RESEARCH

Consequences of nitrogen mineralization dynamics for soil health restoration of degraded tea-growing soil using organic amendments

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ABSTRACT

Understanding of N mineralization dynamics of frequently available organic amendments in the tea (*Camellia sinensis* (L.) O. Kuntze) ecosystem has greater importance in land restoration. Hence, this study focused on assessing the effects of organic amendments on N mineralization and soil quality improvement in tea growing soil. Garden compost (CMP), *Gliricidia* (*Gliricidia sepium* (Jacq.)) leaves (GLI), charged tea waste biochar (CBC), tea waste (TW), and tea waste biochar without charging (RBC) were incubated with soil at a rate of 186 mg N kg^{-1}. Incubated soils were analyzed periodically for soil pH, available NO_3^--N, NH_4^+-N, soil P, and S for 120-d. Microbial biomass C (MBC), protease, urease, phosphatase, and dehydrogenase activities were determined at the end of the incubation. All amendments showed different N mineralization patterns. *Gliricidia*, CMP, and TW released N by 94%, 43%, and 24%, respectively. *Gliricidia* showed the highest peak of NH_4^+-N after 21-d incubation, depicting rapid ammonification. Charged BC and RBC showed N immobilization throughout the incubation period, which finally amounted to 12% and 17%, respectively. *Gliricidia* showed 0.79 mg d^{-1} maximum N mineralization rate and 150 mg kg^{-1} total mineralizable N. The N mineralization was sequenced as GLI > CMP > TW > CBC > RBC. All amendments showed more than 45% increase in MBC, where *Gliricidia* gave the highest (146%) compared to the control. Application of CBC promotes all enzyme activities by > 90% over control. In conclusion, GLI meets the immediate plant N requirement, and CBC significantly improves the degraded soil quality.

Key words: *Camellia sinensis*, enzyme activities, nitrogen mineralization, organic amendments, soil restoration, tea-growing soil.

INTRODUCTION

Soil fertility depletion in current tea (*Camellia sinensis* (L.) O. Kuntze)-growing soils has been experienced by many tea-producing countries, including Sri Lanka (Liyanage et al., 2021), China (Delang, 2018), Iran (Bahrami et al., 2010), India (Sahoo et al., 2016) and Rwanda (Mupenzi et al., 2011), questioning the sustainability of tea cultivation (Liyanage et al., 2021). It has become inevitable in most of the tropical tea-growing soils, mainly due to the continuous removal of nutrients during the harvest, adverse climatic factors, and other natural and anthropogenic factors.

Application of organic amendments is an imperative management technique that may enhance and elevate soil-quality attributes and alter nutrient cycling *via* mineralization or immobilization (Baldi and Toselli, 2014). When nutrient mineralization is accurately measured, it is possible to determine the exact amount of manure without losing the yield and minimizing the risk of environmental pollution. Further, restoration of fertility in degraded soils via organic amendments is a cost-effective and sustainable method, and thus, has received global attention recently (Abbasi et al., 2015).

Nitrogen is the most demanding plant nutrient for tea cultivation because the vegetative growing shoot has been continuously harvested. It is applied based on the potential of the manufactured tea yield. It has been estimated that 120-160 kg N ha^{-1} are removed with 4000 kg ha^{-1} of tea made annually from a tea field (Mohamed and Zoysa, 2008). Further, added N is lost through NH_3 volatilization (30% of added N) (Liyanage et al., 2015), leaching (10-40 kg N ha^{-1} yr^{-1}) and surface runoff losses (50-100 kg N ha^{-1} yr^{-1}).

The rate of mineralization and nutrient release of organic materials determines their suitability as manure. The residue-bound nutrients can become available to the plants in significant quantities over time. Therefore, to optimize N supply to the crop while minimizing N losses, net N mineralization should be synchronized with plant/crop development (Abbasi et al., 2015). However, this is very challenging in tea fertilization with organic manures (Lazicki et al., 2020). The two major stable products of the mineralization of N are NH_4^+ and NO_3^-, thus the measurement of these parameters is used in determining the rate and quantity of N mineralization.

Different empirical models have been used to better explain the dynamics of N mineralization due to its complexity (Mohanty et al., 2011). A first-order kinetic model is the most common model used in describing the N mineralization of both fresh and composted amendments. Some other researchers, in contrast, propose that the decomposition of incompletely composted, heterogeneous, or fresh organic amendments follows a two-pool parallel first-order or occasionally a zero-order model as well (Azeez and van Averbeke, 2010). Nitrogen mineralization is a critical factor that determines soil quality as it directly affects soil microbial activities and enzymatic activities. Because of their influence on nutrient cycling and organic matter decomposition, they are considered the most important microbial-biochemical indicators in soil quality monitoring. Therefore, measurement of these parameters provides the soil health status changes with the addition of organic amendments.

Tea waste, *Gliricidia sepium* (Jacq.), and compost are some of the freely available organic soil amendments in the tea environment. The potential for preparing compost in the tea field itself is greater, as it contains enormous amounts of various residues are available. *Gliricidia* is the most common shade tree in tea gardens, which additionally provides green manure. Tea waste generated from the tea processing system also has the potential to be used as a soil amendment but needs to be studied. Tea waste could also be used as a feedstock to produce biochar along with spent tea, shade tree lopping, and tea pruning materials. Biochar alone is deficient in nutrients for plant growth and hence needs to be fortified with plant nutrients (Pandit et al., 2017).

Although there is an ample amount of information on N mineralization, the trends of N mineralization along with P and S mineralization have not been studied in degraded soils. Studies on the restoration of tea-growing soils with organic amendments are scanty. Understanding mineral N fluctuations in organic matter decomposition over time is useful in managing the plant's N requirement (Marzi et al., 2020). Hence, the objective of this experiment was to study the effects of organic matter incorporation into tea-growing soils on N mineralization and soil quality improvement. It provides scientifically based information for making decisions about crop residue management and its agronomic importance in restoring fertility in degraded tea-growing soil.

MATERIALS AND METHODS

Soil sampling site and physicochemical properties of soil

The soil was collected from a low-elevation tea garden in Banting estate (2°56'05.7" N, 101°34'16.6" E), Selangor, Malaysia, at a depth of 0-15 cm comprising the A_p and A horizons. The sampling area has a warm, humid tropical climate with well-spread 2500 mm average annual rainfall and about 30 °C mean annual temperature. The soil belongs to the Selangor-Briah soil series association and is classified under the Typic Endoaquepts great subgroup having fine textured loamy clay soils, often acidic, derived from Entisols according to the USDA classification. The air-dried soil was then passed through a 2 mm sieve for the experiment as well as to determine the initial soil physical and chemical parameters.

For the analysis of the biological characteristics, the fresh soil sample was preserved at 4 °C. Sampled soil was poor in organic C level (1.43%) as well as in soil fertility.

Characteristics of organic amendments and preparation

Compost (CMP), green manure (*Gliricidia sepium*) (GLI), charged biochar (CBC), tea waste (TW), and tea waste biochar (RBC) were used as organic treatments. Compost was prepared using lawn cuttings and roadside grasses, and tea waste was pyrolyzed at 450 °C under limited oxygen supply for 4 h to produce RBC. Charged biochar was produced by treating the RBC with a nutrient solution containing cow urine, cow dung, sugar molasses, and water at 1:2:2:5 into 20 parts of biochar ratio. Standard protocols were followed to characterize soil (Table 1) and all amendments (Table 2). Briefly, pH and electrical conductivity (EC) of the soil were measured at soil:water suspension ratios of 1:2.5 and 1:5, respectively, using a pH meter (Model HI 2211, Hanna Instruments, Woonsocket, Rhode Island, USA) and electrical conductivity meter (BC3020, Trans Instruments, Singapore). Total C, N, and S in soil and organic amendments were measured using a TruSpec CNS analyzer (Leco, Saint Joseph, Michigan, USA). Organic C was determined based on the modified Walkley-Black oxidation method. To assess exchangeable K, Mg, and Ca, the soil was extracted in 1 M ammonium acetate (pH 7) extraction solution and analyzed using an atomic absorption spectrophotometer (AAS) (AAnalyst 400, PerkinElmer, Waltham, Massachusetts, USA). Soil S was determined using the same extraction followed by turbidity determination using the UV-visible spectrophotometer (Cary 50 Probe, Varian Inc., Palo Alto, California, USA) at 410 nm wavelength. The dry ashing method was followed to determine total P, K, Mg, and Ca in organic amendments. The extracted solution was read by AAS for K, Mg, Ca and P was measured at 882 nm wavelength using a UV-visible spectrophotometer. The NH_4-N and NO_3-N were extracted using 2 M KCl extraction solution and measured by an auto-analyzer (QuikChem FIA+, 8000 series, Lachat Instruments, Hach Company, Loveland, Colorado, USA). The chemicals used for all the analysis were 99.9% pure and analytical grade by Sigma Aldrich Chemie GmbH (Munich, Germany).

Table 1. Some physicochemical characteristics of the soil used. Values are the mean ± standard deviation (n = 3).

Soil property	Soil
Soil texture	Clay loam
Bulk density, g cm^{-3}	1.66
pH (H_2O)	3.56 ± 0.07
pH (KCl)	3.32 ± 0.04
pH ($CaCl_2$)	3.34 ± 0.03
Electrical conductivity, µS cm^{-1}	174.1 ± 8.4
Total C, %	1.87
Organic C, %	1.43
Total N %	0.12
Total S %	0.06
NH_4-N (KCl extractant), mg kg^{-1}	64 ± 3
NO_3-N (KCl extractant), mg kg^{-1}	45 ± 2
NH_4O Ac extractable K, mg kg^{-1}	64 ± 4
Available P (Bray), mg kg^{-1}	144 ± 5
Mg, mg kg^{-1}	73 ± 2
Ca, mg kg^{-1}	172 ± 11

Table 2. Major chemical characteristics of organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW) and raw biochar (RBC) used in the research.

Organic material	Total C	Total N	Total S	Total P	K	Mg	Ca	C/N	C/P	C/S	Moisture
	g/kg										%
CMP	234.3	11.7	1.7	3.4	3.9	0.9	2.0	20	32	65	12.90
GLI	478.0	41.2	3.0	2.3	21.2	2.2	10.3	12	208	159	77.20
CBC	642.7	6.6	1.2	4.9	29.8	2.5	4.1	97	131	536	39.80
TW	466.3	11.4	2.0	2.3	18.0	1.4	2.2	41	203	233	9.50
RBC	667.4	5.8	1.5	5.3	28.7	2.4	3.8	115	126	445	4.10

Soil incubation

The soil was incubated in the laboratory for 120-d with the prepared organic materials at 27-30 °C, 90%-95% humidity, under dark and aerobic conditions to study the N mineralization. Prepared 500 g soil was taken into an incubation flask (750 mL) and then distilled water was added to bring the soil to 55% of field capacity (Masunga et al., 2016). After 14 d, each of the jars received 5 mm sieved treatment materials at a rate of 270 kg N ha^{-1} (186 mg N kg^{-1}) and mixed thoroughly. Then all jars were airtight to prevent desiccation, leaving 1/3 of headspace for air circulation. Five organic amendments and control were triplicated in CRD. Incubation jars were maintained at a 55% water-filled pore space moisture level throughout the incubation period by adding water gravimetrically. Approximately 30 g soil samples were taken weekly for 42 d, then every other week for the next 42 d and finally once a month for analyzing available NH_4^+-N, NO_3^--N, PO_4-P, SO_4^{-2}-S, and pH. Finally, microbial biomass C and enzymes including urease, protease, acid phosphatase, and dehydrogenase activities were determined. Nitrogen mineralization was estimated based on NH_4^+-N and NO_3^--N contents. The net NH_4^+-N and NO_3^--N content at each sampling was determined by getting the difference between the initial values and the final values. Similarly, the net N released from organic material-amended soil was determined by subtracting the values of controls.

N Mineralization kinetics

Nitrogen mineralization data was fitted to three different kinetic models. Firstly, a zero-order model proposed by Addiscott (1983), a first-order model proposed by Stanford and Smith (1972), and a parallel first-order model proposed by Molina et al. (1990) were used to explain N mineralization kinetics:

$$N_{min} = N_0 + kt \quad (1)$$

$$N_{min} = N_0 (1 - e^{-kt}) \quad (2)$$

$$N_{min} = N_f (1 - e^{-kft}) + N_s (1 - e^{-kst}) \quad (3)$$

where N_{min} is the cumulative mineralized N (mg kg^{-1}) at time t (d), N_0 is the amount of total potentially mineralizable N (mg kg^{-1}), and k is the mineralization rate constant (d^{-1}), N_f and N_s denote the active or faster and resistant or slower mineralizable pools of N decomposing at specific rates of k_f and k_s. The sum of N_f and N_s is equivalent to N_0 in the first-order exponential model.

Microbial biomass C

Microbial biomass C (MBC) was determined using the method described by Sparling et al. (1990). Briefly, 20 g fresh soil was fumigated for 30 min with alcohol-free chloroform and then incubated for 10 d in the dark. Carbon was then recovered with 0.5 N K_2SO_4 and syringe filtered using a GF/F filter. In a 75 mL digestion tube, 10 mL filtrate and 10 mL 0.167 N $K_2Cr_2O_7$ were added followed by heating the mixture at 135 °C for 30 min with 20 mL concentrated H_2SO_4. After cooling, the content was volumed up to 75 mL with distilled H_2O and the absorbance was measured at 600 nm using a UV-vis spectrophotometer.

Soil enzyme activities

The activity of dehydrogenase (EC 1.1.1) was measured as described by Casida et al. (1964). Fresh soil was incubated with 3% 2,3,5-triphenyl tetrazolium chloride (TTC) in the presence of $CaCO_3$ under dark at 37 ºC for 24 h. Produced triphenyl formazan (TPF) was extracted with methanol and the optical density was measured at 485 nm. The activity of urease (EC 3.5.1.5) was determined according to Kandeler and Gerber (1988). Fresh soil was incubated with 0.08 M urea solution for 2 h at 37 °C. Then, produced NH_4^+-N was extracted with 1 N KCl to 0.01 N HCl and determined colorimetrically. The potential activity of protease was determined according to Ladd and Butler (1972). In brief, fresh soil was incubated with 5 mL 50 mM Tris-HCl buffer (pH 8.1) and 5 mL 2% sodium caseinate solution for 2 h at 50 °C. Produced tyrosine was extracted with an alkaline reagent and the absorbance at 700 nm wavelength was read after incubating with 33% Folin-Ciocalteu reagent for 1 h. Acid phosphatase activity (EC 3.1.3.2) was analyzed according to Tabatabai (1994). Soil was incubated with *p*-nitrophenyl phosphate (PNP) and toluene for 1 h at 37 °C in a modified universal buffer (MUB-pH 6.5) matrix. After the incubation, 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH were mixed into it and filtered. The optical density of the filtrate was measured at 420 nm.

Statistical analysis

The PROC GLM procedure was used to analyze net N mineralization, MBC, protease, urease, phosphatase, and dehydrogenase activities using SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). The means of treatments were compared using the least significant difference (LSD) technique when significant differences were found at a probability level (p) of 0.05. A nonlinear regression (NLR) and model fitting kinetics were performed using SigmaPlot 14 (Systat Software Inc., San Jose, California, USA) to study the N mineralization dynamics. Relationships between variables were tested by performing Pearson's correlation analysis.

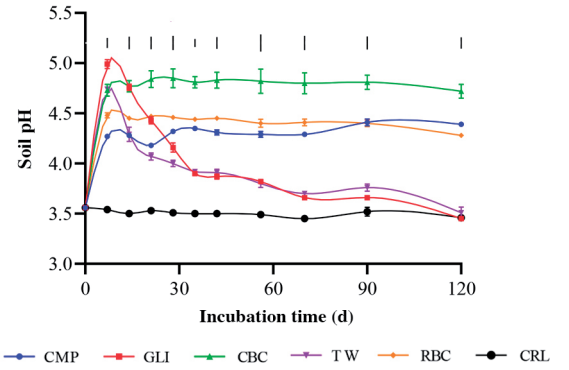
RESULTS AND DISCUSSION

Soil pH changes

Incorporation of organic amendments into the tea-growing soil significantly ($p < 0.05$) affected the soil pH throughout the incubation period of 120 d (Figure 1). All the organic amendment treatments showed increased soil pH compared to the non-amended soil samples, which is in agreement with Abera et al. (2012). Charged BC showed the highest pH throughout the incubation period, though GLI exceeded its pH at the very beginning of the incubation. Despite the sudden increase in pH in GLI amended soil initially, CBC remained at the highest level throughout the incubation period. *Gliricidia* amended soil exhibited a sharp increase in soil pH (4.99 ± 0.05) attributed to the ammonification taking place during the initial decomposition and reached the maximum within 10 d. Thereafter, it decreased gradually, reaching the pH of the unamended soil in 120 d in contrast to the findings of Abera et al. (2012). Rapid nitrification may reduce soil pH in GLI-amended soil (Liyanage et al., 2015). Tea waste also showed a similar pattern, but the maximum pH was less than GLI amounting to 4.73 ± 0.07. Compost, CBC, and RBC showed a similar pattern but were sequenced as CBC < RBC < CMP.

The results of this study were in agreement with the results of several studies that have shown the addition of organic manure caused an increase in the soil pH. Opala et al. (2012) observed the reduction of exchangeable acidity while increase in soil pH with the addition of farmyard manure (FYM) and *Tithonia* leaves with inorganic P sources. According to Abera et al. (2012), this pH increase could be due to the alkalinity of plant residues which produce base cations during hydroxylation of organic matter in their study with legume residue application under different moisture levels. Further, the addition of legume residue under different soil moisture regimes increased the pH rapidly due to the decarboxylation of organic anions from the added residue N during decomposition and/or ammonification.

Figure 1. Soil pH change with the incorporation of different organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW), raw biochar (RBC) and control (CRL) during 120 d incubation period.

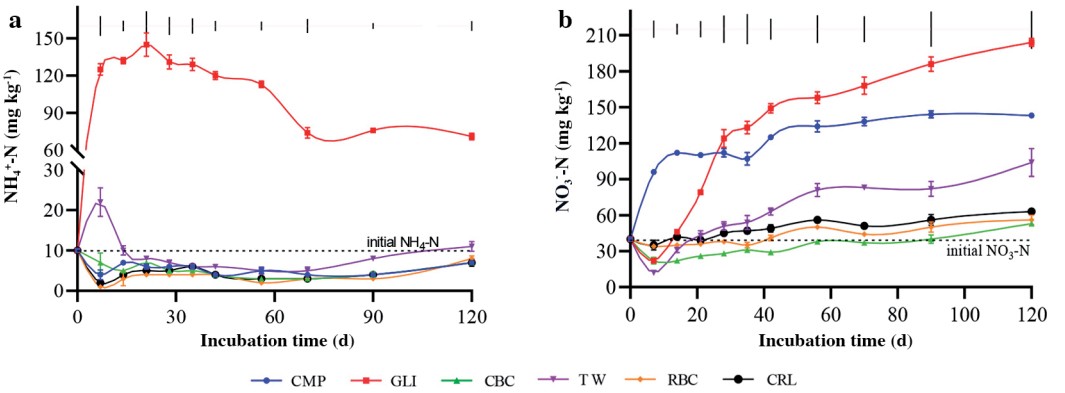


Vertical bars denote the level of significance based on LSD test at $p < 0.05$.

Soil N mineralization and immobilization

Ammonification. Ammonification is a microbe-mediated process that converts complex organic N to ammonium in the soil. Two different patterns of ammonification were observed when the different organic matters were added to the nutrient degraded tea-soil, where GLI showed a very high NH_4^+ release as compared to the other treatments (Figure 2a).

Figure 2. Ammonification (a) and nitrification (b) of different organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW), raw biochar (RBC) and control (CRL) after incorporation to the nutrient degraded tea-growing soil.



Vertical bars denote the level of significance based on LSD test at $p < 0.05$.

Gliricidia amended soil showed a sharp increase of NH_4^+-N within the first 7 d and reached its peak in 20 d incubation, amounting to 143 mg kg^{-1} following a gradual decrease thereafter. The increase of pH and availability of NH_4^+-N could induce the nitrifying bacteria, resulting in a gradual decrease of NH_4^+-N in the GLI amended soil. Rapid ammonification of GLI could be attributed to the robustness of the heterotrophic bacterial activities (Abera et al., 2012). Liyanage et al. (2021) highlighted that the greater N content and lower C/N ratio in GLI accelerates the decomposition and mineralization rapidly even just after incorporation to the soil. Both biochar materials had the lowest NH_4^+-N contents even after 120 d which could be due to the adsorption into biochar apart from the immobilization by microbes (Cheng et al., 2021).

However, all other organic treatments showed ammonification similar to or even below the unamended soil. The tea waste amended soil showed a minute increase of NH_4^+-N 14 d after the incubation and then decreased in 14 d following a similar trend as the unamended soil. In general, ammonification was started within 2 h of incubation and reached its maximum peak at 7 d after incubation depending on the characteristics of the organic matter amended and the soils (Baldi and Toselli, 2014). Though we added the same amounts of N from different organic amendments, the C/N ratio has a greater influence on ammonification (Masunga et al., 2016). The higher C/N ratio in GLI tended to favor microbial transformations. The added organic materials could be classified into two categories based on the observed ammonification patterns: organic matter that undergoes nitrification and organic matter that is subjected to immobilization (Marzi et al., 2020). Except for GLI, all other organic materials were subjected to N immobilization. After 3 mo of incubation, all treatments showed a slight increase in soil NH_4^+-N concentration which may be resulting in re-mineralization. A similar result was observed by Baldi and Toselli (2014) in their commercial organic fertilizer incubation experiment. However, depending on the prevailing adverse soil conditions, the ammonium mineralized from organic amendments might be trapped by soil components or temporarily immobilized by soil microbes, or further converted into nitrate, or even lost as $NH_{3(g)}$ (Azeez and van Averbeke, 2010).

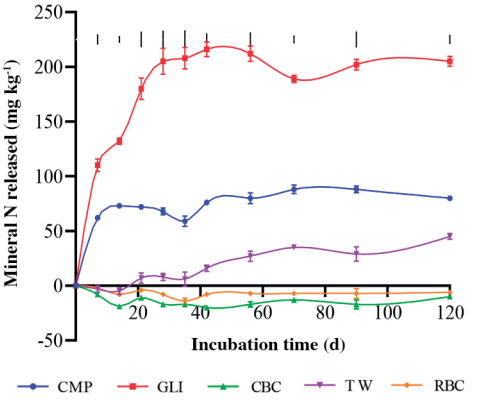
Nitrification. The translation of NH_4^+-N into NO_3^--N was varied significantly ($p < 0.05$) with both incubation time and organic material type (Figure 2b). Initial amounts of 2 N KCl extractable NO_3^--N present in GLI, RBC and TW were negligible, while CBC and CMP had 13 and 89 mg kg^{-1}, respectively. The initial higher NO_3^--N in CMP resulted in a sudden increase of NO_3^--N within the first 7 d as it is an already partially mineralized material. Fourteen days later, the NO_3^--N content in CMP was maintained at a constant rate throughout the incubation period. *Gliricidia* showed rapid nitrification, thus it dominated all the other treatments within the first month of incubation. The greater nitrification in GLI was obvious as it produced the highest NH_4^+-N content during incubation (Figure 2a). Tea waste also followed the same pattern but with lower intensity. Irrespective of the initial NO_3^--N content presented, except for CMP, all other organic amendments showed lower NO_3^--N than the unamended soil within the first 7 d. It illustrates the immobilization of NO_3-N, since the microbes received C source for their energy, resulting in competition for mineralized N. Then all

amended soils started to produce NO_3-N. However, the lowest nitrification was observed in CBC and RBC even below to the unamended soil throughout the experiment period. Higher NO_3^--N observed in unamended soil than CBC and RBC amended soils indicates the immobilization of N, thus creating an unavailability of inorganic N for plant growth particularly when added to such degraded tea-growing soil. Additionally, biochar can adsorb NO_3^- ions from the soil solution aggravating the debt of inorganic N. The predominant inorganic N form in all soil amendments was NO_3^--N (Figure 2b). Mostly, the nitrification was relatively greater and on the other hand, the immobilization of nitrate was low (Abera et al., 2012).

Net N mineralization. Net N mineralized from added organic amendments significantly ($p < 0.05$) varied with the type of material and with incubation time. *Gliricidia* amended soil had the highest mineralized N content whereas CBC showed the lowest mineralization depicting net negative mineralized N (Figure 3). Therefore, the proposed immobilization was highest in CBC amended soil, followed by the RBC amended soils. Tea waste amended soil showed net immobilization until 20 d incubation and thereafter it showed net positive mineralization. The value was, however, lower than GLI and CMP amended soils after 120 d of the incubation period. The fluctuations in this net mineralization with time might be due to the differential proliferation of microbes (population and types), their competition for resources, death of microbes and the subsequent release of earlier held-up N and soil as well as environmental factors (Azeez and van Averbeke, 2010). The ratios of C/N, C/P and N/P of GLI may promote these processes compared to other amendments and control. The increased C content of the other amendments could have enhanced the proliferation of microbes and subsequent use of all the N available for cell metabolism. Therefore, manures with higher C content provide more energy to microorganisms, which promotes their activity, thus utilizing more available N from the soil than released through mineralization processes. Interestingly, CBC showed the highest net immobilization of N, even below RBC despite the fortification of biochar with a solution containing cow dung and cow urine. This revealed that though it undergoes mineralization with increased microbial activities, the released ammonium and nitrates could be re-adsorbed into the porous structure of the biochar. Further, biochar has the capability of accelerating nitrification (Qayyum et al., 2012). Therefore, biochar could retain both NH_4^+-N and NO_3^--N but *via* different mechanisms. Additionally, molasses present in CBC provides readily available C for microbes resulting in increased microbial activities, which again demands more N. Moreover, abiotic immobilization of inorganic N through some chemical processes could also remove mineralized N particularly converting into organic N fraction without existing in microbes (Chen et al., 2014).

As nitrate is the dominant form of N in the soil, the pattern of net N mineralization is similar to that of NO_3^--N mineralization (Figures 2b and 3). It has been documented that generally, 97% of the mineral N present in the soil is mostly in the NO_3^--N form (Marzi et al., 2020). This implies that ammonification is a transitionary stage of the process in this soil and subsequently transformed into NO_3^--N rapidly (Azeez and van Averbeke, 2010). Though the key mineral N supposed to be NH_4^+-N, it undergoes quick immobilization and furthermore, when it is limited, microbes immobilize

Figure 3. Net N mineralization/immobilization of added organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW), raw biochar (RBC) and control (CRL) into the soil during 120 d of the incubation period.



Vertical bars denote the level of significance based on LSD test at $p < 0.05$.

NO_3^--N as well (Abera et al., 2012). Previous studies have confirmed that microbes selectively immobilize NH_4^+-N first and they utilize NO_3^--N after NH_4^+-N gets exhausted (Qayyum et al., 2012). In our study, we also observed similar trends in all treatments. *Gliricidia*, released the highest amount of N at the end of the 120 d incubation. In the case of *Gliricidia,* faster decomposition and mineralization rate was observed as 69% of its C get it mineralized within 90 d after incorporation into the soil (Liyanage et al., 2021). Garden compost had the second highest net N production and it provided mineralized N from the beginning of the incubation. Net N mineralized sequenced as GLI > CMP > TW > RBC > CBC.

Nitrogen mineralization from organic amendments is influenced mainly by the ratios of chemical components such as C to N, cellulose and hemicellulose, lignin, and lignin to N, and polyphenol to N ratios (Mohanty et al., 2011). Since *Gliricidia* has the lowest C/N and the lowest lignified compounds, it mineralized nearly 94% of added N, whereas CMP had 43% N release in which 17% of the mineralization was occurred during the prescribed incubation period. Tea waste is comparatively rich in polyphenolic and cellulose compounds thus, N released at the end of the incubation was 24%. The highest percentage of N released in GLI could also be supported by the priming effects of GLI on organic matter already present in the soil. The biocatalyst activity of GLI could further enhance this priming process. However, both RBC and CBC did not show mineralization; instead, both biochar types exhibited immobilization of 12% and 17%, respectively, during the tested period.

Nitrogen mineralization kinetics

Net N mineralization data was fitted into three models as zero order, first order (exponential) and parallel first-order (double exponential) kinetic models. Both GLI and CMP fitted well with the parallel first-order model with R^2 of 0.91 and 0.92, respectively, demonstrating two different N pools, namely the faster decomposing pool and the slower decomposing pool (Table 3). Two peaks observed in net N mineralization in GLI could be attributed to those two N pools. The first peak which was recorded around the 28 to 42 d period could be due to the readily decomposing N pools, which usually comprise amino acids and peptides. The second peak observed around 100 d after incubation may be due to the decomposition of the resistant pool, which could be affected by the complexity of the protein structure (Baldi and Toselli, 2014; Marzi et al., 2020). *Gliricidia* has a greater amount of faster decomposing organic N compared to CMP and TW. Since CMP is an already partially mineralized material, it has comparatively a higher slow degradable N pool compared to GLI. However, TW did not fit with any of the tested models, maybe due to the initial greater immobilization of mineral N but the release of inorganic N later. Additionally, CBC and RBC also fitted only with the zero-order model, yielding poor model-fitting parameters. The complexity of N mineralization, rapid immobilization and N adsorption into biochar may be the reasons for poor model-fitting data.

However, in contrast to the findings of Qayyum et al. (2012), we observed that none of the models tested were fitted with CBC and RBC data though the zero-order model showed very poor and ambiguous model fitting estimates. The mineralization rates of both biochar types showed negative rate constants. The predominant immobilization throughout the incubation period and thereby drastic reduction of the soil mineral N could produce negative rate constants. Similar results were also observed by Azeez and van Averbeke (2010) in the mineralization of cattle and goat manure.

Table 3. Nitrogen mineralization kinetics after incorporation of organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW) and raw biochar (RBC).

Organic material	Zero order $N_{min} = N_0 + kt$				First order $N_{min} = N_0 (1 - e^{-kt})$				Parallel first order $N_{min} = N_f (1 - e^{-kft}) + N_s (1 - e^{-kst})$					
	N_0	k	R^2	SEE	N_0	k	R^2	SEE	N_r	k_f	N_s	k_s	R^2	SEE
CMP	56.74	0.428	0.842	7.06	89.46	0.073	0.58	11.57	51.87	0.320	68.69	0.011	0.91	6.34
GLI	150.05	0.794	0.436	34.54	217.39	0.079	0.92	12.90	134.62	0.079	82.77	0.079	0.92	14.89
CBC	20.56	-0.125	0.705	3.10	-	-	-	-	-	-	-	-	-	-
TW	-0.72	0.515	0.869	7.64	-	0.001	0.87	7.66	-	0.001	-	0.001	0.87	8.84
RBC	10.03	-0.034	0.067	4.81	-	-	-	-	-	-	-	-	-	-

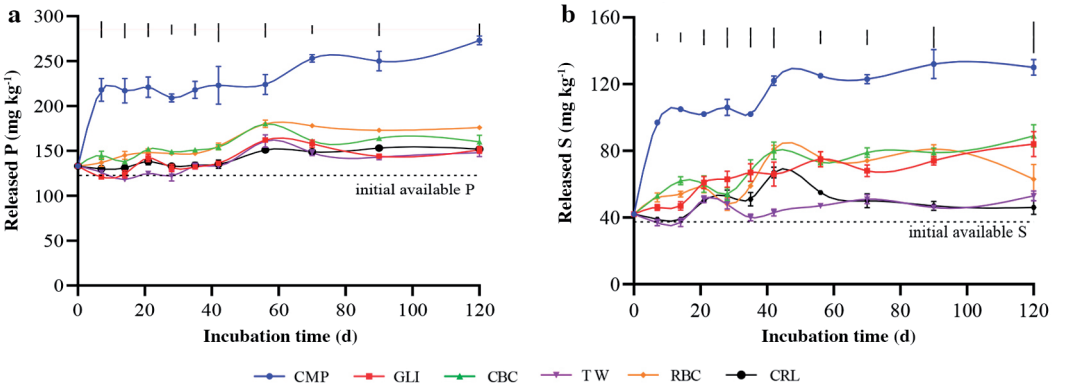
N_{min}: Cumulative mineralized N; N_0: total potentially mineralizable N; kt: mineralization rate constant at time t; N_f and N_s: active or faster and resistant or slower mineralizable pools of N decomposing at specific rates of k_f and k_s; SEE: standard error of estimate.

Soil P mineralization

Net P mineralization from added organic materials in soil differed significantly, where maximum P release (220%) was recorded with CMP followed by RBC (67%), and a minimum (11%) from *Gliricidia* (Figure 4a). In contrast, TW amended soil showed immobilization of available P of 9% when compared to unamended soil. The highest P recorded in CMP may be due to the lower C/P in the amendment and the higher initial P available in the partially mineralized material. During the initial composting, the organic P of readily decomposable organic matter gets mineralized producing inorganic phosphate ion species. Further, CMP could contain P-solubilizing bacteria thus the production of plant-available P could be increased (Erhunmwunse et al., 2019). This result was confirmed by an increase in phosphatase activity (Table 4). Additionally, increased proliferation of microbes under the higher availability of N in GLI might immobilize more P from the soil system. Comparatively, higher C/P and lower total P could be the other reasons for the lower P release in both GLI and TW. Irrespective of the added material, P mineralization remained at a similar level up to 45 d after incubation, and then it exhibited a marginal increasing trend up to 90-d incubation. Despite the higher total P content present in both CBC and RBC (Table 2), they released 23% and 67% P. The released P could be re-adsorbed into the biochar (Cheng et al., 2021) and immobilized by microbes too. Further, increased pH due to the addition of biochar may reduce the P solubilization through neutralization of organic acids. Immobilization and or adsorption of mineralized P were more prominent in CBC than in RBC, undoubtedly due to enhanced microbial activity.

Phosphorous mineralization is highly dynamic. The mineralized component of P could be bound back into organo-complexes apart from the immobilization of microbes (Jalali et al., 2014). Additionally, mineralized P could react with Ca/Mg carbonates or with Fe/Al depending on the pH and thus become unavailable. All these processes are reversible which may result in mineral P returning to the soil.

Figure 4. Changes in P (a) and S (b) mineralization after addition of organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW), raw biochar (RBC) and control (CRL) during 120 d of incubation.



Vertical bars denote the level of significance based on LSD test at $p < 0.05$.

Table 4. Soil microbial biomass C (MBC) and enzyme activities changes with different organic materials compost (CMP), *Gliricidia* (GLI), charged biochar (CBC), tea waste (TW), raw biochar (RBC) and control (CRL) after 120 d of incubation.

Organic material	MBC	Urease	Protease	Phosphatase	Dehydrogenase
	µg C g^{-1}	µg NH_4-N g^{-1} 2 h^{-1}	µg Tyr g^{-1} 2 h^{-1}	µg PNP g^{-1} h^{-1}	µg TPF g^{-1} 24 h^{-1}
CMP	181.6 ± 10.6bc	38 ± 4b	37.4 ± 5.9cd	201.7 ± 11.3a	4.3 ± 0.2b
GLI	251.0 ± 11.6a	51 ± 4a	59.9 ± 7.2b	156.1 ± 11.0bc	2.2 ± 0.2c
CBC	182.6 ± 14.4bc	56 ± 3a	90.2 ± 7.7a	176.4 ± 08.0ab	11.1 ± 1.0a
TW	224.5 ± 26.2ab	34 ± 1b	29.5 ± 5.5cd	185.6 ± 40.5a	9.6 ± 0.7a
RBC	136.7 ± 11.4cd	53 ± 3a	42.9 ± 6.3bc	138.4 ± 10.4c	4.4 ± 0.3b
CRL	93.9 ± 15.0 d	20 ± 1c	18.9 ± 5.2d	100.4 ± 09.5d	1.9 ± 0.2c

Soil weights are in dry weight basis, ± standard error of the mean (SEM).
Means with the same letters are not significantly different within the column according to LSD test at $P < 0.05$.
Tyr: Tyrosine; PNP: *p*-nitrophenyl phosphate; TPF: triphenyl formazan.

Soil S mineralization

The net S mineralization followed a similar pattern to that observed in soil P mineralization. Compost showed the highest S release as it contained a higher amount of mineralized S initially (Figure 4b). However, TW and CRL soils had the minimum S mineralization. Tea waste is rich in C thus having a higher energy source and S could be limited becoming immobilized into microbes. During the first 14 d of incubation, the trend of SO_4^2-S release from TW indicated a reduction of available S indicating a greater S immobilization. Charged biochar and *Gliricidia* showed a gradual increase of available S over time due to the continuous mineralization while other amendments exhibited a fluctuation. The process of mineralization of S from GLI was not interrupted may be due to the initial higher S content of 0.3% (Table 2). However, CBC also behaved similarly though it contained 0.12% of total S.

Soil microbial biomass changes

All organic amendments increased soil MBC by more than 45% over the unamended soil (Table 4). The highest increase of MBC was recorded from GLI amended soil (167%) followed by TW, which showed a 139% increase compared to the control. The application of CBC and CMP had a similar influence on MBC with a 93% increment. The response of MBC to the organic amendment is generally affected by C, N and their ratio of amendment type (Ren et al., 2019). A highly significant correlation was observed between MBC and soil organic C content ($r = 0.78^{**}$). *Gliricidia* has the highest N content and lowest C/N among tested organic materials, hence the proliferation of microorganisms is high resulting in the highest MBC. Further, the amount of readily degradable organic compounds present in the added organic material has a greater influence on MBC (Ren et al., 2019). This could be the reason for increasing the MBC in CBC compared to RBC, where CBC has been charged with sugar molasses which is rich in readily degradable organic compounds. Microbial biomass is a more sensitive parameter to any change in the soil environment than the total organic C. Therefore, as the mineralization proceeds, the MBC could be reduced with time. This could result in a reduced MBC in CMP over TW.

Soil enzyme activities changes

Enzyme activities varied widely among the treatments (Table 4). Soil protease activities ranged from approximately 19 ± 5 to 90 ± 8 μg Tyr g^{-1} 2 h^{-1}. The lowest protease activity was observed in the unamended soils, and interestingly, the highest was observed in CBC amended soil. There was a five-fold increase of soil protease activity in CBC compared to the unamended control. The CBC enriched with cow dung and cow urine which provides the substrate for the enzyme activities and could result the greater protease activity. *Gliricidia* recorded the protease activity as 60 ± 7 μg Tyr g^{-1} 2 h^{-1}, which is again rich in N compounds. A significant positive linear correlation existed between the urease and the protease activities ($r = 0.76^{*}$), meaning that both enzymes react together in N mineralization. Observed protease activities in our experiment are in line with those of Romillac et al. (2019) who compared different organic inputs on soil protease activity.

The urease enzyme catalyzes the hydrolysis of urea and widely used in the evaluation of changes in soil quality. The urease activity was changed with the application of different organic materials. The highest urease activity was found in the CBC amended soil, amounting to 56 ± 3 μg NH_4-N g^{-1} 2 h^{-1}, whereas unamended soil recorded the lowest as 20 ± 1 μg NH_4-N g^{-1} 2 h^{-1} dry soil (Table 4). However, the urease activities in CBC, RBC and GLI amended soils did not vary significantly at $p < 0.05$. As observed in the protease activity, the N enrichment in CBC during nutrient fortification may be the reason for higher urease activity in CBC. All the organic materials recorded significantly higher urease activity levels compared to the control (Table 4) indicating that any kind of increase in organic matter and thereby an increase in microbial population probably results in higher urease activity. A highly significant linear correlation existed between urease activity and soil pH ($r = 0.54^{*}$) confirming that the alkaline pH facilitates urease activity in the soil. Since the urease enzyme is produced by almost all microbial groups as well as some plant roots also as intracellular and extracellular, the N dynamics in the soil are highly influenced by the urease enzyme as it increases the ammoniacal N concentration (Follmer, 2008).

Acid phosphatase activity was the lowest in unamended soil, and it was the highest in compost amended soil (Table 4) ranging from 100 to 201 PNP μg g^{-1} h^{-1} because CMP was already partially mineralized, the initial higher P content may have been the cause of higher phosphatase activity. Similarly, TW also recorded the second highest phosphatase activity and did not vary significantly from CMP. Application of CBC increased phosphatase activity by 75% of when compared to the unamended soil. Further, GLI recorded a 55% increase in enzyme activity compared to the control, which is similar to the mineralization of *Leucaena leucocephala*. Phosphatase activity was significantly correlated ($r = 0.53^{*}$) with the available phosphate released during mineralization.

Soil dehydrogenase (DH) activity reflects the soil's metabolic capacity as it is an intracellular enzyme in microbes. The CBC creates a favorable microclimate for microbes to proliferate comfortably and increase DH activity subsequently (Lehmann et al., 2011). Next to CBC, TW recorded the highest DH activity since it is also rich in labile C (Liyanage et al., 2021). In contrast to our observations of increased DH activity with the addition of biochar, some negative impacts on DH were also recorded depending on the pH, pyrolyzing temperature, and raw materials used to produce biochar. They reported that damages caused by the addition of biochar to microbes and the adsorption of labile C into the porous structure may be the causes of reduced DH activity (Hazrati et al., 2021). The increased DH activity indicates the oxidative capacity of soil microbes, especially in biochar amended soil. However, the addition of GLI had nonsignificant impact on DH activity, which could be due to the low C/N ratio thus the dehydrogenation (transferring of H atoms from organic compounds to electron accepters) component of the mineralization process may have already been completed within 120 d resulting in the low availability of C for microbes.

CONCLUSIONS

Each organic amendment has different N mineralization rates and patterns; thus, recommendations should be material specific. *Gliricidia*, garden compost and tea waste amendments released N by 94%, 43%, and 24%, respectively. Both tea waste biochar without charging (RBC) and charged tea waste biochar (CBC) exhibited 12% and 17% of N immobilization, resulting in the reduction of N availability to plant uptake; therefore, biochar cannot be incorporated into soil to improve the soil fertility in short term. Despites the initial immobilization of N in CBC, the results of all enzyme activities and microbial biomass C suggest that the application of CBC has remarkable benefits in improving soil quality in fertility degraded soils.

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