



Research Article

Effect of Urea Fertilizer on the Biochemical Characteristics of Soil

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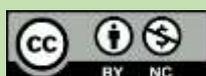
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Abstract

Urea-potash mixture was added to the manured soil at three different concentrations equivalent to 0.8, 1.6 and 2.4 g urea per 10Kg of soil. Nitrate and nitrite N concentration in the soil increased within 24h after addition of urea. The nitrate N content in soil without urea was 17 µg and in urea fertilized soils, it ranged from 39.9-47 µg/g of soil after 19h. Increase in total mineralizable N was around 67- 160% in urea fertilized soils in comparison to the control. Percent conversion of urea to nitrate and nitrite N decreased at higher concentrations of the fertilizer. Addition of biochar to urea amended soil did not bring about significant change in the available N content. Decrease in total mineralizable N and accumulation of available P was observed over the period of 15 days. Addition of urea resulted in acidification of the soil. Acidification of the soil could be correlated with increase in acid phosphatase concentration. The soil amended with biochar exhibited significant buffering capacity in the region of pH 7.4-9.

Keywords: Urea; nitrate N; mineralizable N; acid phosphatase; biochar

Introduction

The practice of enriching the soil with inorganic or organic nutrients in order to increase the output has led to development of various fertilizers. Being a cheap and easily assimilable form of N, urea is an extensively used fertilizer in world agriculture. Addition of urea to soil compensates for the nitrate leached from the soil. It is generally added along with potassium salts such as potassium oxide, potassium sulfate or potassium chloride. Application of urea-potash mixture is known to increase the crop yield to a significant extent as it gets rapidly hydrolyzed to

ammonium (Berhe *et al.*, 2019). Nitrifying bacteria convert ammonium to nitrate releasing hydrogen ions (H⁺). While assimilating the nitrates, plants release hydroxide ions thereby neutralizing the protons. Urea is reported to have adverse effects on seed germination and seedling growth. The effect is attributed to the breakdown products of urea (Smiley and Cook 1973; Bremner and Krogmeier, 1988). Accumulation of protons consequent to formation of nitrate and the inability of the seedlings to assimilate nitrate efficiently may not be able to circumvent acidification of

the soil. Urea-potash mixture is therefore added after the seedlings have attained 75-80% of their final height. Very often the use of this fertilizer exceeds the dose of around 110 Kg urea recommended per acre for paddy/ wheat cultivation which is equivalent to around 122g urea in 1000Kg of soil (Grover *et al.*, 2014; http://agripb.gov.in/pub/pdf/fertilizer_application_for_rice.pdf). Inordinate application of urea is reported to affect the macro fauna of the soil. Continuous and excessive use of urea may also lead to alteration of soil pH and induce alteration in the microbial population and biochemical parameters (Oim and Dynoodt, 2008). Phosphorus is one of the nutrients as availability of this macronutrient is reported to be affected by soil pH to a significant extent. Burnt residues of partly decomposed plant litter are reportedly rich in humus and ash minerals. Humus rich matter is known to affect microbial growth and water holding capacity of the soil which may influence the leaching of ions. The burnt plant residue matter (biochar) therefore, can alter the physical and biochemical properties of the soil. It is reported that biochar influences the availability of phosphorus and nitrogen. Biochar is known to be alkaline in nature (Wang *et al.*, 2018; Gao *et al.*, 2019). As application of urea tends to acidify the soil, it would be interesting to study the effect of simultaneous application of biochar along with urea.

The objective of the present investigation was to study the effect of urea on various chemical and biochemical parameters of the soil. Effect of burnt plant residues on these parameters in soils amended with urea was also studied.

Materials and Methods

All the chemicals and reagents used for the study were of analytical grade.

Garden soil (freshly furnished with cow manure, well mixed and sieved) procured from the nursery was employed for study. Burnt plant residues were collected from a local farm, and the partially burnt twigs were removed. The BPR was ground using pestle and mortar and used.

Experimental Design

Garden pots each filled with around 10 Kg of soil (6Kg soil was overlaid with 4Kg of soil containing the urea/urea + biochar) were used for the study. Urea- potash mixture was prepared by mixing urea and K₂O in the ratio of 2:1. Sets U1, U2, U3 were prepared by adding 1.2g, 2.4 and 3.6g urea-potash mixture respectively to the top layer. Sets AU2 and AU3 contained 2.4g and 3.6g urea-potash respectively in addition to 20g of BPR each. Control sets were maintained with 10Kg of soil without fertilizer and BPR. Watering was done every evening by sprinkling the top layer with 750ml of water every day.

Sample Collection

Sample collection was carried out in the morning at around 10.00 a.m. on Day 1 (at 18h) and on the 15th day. Soil was sampled from 4 spots (≈10g each) at a depth of around 70-80mm, pooled and ground with pestle and mortar. For enzyme assay, the samples were stored in air-tight containers at -20°C until assay. The samples dried in an oven at 40°C to constant weight were subjected to analysis of chemical parameters.

Analytical Methods

The soil samples were extracted in suitable extracts (unless mentioned otherwise) and appropriate dilutions of the extracts were used for the estimations.

Estimation of Available Phosphorus

Soil, 1g was extracted for 30min in 20ml 0.5 M solution of sodium bicarbonate at pH 8.5 (Olsen's method). The suspension was filtered through Whatman no 1 filter paper. The filtrate was analyzed for phosphorus using molybdate and stannous chloride. To 1.9ml of the appropriately diluted sample, 0.5ml of ammonium molybdate solution (0.15% in 4N HCl) was added, followed by addition of 0.1ml of stannous chloride (0.07g dissolved in 0.175ml HCL and diluted to 10ml with D/W). Absorbance was read at 700nm. Method was calibrated using KH₂PO₄.

Estimation of Available Nitrate and Nitrite Nitrogen

The soil sample, 1g was extracted with 5ml of 1% sodium carbonate. Further extraction was carried out twice with 7.5ml of the extractant. The filtrates obtained by filtration of the extracts through Whatman filter paper were pooled and analyzed for nitrate and nitrite nitrogen.

Nitrate N was estimated by adding 75ul of the sample to 85ul of Salicylic acid (5% w/v in sulfuric acid) followed by addition of 2ml of 2N NaOH after 20min. The tubes were cooled and read at 405nm. Method was standardized with lead nitrate as the standard.

Nitrite N was estimated by adding 0.05ml of benzidine (0.05%) to 0.2ml of the sample, followed by addition of 0.05ml of 2M HCl. After 4min, 0.1ml resorcinol (5%), 0.1ml NaOH (2M) and 0.7ml D/W were added to the tubes. Absorbance was read at 450nm. The method was standardized with sodium nitrite (<https://www.ucltomars.org/resources/crews/2018.190/soil-chemical-composition.experimental-plan.pdf>).

Estimation of Total Available Nitrogen by Alkaline Permanganate Method

5g soil sample in the digestion tube was placed into the distillation unit (KJELO PLUS automatic Nitrogen/protein estimation system) and digested with 25ml each of 0.32% KMnO₄ solution 2.5% of NaOH (automated addition). The sample was heated and the liberated ammonia was collected in 20ml of 2.5% of boric acid at the receiving end of the unit. Five drops of mixed indicator (0.07% Methyl red,

0.1% Bromocresol green in ethanol) was added and titrated with 0.02N H₂SO₄ to a color change from green to pink. Milligrams of total available nitrogen in one gram of soil was calculated by multiplying the titration reading with a factor of 0.05595.

Effect on soil pH:

Soil 4g suspended in 10ml D/W was mixed by vortexing and the tubes were kept undisturbed for 15min. The pH of the upper layer was measured using digital pH meter (Okalebo et al., 2002).

Buffering capacity of the soil was studied by measuring the pH after addition of 40µl 0.01N NaOH/HCl to the suspension. Increments of NaOH/HCl were added, followed by measurement of pH.

Enzyme assays:

For assay of acid phosphatase, 0.3g soil was suspended in 0.75ml of 0.1M acetate buffer, pH 5.0 and mixed using a vortex mixer. Para nitro phenyl phosphate (0.1M), 0.1ml was added to the tubes and the tubes were incubated for 1h at 37°C. The contents were mixed by inverting the tubes once every 15min during the incubation. Tubes were centrifuged at 5000rpm for 5min. To 0.6ml of the supernatant, 0.3ml of 2M NaOH was added. A control with 0min of incubation was prepared in a similar manner. Absorbance was read at 405nm. Unit of activity is defined as the micromoles of nitrophenol released in one hour by one gram of the soil sample at 37°C under the defined conditions. Alkaline phosphatase assay was carried out by the same method using 0.1M glycine-NaOH buffer (pH 9).

Sulfatase activity was measured using 0.1M phosphate buffer, pH 7.0 by the same method using p-nitro phenyl sulfate as the substrate.

Enzyme unit is defined as micromole of para nitrophenol released in one hour by the enzyme under the defined conditions of assay.

Results and Discussion

Fertilizers, mineral or organic are applied to the soil to enrich its nutrient content. The diverse microbial populations partake in transformation of these nutrients from one form to the other, thus influencing the soil pH and other biochemical parameters. Nitrogen is the major nutrient required for plant growth and hence is required to be replenished in abundance in the form of manure and fertilizer. Urea is one of the widely used nitrogenous fertilizers which are added to the soil along with potassium salts. Frequent and excessive use of urea is reported to affect soil characteristics. Although it is reported that urea undergoes slow hydrolysis in presence of moisture, microbial ureases are the major catalytic agents which

breakdown urea to release ammonia and CO₂. Nitrifying bacterial flora tend to oxidize ammonia to nitrite, which is further converted to nitrate with concomitant release of protons (Ghaly and Ramakrishnan, 2013). In the present investigation, an attempt was made to investigate the effect of urea on nitrate and nitrite content. One of the age-old practices in agriculture is piling the plant leaves and twig residues (partly digested by cattle excreta) in small heaps across the field followed by burning. The burning process is believed to prevent growth of the unwanted plants/weeds and provide minerals in readily assimilable forms. Several theories have been presented about the pros and cons of this practice (Xu et al, 2012; Windeatt et al., 2014; Edem and Udo-Inyang, 2016). A previous study in our laboratory had shown that the biochar residues affected pH changes in the soil (unpublished results). Therefore, in the present investigation, an attempt was made to study the influence of BPR in soils containing higher content of urea. Mixture of urea and K₂O was added at three different concentrations 1.2, 2.4 and 3.6 g/Kg accounting for 0.8g, 1.6g and 2.4g of urea respectively in the upper layer of the soil. As ureases are known to be highly active, the samples were collected at 19h and analyzed. Results of estimation of nitrate and nitrite N are presented in Fig. 1. Estimation of nitrite was carried out based on the tetrazotization of benzidine with nitrite (Nagaraj et al., 2016). Nitrite and nitrate N increased within 24h, after addition of urea. In the samples collected on 15th day, nitrite concentration decreased with concomitant increase in nitrate N. Decrease in nitrite content on day 15 was more pronounced in samples (U3 and AU3) to which 2.4g of urea had been added. Results for AU2 (U2 amended with BPR) and AU3 (U3 amended with BPR) showed that addition of soil with BPR did not increase nitrate/nitrite N content. Addition of urea in excess (U2 and U3) did not affect an increase in Nitrate N to a significant extent. Apart from nitrite and nitrates, ammonium salts also contribute towards the available nitrogen content of the soil. As seen in Table 1, total available (mineralized) N content was higher than the sum of nitrate and nitrite N in all the soil samples. The difference was more in the samples collected on day 1. It appears that the ammonium contributed by manure as well as urea may be contributing towards the other mineralizable forms of N. The results indicated in U1, around 40% of the added nitrogen (0.8g urea ≈ 0.37g nitrogen) was converted to nitrate and nitrite nitrogen. In U2 and U3, around 21% and 18% of the added N was converted to nitrate and nitrite N. Build-up of available nitrogen did not occur over the period of 15 days. Loss of N due to volatilization of ammonia or leaching of minerals to the lower layers could be the possible reasons for the reduced amount of total available N recorded on day 15.

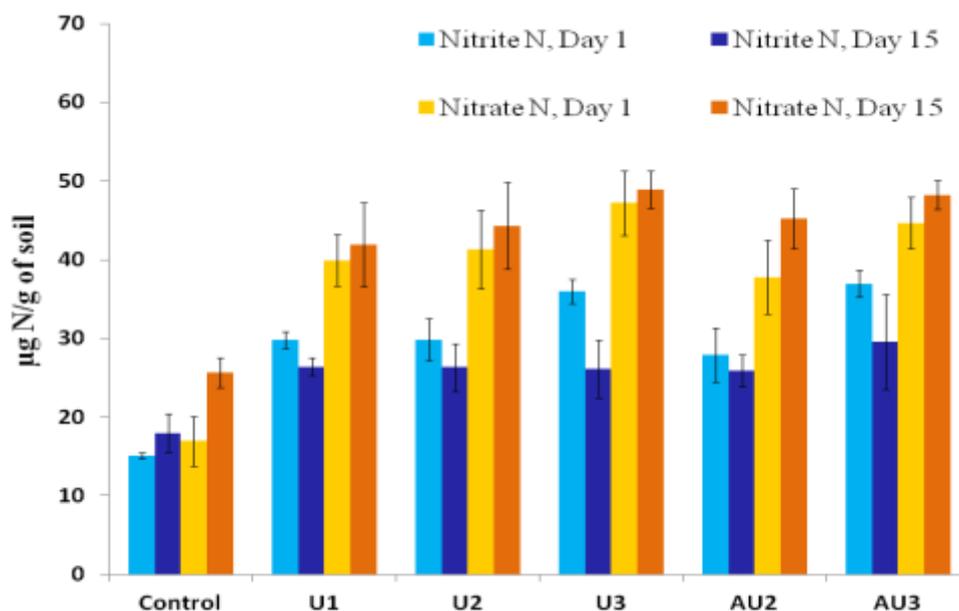


Fig. 1: Effect of urea on the concentration of nitrate and nitrite nitrogen in soil on day 1 and day 15

Table 1: Summary of nitrate and nitrite N formed in relation to total mineralizable N

Soil	Day 1, N content mg/g		Day 15, mg/g	
	Nitrite N+ Nitrate N	Total available N	Nitrite N+ Nitrate N	Total available N
Control	0.0321	0.084	0.0436	0.0698
U1	0.0697	0.14	0.0684	0.0839
U2	0.071	0.168	0.0706	0.0978
U3	0.0831	0.224	0.0751	-
AU2	0.0657	0.196	0.0711	0.0839
AU3	0.0816	0.238	0.0778	0.0839

Volatilization of ammonia is influenced by soil pH, temperature and biochemical properties of the soil. Reduction of nitrate to ammonia is facilitated by denitrifying microbes. Thus a balance between nitrification and denitrification processes can also affect the nitrogen content in soil. Higher pH and temperatures lead to volatilization of nitrogen (Whitehead *et al.*, 1991; Rochette *et al.*, 2013). The pH however, was lower in the soil samples to which urea had been added (Table 2). The pH decreased to a significant extent in all the samples over the period of 15 days. Addition of urea is known to acidify soils (Tong and Xu, 2012). Urea added soils were acidified to a greater extent than the control soil. Nitrate being an easily assimilable form of N, is taken up by nitrate transporters in the root cells using a proton gradient leading to release of hydroxyl ions. Thus acidification of the soil which occurs during nitrate formation is circumvented by nitrate uptake in farmed soil. The pH of soil is one of the primary concerns as it is known to influence the availability of nutrients to the plants (Sims and Patrick, 1977; Xiang *et al.*, 2009; Jensen, 2010). While ammonia uptake by plants is best at neutral

pH, nitrate uptake is reportedly optimum at lower pH values. Optimum phosphorus availability is at pH 6.5-7.0. Below pH 6.5, it gets insolubilised as phosphates of Al/Fe or gets immobilized onto clay. At high pH, phosphorous availability decreases as it reacts with calcium and becomes inaccessible. At lower pH availability of K, Ca and MG reduces as they get leached out. At pH below 6, aluminum becomes more and more accessible to plants leading to toxic effects. As pH rises, micronutrients such as boron, Zn and Fe tend to precipitate and become unavailable. Thus balancing pH in and around neutrality is required for optimal crop yield in most cases. The pH of cattle manure is relatively alkaline and amendment of soil with manure is known to increase the soil pH. Reclamation of the soils by replenishing with cattle manure serves not only to recharge the soil with nutrients, but also may help to restore the acidic soils to near neutral pH (Ayeni and Adeleye, 2012). In the present study, the soil used for the study had been amended with cow manure. The pH of the soil was 7.4 on day1. However, the fractions of the same soil to which urea had been added showed acidification within 24 hours. Over the

period of 15 days, all the samples including the control soil became acidic. The pH of the biochar (10% suspension) was around 8.9. Addition of biochar to urea containing soils raised the pH by 0.18-0.27 units on day1. However, it must be noted that on day 15, the biochar amended soil samples AU2 and AU3 were more acidic than their respective urea controls U2 and U3. The results therefore imply that dumping the unfarmed soil with urea and biochar can lead to acidification of the soil in the long run. The alkalizing/neutralizing ability of the manure/biochar appears to be a short term effect.

The pH can also affect the phosphatase activity in soil. Input of nitrogenous fertilizers is reported to accelerate recycling of P (Marklein. and Houlton, 2011). Phosphatases are the microbial enzymes which tend to cleave the phosphate groups from their organic complexes, rendering the P available for plants Piotrowska-Długosz and Wilczewski, 2014. Organic P is reportedly a good predictor of Phosphatase (Margalef *et al.*, 2017). Organic phosphates are soluble at acidic side of the pH, thereby increasing the availability of substrates for phosphatases. In all the soil samples enriched with urea, pH had become acidic and the activity of acid phosphatase was found to be significantly higher than the control. The acid phosphatase activity in biochar added samples AU2 and AU3 was less than their respective urea controls U2 and U3 (Table 2). Phosphorus content in AU2 and AU3 was 7-13% higher than the urea controls. Interestingly, the alkaline phosphatase content decreased over the period of 15 days, while the activity of acid phosphatases increased. These results could be correlated to the change in pH, as the pH became acidic over the period of 15 days. Acid phosphatases are known to predominate in acidic soils while alkaline phosphatases tend to dominate at higher pH values. Reduction in alkaline phosphatases was however significant in control and biochar amended soils. Accumulation of available P occurred in the period of 15 days. Urea enriched soils

contained higher content of available P which can be correlated to increased phosphatase activity in these samples. The results shown in Table 2, for U1, U2 and U3 indicated that addition of urea in excess did not influence the P or phosphatase content to a significant content.

Aryl sulfatase activity in the soil was measured using para nitro phenyl sulfate as the substrate (Baligar and Wright, 1991). The activity of the enzyme was found to be very low and hence incubation was carried out for 5h. The day 1 samples showed activity ranging from 0.019 to 0.032 U/g. The control samples exhibited 0.022 U/g of activity. Meaningful correlation could not be established between the activity and urea/ biochar concentration in the samples. Sulfatase activity was found to diminish with time, as the samples collected on day 15 exhibited 0.0009-0.0032 U activity per gram of soil.

Mineral composition and concentration can affect the buffering power and hence, buffering capacity of the soil samples was studied. The results are presented in Fig. 2. Biochar added soil samples collected on day 1, showed good buffering potential in the alkaline range. Around 440 µl of 0.01N NaOH was required to change the pH of the sample from 7.6 to 8.8 in AU2 and AU3. Addition of 160 and 120µl of NaOH brought about a change in pH from 7.8 to 9.0 for U2 and U3 respectively. In the acidic side of the pH, the soil samples did not exhibit much resistance to change in pH on addition of HCl. To bring about reduction in the pH by 2 units, around 100-110 µl of 0.01N HCl was added to all the soil suspensions with the exception of U2 and U3 which required addition of around 125 and 150 µl respectively. As the time progressed, the ability of the soil to resist changes in pH decreased in the alkaline region. Around 100 -120 µl of 0.01N NaOH was sufficient to increase the pH from 5.6 to 9.0 in all the samples. Sixty to seventy five microlitres of 0.01N HCl was required to alter the pH of the soil samples by 1 unit of pH

Table 2: Effect of urea and biochar on the buffering capacity of the soil

Soil	Day 1				Day 15			
	pH	µg P/g	Acid Phosphatase	Alkaline Phosphatase	pH	µg P/g	Acid Phosphatase	Alkaline Phosphatase
Control	7.41	39.73	0.277	0.48	5.7	52.36	0.458	0.115
U1	6.2	40.65	0.431	0.493	5.28	55.98	0.579	0.396
U2	6.31	38.71	0.45	0.542	5.25	55.52	0.534	0.399
U3	6.4	37.96	0.497	0.524	5.39	54.9	0.55	0.384
AU2	6.49	41.63	0.405	0.596	5.04	56.49	0.567	0.162
AU3	6.67	42.97	0.421	0.565	5.05	57.37	0.591	0.148

Enzyme activity is represented as units of activity per gram of soil

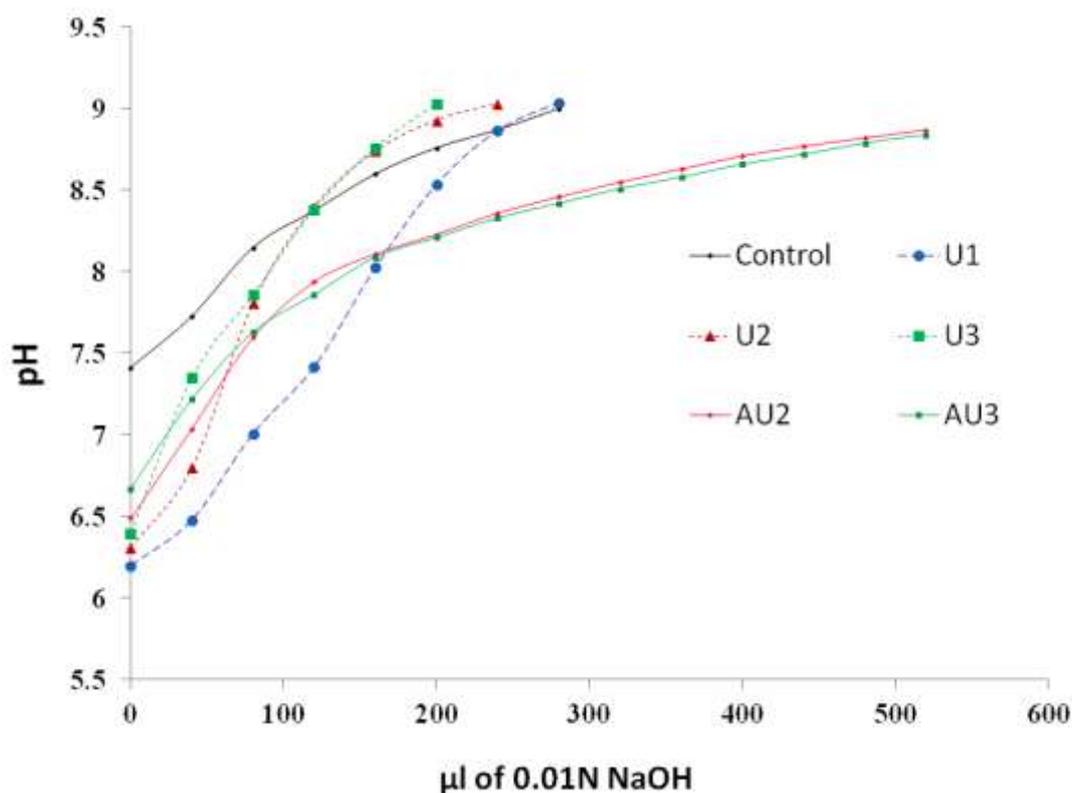


Fig. 2: Effect of urea and Biochar on the buffering capacity of soil samples collected on day 1

Conclusion

Urea addition at levels of 0, 0.8, 1.6 and 2.4 g to the soil resulted in formation of 17, 39.9, 41.3, 47 µg of nitrate N per g of soil on day 1. Thus, increase in concentration of urea did not result in proportional increase in the nitrate content of soil. Acidification of the soil was observed within 24h and continued thereafter. Urea added soils showed higher acid phosphatase activity. Build-up of available P occurred over the period of 15 days. Amount of P in urea containing soil was found to slightly higher (6-9%) than in control. Decrease in soil pH appears to correlate with increased acid phosphatase activity. Biochar was found to resist changes in pH in the alkaline region. Thus burnt plant residues may help to prevent alkalization of soils.

Author's Contribution

All authors contributed equally in every steps. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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