Activities of Effective Microorganism (EM) on the Nutrient Dynamics of Different Organic Materials Applied to Soil

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Abstract: A 56 days incubation study was set up to investigate the effects of combined application of effective microorganism (EM) with composted or fresh organic materials on soil nutrient dynamics. Treatments include; Water (W) as Control, EM, Kraal manure (KM) + W, KM + EM, Lawn clippings (LC) + W, LC+EM, Commercial compost (CC) + W and CC + EM. A CO₂ efflux study was also set up with the same treatments. The evolution of CO₂-C was significantly (P>0.05) higher with the application of lawn clippings and kraal manure compared to the commercial compost. Combined application of EM with organic materials led to a 24% higher C loss after 56 days of incubation compared with sole application of EM. The decomposition rate constant (k) was significantly (P>0.05) higher when fresh organic materials were applied compared to commercial compost, While the application of EM further increased the rate constant. Net mineralization of N was 51% and 99% higher with the integrated application of EM with kraal manure and lawn clippings respectively; compared to their sole application. While net mineralization of N was reduced by 30.1% where EM was applied with commercial compost compared with sole application of commercial compost. Integrated use of EM with organic materials as soil amendments, stimulated the mineralization of nutrients, but its application with Fresh organic material was more beneficial than with compost. Sole application of EM to soil resulted in immobilization of N and slight mineralization of the soil organic matter pool.

Key words: Effective microorganism • Organic materials • Compost • Mineral N • Soil nutrients

INTRODUCTION

The use of Effective microorganism (EM) as amendment in agriculture, especially for soil health and control of plant disease has been reported by some authors [1-3]. EM is a liquid microbial inoculant that contains assorted culture of beneficial fermentative microorganism such as lactic acid bacteria (Lactobacillus Spp), yeast (Saccharomyces spp), photosynthetic bacteria (Rhodopseudomonas spp), actinomycetes and fermenting fungi [4, 3]. Application of EM has been reported to result in rapid proliferation of its constituent beneficial microorganism and subsequent suppression of soil borne pathogens. The beneficial organisms have also been ascribed with the ability to encourage the mineralization of soil organic matter [5], which is the main mechanism through which EM could benefit soil health and plant nutrition. The use of EM as soil amendment could play an important role in plant nutrition in the organic agriculture movement as well as under the integrated soil fertility system. Since the sole use of mineral fertilizer has been reported to lack the capacity to sustain productivity under the continuous intensive cropping system found in most commercial farms [6]. Long term use of mineral fertilizer without organic amendments has been reported to result in reduction in soil base saturation and increased acidity over [7], while the application of organic material has been reported to improve soil physical properties, nutrient supply and crop yield over time [8]. The application of organic materials only as source of nutrient for crop is often not effective in the short run due to their low nutrient content and the time required for decomposition and mineralization to take place naturally. Yet, continuous application is known to yield some benefits in the long run.
The combined use of organic materials with EM inoculums has been reported as a way to stimulate quick decomposition and mineralization of nutrient from applied organic amendments [3, 2]. The mechanism of EM activities for rapid nutrient release from organic amendment involves the rapid proliferation of its “effective and beneficial” microorganism content within the soil system. This results in consumption of C, N and other nutrient elements by the microbes and their subsequent release for plant use. This depicts the known microbial immobilization and mineralization of nutrients for plant use. The duration of this phenomenon in a system will either allow or debar the utility of organic materials as source of plant nutrients in the short-run. The duration is known to be affected by the chemical characteristics of the organic materials viz., C:N, Lignin, polyphenols, total N and climatic variables such as moisture, temperature, relative humidity etc [9-11]. Thus, there is a need to determine the immobilization/mineralization time lag, when EM is applied with conventional organic materials. This will provide information that could be used to synchronize the time of organic materials and EM application with that of plant nutrient need.

The use of EM as soil amendment for crop production has been observed among some commercial farmers in South Africa. But, the effectiveness of this emerging practice to crop productivity in South Africa has not been reported. A preliminary field study at the University of Fort Hare did not detect substantial contribution to crop yield by the recommended application of EM in combination with commercial compost. This was attributed to the low quality C constituent in the compost used, which is typical of most matured compost. Composted organic wastes are low in soluble C and may not be able to effectively support proliferation of decomposer community.

Our study was set up to examine the effects of EM application on the dynamics of nutrient release from fresh organic waste compared with composted materials applied to soil.

**MATERIAL AND METHODS**

**Incubation Study:** A 52 days incubation experiment was conducted at the University of Fort Hare (UFH), Alice, South Africa. The study was a factorial experiment, laid out in randomized complete block design. Two factors considered were; (a) Organic amendments at three levels viz., kraal manure, lawn clippings and commercial compost (b) application of effective microorganism (EM) at two levels i.e. EM applied and EM not applied. The factorial combinations resulted in the following treatments, (a) Soil only [Control]; (b) Soil + EM; (c) Soil + Kraal manure; (d) Soil + Kraal manure + EM; (e) Soil + Lawn clippings; (f) Soil + Lawn Clippings + EM; (g) Soil + Commercial Compost; (h) Soil + Commercial compost +EM.

The soil used for the study was collected at depth (0-15 cm) from a frequently cropped field at the UFH teaching and research farm. The soils of the area was classified as Dystric Regosols or Cambisols according to the USDA soil classification system [30]. The chemical properties of the soil include; pH = 6.12, EC = 89.8 uS/cm, Organic C = 1.09 g/kg, NO3-N = 1.23g/kg, NH4-N = 21.36 g/kg, Total N = 0.079g/kg, available P = 0.425 g/kg and exchangeable K = 0.5 g/kg. Kraal manure was collected from the goat rearing section of the UFH research farm. The lawn clippings were from the UFH cricket field. The EM stock solution was procured from EMROSA PYT, a commercial EM marketer in South Africa. The physico-chemical characteristics of the organic materials are presented in Table 1.

The soil were air-dried and sieved through a 2mm mesh. One hundred gram of air dried soil was weighed into 350ml PVC bottles. The organic materials were oven dried at 65°C and crushed in a hammer mill fitted with 1mm mesh. The materials were applied to the soil at the rate of 50 t ha⁻¹ (w/w basis). Stock solution of EM was diluted in distilled water at ratio 1: 500 [12] and applied to the EM designated experimental units at 80% of the soil field capacity. Water was added to other treatments at the same moisture level. The mixtures were kept in plastic bottles with slightly closed lids in such a way that exchange of gas was possible to prevent anaerobia. The treatments were incubated at 30°C, with regular maintenance of moisture content at 80% field capacity by occasional supplementary application of water and EM solution after moisture determination. The treatments were replicated three times and destructive sampling was done.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Kraal Manure</th>
<th>Lawn clippings</th>
<th>Commercial compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.97</td>
<td>6.69</td>
<td>4.33</td>
</tr>
<tr>
<td>EC (us/cm)</td>
<td>2.23</td>
<td>2.70</td>
<td>2.37</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>38.5</td>
<td>29.9</td>
<td>37.6</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>3.02</td>
<td>3.89</td>
<td>1.92</td>
</tr>
<tr>
<td>Total P (g/kg)</td>
<td>1.07</td>
<td>1.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Total K (g/kg)</td>
<td>0.45</td>
<td>0.60</td>
<td>0.04</td>
</tr>
<tr>
<td>Polyphenol (%)</td>
<td>-</td>
<td>0.7</td>
<td>0.98</td>
</tr>
</tbody>
</table>
at 0, 1, 2, 4 and 8 weeks of incubation. A portion of each sample was stored at 4°C in a refrigerator and the others were air dried for chemical analysis. The soil organic C, pH, EC, total N, available P and exchangeable K were determined in the dry samples, while mineral N (NH₄-N + NO₃-N) were determined in the wet samples.

**Soil Analysis:** Soil pH and EC were measured in water at ratio 1:2.5 [13]. Total N was determined colorimetrically from the digest of sulphuric acid, selenium powder and salicylic acid [13]. Organic carbon was determined in the soil sample by complete oxidation with sulphuric acid and aqueous dichromate mixture. The digest were then back titrated for the residual potassium dichromate with ferrous ammonium sulphate [13]. Mineral N (NH₄-N and NO₃-N) was extracted from the wet soil samples by shaking with a 0.5 M K₂SO₄ solution at the ratio of 1:10 (w/v) for one hour and filtered through Whatman 42 filter paper. [13]. The nitrate concentration was determined in an aliquot of the filtrate, after the development of a yellow colour using 5% salicylic acid in concentrated sulphuric acid. The absorbance was read on a spectrophotometer and concentration calculated against known standards. The ammonium concentration was also determined in an aliquot of the filtrate on a spectrophotometer after the development of a blue colour using sodium nitroprusside as described by [13]. Available P was determined colorimetrically using the molybdate blue method in AMBIC-2 extract [14].

**Carbon Mineralization Study:** A carbon mineralization study was set up with the same treatments along side with the incubation experiment. The treatment mixtures were placed in a Kilner jar, with a beaker containing 100ml of 1N NaOH placed in the middle of the jar to trap the mineralized CO₂ from the mixture.

The jars were sealed air-tight and incubated at 30°C for 56 days. The beakers with NaOH were retrieved daily in the first week and subsequently on weekly basis. At each retrieval time, the jars were left open for up to one hour to prevent the development of anaerobic condition. The amount of CO₂-C trapped was determined by back titrating the unused NaOH with 1N HCl after the addition of 5ml BaCl₂(to precipitate the carbonates) and 3 drops of phenolphthalein indicator.

The net CO₂-C was obtained by subtracting the value of the control from that of the treatments, using the relationship reported by [15].

\[
\text{CO}_2 = (B-V) \text{ NE}
\]

Where:
- B = Volume of HCl used to titrate NaOH in the beaker to the end point of the control
- V = Volume of acid used to titrate NaOH in the beaker to the end point
- N = Normality of the acid.
- E = Equivalent weight (to express data as C, E=6, while as CO₂ = E= 22)

The mineralized CO₂-C recorded for the first week was the cumulative value obtained daily for that week.

**Calculations and Statistical Analysis:** The apparent loss of added organic C was estimated by subtracting the total CO₂-C released in a given treatment from the control. The estimated percentage C remaining in the treatments with organic material was fitted to a simple exponential decay function reported by [31] to calculate the decomposition rate constant (k). The equations have coefficient of determination (R²) which ranged from 0.85 to 0.99.

The decomposition of organic materials follows a first order kinetic equation:

\[
\frac{dy}{dt} = ky
\]

On integration the equation yields

\[
y = Y_0 e^{kt}
\]

y = proportion of C remaining.
- t = Time
- k = decomposition rate constant

All data set were subjected to an F-test for factorial analysis using the generalized linear model, means were separated using LSD at (0.05) [29].

**RESULTS**

**Carbon Dioxide Evolution:** Figure 1 showed the CO₂ fluxes in the treated and control soil across the incubation period.

Highest peak was observed after day 1 of incubation while the emission decreased till day 14 after which there was another peak at day 28 of incubation (Figure 1a). The CO₂-C fluxes from soil with organic materials were
Fig. 1: The effects of different organic materials and EM application on CO$_2$-C fluxes during a 56 day incubation period. Bars represent LSD(0.05)

Fig. 2: Influence of organic materials and EM on the cumulative CO$_2$-C released from incubated soil. Bars represent LSD (0.05)
Fig. 3: Relative loss of the total C after a 56 days incubation period (a) from application of different organic materials (B) from application of EM. Bars represent LSD (0.05).

Fig. 4: Changes in NH₄-N, NO₃-N and Mineral N content of soil treated with various organic materials and EM during the 56 day incubation.
consistently higher at all sampling time compared with the control (Soil only). Application of commercial compost resulted in significantly (P>0.05) lower CO$_2$-C fluxes at the day 7 and 56 of incubation compared to lawn clippings and kraal manure. Similar trend was observed among the organic materials when EM was applied, although, the CO$_2$-C fluxes were significantly higher (P>0.05) at all sampling period with addition of EM compared to sole application of the organic materials (Figure 1b).

The cumulative percentage of C released, from the total C added to soil by the treatments (EM + organic materials) are shown in figure 2. Total C released were significantly (P>0.05) different at all sampling period between the control (Soil only) and treatment with organic material. At day 56 of incubation the application of kraal manure resulted in 10.24% increase in the cumulative amount of C released compared to the commercial compost (figure 2a). At day 56 of incubation, application of EM also resulted in 24% higher cumulative C (Figure 2b). Application of EM to soil only resulted in 44% increase in cumulative C release at day 56, while kraal manure had 21%, lawn clippings 8% and commercial compost 38%.

The relative loss of added C varied significantly (P>0.05) among the organic matter treatment (Figure 3a). The percentage C loss from the initial among the organic materials treatment ranged between 18% – 27%. Application of lawn clippings resulted in 27% loss of the initial C which was significantly (P>0.05) higher than other treatments. Application of EM also led to significant (P>0.05) increase in the loss of added C.

The decomposition rate constant ($k$) varied significantly (P>0.05) among the organic materials applied (Table 2). Rates were higher in the lawn clippings treated soils ($k$= 0.000327) than in the kraal manure and commercial compost treated soil (0.000217 and 0.000268). The decomposition rate constant was slightly higher where EM and organic materials were jointly applied to soil than sole application of organic materials; Kraal manure + EM increased by 8%, LC + EM 12% and CC + EM 27%. Application of EM to soil resulted in reduced rates constant (0.000189) compared with soil only without EM (0.000329).

### Nitrogen Mineralization from the Added Organic Materials:

Immobilization of N was observed in all treatments from day 1 till day 14 of incubation, with the exception of control treatment where mineralization of N was observed from day 1 till day 7 of incubation. (Figure 4). Net mineralization of N increased with the application of organic materials; Kraal manure had 113% higher mineral N, lawn clippings 106% and commercial compost 110% relative to the control (Table 3). The application of EM also had significant (P>0.05) positive effect on the net mineralization of N. The application of EM with Kraal manure and lawn clippings resulted in 51% and 99% respective higher net mineralized N than the sole application of the organic materials. This was in contrast to the observed 30.1% reduction in net mineral N when commercial compost was applied with EM compared with sole application of the commercial compost (Table3).

Application of organic material and EM had significant effect on the accumulation of NO$_3$-N (i.e. nitrification) in the soil. Rapid nitrification was observed in soils treated with lawn clippings and commercial compost, although the nitrification was short lived (between day14 - 28 of incubation) while the kraal manure treated soil took off slowly from day 14 and lasted till day 56 of incubation. Thus nitrification was significantly

### Table 2: The decomposition rate constant ($-k$) of different organic materials combined with or without EM during the 56 days incubation

<table>
<thead>
<tr>
<th>Organic material</th>
<th>Without</th>
<th>With EM</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.000329</td>
<td>0.000189</td>
<td>0.000259</td>
</tr>
<tr>
<td>Soil + Kraal manure</td>
<td>0.000208</td>
<td>0.000226</td>
<td>0.000217</td>
</tr>
<tr>
<td>Soil + Lawn Clippings</td>
<td>0.000306</td>
<td>0.000348</td>
<td>0.000327</td>
</tr>
<tr>
<td>Soil + Commercial Compost</td>
<td>0.000226</td>
<td>0.000310</td>
<td>0.000268</td>
</tr>
<tr>
<td>Mean</td>
<td>0.000267</td>
<td>0.0002683</td>
<td></td>
</tr>
</tbody>
</table>

LSD (0.05): Organic materials, 0.0000545; EM application, 0.0003; EM x Organic materials, 0.0000345

### Table 3: Net nitrogen mineralization and net nitrification (mg/kg) of soil amended which different organic material and EM after 56 days of incubation

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Net mineralization</th>
<th>Net nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic materials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>soil</td>
<td>0.030</td>
<td>0.0128</td>
</tr>
<tr>
<td>Soil + Kraal manure</td>
<td>0.064</td>
<td>0.0195</td>
</tr>
<tr>
<td>Soil + Lawn Clippings</td>
<td>0.062</td>
<td>0.0055</td>
</tr>
<tr>
<td>Soil + Commercial Compost</td>
<td>0.063</td>
<td>0.0027</td>
</tr>
<tr>
<td>LSD</td>
<td>0.0385 ns</td>
<td>0.0122***</td>
</tr>
<tr>
<td>EM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not applied</td>
<td>0.047</td>
<td>0.0094</td>
</tr>
<tr>
<td>Applied</td>
<td>0.063</td>
<td>0.0108</td>
</tr>
<tr>
<td>LSD</td>
<td>0.0272***</td>
<td>0.005**</td>
</tr>
</tbody>
</table>
Table 4: Coefficient of determination ($R^2$) of the simple linear regression relating some independent variables with biochemical characteristics of a soil treated with organic materials and EM

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>pH</th>
<th>EC</th>
<th>Total N</th>
<th>Organic C</th>
<th>Available P</th>
<th>Mineral N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ fluxes</td>
<td>-0.634***</td>
<td>0.067***</td>
<td>0.256***</td>
<td>0.450***</td>
<td>0.702***</td>
<td>-0.340***</td>
</tr>
<tr>
<td>Decomposition rate constant (k)</td>
<td>0.058</td>
<td>0.193***</td>
<td>0.270***</td>
<td>-0.184***</td>
<td>0.420***</td>
<td>0.042</td>
</tr>
<tr>
<td>Net N mineralization</td>
<td>0.063</td>
<td>0.181***</td>
<td>0.063</td>
<td>0.399***</td>
<td>0.084</td>
<td>-0.310***</td>
</tr>
<tr>
<td>Net nitrification</td>
<td>-0.006</td>
<td>-0.194***</td>
<td>-0.276</td>
<td>0.166***</td>
<td>-0.235</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Note *** = Significant at P>0.01, ** = Significant at P>0.05, * = Significant at P>0.10

DISCUSSION

Mineralization of Organic Carbon: Results from our study showed that the amendment soil with all the organic materials used in this study positively influenced C mineralization at all sampling period. The observed early climax (day 1-3) for CO$_2$-C efflux was due to the priming effects of the added organic materials, which is known to occur at the active phase of organic C release [16, 17]. The difference in carbon mineralization between the control and soils treated with organic materials at day 14 and day 42 of incubation could also be attributed to the biochemical properties of the added organic materials viz., C: N ratio, polyphenol, lignin etc. These properties are known to regulate the decomposition and mineralization of organic materials added to soil and could determine the contribution of such material to plant nutrition in the short-run [18]. Furthermore, we speculated that the lower efflux of CO$_2$-C from the commercial compost treated soils, compared with kraal manure and lawn clippings are due to rapid mineralization of the readily hydrolysable C in the fresh materials; this is known to be low in stable compost. Application of EM in turn improved CO$_2$-C efflux in all treatments mainly due to an integration of its beneficial microorganism content [3]. This resulted in further breakdown of the organic materials and mineralization of their nutrient content; similar observation was reported by [19]. The higher percentage increase in cumulative C released when EM was applied to Soil + EM (44%) and Commercial compost + EM (38%) compared with Kraal manure + EM (21%) and lawn clippings + EM (8%) gives an indication of the potentials of EM application to stimulate the mineralization of the more recalcitrant C in the stable organic matter pool of the soil and commercial compost. [17] reported that the mineralization of the non-readily hydrolysable C in the soil might be independent of the added organic materials and such could become slowly available to the microorganisms [20].

The observed significant difference in decomposition rate constant (k) for soil with organic materials was related to the availability of hydrolysable C and other chemical characteristics of the materials [17]. Higher k values observed with application of lawn clippings was attributed to its C and N constituent that was higher compared to other organic materials used in the study. The general effects of EM application on decomposition rate was low; however, the observed substantial increase with application of individual organic material gives further clue to the relative effect of EM on degradation of the recalcitrant fraction of organic matter. The 27% increase in k observed when commercial compost + EM was applied compared with Kraal manure + EM and lawn clippings + EM implied that EM led to degradation of the more stable organic C pool. The observed reduction of k where EM was applied to soil only could be attributed to lack of sufficient easily hydrolysable C pool. But much more, it depict the slow pace for degradation of the soil organic matter pool, since the cumulative respired C was higher at the close of the study. Similar observation was reported by [21].
Mineralization and Nitrification of N: Results from our study showed that N mineralization and amonification had similar trend and were closely related up till day 14 of incubation, this observation agreed with other reports [22, 17, 23]. The higher mineral N from the application of kraal manure, lawn clippings and their separate combination with EM, further confirm the suitability of fresh organic materials as a more suitable soil amendment to be combined with EM, compared to the commercial compost with a more stable biochemical properties. Moreover, these materials also exhibited a high degree of ammonification concurrently with mineralization; which signified the occurrence of N immobilization and rapid remineralization over time [17]. The application of EM increased amonification and mineralization of N across the sampling period suggesting that its application stimulated the proliferation of wide range of microbial groups including the ammonium producers.

The net mineralization of N among the applied organic materials were not significantly different from one another, but substantially higher in other treatments than the control, this showed that the organic N content of the applied materials contributed to N availability in the soil. Other factors as the soil C:N ratio, soil macro fauna, soil microbial activities and soil pH has been reported to contribute to N mineralization when organic materials are added to the soil [24, 17].

The observed higher nitrification with the application of Kraal manure, could be attributed to the quality of the added C. [25] reported a strong interaction between heterotrophic microorganism and the nitrifiers when materials with rapidly hydrolysable C are applied to the soil. Our result on the net nitrification in the soil only treatment compared with commercial compost and lawn clippings, further confirm that nitrification could be constrained by the application of materials with high content of slowly hydrolysable C and high C:N ratio [24].

Relationship Between Measured Variables: The characteristic of the organic materials added in our study had significant relationship with the chemical properties of the soil. The observed correlation of the CO₂-C fluxes and pH indicated the role of soil properties on C mineralization from the added organic materials, an increase in pH of the soil has been reported to favor C and N mineralization. [26]. Increase in pH could also be enhanced by the initial ammonification process which was due to the addition of organic materials and EM. The observed relationship of CO₂-C efflux to the C and total N has been reported by many authors [19, 27, 17].

The $k$ values have been observed by many authors to correlate well with the biochemical properties of the added organic materials [9, 18, 28]. The observed relationship with $k$ was brought about by the properties of the treatment mixtures and the heterogeneity of the decomposing microbes which was further enhanced by the application of EM [28].

In conclusion, our results showed that the decomposition and mineralization of the added organic materials to soil, was influenced positively by the application of EM. Although, the magnitude of effects was small considering the total organic pool but substantial (27%) when considered as proportion of the applied organic material. The quality of the applied C in the material also plays a major role in the effectiveness of EM as a soil amendment. While the fresh organic materials could supply higher quantity of easily hydrolysable C amenable to quick mineralization, the composted materials contains more of stable C which degrades slowly, although the labile mineral N content of the later could be higher. Application of EM to soil only may not be effective in the short run as the rate of decomposition of the stable organic pool will be low. However, the residual effect of consistent application with fresh organic material over seasons could be a sustainable way to recycle organic nutrient.

Further studies are required to evaluate the effectiveness of EM on different soil type and climatic conditions. A long term study (2-5 years) may be necessary to validate the potentials of EM as a veritable tool for organic agriculture crop production in South Africa.

REFERENCES


