol kg^{-1}), organic matter (8.9 g kg^{-1}), bacteria (1.58×107 g^{-1}), fungus (0.89×106 g^{-1}), actinomyces (0.79×106 g^{-1}) and exchangeable Al (58 μmol g^{-1}).

The containers for soil incubation used in this study were wax columns 90 mm in height with a cylindrical cavity having an inter diameter of 70 mm. The wax columns were prepared using the method as outlined by Akinremi (1990), modified from Khasawneh and Soileau (1969). The wax was closed on the bottom with a set of two pieces of filter paper. A fresh soil sample of 463.9 g was packed into the wax column by gentle tapping to obtain bulk density of 1.34 g cm^{-3}. Another filter paper, slightly smaller than the diameter of the soil cylinder, was placed on the top of the soil to separate the soil from fertilizer. The filter papers covered on the bottom of the soil were then abandoned, and the top and bottom of the packed wax blocks were covered with parafilm to prevent moisture loss (Fan and MacKenzie, 1993). Finally, they were allowed to equilibrate for 48 h at 25ºC before fertilizers were applied.

FA was made in Shanghai Tongwei Biological Science and Technology Co., Ltd. It was extracted

from weathered coal and consisted of FA ≥ 95%, C 54.82%, H 2.29%, O 41.14%, N 0.66%, S 1.09% and pH 6.5. Approximate molecular weight and molar concentration of FA are 1032 and 0.95 M. Four treatments were applied in this study: 1) Only P, 2) P+FA, 3) P+2FA, 4) P+3FA. P indicated the added dose of 0.19 KH_2PO_4 (equivalent to 0.1 g P_2O_5) and FA showed the applied dose of 0.1 g FA. Blank incubation was also carried out in which FA and KH_2PO_4 were not used. The fertilizer and FA were sprinkled uniformly as finely ground materials (<1 mm) on the surface of the filter paper on the soil cylinder. The wax blocks were then incubation vertically at 25ºC.

After 21 days of incubation, the wax columns were horizontally sectioned from the top into 18 slices with every slice 5 mm thickness using a sharp knife after removing the filter paper and fertilizer. The microorganism population of fresh soil was determined with surface planting technique, modified from Estermann and McLaren (1961). Then 0.1 mL of appropriately diluted soil sample was planted on 10% tryptic soy agar for the number of soil bacteria, on Czapek-Dox medium with rose bengal for the number of soil fungi and on Gause's No. 1 synthetic medium for the number of soil actinomycetes. Colonies were counted after incubation at 28ºC for 3 days for growth of bacteria, at 22ºC for 7 days for growth of fungi and at 28ºC for 14 days for growth of actinomycetes. The other analyses for soil samples were made on air-dried soil, ground, sieved through 2 mm sieve. The pH of soil water suspension (1:5, w/v) was determined with the help of a pH meter by the method described by McClean (1993). Available P was extracted with 0.5 M $NaHCO_3$ extractant and determined with ascorbic acid-ammonium molybdophosphate blue method (Liu, 1996). Cation exchange capacity was measured using the method of Chapman (1965). Briefly, 4 g of soil sample is mixed with an excess of sodium acetate solution and subsequently washed with isopropyl alcohol and an ammonium acetate solution is then added. The concentration of displaced sodium is then determined by atomic absorption spectrophotometry. The organic matter content was measured according to the method described by Liu (1996) in which soil sample was digested with $K_2Cr_2O_7$-H_2SO_4 and subsequently titrated with $FeSO_4$. The exchangeable Al concentration was estimated by leaching soil with 1 M KCl and 0.5 M NaF was added to filtrate to release the HO ions bound to Al. The Al content was determined by titrating the released HO with 0.5 M HCl (Lu, 1999).

All experiments were repeated three times. Values presented are means. The effects of the treatments were tested by one-way analysis of variance (ANOVA). Means were compared between the treatments using the LSD (least significant difference) test at the 0.05 probability level.
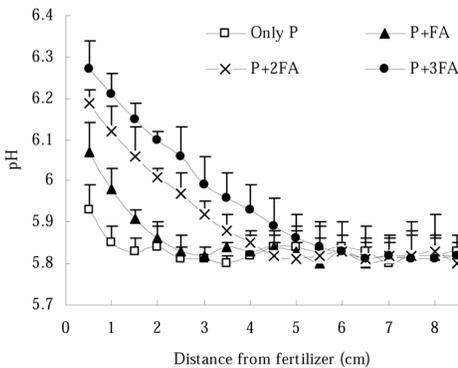
3. Results

Microsites changes of pH and available P were presented in Figure 1 and Figure 2 as KH_2PO_4 alone or in combination with different levels of FA were applied to soil. The highest values of pH and available P were occurred at soil surface and decreased with distance from fertilizer. The decrease rates of both pH and available P was much higher in Only P treatment than in FA treatments where pH and available P reached the background levels of 5.83 and 2.4 mg kg^{-1} centered at about 1.5, 2.5, 4, 6 cm and 3.5, 4.5, 5.5, 6.5 cm, respectively for treatments of Only P, P+FA, P+2FA and P+3FA. A positive correlation between FA amount and pH and available P was found, in this case, pH and available P concentration were P+3FA > P+2FA > P+FA > Only P.
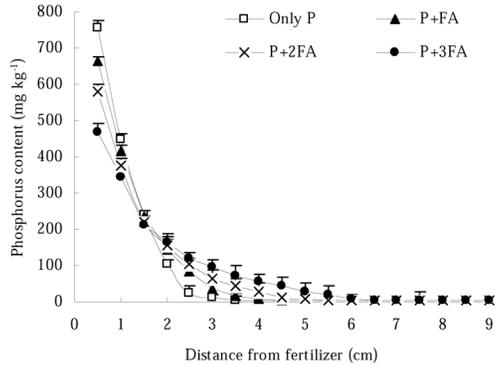
Contrary to pH and available P, the lowest concentrations of exchangeable Al were located at soil surface and increased with soil depth (Figure 3). The increase rate decreased as FA application quantity increased. As a consequence, exchangeable Al concentration recovered to the background level of about 58 μmol g^{-1} were located at depths of 3, 5 and 6.5 cm for treatments P+FA, P+2FA and P+3FA, respectively. Although exchangeable Al concentration also decreased to some extent in Only P treatment, differences were not observed between soil

depths. A conclusion was drawn that the intensity of influence on exchangeable Al content in soil depended on the amount of FA application.

Figure 4 and Figure 5 suggested that organic matter content and CEC in Only P treatment always remained the original status of 8.7 g kg^{-1} and 11 cmol kg^{-1} whatever soil depth increased or decreased. Compared to Only P treatment, organic matter content and CEC near the site of fertilizer were increased by 32.2, 42.7, 47% and 153.9, 240.9, 333% and decreased with soil depth ranging from 0–3, 0–4, 0–6.5 cm and 0–4, 0–6.5, 0–7.5 cm, respectively for P+FA, P+2FA and P+3FA treatments. The highest population of microorganism was also appeared at the soil surface and decreased with soil depth (Table 1). For Only P treatment, although the populations of bacteria, fungus and actinomyces had a certain increase, variations were only defined in the depth of 0–1.5 cm. FA application contributed to the increase in microbial population, particularly P+3FA treatment in which populations of bacteria, fungus and actinomyces were 1.9, 2.3 and 2.9 times than those of only P treatment.
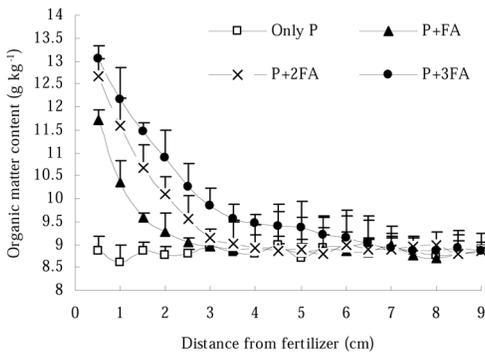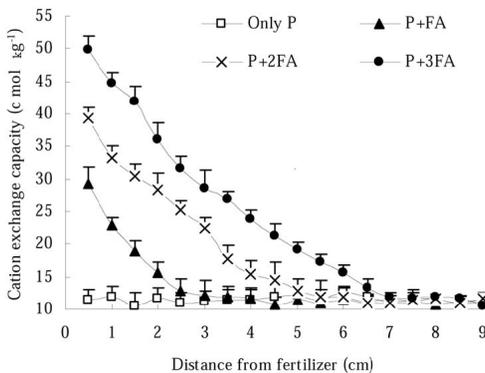

Figure 2. The effect of KH$_2$PO$_4$ alone and in combination with different levels of FA on phosphorus content. Treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH$_2$PO$_4$ and FA per column (i.e., 0.19 g KH$_2$PO$_4$, 0.19 g KH$_2$PO$_4$ + 0.1 g FA, 0.19 g KH$_2$PO$_4$ + 0.2 g FA and 0.19 g KH$_2$PO$_4$ + 0.3 g FA).

Figure 1. The effect of KH$_2$PO$_4$ alone and in combination with different levels of FA on pH. Treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH2PO4 and FA per column (i.e., 0.19 g KH$_2$PO$_4$, 0.19 g KH$_2$PO$_4$ + 0.1 g FA, 0.19 g KH$_2$PO$_4$ + 0.2 g FA and 0.19 g KH$_2$PO$_4$ + 0.3 g FA).
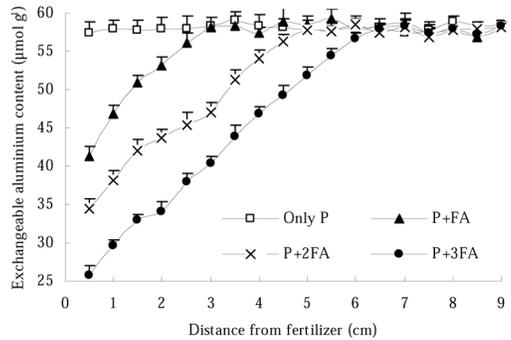
Figure 3. The effect of KH$_2$PO$_4$ alone and in combination with different levels of FA on exchangeable Al content. Treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH$_2$PO$_4$ and FA per column (i.e., 0.19 g KH$_2$PO$_4$, 0.19 g KH$_2$PO$_4$ + 0.1 g FA, 0.19 g KH$_2$PO$_4$ + 0.2 g FA and 0.19 g KH$_2$PO$_4$ + 0.3 g FA).

Figure 4. The effect of KH_2PO_4 alone and in combination with different levels of FA on organic matter content. Treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH_2PO_4 and FA per column (i.e., 0.19 g KH_2PO_4, 0.19 g KH_2PO_4 + 0.1 g FA, 0.19 g KH_2PO_4 + 0.2 g FA and 0.19 g KH_2PO_4 + 0.3 g FA).

Figure 5. The effect of KH_2PO_4 alone and in combination with different levels of FA on cation exchange capacity. Treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH_2PO_4 and FA per column (i.e., 0.19 g KH_2PO_4, 0.19 g KH_2PO_4 + 0.1 g FA, 0.19 g KH_2PO_4 + 0.2 g FA and 0.19 g KH_2PO_4 + 0.3 g FA).

4. Discussion

Generally, KH_2PO_4 was transformed into HPO_4^{2-}, $H_2PO_4^-$, PO_4^{3-} and K^+ ions in soil solution when it was solely added into soil. In acid soil, the processes of dissolution and hydrolysis, on one hand, caused base cation of K^+ directly to neutralize H_3O^+ rich in soil solution, and on the other hand, resulted in the transfer of H^+ to HPO_4^{2-}, $H_2PO_4^-$ and PO_4^{3-} to form $H_2PO_4^-$ and H_3PO_4. The transformation was in fact a consumption of protons. Although H_3PO_4 is a medium strong acid, its formation did not add to the background acidity of soil. This was attributed to the replacement of normal weak acids by it and weak acids were suppressed to dissociated (Sott *et al.*, 2011). Therefore, there was no net increase in the rate of acidity of soil acidification. Microbes in soil were also beneficial for acid consumption. They could take up SO_4^{2-}, NO_3^- and PO_4^{3-} in soil solution to ameliorate soil acidification by removing mobile anions. The assimilation of anions was really a H^+ consumption reaction (Sott *et al.*, 2011). If the above mentioned acid neutralizing processes failed to maintain the soil pH above 5, Al dissolution becomes an important acid neutralizing reaction (Cronan *et al.*, 1989). Due to the original soil pH was 5.83 and thus buffer effect of Al on pH was very little.

The acid consumption was almost simultaneous with acid recovery (Dahlgren *et al.*, 1990). The previously adsorbed H^+ and Al^{3+} again released into soil solution and exchangeable Al^{3+} hydrolysis became the leading source of H^+. The H^+ newly entered soil solution continued to be neutralized and a new equilibrium reached once again as H^+ was depleted. Figure 1 suggests that the pH in the soil of 0-1.5 cm in the treatment of only P was higher than original pH of 5.83, this meant KH_2PO_4 input in this microsite was exceeded the soil buffering capacity.

FA contains carboxylic, phenolic-OH and enolic groups which can dissociate to develop negative charges (Plaza *et al.*, 2006) and inevitably increase negative charge on soil particles (Naidu, 1990).

Table1. The effect of KH_2PO_4 alone and in combination with different levels of FA on microbial population.

Soil depth (cm)	Bacteria (×10⁷)				Fungus (×10⁶)				Actinomyces (×10⁶)			
	Only P	P+FA	P+2FA	P+3FA	Only P	P+FA	P+2FA	P+3FA	Only P	P+FA	P+2FA	P+3FA
0.5	2.35[a]	3.62[a]	4.13[a]	4.47[a]	1.15[a]	1.95[a]	2.48[a]	2.69[a]	0.94[a]	1.85[a]	2.40[a]	2.81[a]
1	1.71[b]	3.14[b]	3.72[b]	4.12[b]	0.93[b]	1.42[b]	2.06[b]	2.38[b]	0.85[b]	1.36[b]	2.03[b]	2.48[b]
1.5	1.65[c]	2.68[c]	3.36[c]	3.84[c]	0.88[bc]	1.14[c]	1.72[c]	2.12[c]	0.80[c]	1.09[c]	1.75[c]	2.19[c]
2	1.62[cd]	2.25[d]	3.01[d]	3.56[d]	0.89[bc]	1.06[d]	1.44[d]	1.89[d]	0.77[c]	0.95[d]	1.56[d]	1.90[d]
2.5	1.58[de]	1.93[e]	2.69[e]	3.32[e]	0.91[bc]	0.95[e]	1.21[e]	1.63[e]	0.79[c]	0.82[e]	1.41[e]	1.76[e]
3	1.60[cde]	1.69[f]	2.44[f]	3.10[f]	0.88[b]	0.90[f]	1.05[f]	1.34[f]	0.80[c]	0.76[f]	1.28[f]	1.52[f]
3.5	1.57[de]	1.58[g]	2.20[g]	2.93[g]	0.88[bc]	0.88[g]	0.93[g]	1.20[g]	0.80[c]	0.78[fg]	1.17[g]	1.35[g]
4	1.61[cde]	1.56[g]	1.97[h]	2.65[h]	0.87[bc]	0.87[g]	0.87[h]	1.13[h]	0.79[c]	0.78[fg]	1.04[h]	1.22[h]
4.5	1.58[de]	1.59[g]	1.85[i]	2.41[i]	0.86[bc]	0.89[g]	0.89[h]	0.95[i]	0.78[c]	0.79[fg]	0.94[i]	1.10[i]
5	1.59[de]	1.59[g]	1.68[j]	2.28[j]	0.89[bc]	0.88[g]	0.88[h]	0.88[ij]	0.77[c]	0.80[fg]	0.87[ij]	1.03[i]
5.5	1.59[de]	1.57[g]	1.57[k]	1.93[k]	0.90[bc]	0.89[g]	0.87[h]	0.90[ij]	0.79[c]	0.80[fg]	0.79[j]	0.95[i]
6	1.60[cde]	1.58[g]	1.59[k]	1.74[l]	0.92[bc]	0.89[g]	0.88[h]	0.89[j]	0.79[c]	0.79[fg]	0.80[j]	0.87[k]
6.5	1.58[de]	1.56[g]	1.58[k]	1.58[m]	0.89[bc]	0.87[g]	0.90[gh]	0.87[j]	0.78[c]	0.78[fg]	0.78[j]	0.79[l]
7	1.58[de]	1.57[g]	1.56[k]	1.60[m]	0.88[bc]	0.86[g]	0.88[h]	0.86[j]	0.77[c]	0.78[fg]	0.79[j]	0.78[l]
7.5	1.59[de]	1.58[g]	1.59[k]	1.58[m]	0.87[bc]	0.86[g]	0.89[gh]	0.88[j]	0.77[c]	0.79[fg]	0.78[j]	0.80[l]
8	1.57[de]	1.58[g]	1.58[k]	1.58[m]	0.89[bc]	0.88[g]	0.90[gh]	0.90[j]	0.79[c]	0.80[fg]	0.79[j]	0.78[l]
8.5	1.58[de]	1.61[g]	1.59[k]	1.57[m]	0.88[bc]	0.89[g]	0.88[h]	0.88[j]	0.79[c]	0.80[fg]	0.81[j]	0.79[l]
9	1.57[de]	1.60[g]	1.59[k]	1.57[m]	0.86[c]	0.88[g]	0.87[h]	0.88[j]	0.80[c]	0.80[fg]	0.79[j]	0.81[l]

The results are expressed as means (n = 3) and treatments Only P, P+FA, P+2FA and P+3FA respectively indicate application amount of KH_2PO_4 and FA per column (i.e., 0.19 g KH_2PO_4, 0.19 g KH_2PO_4 + 0.1 g FA, 0.19 g KH_2PO_4 + 0.2 g FA and 0.19 g KH_2PO_4 + 0.3 g FA). Different lowercase letters in the each vertical column show microbe population differences of different depths in the same treatment. All differences between means are statistically different according to LSD test at ($P<0.05$).

This was the main reason for increasing soil CEC. The greater CEC implied that soil could provide more cation exchange sites to take up more H^+ and Al^{3+} (Xu, *et al*., 2012) in agreement with date derived from Figure 1 and 3, illustrating, based on the effects of KH_2PO_4 on pH and Al content, CEC elevation further increased pH and decreased Al content and the extent to which they elevated and reduced was highly correlated with CEC.

FA acted as a part of carbon reservoir in the biosphere (Grinhut *et al*., 2007), which was necessary for the livelihood of soil microorganisms. Results presented in Figure. 4 and Table 1 showed that FA addition directly caused the increase in organic matter content and successively raised microbial population. With regard to pH, acidity might have resulted in death to some microorganisms, particularly in bacterial, fungal and actinomycetic communities sensitive to acidity (Blagodatskaya and Anderson, 1999). Raising the pH by FA in combination with KH_2PO_4 could ameliorate the acidic harm to microbial community

and which might be another reason for the increase in microorganism population. Correlation analysis indicated that bacteria was the most correlated ($R^2=0.928$) with FA application amount, fungus the least ($R^2=0.745$) and actinomyces of intermediate correlation ($R^2=0.838$).

The decrease in Al content caused by FA addition reduced the formation of aluminum phosphate and thus improved phosphorus availability (Hu *et al*., 2011). Besides, the reduction of soil acidity decreased phosphorus adsorption by OH^{2+} formed by protonation of OH^- on soil particle surface (Chen, 2004) and more microorganisms also accelerated organic phosphorus compound to be hydrolyzed (Hu *et al*., 2011), which could be regarded as an effective supplementary to phosphorus availability.

In the present study, the application rates of FA were equivalent to approximate 260, 520 and 780 kg ha^{-1} and 780 kg ha^{-1} of FA combined with phosphorus fertilizer was the best option for improvement in phosphorus availability. However, what effect will

occur if the quantity of FA application continues to be increased and that merits further study.

5. Conclusion

KH_2PO_4 application alone could lessen acidity of acid soil but mitigating effect was weaker and therefore phosphorus availability was not significantly increased. Due to FA contained larger functional groups that contributed to CEC and so FA application stimulated soil particle to absorb more H^+ and Al^{3+} and following to decreased phosphorus adsorption and precipitation. This was the main mechanism for the increased phosphorus availability. Results revealed phosphorus availability was positively correlated with FA application amount, soil CEC and pH and was negatively related with exchangeable Al content.

References

Aitken, P. L., Moody, P. W. 1994. The effect of valence and ionic strength on the measurement of pH buffer capacity. Aust. J. Soil Res. 32, 975-984.

Akinremi, O. O. 1990. The Diffusive Transport of Phosphate and Associated Cation in Soil and Soil-Like System. Doctoral thesis, Manitoba University.

Blagodatskaya, E.V., Anderson, T.H. 1999. Adaptive responses of soil microbial communities under experimental acid stress in controlled laboratory studies. Applied Soil Ecol. 11, 207-216.

Brannon, C.K., Sommers, L.E. 1985. Preparation and characterization of model humic polymers containing organic phosphorus. Soil Biol. Biochem. 17(2), 213-219.

Campitelli, P.A., Velasco, M.I., Ceppi, S.B. 2003. Charge development and acid–base characteristics of soil and compost humic acids. J. Chil. Chem. Soc. 48, 91-96.

Ceppi, S.B., Velasco, M.I., Pauli, C.P. 1999. Differential scanning potentiometry: Surface charges development and apparent association constant of natural humic acids. Talanta. 50, 1057-1063.

Chapman, H.D. 1965. Cation exchange capacity. In: C. A. Black (ed). Method of Soil Analysis, American Society of Agronomy, America, pp: 891-901.

Chen, G.C., He, Z.L., Wang, Y.J. 2004. Impact of pH on microbial biomass carbon and microbial biomass phosphorus in red soils. Pedosphere. 14, 9-15.

Cronan, C.S., April, R., Bartlett, R.J., Bloom, P.R., Driscoll, C.T. 1989. Aluminum toxicity in forests exposed to acidic deposition: the Albios results. Water, Air, Soil Pollut. 48, 181-192.

Dahlgren, R.A., McAvoy, D.C., Driscoll, C.T. 1990. Acidification and recovery of a spodosol Bs horizon from acidic deposition. Environ. Sci. Technol. 24, 531-537.

Eghball, B., Sander, D.H., Skopp, J. 1990. Diffusion, adsorption and predicated longevity of banded phosphorus fertilizer in three soils. Soil Sci. Am. J. 54, 1161-1165.

Estermann, E.F., McLaren, A.D. 1961. Contribution of rhizosplant organisms to the total capacity of plants to utilize organic nutrients. Plant Soil. 3, 243-260.

Fan, M. X., MacKenzie, A. F. 1993. Interaction of urea with triple superphosphate in a simulated fertilizer band. Fert. Res. 36, 35-44.

Gao, Y. F., Sun, L.R. 2011. Investigation of fulvic acid efficiency. Shanxi Agricultural Science. 6, 100-103.

Grinhut, T., Hadar, Y., Chen, Y. 2007. Degradation and transformation of humic substances by saprotrophic fungi: processes and mechanisms. Fungal Biol. Reviews. 21, 179-189.

Hu, A.T., Zhou, L.X., Zhou, Q.S., Yang, C.G., Zhang, C.L. 2011. Plant Nutrition Science. China Agricultural University Publishing House, Beijing.

Kamprath, E.J. 1971. Potential detrimental effects from liming highly weathered soils to neutrality. Florida Soil and Crop Sci. Soc. Proc. 31, 200-203.

Khasawneh, F. E., Soileau, J. M. 1969. Soil preparation and sampling techniques for studying ion movement. Soil Sci. Soc. Am. Proc. 33, 476-477.

Lee, Y.S., Bartlette, R.J. 1976. Stimulation of plant growth by humic substances. J. Am. Soil Sci. Soc. 40, 876-879.

Linchen, D.J. 1978. HA-Fe uptake by plants. Plant and Soil. 50, 663-670.

Lindsay, W. L., Frazier, A.W., Stephenson, H. F. 1988. Identification of reaction products from phosphate fertilizers in soil. Soil Sci. Soc. Am. J. 52, 1793-1796.

Liu, C.S., Yang, S.X. 1996. Agricultural Chemistry and Analysis. China Agricultural University Publishing House, Beijing.

Lu, R.K. 1999. Analytical Methods for Soil and Agro-Chemistry. China Agricultural Science and Technology Publishing House, Beijing.

McClean, E.O. 1982. pH and lime requirement. In: R. H. Miller, D. R. Keeny (ed). Methods of Soil Analysis, American Society of Agronomy, Madison, WI, pp: 209-223.

Moody, P.W., Aitken, R.L. 1997. Soil acidification under some tropical agricultural systems. Aust. J. Soil Res. 35, 163-173.

Naidu, R., Syers, J.K., Tillman, R.W., Kirkman, J.H. 1990. Effect of liming on phosphate sorption by acid soils. J. Soil Sci. 41, 165-175.

Nelson, P.N., Su, N. 2010. Soil pH buffering capacity: a descriptive function and its application to some acidic tropical soils. Aust. J. Soil Res. 48, 210-217.

Plaza, C., Brunetti, G., Senesi, N., Polo, A. 2006. Molecular and quantitative analysis of metal ion binding to humic acids from sewage sludge and sludge amended soil by fluorescence spectroscopy. Environ. Sci. Technol. 40, 917-923.

Rengel, Z. 2003. Handbook of Soil Acidity. Marcel Dekker, New York, USA.

Robson, A,D., Abbott, L.K. 1989. The effect of soil acidity on microbial activity in Soils. In: A. D. Robson (ed). Soil Acidity and Plant Growth, Academic Press, Sydney, pp: 167-203.

Sott, L.M., Benjamin, Z.H., Randy, A.D. 2011. Increased forest ecosystem carbon and nitrogen storage from rich bedrock. Nature. 447, 78-81.

Xu, R.K., Zhao, A.Z., Yuan J.H., Jiang, J. 2012. pH buffering capacity of acid soils from tropical and subtropical regions of China as influenced by incorporation of crop straw biochars. J. Soils Sediments. 12, 494-502.