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Changes in biochemical properties of cow manure during processing by earthworms (*Eisenia andrei*, Bouché) and the effects on seedling growth

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Summary

The biochemical changes in fresh cow manure caused by the earthworm *Eisenia andrei* (Bouché) were measured over a period of four months, under controlled laboratory conditions. Earthworms were introduced into each of four plastic containers (0.4×0.27×0.15 m) containing fresh cow manure (2500 g), and four containers containing manure but without earthworms served as controls. Earthworms reduced the pH and decreased the moisture content in the manure. The C:N ratio of the manure with or without earthworms decreased progressively from 36 to 21. The ash and total nitrogen contents increased greatly for a few weeks after the introduction of earthworms, reflecting a rapid breakdown of carbon compounds and mineralization of nitrogen by the earthworms. CO_2 evolution decreased rapidly (44 %) one week after the introduction of earthworms, and continued at a lower rate throughout the 17 weeks (51 % reduction as compared to 22 % without earthworms), indicating increasing stability of the organic matter. Earthworms reduced microbial biomass early in the process, but enhanced nitrogen mineralization and increased the rates of conversion of ammonium-nitrogen into nitrate. The major general effect of earthworms on the organic wastes was to accelerate the maturation of the organic wastes as demonstrated by enhanced growth of lettuce and tomato seedlings.

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Introduction

The ability of some earthworm species to consume a wide range of organic residues such as sewage sludge, animal wastes, crop residues, and industrial refuse has been well-established (Mitchell et al. 1980; Edwards et al. 1985; Chan & Griffiths 1988; Hartenstein & Bisesi 1989). The earthworm species most commonly utilized for the breakdown of organic wastes are *Eisenia fetida* (Savigny) and its related species *Eisenia andrei* (Bouché). Their biology and main environmental requirements have been studied extensively (Hartenstein et al. 1979; Kaplan et al. 1980; Reinecke & Venter 1987; Venter & Reinecke 1988; Reinecke & Viljoen 1990; Haimi 1990; van Gestel et al. 1992; Domínguez & Edwards 1997). These two species are prolific, have a wide temperature tolerance, and can grow and reproduce well in many kinds of organic wastes with a wide range of moisture content.

Over the last few years, as regulations for field application and disposal of animal manure has become more rigorous, the interest in using earthworms as an ecologically sound system for manure management has increased tremendously. Various researchers have examined the potential utilization of earthworm-processed wastes, commonly referred to as vermicomposts, in the horticultural and agricultural industries. Whether used as soil additives or as components of horticultural media, vermicomposts usually enhanced seedling growth and development, and increased productivity of a wide variety of crops (Edwards & Burrows 1988; Wilson & Carlile 1989; Mba 1996; Buckerfield & Webster 1998; Edwards 1998; Subler et al. 1998; Atiyeh et al. 1999). Enhancement in plant growth and productivity has been attributed to the physical and chemical characteristics of the processed materials. Vermicomposts are finely-divided peat-like materials with high porosity, aeration, drainage, and water-holding capacity (Edwards & Burrows 1988). They have a large surface area, providing strong absorption capability and retention of nutrients (Shi-wei & Fu-zhen 1991). They contain nutrients in forms that are readily taken up by the plants such as nitrates, exchangeable phosphorus, and soluble potassium, calcium, and magnesium (Orozco et al. 1996).

Most of the research on utilization of earthworms in waste management has focused on the final product, i.e. the vermicompost. There are only few literature references that have looked into the process, or examined the biochemical transformations that are brought about by the action of earthworms as they fragment the organic matter, resulting in the formation of a vermicompost with physicochemical and biological properties which seem to be superior for plant growth to those of the parent material. It has been reported that the storage of organic wastes over a period of time could alter the biochemistry of the organic matter and could eventually lead to the stabilization of the organic waste (Levi-Minzi et al. 1986). Nevertheless, we hypothesize that adding earthworms to the organic wastes would accelerate the stabilization of these wastes in terms of decomposition and mineralization of the organic matter, leading to a more suitable medium for plant growth.

The main objective of this experiment was to monitor the primary changes in the biochemical properties of cow manure during processing by earthworms (vermicomposting) under controlled environmental conditions. To be compatible with agricultural uses and to avoid possible adverse effects on plant growth, organic wastes should be transformed into a humus-like material and be sufficiently stabilized for plant growth (Saviozzi et al. 1988). Accordingly, we grew lettuce and tomato plants in the vermicomposted material to assess the suitability of the product as a plant growth medium.

Materials and Methods

Recently-deposited cow manure was collected from the Waterman Dairy Farm at the Ohio State University, Ohio. Approximately 2500 g of the manure (77 % moisture content) was placed into each of 8 plastic containers (0.4 × 0.27 × 0.15 m). Earthworms, *Eisenia andrei*, were added into four of the containers, while the other four containers without earthworms served as controls. The number of earthworms introduced initially was 250 per container (ca. 65g), including juveniles and adults. All containers were covered with lids perforated for aeration, and maintained in the laboratory at 24±2°C for a period of four months. At intervals of 1, 2, 4, 6, 8, 10, 14, and 17 weeks, 100 g of manure was removed from each container, mixed carefully, and analyzed to determine moisture and ash content, pH, C:N ratio, rate of biological activity (basal respiration), and nitrogen concentrations (total N, microbial biomass N, ammonium-N, and nitrate-N). The earthworms were removed from each sample by hand sorting and returned to their corresponding containers. The moisture contents of samples of manure were determined by drying at 60°C for 3 days and the ash contents by heating at 550°C for four hours. The pH was recorded from a suspension of the material in double-distilled water, in the ratio of 1:10 (w:v), after one hour of incubation. Total nitrogen and C:N ratios were measured on oven-dried (60°C) and ball-milled subsamples using a Carlo Erba NA 1500 C/N analyzer. Microbial activity was assessed by measuring the rate of CO_2 evolution from the sample during a 24-hour incubation. The evolved CO_2 was trapped in 0.05 M NaOH and subsequently measured by titration with HCl to a phenolphthalein endpoint, after adding excess $BaCl_2$ (Anderson 1982). Microbial biomass N was assessed using a chloroform fumigation-direct extraction method (Brookes et al. 1985). Mineral N concentrations (NH_4-N and NO_3-N) were determined colorimetrically in 0.5 M K_2SO_4 extracts in the ratio of 1:10 (w:v) manure to extractant, using the modified indophenol blue technique (Sims et al. 1995) with a Bio-Tek EL211sx automated microplate reader.

After four months, the vermicomposted manure was used in plant growth bioassays, to provide an index of maturity and utility of the organic waste. Rates of growth of lettuce and tomato seedlings in the vermicomposted manure were compared with those of plants grown either in the control manure with no earthworms or in a commercial potting medium that is commonly used in horticulture (Pro-Mix®, by Premier Co.). The plant growth bioassays were done in the Biological Sciences Greenhouses at the Ohio State University. In the first bioassay, lettuce seeds ('Grand Rapids TBR') were grown in each of three plug trays (30 plugs/tray) containing either vermicomposted manure, control manure, or Pro-Mix. The trays were irrigated daily with water. The lettuce plants were grown for nine weeks after which the average plant heights, the length of the longest root, the root and shoot dry weights, as well as root to shoot ratios were determined. In the second growth trial, tomato seeds ('Red Cherry Large') were sown into each of 20 small pots (5.6 cm in diameter) containing vermicomposted manure, control manure, or Pro-Mix. Half the pots were watered daily with a full strength fertilizer solution (Hoagland solution) (Hoagland & Arnon 1947) to eliminate nutrient limitations. The other half received water only, containing no fertilizer. All pots were watered to saturation (i.e. until water leached from the bottom of the pot). Plants receiving the fertilizer solution grew faster than those receiving water only, so the fertilized tomato seedlings were sampled earlier (five weeks after germination) than the unfertilized ones (twelve weeks after germination). At weekly intervals, the heights, numbers of leaves, and stem diameters of the seedlings were recorded. At the end of the growth period, the leaf area, root and shoot dry weights, as well as root to shoot ratios of the tomato plants were measured.

Data were analyzed statistically using a one and two-factor ANOVA in a general linear model (GLM) using SAS (SAS Institute Inc. 1990). The means of each of the biochemical parameters at each sampling date were adjusted for multiple comparisons and were separated statistically using Dunnett's multiple range test with the manure without earthworms set as the control. For the plant growth bioassays, Tukey's multiple range test was used to determine significant differences in plant growth among the different media. Significance was defined as $P \leq 0.05$, unless otherwise indicated.

Results

Biochemical changes in the substrate

The moisture contents of the manure increased with time, irrespective of the presence or absence of earthworms (Fig. 1). However, the percentage increase was less with

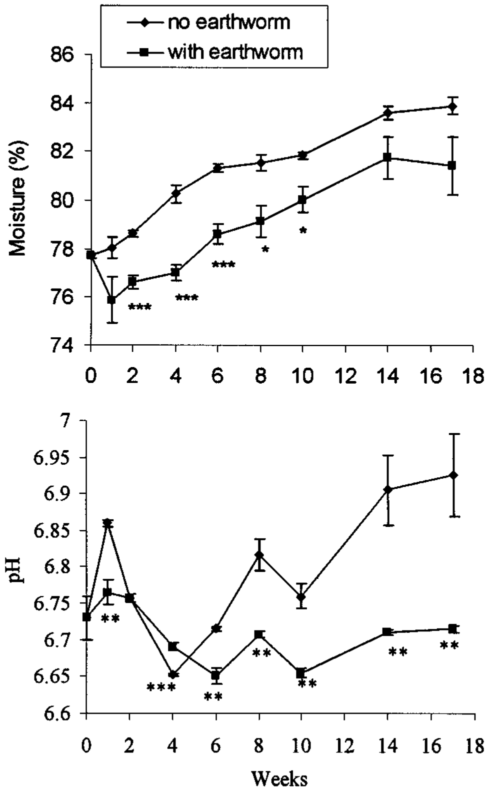


Fig. 1. Changes in the moisture content (%) and pH value of cow manure in presence and absence of *Eisenia andrei* (mean ± standard error). Means within the same date followed by *, **, and *** are significantly different at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

earthworms (from 77 % to 81 %) than without them (from 77 % to 84 %) although the differences were not statistically significant.

There were only very slight changes in pH values in both treatments (Fig. 1). The pH values in earthworm containers decreased slightly after week 4, whereas with no earthworms, the pH value of the manure increased and moved towards neutrality. The differences in pH resulting from addition of earthworms were highly significant over the whole period of the experiment.

The C:N ratio, one of the most widely used indices for maturity of organic wastes, decreased progressively with or without earthworms from 36 to 21 (Fig. 2). There was no difference in the C:N ratios between the two treatments after 17 weeks. This overall decrease in the C:N ratio was associated with an increase in total nitrogen during the four months of study (Fig. 2). However between week 2 and week 10, the decreases in the C:N ratio and the increases in total nitrogen were significantly greater in the manure with earthworms.

There was no difference in the ash content between the two treatments after 17 weeks (Fig. 3). The ash contents in both treatments increased significantly with time from 6.7 % to 12 %. However, earlier in the process (week one and week 6), ear-

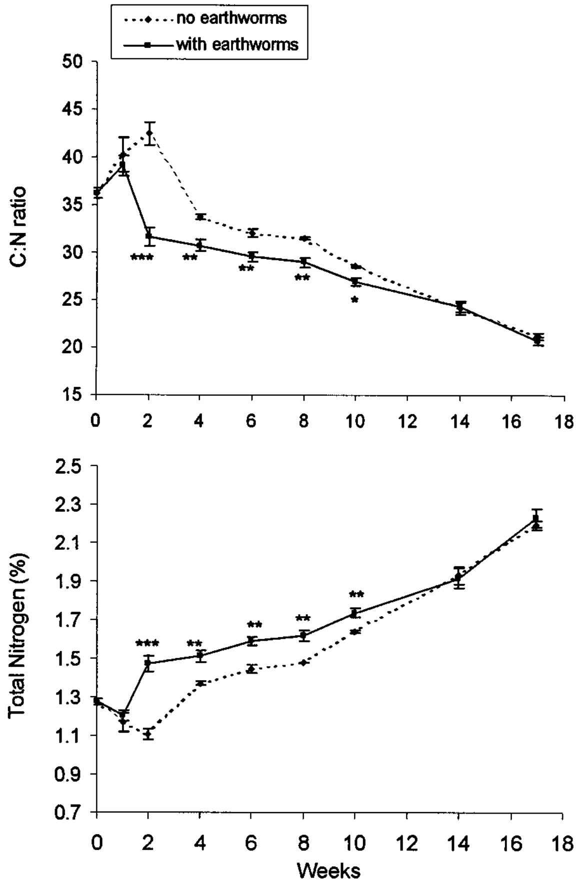


Fig. 2. Changes in the C:N ratio and total nitrogen content of cow manure in presence and absence of *Eisenia andrei* (mean ± standard error). Means within the same date followed by *, **, and *** are significantly different at P≤0.05, P≤0.01and P≤0.001, respectively.

thworms increased the ash content of the manure at times but only by 5 % (week one) and 8 % (week 6).

Respiration rates decreased rapidly (44 %) one-week after the introduction of earthworms (Fig. 4). The trend of decreasing CO_2 evolution in the manure with earthworms continued throughout the 17 weeks (51 % reduction as compared to 22 % without earthworms), indicating greater stability of the organic matter exposed to earthworms in time.

Microbial biomass nitrogen (Fig. 5) increased progressively in both treatments,

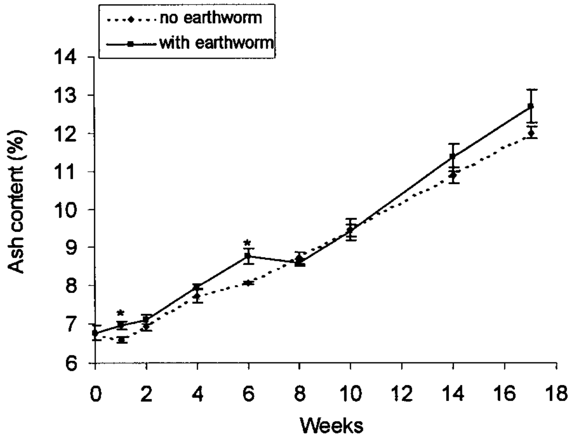


Fig. 3. Changes in ash content of cow manure in presence and absence of *Eisenia andrei* (mean ± standard error). Means within the same date followed by * are significantly different at P≤0.05.

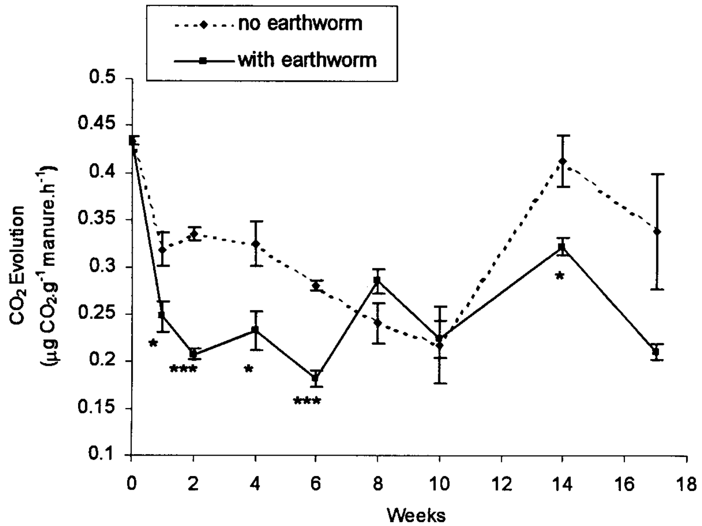
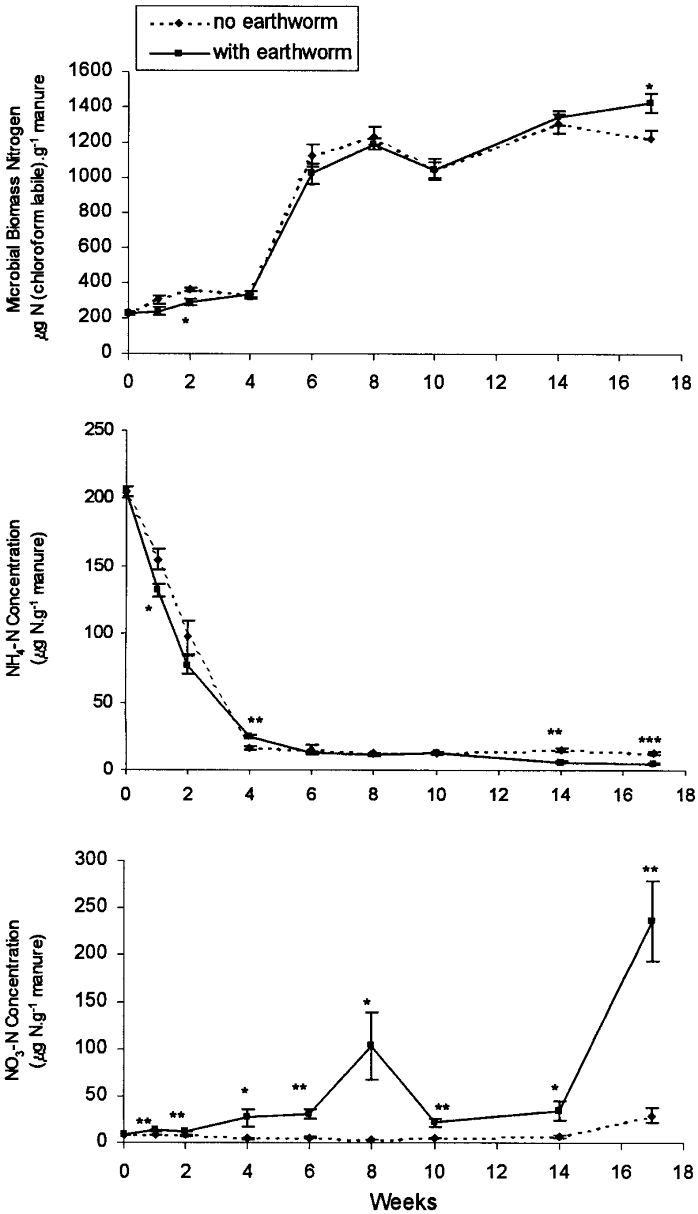


Fig. 4. Rate of CO2 evolution from cow manure in presence and absence of *Eisenia andrei* (mean ± standard error). Means within the same date followed by *, and *** are significantly different at P≤0.05 and P≤0.001, respectively.

from 226 µg.g-1 initially to 1225 µg.g-1 (without earthworms) and 1428 µg.g-1 (with earthworms). Although by week 17 earthworm introductions resulted in a greater microbial biomass, the earthworms decreased the microbial biomass nitrogen earlier in the process.

The ammonium-nitrogen concentration (Fig. 5) decreased rapidly in both treatments within the first four weeks, dropping from initial levels of 200 µg.g-1 to about 16 µg.g-1 and 25 µg.g-1 both without and with earthworms, respectively. The earth-

Fig. 5. Changes in microbial biomass nitrogen, ammonium-nitrogen, and nitrate-nitrogen concentrations in cow manure in presence and absence of *Eisenia andrei* (mean ± standard error). Means within the same date followed by *, **, and *** are significantly different at P<0.05, P<0.01,and P<0.001, respectively



worm introductions caused a more rapid loss of ammonium – nitrogen (1.5 fold over control) after week 1. The concentrations of ammonium did not change between week 6 and week 17. However in the manure with earthworms, ammonium concentrations continued to decrease, reaching 5 µg.g^{-1} after 17 weeks compared to 13 µg.g^{-1} with no earthworms. Concentrations of nitrate-nitrogen (Fig. 5) were significantly more in the manure with earthworms, throughout the whole experimental period. Earthworms increased the nitrate-nitrogen concentration 28 fold after 17 weeks compared to only a 3-fold increase in the control. The largest nitrate concentrations occurred in week 8 and week 17, in treatments with earthworms.

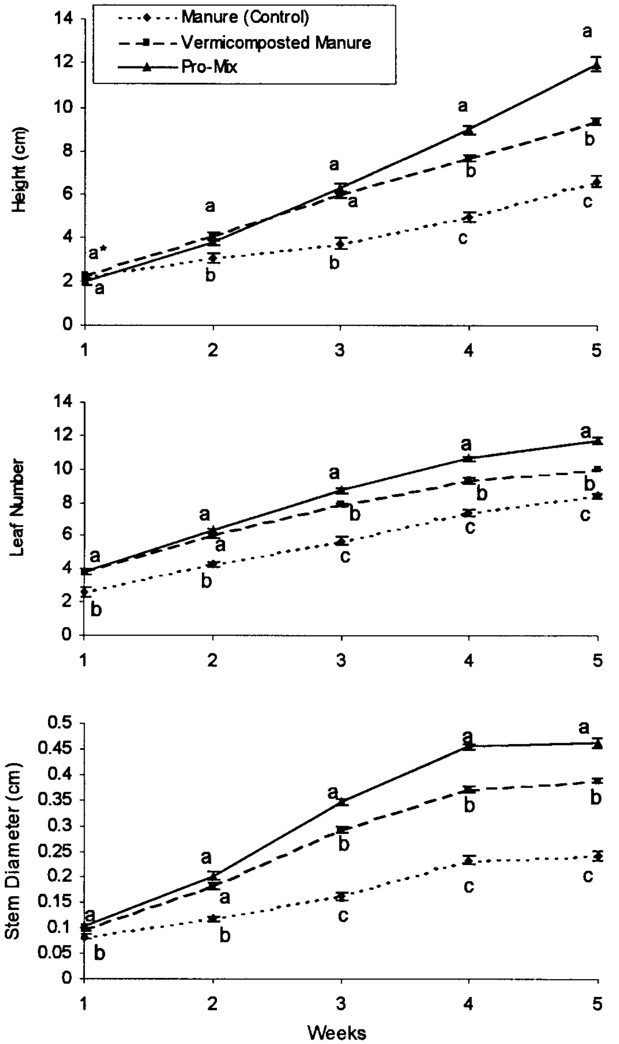


Fig. 6. Effect of manure, vermicomposted manure, and a commercial potting medium (Pro-Mix) on the growth of fertilized tomato plants (mean ± standard error). *Means within the same date followed by the same letter are not significantly different at P≤0.05

Table 1. Growth (mean ± standard deviation) of lettuce plants in manure, vermicomposted manure, and in a commercial potting medium (Pro-Mix)

	Height[a] *(cm)*	*Root length (cm)*	*Shoot dry weight (g)*	*Root dry weight (g)*	*Root : shoot ratio*
Manure (control)	2.9+0.5 b	5.7+0.9 c	0.01+0.004 c	0.003+0.001 c	0.36+0.18 b
Vermicompost	5.9+0.8 a	8.8+1.6 b	0.02+0.008 b	0.010+0.003 b	0.46+0.12 a
Pro-Mix	5.6+0.6 a	11.7+2.0 a	0.06+0.010 a	0.019+0.006 a	0.34+0.09 b

[a] Means within the same column followed by the same letter are not significantly different at $P \leq 0.05$

Table 2. Growth (mean ± standard deviation) of fertilized and unfertilized tomato seedlings in manure, vermicomposted manure, and in a commercial potting medium (Pro-Mix)

	Leaf area[a] (cm^2)	*Shoot dry weight (g)*	*Root dry weight (g)*	*Root : shoot ratio*
With Fertilizer				
Manure (control)	30.7+9.9 c	0.14+0.05 c	0.03+0.01 c	0.23+0.03 a
Vermicompost	93.0+9.7 b	0.37+0.04 b	0.06+0.01 b	0.17+0.02 b
Pro-Mix	186.1+19 a	0.79+0.11 a	0.18+0.03 a	0.22+0.04 a
No Fertilizer				
Manure (control)	20.3+4.1 b	0.13+0.05 c	0.05+0.01 c	0.40+0.07 b
Vermicompost	46.8+7.2 a	0.35+0.05 a	0.12+0.03 a	0.34+0.04 a
Pro-Mix	15.6+1.2 b	0.18+0.02 b	0.09+0.01 b	0.52+0.08 a

[a] Means within the same column followed by the same letter are not significantly different at $P \leq 0.05$

Plant growth bioassays

The effects of vermicomposted manure, control manure, and the commercial medium (Pro-Mix) on the growth of lettuce and tomato plants are summarized in Table 1 and Table 2. With both crops, the slowest plant growth occurred in the manure with no earthworms although the fastest growth was in the commercial medium.

The heights of lettuce plants grown in the vermicomposted manure were not always greater and were not significantly different from those of plants grown in the commercial medium (Table 2). Lettuce root lengths and shoot and root weights were

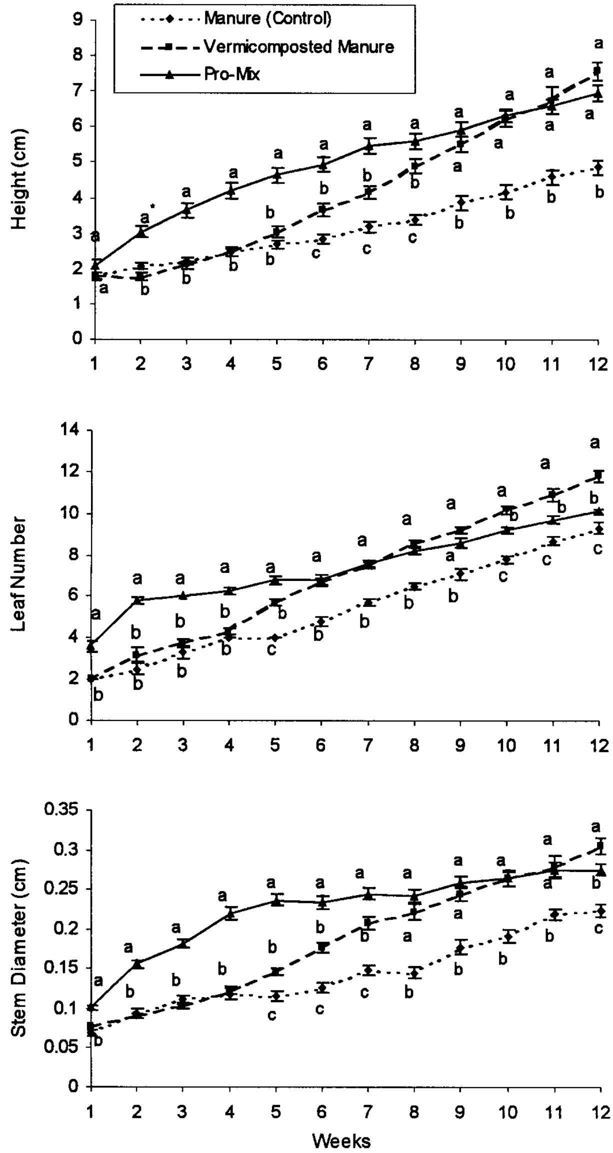


Fig. 7. Effect of manure, vermicomposted manure, and a commercial potting medium (Pro-Mix) on the growth of unfertilized tomato plants (mean ± standard error). *Means within the same date followed by the same letter are not significantly different at P≤0.05

greatest in Pro-Mix, and least in the vermicomposted manure. The root to shoot ratios of plants in the vermicomposted manure were significantly larger than those of plants in the control manure or in Pro-Mix.

Tomato plants, which received nutrient solution, were bigger and grew faster than those receiving water only. The growth parameters (heights, number of leaves, and stem diameters) and yields (leaf area and shoot and root weights) of plants receiving nutrient solution were greatest in Pro-Mix, followed by those in vermicomposted manure, then by those in the control manure (Fig. 6, Table 2). On the other hand, tomato plants receiving no nutrient solution were taller, had more leaves, and greater stem diameters in the Pro-Mix than those in other treatments, at the early stages of growth (Fig. 7). After week 8, the growth of plants in the vermicomposted manure equaled that of plants in Pro-Mix, with better plant growth in the vermicomposted manure than in Pro-Mix (Fig. 7, Table 2).

Discussion

The results suggest that earthworms play a significant role in processing cow manure, since the earthworm activity accelerated the process of manure decomposition and stabilization and promoted biochemical characteristics that were favorable for plant growth.

The vermicomposted manure was much darker in color and had been processed into a much more homogeneous mass after four months of earthworm activity, whereas the material without earthworms remained in compact clumps. Hartenstein and Hartenstein (1981) and Albanell et al. (1988) reported increased drying rates of organic wastes containing earthworms. However in our experiment, there were increases in moisture content in the manure over time, even in the presence of earthworms. The differences in the types of organic waste tested and the experimental conditions may explain these discrepancies. We found that evaporation was minimized by covering the experimental units with plastic lids whereas the experimental units used by Hartenstein & Hartenstein (1981) and Albanell et al. (1988) were kept open. Nevertheless, there was a clear difference in the rates of evaporation from the materials with and without earthworms, with less moisture in the manure with earthworms two weeks after the introduction of earthworms than in the manure with no earthworms. This could be because the vermicomposted material has a larger surface to volume ratio (Haimi & Huhta 1987) compared to that of the control, a property that enhances both aeration and evaporation. Vermicomposted manure also had a lower pH, which may be due to the accumulation of organic acids from microbial metabolism or from the production of fulvic and humic acids during decomposition (Albanell et al. 1988; Chan & Griffiths 1988).

Earthworms had marked effects upon rates of overall manure stabilization (the degree to which organic waste has been decomposed) and rates of mineralization. According to Senesi (1989), a decline of the C:N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of the organic waste. However, we found that the C:N ratios of the manure in both treatments fell from 36 to 21. Levi-Minzi et al. (1986) reported that the C:N ratio of farmyard manure decreased after storing for a period of three months. However in our experiment, earthworms initially accelerated the decrease in the C:N ratio significantly, demonstrating much more rapid decomposition and rates of mineralization of the organic matter, accompanied by increases in ash concentrations and total nitrogen contents during the first few weeks. Similar results were reported by Vinceslas-Akpa & Loquet (1994) who found that the C:N ratio of pruning wastes decreased from 62 to 27 within a period of 8 months, reflecting changes in the form and properties of the organic constituents of the waste, with earthworms, as well as the greater level of nitrogen than when there were no earthworms. Hartenstein & Hartenstein (1981) reported increases in the ash contents of activated sludge containing no earthworms by 23% during the first four weeks of their study, whereas with Eisenia fetida, the rates of mineralization increased by 130 %. Another parameter that demonstrates rapid stabilization of manure with earthworms is the respiration rate (i.e. CO_2 production). Within the first six weeks of processing, earthworms, and the microbial activity they promoted, seemed to destroy most of the easily biodegradable substances rapidly, as was indicated by the rapid reduction in the amounts of CO_2 evolving from the manure. Flushes of CO_2 production in week 8 and week 14 in manure containing earthworms might possibly be attributed to the decay of dead earthworm biomass, that might have been incorporated as fresh organic matter into the substrate, although overall earthworm mortality was low during the experiment.

Earthworms also had a great impact on nitrogen transformations in the manure, by enhancing nitrogen mineralization, so that mineral nitrogen was retained in the nitrate form. This suggests that *Eisenia andrei* produced conditions in the manure that favored nitrification, resulting in the rapid conversion of ammonium-nitrogen into nitrates. Similar results were reported by Hand et al. (1988) who found that *Eisenia fetida* in cow slurry increased the nitrate-nitrogen content of the substrate. It is important to note that part of the high nitrate concentrations, recorded in weeks 8 and 17, could be due to the decomposition of some of the earthworm biomass. Some denitrification due to increases in moisture content of the material, and some microbial biomass immobilization of nitrogen might have also occurred in the manure with earthworms, indicated by the decreases in nitrate content between weeks 10 and 14.

Earthworm activity in soils usually enhances microbial numbers and biomass (Edwards & Bohlen 1996). However, earthworm activity in the cow manure did not have much effect on the microbial biomass. Satchell (1967) pointed out that the effect of earthworms in readily degradable organic matter, which already contains a high population of microorganisms, is likely to be less significant than in soil. The only effects of earthworms on microbial biomass in cow manure occurred early in the study, when earthworms seemed to be grazing on the microorganisms, and at the end where microbial biomass was increased, possibly due to rapid proliferation of microorganisms caused by increases in the surface area of the substrate.

An acceptable level of maturity of organic wastes, which implies potential for the development of beneficial effects when they are used as growth media, can be determined by plant growth bioassays (Chen & Inbar 1993). In our experiment, vermicomposted manure, stimulated the growth of both lettuce and tomato plants in contrast with the manure from which it was derived. This suggests that earthworms had enhanced the maturity of the organic wastes. Similarly, Wilson & Carlile (1989) reported better growth of tomatoes, lettuce, and peppers in vermicomposted duck wastes than in unprocessed wastes. The enhancement in plant growth could be due to the more favorable physicochemical characteristics of the processed waste and the higher content of nitrate -N a form of nitrogen that is readily available for plant uptake. However, the physical structure of the vermicomposted manure was not as good as that of the standard commercial potting medium; the vermicomposted manure showed a tendency to form a very hard block within the plant pot, which eventually pulled away from the sides of the pot and showed signs of cracking. It seemed also that none of the unamended media could supply all nutrients necessary for plant growth, since tomato growth was faster when plants were fertilized. But as nutrients became more and more limiting, plants in the vermicomposted manure outgrew those in Pro-Mix, demonstrating the ability of the vermicomposted manure to act as a slow-release fertilizer. These conclusions are supported by Handreck, (1986) who concluded from a survey of vermicomposts that some may not provide sufficient nutrients for plant growth. Sikora & Azad (1993) reported greater wheat yields using industrial-wastes compost-fertilizer combinations than with the wastes or fertilizer alone.

In summary, we demonstrated that earthworms could alter the biochemistry of cow manure considerably and accelerate the stabilization and maturity of the organic waste. Our laboratory-scale experiment of processing cow manure by earthworms may not fully duplicate large-scale commercial conditions, but provides valuable insight about the process and the changes brought about by earthworm activity. It appeared that the first few weeks after introduction of earthworms to the manure were the most critical. During this period, most of decomposition and stabilization of manure by earthworms occurred although NO_3-N and pH did not change much. The C:N ratio decreased significantly and the ash and total nitrogen contents increased. As the vermicomposting process progressed, biological activity began to slow down, due to a depletion of readily available organic matter, and most of the nitrogen was converted into the nitrate form. The final product, in contrast to the manure from which it was derived, was more mature and stabilized which was demonstrated by the increased plant productivity it produced.

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