# Organic residue valorization for Ethiopian agriculture through vermicomposting with native (*Eudrilus eugeniae*) and exotic (*Eisenia fetida* and *Eisenia andrei*) earthworms

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#### Abstract

Vermicomposting has recently been shown to be an efficient recycling technology that would improve the quality of the final product by stimulating the microbial community. However, the efficacy of vermicomposting process, as well as the microbial biomass and community structure during this bioconversion process, depend on earthworm species and type of substrate ingested. Therefore, this study investigated the potential of native (*Eudrilus eugeniae*) and exotic (*Eisenia fetida* and *Eisenia andrei*) earthworms in Ethiopia on nutrient

transformation and microbial dynamics and enzymatic activity during vermicomposting of substrates from mixed agricultural wastes. Earthworm inoculation transformed the wastes into a nutrient-rich humified vermicompost with a significant reduction in pH, TOC and C:N ratios (twofold reduction compared to non-composted substrates). An increase in worm growth and cocoon production were shown in substrates of cow manure mixed with soybean and banana residues (CM+SB) after gut transit through Eudrilus eugeniae and Eisenia fetida. Cow manure mixed with maize and soybean residues (CM+MS) were found suitable for Eisenia andrei. The same substrate and earthworm species resulted in the highest nutrient concentrations (N, P, K, and S) and higher C loss (up to 77% of the initial C) with lower N loss (< 9%) compared to non-composted substrates. A considerable increase in microbial biomass,  $\beta$ -glucosidase and dehydrogenase activity (two times more than control) was obtained in substrates of CM+SB using Eudrilus eugeniae. The PLFA analysis indicated that earthworms increased Grampositive and Gram-negative bacteria, actinomycetes, AMF, fungi18:1, and protozoan population, while they reduced fungal community (fungi18:2 and fungi18:3) compared to uninoculated control. In conclusion, vermicomposting earthworms could potentially valorize substrates from agricultural wastes into nutrient-rich and microbially improved vermicomposts.

*Keywords:* Earthworm species, vermiculture, bioconversion, agricultural wastes, PLFA profile

## 1. Introduction

The application of organic amendments to improve soil fertility and increase crop yields is gaining importance among smallholder farmers in sub-Saharan Africa, particularly in Ethiopia, due to the increasing lack of access to chemical fertilizers for subsistence farmers [1,2]. Unlike the agricultural intensification through high chemical inputs, which could result in loss of soil biodiversity [3], organic amendments restore soil biodiversity and increase soil organic matter content. Despite such advantages of organic amendments, it is not widely practised by subsistence farmers due to limited availability of organic materials and lack of skills for transforming the organic materials into composts and organic fertilizers [4]. Vermicomposting is a bio-oxidative composting process for recycling various types of organic wastes [5], which in general can be easily adopted by farmers. It is largely the biological breakdown and stabilization of organic matter mediated by the combined action of both earthworms feed on organic substrate and microbes [6-8] which result in increasing the surface area that would stimulate rapid microbial growth and convert locally available biowastes into high-quality vermicast [9,10].

Various vermicomposting earthworms have been identified as potential candidates for organic waste decomposition [11-13]. Eisenia fetida and Eisenia andrei are native to Europe and have been imported and humanintroduced to various areas around the world, being the most commonly used species for vermicomposting under a variety of environmental conditions [14]. Eudrilus eugeniae is a native earthworm species widely distributed in the tropics and subtropics [15]. Studies have shown that exotic species (such as Eisenia fetida) were more efficient than native Indian worms (Lempito mauritii) in vermicomposting of various waste streams [16,17]. Native earthworms (*Perionyx ceylanesis*) were found to significantly increase the microbial population of vermicompost compared to exotic worms (E. eugeniae) during vermicomposting of coffee pulp in India [18]. To date, no study has evaluated the performance of exotic and native earthworms in converting locally available agricultural wastes into valuable vermicompost in Ethiopia. Our previous study on assessment of locally available wastes and farmers perception towards vermicomposting [4] showed that a lack of knowledge about the potential use of native earthworms is one of the main reasons for the poor adoption of vermicomposting in Ethiopia. Thus, it is crucial to investigate whether native worms are more efficient than exotic ones in bioconversion of locally available organic wastes.

Several factors influence the survival and performance of earthworms in converting organic wastes into vermicompost. The primary factors that

affect the performance and reproduction of earthworms are temperature and moisture content [19] and the feedstock quality [13,20]. Different feed substrates have varying palatability and properties that can significantly influence earthworm growth and reproductive potential [21] and the production of high-quality vermicompost [22,23]. For example, feeding substrates with higher N concentrations enhanced the earthworm reproduction compared to the feedstock with lower N concentrations [24]. The quality of the initial substrate and the type of earthworm species also influence the composition of the microbial community in the final vermicompost [25–27], and such a shift in microbial populations could strongly influence the mineralization dynamics of C and N [28–30]. Therefore, identifying the best performing earthworm species and the appropriate composition of feedstock that results in high-quality vermicompost is crucial to increasing the adoption of vermicomposting by smallholder farmers in Ethiopia.

We investigated the quantities, availability, and biochemical composition of locally available crop residues and animal manures in the western part of Ethiopia, where vermicomposting is rarely practiced [4]. The study showed that farmers in the region have sufficient quantity of organic materials which significantly vary in availability period and biochemical composition. Given that the growth and reproduction rate of the earthworms and quality of the subsequent vermicompost depends on the substrate quality, it is crucial to make the right substrate mix considering the availability of organic materials in the region. It is also important to investigate the potential of native earthworm species in comparison to the exotic ones on vermicomposting. Introduction of vermicomposting technology to the region would help the farmers to valorise the locally available agricultural wastes into high-value biofertilizer rich in diverse microbial communities and plant nutrients [31]. We hypothesized that native earthworm species perform better than the exotic species in terms of growth and reproduction with impacts on the quality of vermicompost. We also hypothesized that earthworm performance and the quality of vermicompost varies depending on the composition of the locally available mixed feedstock. To test these hypotheses, we set up a vermicomposting experiment that lasted for 90 days during which we evaluated the vermicomposting potential of a native (*E. eugeniae*) and two exotic (*E. fetida* and *E. andrei*) earthworms using six different combinations of locally available agricultural wastes.

#### 2. Material and methods

### 2.1. Substrates used for vermicomposting and experimental design

The feedstocks were obtained from farmer's homesteads and farmlands of the Assosa agricultural research center. These different animal manures and crop residues were selected previously based on availability and biochemical composition using farmer's household survey [4]. The native earthworm (E. eugeniae) was found in and collected from various banana cultivation habitats in the study region. Eisenia andrei is an exotic earthworm previously collected at the research center and identified as a vermicomposting earthworm (EW) and E. fetida, a common exotic earthworm, was obtained from a previously cultivated space. The three selected crop residues (maize, banana and soybean) were mixed first in pairs in equal proportions (50% w/w) (maize and soybean, maize and banana and soybean and banana residues) (Table 1). The final substrate mix in each box was prepared by mixing the animal manure (AM) with the mixture of crop residues (CR) at a ratio (mass based) of 2.3:1, that is 2.1 kg (dry weight) of cow or donkey manure and 0.45 kg of each of the two mixed crop residues. A total of 72 vermicomposting units were prepared with 24 different mixed substrates in triplicate (i.e. 3 CR \* 2 AM \* 4 EW \*3 replicates) as follows. Seventy-two rectangular plastic containers (length 50 cm, width 30 cm, and depth 20 cm) were filled with a total weight of 3.0 kg (dry weight) of each mixed substrate prepared. After pre-composting for two-weeks maintaining moisture, 200 nonclitellated earthworms of the respective species were added. Each control treatment box was also filled with the respective combination of CR and AM and pre-composted under the same conditions but without earthworm inoculation. All boxes were kept in a dark vermicomposting room at room temperature (22 to 25°C). Moisture content for all treatment boxes was maintained at approximately 70% w/v (fresh material to water) by spraying tap water. The vermicomposting experiment was conducted as a factorial RCBD experiment in triplicate and lasted 90 days. Vermicompost samples were collected from each box to determine selected physicochemical and biological parameters at the end of the vermicomposting process.

# Table 1.2.2. Earthworm growth data collection

Substrates in each box were evaluated after 30, 60 and 90 days (end of the composting) to assess earthworm reproductive status. To this end, in each box the earthworms and cocoons were hand-sorted by systematically turning the substrate, and examined for clitellum development, and then counted and weighed to determine the total number and weight of cocoons, clitellated earthworms, juvenile and adult earthworms and total earthworm biomass [32]. The maximum individual growth rate (mg worm<sup>-1</sup> day<sup>-1</sup>) was also calculated [33] based on the earthworm biomass data obtained as follows (Eq.1):

#### Eq.1.

In addition, the initial and final total C and total N concentrations were used to calculate the C and N mass balances at the end of the vermicomposting process (Eq. 2):

# (2)

where Qi and Qf are the total dry biomass of each reactor (box) at the start and end of the vermicomposting process, respectively, and Ci and Cf are the total carbon or nitrogen concentrations before and at the end of the vermicomposting process, respectively.

#### 2.3. Chemical analysis for vermicompost samples

The pH was determined using a water suspension of the vermicompost in the ratio of 1:10 (w/v) using a pH meter (PHS-1705 benchtop pH meter, BOQU Instrument Co., Ltd, Shanghai, China). Total organic carbon content was determined using the chromic acid oxidation method (Walkley-Black method), while total nitrogen was analyzed using the Kjeldhal method after the sample digestion with concentrated H<sub>2</sub>SO<sub>4</sub>. A wet di-acid digestion using conc. HNO<sub>3</sub> and conc. HClO<sub>4</sub> (mixed 9:4 v/v) procedure was used to determine the total P, K and S [34]. Total P and S were analyzed spectrophotometrically with molybdenum in sulphuric acid and turbidimetric (formation of BaSO<sub>4</sub> precipitate) method, respectively, whereas total K was analyzed using a flame photometer (PFP-7 Flame Photometer, Buck Scientific Instruments LLC., Norwalk, USA).

#### 2.4. Microbial biomass carbon and enzyme analyses

Microbial biomass C (MBC) was measured by fumigating the soil mixtures with chloroform for 24 h in the dark and subsequent extraction with 0.5  $MK_2SO_4$  (1:3 fresh soil to extraction solvent ratio). The C contents of the

fumigated and non-fumigated extracts were determined with a TOC analyzer (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan). MBC was estimated as the difference between the organic C extracted from the fumigated and that from the non-fumigated sample, multiplied by the  $K_2SO_4$  extract efficiency factor for microbial C ( $K_{EC}$ = 2.64) for vermicompost sample was assumed [35,36]. Dehydrogenase enzyme activity was measured by estimation of the rate of reduction of triphenyl-tetrazolium chloride (TTC) (1.5%) to tri-phenyl-formazan (TPF). A 5 g of vermicompost sample from each treatment was incubated at 37°C using TTC for 24 h in the dark and the amount of formazan formed was extracted with methanol and then the color intensity of the filtrates was measured at 485 nm with a Hitachi 150–20 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The activity of  $\beta$ -glucosidase was assessed by determination of the released *p*-nitrophenol, after incubation of 1g vermicompost samples with *p*-nitrophenyl glucoside (0.025M) substrate for 1 h at 37 °C and the liberated *p*-nitrophenol was measured with a Hitachi 150–20 spectrophotometer at 400nm [37].

### 2.5. PLFA and GC-MS analysis

Extracted phospholipid fatty acid (PLFA) and changes in microbial community structure were determined based on microbial membrane PLFA using a procedure detailed by Moeskops et al. [38]. 1 g of freezedried vermicompost sample was weighed into glass tubes and extracted

with a mixture of 3.6 ml phosphate buffer (P-Buffer) pH 7.0, 4 ml chloroform, and 8 ml methanol to extract total lipids. The extracted lipids were drained from separatory funnels and dried under N<sub>2</sub> gas. The dried lipids were further separated into three lipid classes using Solid Phase Extraction (SPE) cartridges (Chromabond, Macherey–Nagel GmbH, Duren, Germany). Neutral and glycolipids were discarded but phospholipids collected in methanol were kept for analysis. The PLFAs were transformed into fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. The FAMEs were dried under N<sub>2</sub> gas and later re-dissolved in hexane containing nonadecanoic acid methyl ester (C19:0) as an internal standard FAME. Then, the extracted samples were quantitatively analyzed by gas chromatography mass spectrometry (GC-MS) with a Thermo Focus GC coupled to a Thermo DSQ MS (Thermo Fisher Scientific Inc., Waltham, USA) in electron ionization mode. The sums of specific markers of PLFAs for selected microbial community structures were used for calculation [38]. PLFAs iC15:0, aC15:0, iC16:0, iC17:0, and aC17:0 were representative for Gram-positive (G+) bacteria, and PLFAs C16:1 $\omega$ 7c, C18:1w7c, and cyC17:0 for Gram-negative (G-) bacteria. PLFAs C15:0, C17:0, and cyC19:0 were considered as markers for general bacteria, and PLFAs 10MeC16:0 and 10MeC18:0 as markers for actinomycetes, PLFAs C18:2w6,9c, C18:1w9c (fungi18:1), C18:2c9,12 (fungi18:2) and C18:3c9,12,15 (fungi18:3) were considered as indicators of fungi [39], C16:1c11 as signature fatty acid for arbuscular mycorrhizal fungi (AMF) [40], and PLFAs C20:4ω6,9,12,15c and C20:5ω3,6,9,12,15c of protozoa [39,41]. The ratio bacteria PLFA (Gram+ and Gram) to C18:2c9,12 suggested as an index of bacterial:fungal biomass ratio were calculated by dividing the respective sums of the biomarker fatty acids.

## 2.6. Statistical analysis

Three-way ANOVA model was run for data on earthworm growth and biochemical characteristics of vermicomposts using PROC GLM of SAS statistical software version 9.40. Assumptions of ANOVA such as normality, homogeneity and independence of experimental error were tested before data analysis. All main and the interaction effects between the three factors (type of animal manure, crop residue, and earthworm species) were determined via F- tests and means were separated using Tukey's procedure (P < 0.05). Mean comparison for the three-way interaction effects (for each experimental period) were done using SAS PROC MIXED procedure, which provided a more useful output for computing the Least Significant Difference (LSD). The output provided the standard errors of a difference (SED) and the error degree of freedom (DF) to further compute the LSD using the tabular t-value. Standardized fatty acid biomarker concentrations (nmol g<sup>-1</sup> vermicompost) were further analysed with principal component analysis (PCA) to assess whether the combination of earthworm and feedstock are clustered in relation to the functional microbial groups using R statistical software version 4.1.1.

# 3.1. Cocoon production and earthworm growth performance during vermicomposting

The factorial analysis of variance showed a significant (P < 0.001) threeway interaction (AM × CR × EW) on earthworm growth and reproductive parameters such as cocoon production, number of clitellated worms, and earthworm biomass and growth rate at day 30 (Fig. 1 a-d) (Table S.1a). Eisenia fetida had the highest number of cocoons in substrates of cow manure mixed with maize and soybean residues (CM +MS) (680 cocoons) as well as in cow manure mixed with soybean and banana residues (CM +SB). The same is true for *E. andrei* fed on CM +MS and CM +SB. However, E. eugeniae showed high cocoon production in both CM +SB and DM +SB substrates. Likewise, the number of clitellated and biomass of earthworms were always larger with E. andrei and E. fetida fed on CM +SB and CM +MS substrates than the respective vermireactors. Similar to the number of cocoons, the lowest number of clitellated worms, biomass and growth rate were found in *E. eugeniae* fed on DM +MB substrate. On  $60^{\text{th}}$  days of the experiment, a significant (p < 0.001) three-way interaction was found between mixed substrates and earthworm species only for the number of cocoons and clitellated worms (Fig. 2 a-b). The maximum mean number of cocoons and clitellated worms were recorded

by *E. andrei* in both CM+SB and CM+MS substrates, followed by both *E. fetida* and *E. eugeniae* in the same substrate.

Unlike the 30<sup>th</sup> and 60<sup>th</sup> days, there was no significant three-way interaction between AM, CR and EW for all earthworm growth and reproduction parameters at the end of the experiment (the 90<sup>th</sup> day), but there were significant two-way interactions (AM × CR, AM × EW, and CR × EW). Hence, the pattern of earthworm growth and reproduction for selected two-way interaction (i.e. CR × EW) across the experimental periods (30, 60 and 90 days) is shown in Figure 3. The CR × EW interaction was selected based on its consistent significance across the three growth periods (Table S.1a-c). The interaction between CR × EW showed a consistent pattern of influence (maximum at day 60 and then gradually declined to the end) on earthworm reproduction except cocoon production (Fig. 3 a-d). The highest cocoon production was observed after the 30 days of the experiment. Results also showed the peak of earthworm growth was observed at 60<sup>th</sup> day and followed by a decline until 90<sup>th</sup> day (end).

## **3.2.** Chemical properties of vermicompost samples

After vermicomposting, nutrients concentration (total N, P, K, and S) were found increased, whereas total organic carbon (TOC) and C:N ratio decreased markedly in earthworm inoculated vermireactors, compared

to non-composted control. Except pH and total K, the amount of TOC, C:N ratio, total N, P, and S concentration differ with respect to substrates consumed for each employed earthworm species, resulting in significant three-way interaction between factors (Table 2; Fig. 4a-d). Significantly the lowest TOC and C:N ratio using both *E. eugeniae* (17.76% and 13.90) and Eisenia fetida (16.30% and 14.08) were exhibited in substrates of CM+SB (Fig. 4a). Vermicompost had significantly higher nutrients (N, P, K and S) concentration than the initial feedstock materials. E. eugeniae (native worm) fed on substrate of CM+SB had maximum N concentration (1.28%) followed by with the same substrate using *E. fetida* (1.16%), whereas E. andrei had shown high N concentration in substrate of CM+MS (1.02%) (Fig. 4b). Similarly, the highest total P and S concentration (13 and 3 times more than the initial concentration, respectively) was found in vermicomposts (Fig. 4c and ds (Fig. 4c and d). Nutrient balances were calculated to evaluate the C and nutrients loss in relation to the initial composition of substrates (Table S.2). Irrespective of substrate ingested, earthworm activities significantly increased total C loss in all vermireactors (Fig. 5a). The highest significant C loss (77.3% of the initial C) recorded in casts of *E. eugeniae* fed on CM+SB substrates, followed by both E. andrei and E. fetida fed on CM+MS. The cumulative N loss in the final vermicompost reduced considerably as compared to the control (substrates without earthworm) which lost up to 55% of the initial total N (Fig. 5b). Unlike C loss, the lowest N loss was observed in vermicomposts of E. eugeniae fed on CM+SB, followed by E. andrei and

*E. fetida* fed on CM+MS. No losses were recorded for other nutrients such as (P, K and S).

#### 3.3. Microbial biomass and enzymatic activity

Regardless of earthworm species and crop residues, cow manure vermicompost had significantly higher activities of  $\beta$ -glucosidase, dehydrogenase and MBC by 31, 53 and 62%, respectively, than donkey manure vermicompost (Table 2). The CM+SB vermicompost significantly increased  $\beta$ -glucosidase activity and MBC by 57 and 64%, respectively, compared to DM+SB vermicompost regardless of the animal manure and EW species. Dehydrogenase activity varied by three-way interaction between factors (Table 2). Inoculation of *E. eugeniae* in substrates of CM +SB resulted in a significant twofold increase in dehydrogenase activity compared the corresponding non-composted treatments (CM +SB without earthworm) (Fig. 6).

#### Table 2.

#### 3.4. PLFA biomarkers of the microbial community

The total concentration of PLFAs in the finished vermicompost differed significantly (p < 0.05) between the type of animal manure and mixed crop residues (Table 3), while earthworm species did not significantly affect the total PLFA concentration. Substrate consisting of cow manure caused significantly higher individual PLFA biomarkers or total PLFA concentration than the corresponding donkey manure. The presence of

earthworms significantly decreased the concentration of PLFA biomarkers for Fungi18:2 (C18:2 $\omega$ 9,12) and Fungi18:3 (C18:3 $\omega$ 6,9,12) compared to the uninoculated control. The relative concentration of protozoan biomarkers was significantly higher in the presence of earthworms relative to control without earthworms (Table 3). There was no significant three-way or two-way interaction effects on the concentration of total PLFA and individual PLFA biomarkers except one of the fungal PLFA biomarkers (18:3).

#### Table 3.

A clear separation between the samples was found along the first principal component (PC1) accounted for between 45.1 and 78.4 %, and the second component (PC2), between 11.7 and 27.1 of the variances as a function of the type of animal manure, crop residues and earthworm species (Fig. 7a-d). The PCA for the type of animal manure (Fig. 7a) resulted in overlapping of cow and donkey manure. However, most of the individual biomarkers were aligned with individual scores of cow manure. PCA on crop residue showed that soybean with banana residue separated by PC2 from the other two residues (Fig. 7b). Biomarkers of protozoa, fungi 18:1 and B:F ratio were aligned with individual score of soybean with banana residue, while the fungal biomarkers (fungi 18:2 and fungi 18:3) were aligned with maize with banana residue. PCA for each earthworm species excluding uninoculated control (Fig. 7c) showed that most of the biomarkers of bacteria (Gram+ and Gram-), AMF, actinomycetes, fungi18:2 and total PLFA were mainly aligned with *E*.

*eugeniae* than the corresponding exotic earthworms (*E. fetida* and *E. andrei*). With the uninoculated control (Fig. 7d) included in the PCA model, PC2 separated all the three-earthworm species from the uninoculated control. The fungal biomarkers (fungi 18:2 and fungi 18:3) were aligned with individual scores of the uninoculated control.

#### 4. Discussion

#### 4.1. Earthworm reproduction and growth performance

Vermicomposting of different combinations of substrates (mixed crop residue with animal manure) significantly (p < 0.05) affected the growth and reproduction of the three-earthworm species employed. A clear difference in cocoons production, number of juveniles and adults, and worm biomass and growth rate were observed among the different substrate combinations and during the vermicomposting process indicating the preference of earthworms for a specific type of substrate mixture. Particularly during the first 30 days, the cocoon production, the number of clitelleated worms and earthworm biomass were higher in substrates with cow manure than donkey manure indicating the preference of earthworms for the cow manure (Fig 2a-d). Similarly, Garg et al. [11] also reported lower cocoon production and earthworm growth potential with donkey manure compared to cow, sheep and horse

manures. This could be related to the initial lower biochemical quality of donkey manure (higher lignin and polyphenol) as reported in our previous study [4], that likely affecting its palatability to earthworms and thus suitability for vermicomposting. The earthworm species showed variation in growth and reproduction in response to the composition of the substrates. For example, the exotic earthworm species (E. fetida and E. andrei) performed better than the native worm in substrates of CM+SB and CM+MS. Interestingly, E. eugeniae (native worm) performed better in substrates consisting of soybean and banana residues than maize and banana as well as maize and soybean residues irrespective of animal manure (Fig. 2a-d), which could be due to the difference in substrate quality. Regardless of earthworm species, the highest number of cocoons after 30 days in CM+SB and CM+MS might be due to the high N content of soybean straw in the mixed substrates, given that feedstock N content is an important factor influencing the cocoon production [42] because nutritious substrates provide energy and protein for the onset of cocoon production.

After an increase in cocoon number (after 30 days), then a large worm biomass and growth (after 60 days), and later a decline in growth were observed at the end of the experiment (90 days). Earthworm biomass and cocoon production rate are important factors to express earthworm's fecundity [15,43] and to compare the growth rates of worms which could differ with the substrate. The peak worm growth rate and biomass of earthworms on the 60<sup>th</sup> day might be due to the consumption of suitable substrates resulting in rapid cocoon hatchlings [6,43]. A consistent trend of decreasing cocoon and earthworm numbers and worm biomass after the 60<sup>th</sup> day in all feed substrates may be due to the aging of the culture organic substrates [15,44].

#### 4.2. C and nutrient concentrations and balances of vermicompost

The vermicomposting process caused a significant transformation in the chemical composition of the initial substrate materials and resulted in the formation of dark and homogenous vermicompost than uninoculated control. The shift in pH towards neutrality during vermicomposting might be due to the neutralization reaction between carboxylic and phenolic groups in humic acids and ammonium ions [45,46]. The decrease in the C:N ratio could also be due to respiratory activities of earthworms and associated microflora and simultaneous increases in the N concentration of the composts [47]. Vermicomposting earthworms significantly increased total C loss (especially in substrate of CM+SB using E. eugeniae accounted for over 77% of initial C loss) compared to non-composted treatments (Fig. 5a). In addition to direct earthworm activity such as ingestion and fragmentation of substrate, the higher C loss after vermicomposting as compared to the composting without earthworms could also be due to the stimulation of decomposing microbes by the earthworm casts [48]. Similar to a lower OC and C:N ratio, E. eugeniae

and E. fetida resulted in the maximum N content (1.28% N and 1.16% N, respectively) in a substrate of CM+SB, while E. andrei (1.02% N) in CM+MS. Regardless of the earthworm species used, N losses from the uninoculated substrates in the control (especially substrates consisting of donkey manure) were the highest, averaging up to 48% of the initial total N content, compared to N losses in vermicomposts. The lowest N losses observed in CM+SB substrate with E. eugeniae (9% loss) and in CM+MS with both E. fetida and E. andrei (18 and 21% losses, respectively) (Fig. 5b) might be due to their highest total N content. Nigussie et al. [49] reported that during vermicomposting, effective N loss reduction through less N<sub>2</sub>O emission could be pronounced due to availability of mixed residues in earthworm feed substrates. The availability of mixed residues may have reduced nutrient loss in our study. Overall, the lowest total Closs with high N loss were observed in non-composted treatments in our study. Domínguez et al. [10] as well as Gómez-Brandón et al. [36] reported that the enhanced N concentration in the presence of earthworms is mainly due to the direct excretion of excess N through their casts.

Vermicomposting using earthworms could therefore result with a high total N concentration in vermicomposts with effective reduction of N losses that are unlikely to occur in the uninoculated control. Earthworm activity through gut digestion and mucus excretion [50] have been shown to enhance N mineralization of the OM and enriched the total N content of vermicompost [9,51]. The N loss reported in the present study (9%) during vermicomposting was less than a previous study on conventional and vermicomposting conditions by Nigussie et al. [49]. Unlike the C and N, no losses in vermicomposts were observed for P, K and S which could be due to the fact that no leaching occurs. Thus, the increased P and K concentrations in vermicomposts could be largely due to dry matter losses, as essentially the same mass of P, K and S could be present in less dry matter. Therefore, vermicomposting allows farmers to reduce the volume and weight of organic material that must be transported to their fields while maintaining equivalent nutrients (N, P, K and S) value of the stabilized vermicompost.

#### 4.3. Microbial properties

Irrespective of earthworm species, CM+SB resulted in significantly higher microbial biomass C and  $\beta$ -glucosidase compared to the other mixed substrates. Earthworms could cause an increase in microbial biomass during the vermicomposting process, particularly during the early phase of the vermicomposting process [52] while it could decrease due to the aging of vermicompost when earthworms have left the organic substrate [53,54]. The non-significant difference in MBC between earthworm casts and non-composted at the end of vermicomposting could be due to a rapid degradation of OM during the initial period or exhaustion of available C sources by inhabiting microbial communities [55]. Aira et al. [30] also suggested that a decrease in microbial biomass could be

attributed to the digestion of earthworms on selected microbes during vermicomposting. However, Lv et al. [56] found an increase in microbial activity in the final vermicompost which could be due to the availability of high content of substrates for microbial metabolism. During vermicomposting, the activity of  $\beta$ -glucosidase can be an indicator of the rapid loss of organic matter [55]. This extracellular enzyme takes part in the decomposition of lignocellulose and it plays a role in the carbon cycle by catalyzing the conversion of disaccharides into glucose, which are subsequently used by soil microbes as a source of energy [38,57]. Villar et al. [58] demonstrated the action of earthworms on  $\beta$ -glucosidase activity depends on the type of substrate ingested. Our study also showed that earthworms could increase the activity of microbes, as indicated by their dehydrogenase activity. The highest dehydrogenase activity was obtained in CM+SB using E. eugeniae (Fig. 6), which could be due to the high content of water-soluble C used for microbial growth [55] and the aerobic pathways of the microflora in the earthworm gut. In agreement with previous reports by Lv et al. [56] that demonstrated higher microbial activity in the final vermicompost, our results on microbial activity were higher in worm casts as evidenced by higher dehydrogenase.

The nature of feedstock and earthworm species used could significantly influence the community structure and the abundance of the microbial population [59]. Cow manure increased the abundance of the individual

microbial communities more than donkey manure. This is consistent with previous studies that have shown that the microbial community found in the cast varied depending on the animal manure ingested by the earthworm [60] (Table 3). The results of PCA indicated that the composition of the microflora communities differed between treatments applied. PCA biplot analysis also separated soybean with banana residue from the other mixed residues mainly by biomarkers of protozoa and fungi18:1 as well as B:F ratio (Fig. 7b), suggesting that the highest microbial community with soybean and banana residues was dominated by bacteria, while maize and banana residue aligned with fungal community (fungi18:2 and 18:3) showing the initial difference in C:N ratio between crop residues affected the microbial composition; and it could also be due to the initial microorganisms present on the plant materials, phyllosphere microbiome [61]. Whereas the uninoculated (noncomposted) control separated from earthworms by fungal community (fungi 18:2 and 18:3). This indicates the significant effect of earthworms on changing the composition of microbial communities by decreasing the fungal community (fungi 18:2 and 18:3) in the final vermicomposts. In addition to the fungi, the earthworm activity had also an impact on protozoa, as both are important for its nutrition [46,62]. The results of the current study are consistent with the general view that earthworms can selectively graze fungal species that can be digested by earthworms [54] and support the findings of [63] who documented that the earthworms selectively promoted fungi18:1 and protozoan abundance.

Domínguez et al. [19] also observed significant changes (richness and evenness) in bacterial community composition in vermicomposts. Unlike the saprotrophic fungal biomarkers, the earthworms did not significantly change the abundance of AMF. However, the presence of cow manure significantly increased AMF than donkey manure regardless of the CR and EW. As AMF are obligate symbionts with roots, they may not survive long without active C supply by plants. Part of the high concentration of PLFA 16:1 $\omega$ 5 recorded in the current experiment could be from sources other than the active AMF, such as the G- bacteria and lipid stabilized on soil minerals and microbial necromass [64]. Despite the frequent use of PLFA  $16:1\omega 5$  as a proxy to estimate active AMF abundance in soil, studies have challenged its specificity and instead recommended NLFA 16:1w5 [64], while others considered it as a biomarker for AMF arguing that most of the PLFA 16:1 $\omega$ 5 in the soil comes from AMF cells [65]. Overall, the function and changes in microbial community composition resulted from vermicomposting are evidence of the beneficial effects of vermicompost as a soil amendment essential for plant growth and disease suppression, especially in areas subject to improper agricultural practices such as Ethiopian agriculture.

## 5. Conclusion

The vermicomposting potential of native and exotic earthworms is an alternative technology for bioconversion of various agricultural wastes

and the production of valuable vermicompost with improved chemical and microbiological properties. The substrates CM +SB and CM +MS (for E. fetida and E. andrei) as well as CM +SB (for E. eugeniae) result in best palatability for effective earthworm growth and reproduction, offering adequate energy to sustain earthworm population and biomass during the vermicomposting process. Substrate biomass loss caused by earthworm activity resulted in the production of nutrient-rich vermicompost and increased nutrient content while reduced N loss than non-composted. The PLFA assay also showed that earthworms were able to enhance the composition of the microbial communities by decreasing the fungal populations (fungi18:2 and 18:3) and tend to increase biomarkers of Gram-positive and Gram-negative bacteria, actinomycetes, fungi18:1 as well as protozoa compared to uninoculated control. The study showed the potential of native and exotic earthworms in optimizing of a wider range of mixed substrates for vermiculture and producing high-quality vermicompost at the farm level. The valorization of underutilized agricultural wastes through vermicomposting is extremely relevant to the local situation in Ethiopia, given the severe pressure on soil resources and the rapidly declining soil fertility in many areas. Adopting vermicomposting and its application by smallholder farmers could help farmers improve soil and crop productivity and ensure food security. Future studies should also evaluate the potential contribution of various vermicompost sources as biofertilizers and biopesticides.

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**Figure captions** 

**Fig. 1.** Interaction effect (three-way) of animal manure and crop residues on mean values (n=6) of (a) cocoon production (LSD=152.29), (b) number of clitellated earthworms (LSD=71.61), (c) earthworm biomass (LSD=29.97) and (d) growth rate (LSD=5.00) for each earthworm species on 30<sup>th</sup> days of vermicomposting (CM- Cow manure; DM- Donkey manure; MS- Maize with soybean residue; SB- Soybean with banana residue; MB- Maize with banana residue).

**Fig. 2.** Interaction effect (three-way) of animal manure and crop residues on mean values (n=6) of cocoon production (LSD=67.39) and number of clitellated earthworms (LSD=41.59) for each earthworm species on 60<sup>th</sup> days of vermicomposting (CM- Cow manure; DM- Donkey manure; MS-Maize with soybean residue; SB- Soybean with banana residue; MB-Maize with banana residue).

**Fig. 3.** The dynamics in cocoon and earthworm growth and production of the different earthworm species over time as affected by the type of mixed crop residue. Values show means and error bars are standard deviation (n=9) (CM- Cow manure; DM- Donkey manure; MS- Maize with soybean residue; SB- Soybean with banana residue; MB- Maize with banana residue).

**Fig. 4.** Interaction effect (three-way) of animal manure and crop residues on mean values (n=6) of C:N ratio (LSD=5.24), total N (LSD=0.1379), P (LSD=0.1852) and S (LSD=563.36) of the final vermicompost (90<sup>th</sup> day) produced from each earthworm species (CM- Cow manure; DM- Donkey manure; MS- Maize with soybean residue; SB- Soybean with banana residue; MB- Maize with banana residue).

**Fig. 5.** Interaction effect (three-way) of animal manure and crop residues on mean values (n=6) of C (LSD=3.33) and N (LSD=7.48) losses of the final vermicompost (90<sup>th</sup> day) produced from each earthworm species (CM-Cow manure; DM- Donkey manure; MS- Maize with soybean residue; SB-Soybean with banana residue; MB- Maize with banana residue).

**Fig. 6.** Interaction effect (three-way) of animal manure and crop residues on mean values (n=6) of on dehydrogenase activity (LSD=466.80) of the final vermicompost (90<sup>th</sup> day) produced from each earthworm species (CM- Cow manure; DM- Donkey manure; MS- Maize with soybean residue; SB- Soybean with banana residue; MB- Maize with banana residue).

**Fig. 7.** Principal component analyses (PCA) biplot for PLFA biomarkers in vermicomposts and non-composted substrate samples as affected by animal manure (a), crop residue (b), earthworm species (c) and earthworm species including non-inoculated control (d)