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Nutrient dynamics and assessment of nitrogen-fixing bacteria during vermicomposting of leaf litter of neem (*Azadirachta indica*) using two epigeic earthworms

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Abstract

The soil is a natural dynamic body with properties derived from combined effects of climate and biotic activity. It is a porous medium made up of minerals, water, chemicals, organic matter, and micro-organisms which supports plant growth and development. On the other side, the leaf litter from the plants upon decomposition release nutrients and helps in nutrient recycling. In nature, leaf litter is processed by soil organisms of which earthworms are one of the major contributors in nutrient cycling. In the present study, an attempt has been made to study the nutrient dynamics while vermicomposting neem leaf litter employing two epigeic earthworm species, *Eisenia fetida* and *Eudrilus eugeniae*. In addition, an assessment has been made on the presence and quantification of the nitrogen (N_2) fixing bacteria during the vermicomposting of leaf litter. From this study, it was found that the nutrients were higher in the earthworm worked leaf litter than the control. It was also found that the nutrients were higher on the 30th day from the start of the vermicomposting process, and tend to reduce by the 60th day with further fall on the 90th day.

Key words: Earthworms, vermicomposting, nutrients, neem, *Azadirachta indica*

Introduction

Soil is a system comprised of various components in different proportions that supports plant growth. But the system is not as simple as it seems, so there arises a need to study the dynamic aspect of the soil. Soil supports different types of vegetation. Litter fall is an essential mechanism from vegetation to soil for the flow of organic matter as well as for nutrients. The main biogeochemical cycling processes are plant uptake of nutrients and their return by litter fall, stem flow, and through fall (Switzer and Nelson, 1972). Litter fall preserve soil fertility and increase agricultural productivity. Fertilizers have allowed faster growth on less crop area compared to without use of fertilizers; thus, they are important key elements in agricultural production worldwide. Agricultural growth will be highly demanding as the population grows and fertilizers have a crucial role (Gellings and Parmenter, 2004). However, overuse of pesticides and fertilizers, particularly in fruits and vegetable plants, results in residue above levels of safety (Agnihotri, 1999) and in the long run has negative effects on the fertility of soil. Therefore, the need for natural processes/ products for enhancing the plant growth becomes essential. The process/ product should be in such an entity that its production or application would not cause any negative impact on the environment and be in harmony with nature. As evident and established by now, vermicomposting is one such process. Vermicomposting of commonly available neem leaf litter using two earthworm species *Eisenia fetida* and *Eudrilus eugeniae* has been reported earlier (Gajalakshmi and Abbasi, 2004 and Nayeem-Shah *et al.*, 2015). In the present study, an attempt has been made to understand the pattern of nutrient dynamics in terms of macro and micronutrients of the soil during vermicomposting of neem litter. In addition, presence and enumeration of nitrogen fixing bacteria during the vermicomposting process has been investigated.

Materials and methods

Soil samples were collected within Pondicherry University campus, Puducherry, where neem (*Azadirachta indica*) plantation is more prevalent. A sampling plot of 1 x 1 m was marked in these sites and the soil samples were collected from the surface and subsurface at depths of 0-5, 5-10 and 15-30 cm, respectively. A stainless steel scoop was used for collecting the samples. The soil samples were made to pass through > 2 mm sieve for uniformity before using for the experiment. Leaf fall from the trees was collected using litter mesh. A 4 x 3 feet mesh was tied to 1 feet tall sticks to make a 4x3 feet square segment for the collection of leaf litter. Leaf litter collection was done for a month and quantified by weight and used for the experiment. The experiments were made through three different trials *viz.,* (i) Soil with neem leaf litter (Azll + S) (ii) 100 g leaf litter mixed with the soil and inoculated with the earthworm *Eudrilus eugeniae* (Azll + S + Ee) (iii) 100 g leaf litter mixed with the soil and inoculated with the earthworm *Eisenia fetida* (Azll + S + Ef). A reactor with only soil was maintained as control. The experiments were done in triplicates. The earthworms used for the study were collected from the cow dung fed culture maintained in the laboratory. All the reactors were kept at room temperature under shade. At the start of the experiment and every 30 days, the earthworms were weighed and counted. This quantification study helped in understanding the health status and the growth of the worms in the vermireactor. The vermicast, composted material, released

by earthworms was harvested every 30 days after the start of the reactor for three months. The vermicast was sieved and processed to analyse nutrient profile. Carbon, nitrogen contents and elemental analysis was done by using the CHNS analyser and Wave Length Dispersive X-Ray Fluorescence Spectrometer (WD-XRF), respectively. Soil from the control reactor was collected, sieved with 0.075 mm mesh sieve. About 2 g of each soil sample was added with 0.2 g of crystalline boric acid and completely ground with mortar and pestle into a fine powder for the elemental analysis.

The microbial study was made to assess the vermicompost for its plant growth-promoting ability by testing the presence of nitrogen fixing bacteria. One gram of control soil, vermicompost from *E. fetida* and *E.eugeniae* were weighed and suspended in 100 mL of sterile distilled water in 3 different conical flasks. The suspension was well mixed. 1 mL of each suspended solution was diluted with 9 mL of sterile distilled water and serially diluted up to to 10^{-5} dilutions and plated on the Jensen's medium (Jensen, 1942) by pour plate technique. All the inoculated plates were incubated for 72 hours at room temperature. The same was repeated for all the samples collected at the end of three harvests. The nitrogen-fixing bacteria grown in Jensen's medium were recorded and compared.

Statistical analysis: The results of the data were statistically evaluated and analyzed through one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, respectively. They were evaluated by Graph Pad Prism software (version 5.0) and the results are expressed as Mean ± SD n=3 all groups and value of $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ were considered for the significance and interpretation of the results.

Results and discussion

Quantification of zoomass: The earthworm mass of *E. eugeniae* and *E. fetida* was measured using the physical balance and recorded the worm weight periodically at every 30 days interval up to 90 days. The results (Table 1) indicate that the earthworms were healthy and actively feeding on the neem leaf litter. The increase in the zoomass clearly reveals that the worms have stabilized themselves in the substrate provided and were able to reproduce too. The cocoons were also spotted from the 60th day.

Table 1. Zoomass of *Eisenia fetida* and *Eudrilus eugeniae* during vermicomposting process

Mass of the worms at the start of the process	Mass of the worms during the vermicomposting process (g)		
	30th day	60th day	90th day
Eisenia fetida			
20.6	21.1	27.5	39.6
21.5	19.6	28.4	40.4
20.4	20.4	27.6	39.9
Eudrilus eugeniae			
22.4	18.0	28.5	39.9
23.4	16.9	28.4	39.8
21.5	16.9	26.7	38.1

Nutrient dynamics in the neem leaf litter compost: Before the start of the experiment, the substrates were analysed for elemental composition with respect to organic carbon, macronutrients and micronutrients and the results are presented in Table 2.

Table 2. Nutrient profile of the natural soil and leaf litter before the start of the experiment. Values are mean of three replicates ± standard error

Soil nutrients	Control soil	*A. indica*-leaf (powdered) (Mean ± SD)
C (%)	2.02 ± 0.05	42.46 ± 0.19
N (%)	0.18 ± 0.01	2.80 ± 0.06
P (%)	0.11 ± 0.00	1.42 ± 0.01
K (%)	4.53 ± 0.07	4.80 ± 1.06
Ca (%)	3.08 ± 0.06	5.51 ± 1.35
Mg (%)	0.91 ± 0.02	3.56 ± 0.12
Fe (%)	18.37 ± 0.57	7.68 ± 0.17
Mn (%)	0.42 ± 0.03	0.39 ± 0.01
C:N	10.85 ± 0.86	15.14 ± 0.30

The soil nutrients were found to be more in the neem leaf litter powder than the control soil, except for Fe and Mn. The nutrient profile of the composted leaf litter materials included C, N, P, K, Ca, Mg, Fe, Na, Zn, and Mn which were analysed every 30 days up to 90 days. The nutrients tended to be higher on the 30th day and got reduced on the 60th and 90th day. The reduction on the 90th day may be attributed to less availability of the substrate for the earthworms. The elemental composition of the control soil, soil with leaf litter, and soil with leaf litter and earthworms is given in Table 3. Comparatively, *E. fetida* showed better degradation efficiency of neem leaf litter than *E. eugeniae*.

Total organic carbon was higher in all the reactors than the control soil. The maximum increase of organic carbon was recorded in reactors inoculated with earthworms *viz.,* 5.29 and 5.54 % in Azll+S+Ee and Azll+S+Ef, respectively, on 30th day. This was followed by decrease in carbon as the days progressed on the 60th and 90th days. The decrease in carbon content is due to the respiratory function and assimilation process of the microbial biomass as well as the earthworms (Garg and Kaushik, 2005; Elvira *et al.,* 1998). In case of reactors with leaf litter and without earthworms, the carbon increased up to 60th day, but there was a fall on the 90th day whereas in control, there was increase of carbon from the 30th day to 90th day.

The macro and micronutrient composition of the control soil, reactors with only leaf litter and reactors with soil amended with leaf litter and earthworms are given in Table 3. All the macronutrients were significantly enriched in the soil with leaf litter than the control soil. There was a net increase in total N contents in all reactors. Compared to the initial phase, nitrogen showed a rise in the initial 30 days followed by a decrease towards 90 days. The total N content at the end of 90 days was 0.16 % in control soil, 0.26 % in reactors with *E. fetida* and 0.32 % in reactors with *E. eugeniae.* It can be seen that there was high nitrogen content from four to five folds increase in earthworm worked soil than in control. This may be attributed to augmentation of the nitrogenous excretory products, mucus, body fluids and enzymes of the microbial biomass and the earthworms onto the soil (Suthar, 2007).

The trend of phosphorous and potassium content was similar to N except in few instances. The P content increased on 30th and 60th days in all the reactors (Table 3). On the 90th day, except control

Table 3. Macro and micronutrients of the soil in different experiments. All values are represented in %.

Treatment	Sampling day	C	N	P	K	Ca	Mg	Cu	Fe	Mn	Na	Zn
Control soil	30th day	1.20±0.08	0.11±0.02	0.10±0.06	1.20±0.01	0.91±0.08	0.21±0.08	0.04±0.02	9.67±0.41	0.17±0.03	0.27±0.02	0.04±0.02
	60th day	1.39±0.03	0.14±0.03	0.11±0.02	1.23±0.02	0.94±0.06	0.22±0.02	0.04±0.04	9.63±0.39	0.16±0.02	0.26±0.02	0.03±0.01
	90th day	1.45±0.34	0.16±0.02	0.16±0.00	1.37±0.02	0.95±0.11	0.24±0.00	0.04±0.01	9.22±0.18	0.15±0.06	0.25±.0.01	0.02±0.02
Az LL+S	30th day	1.32±0.55	0.22±0.2	0.12±0.01	1.06±0.00**	0.51±0.01*	0.22±0.01	0.06±0.01	15.57±1.39***	0.24±0.04	0.28±0.08	0.02±0.04
	60th day	2.42±0.12**	0.29±0.03**	0.14±0.02	1.15±0.03**	1.04±0.05	0.19±0.01	0.04±0.03	12.26±1.08**	0.20±0.02	0.21±0.01	0.02±0.01
	90th day	1.29±0.08	0.13±0.01	0.12±0.01	1.03±0.03***	0.62±0.05	0.18±0.03	0.02±0.02	9.17±1.02***	0.13±0.03	0.19±0.02	0.01±0.01
AzLL+S+Ec	30th day	5.29±0.16***	0.47±0.06**	0.14±0.04	2.39±0.06***	3.74±0.23***	0.49±0.02***	0.04±0.01	10.74±0.05	0.21±0.09	0.29±0.08	0.05±0.02
	60th day	4.83±0.49***	0.41±0.03***	0.15±0.04	2.16±0.03***	3.13±0.13***	0.46±0.00***	0.04±0.02	10.45±0.04	0.20±0.01	0.27±0.04	0.03±0.01
	90th day	3.07±0.36**	0.26±0.03**	0.14±0.05	2.03±0.07***	2.53±0.31***	0.41±0.05***	0.04±0.02	9.99±0.15***	0.17±0.06	0.26±0.05	0.03±0.01
AzLL+S+Ef	30th day	5.54±0.18***	0.57±0.02**	0.13±0.02	2.06±0.03***	3.84±0.11***	0.51±0.06***	0.05±0.02	10.69±0.34	0.19±0.02	0.30±0.01	0.04±0.06
	60th day	4.75±0.37***	0.49±0.04***	0.14±0.03	2.49±0.01***	3.17±0.05***	0.48±0.08***	0.04±0.01	10.12±0.26	0.18±0.04	0.28±0.01	0.03±0.02
	90th day	3.46±0.51***	0.32±0.04***	0.14±0.04	2.38±0.02***	2.63±0.20***	0.41±0.04***	0.04±0.02	9.58±0.16*	0.16±0.03	0.28±0.09	0.03±0.01

Values are expressed as Mean±SD, n=3, One way ANOVA followed by DMRT. *, **and *** indicates *P* value < 0.05, 0.01 and 0.001, respectively *vs* control soil.

there was fall in phosphorous in reactors with/ without earthworms. It is generally reported that earthworms have a great impact on phosphorous mineralization due to the presence of phosphate solubilizing microorganisms (Le Bayon and Binet, 2006). However in this study, the phosphorous content was less in almost all the reactors. In case of potassium, the difference in the trend was only in the case of reactor with *E. fetida* where there was a fall on the 60th day but there was increase in all other reactors. On the 90th day, there was increase in potassium in control and reactors with soil augmented with leaf litter; whereas there was fall in reactors with leaf litter and both species of earthworms. The potassium content was double in worm processed soil than the control.

With respect to secondary elements, calcium fell exactly in line with the same pattern as that of potassium whereas magnesium content decreased in all the reactors and the control (Table 3). Calcium was seven to eight times higher in worm worked soil than the control and four times higher in reactor with leaf litter but no earthworms on the 30th day, marking the contribution of the microorganism in the soil. This increase is related to the catalytic activity of the enzymes in calciferous glands of earthworms generating calcium carbonate on the fixation of carbon dioxide (Padmavathiamma *et al.*, 2008). Magnesium was two folds higher in reactors with earthworms than the control on all the days of sampling. It is interesting to note that the micronutrients copper, iron, manganese, sodium and zinc followed the same pattern of continuous decrease from the 30th day to the 90th day in all the reactors including the control (Table 3). There was no significant difference in the worm processed soil and the control soil on all the days with respect to copper, iron, manganese and sodium; but in case of zinc, its content was two to three times greater in earthworm processed soil compared to control and reactor with leaf litter and soil but no earthworms.

The C: N is a key indicator of whether net mineralization or immobilisation will occur during decomposition. But since litter mass had C: N levels of less than 30:1, it can be concluded that the mineralization occurred during litter decomposition. The C: N ratio in soil in reactors with *E. eugeniae* was 11.48 and this is higher than in reactor with *E. fetida* (9.92) on the 90th day of decomposition.

Nitrogen-fixing bacteria: The leaf litter degradation is facilitated by the microorganism in the earthworms gut by the mutual interplay between earthworms and microbes (Edwards and Fletcher, 1988). Although earthworms consume fungi with organic substances to meet their protein and nitrogen requirements, the fungal population transform the vermicast to almost equal or better than the preliminary substrates (Edwards and Bohlen, 1996). The micro-organisms not only mineralize complicated materials but also synthesize biologically active substances that are useful for plant growth. In this study, soil samples and vermicast from all reactors were tested for nitrogen-fixing bacteria during the composting of leaf litter of neem. The total nitrogen-fixing bacteria were enumerated using Jensens medium. There were "too numerous to count" of nitrogen-fixing bacteria in the dilutions 10^{-3} and 10^{-4} on 30th day. The N_2 fixing population gradually decreased to 45 x 10^{-3} and 36 x 10^{-4} in *E. eugeniae* generated vermicompost and 47 x 10^{-3} and 41 x 10^{-4} in vermicompost derived from *E. fetida* in the 10^{-3} and 10^{-4} dilutions on 60th day, respectively. The count further reduced to 33 x 10^{-3} and 28 x 10^{-4} in *E. eugeniae* based vermicompost and 30 x 10^{-3} and 25 x 10^{-4} in vermicompost of *E. fetida* in the 10^{-3} and 10^{-4} dilutions on the 90th day samples, respectively. The earthworms secrete mucus in order to maintain moisture of its body surface and this mucus has a great influence in colonization of nitrogen fixing bacteria in the vermibeds (Satchell, 1967).

The vermicomposting of neem leaf litter employing two epigeic earthworm species *viz., E. fetida* and *E. eugeniae* showed better nutrient profile through an increase of the macro nutrients and micronutrients in the worm processed material than in the control. This study indicates that the nutrient release is higher during the initial 30 days of vermicomposting the neem leaf litter. This study also reveals the presence of higher nitrogen fixing bacterial population in the worm worked neem

leaf litter substrate than the soil control.The findings of this study would provide solution for effective conversion of neem leaf letter into nutrient rich organic manure towards sustainable agriculture.

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