Characterisation of Chamaecytisus tagasaste, Moringa oleifera and Vachellia karroo Vermicomposts and Their Potential to Improve Soil Fertility

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Abstract: Poor soil fertility and land degradation limit crop production among smallholder farmers. The practice of agroforestry with leguminous trees has proven to be sustainable as it bolsters nutrient supply through nitrogen fixation and nutrient cycling. The beneficiation of agroforestry species could add even more value by using tree based waste materials as mulch or vermicomposting. A study was conducted to investigate the impact of vermicomposting on chemical and biological characteristics of three agroforestry species; Chamaecytisus tagasaste, Vachellia karroo and Moringa oleifera. Eisenia fetida earthworms were added to the leaves and small twigs of the three trees in worm composting bins. The worms were allowed to feed on the feedstocks for six weeks under laboratory conditions. The results showed that vermicomposting significantly enhanced macronutrient nutrient content in all the three feedstocks. The findings also showed that the quality of the vermicompost depends on the feedstock type. M. oleifera had the best quality vermicomposts with a significantly higher composition of macronutrients which ranged between 50 and 170% higher for Ca, K, Mg and P. Vermicomposting increased Mo while other micronutrients such as Zn, Mn, Fe and B significantly decreased with vermicomposting time. In addition, vermicomposting increased E. fetida reproduction with more than a 450% increase in earthworm numbers in all three feedstocks. In conclusion, vermicompost have potential to be used to improve soil fertility and thus reduce the use of synthetic fertilisers in crop production.

Keywords: agroforestry; macronutrients; micronutrients; soil fertility; vermicompost

1. Introduction

Agroforestry is a land use practice in which selected woody perennial species, mainly trees and shrubs are grown in association with herbaceous crops, pastures or livestock where both ecological and economic interactions occur between the woody species and the other components [1]. Agroforestry trees have multiple benefits such as bolstering nutrient supply through nitrogen (N) fixation and nutrient cycling [2], improved soil health, structure and water infiltration [3] and enhanced carbon (C) storage both above-ground and below-ground [4]. The trees and shrubs contribute to food security directly in the form of fruits, seeds and other edible parts or indirectly by maintaining and restoring soil fertility and water resources, which subsequently increase agricultural production [5].

Though agroforestry is a resilient and sustainable system for resource-poor farmers, its adoption as an intervention strategy is not widespread in South Africa [6]. The adoption of agroforestry in farming systems in conjunction with other conservation agriculture techniques is being promoted.
as one of the location specific strategies, and an affordable science based approach to improve land care among smallholder food production in Limpopo Province [6]. Reconnaissance surveys have established that only passive agroforestry systems exist in Limpopo province [6]. The main agroforestry species that have been extensively utilised include tree legumes such as Acacia spp. ((Carl.) Martius), Faidherbia albida ((Delile) A. Chev.), Sesbania sesban ((Elmer) Merr.), Gliricidia sepium ((Jacq.) Steud) and Chamaecytisus tagasaste ((L.). These species provide high quality biomass and residue that can be used for soil fertility improvement. The low C/N ratio of C. tagasaste foliage could be an indication that its incorporation may improve soil N and increase yields of non-leguminous crops such as maize [7]. The foliage of C. tagasaste contains 18–22% crude protein which provides a good source of calcium, vitamins and minerals [8]. Large volumes of organic materials generated could pose a problem for safe disposal. Beneficiation of agroforestry species has added more value to the concept of agroforestry by introducing the use of tree based waste material as mulch or vermicompost among others [9].

Agroforestry tree species such as Vachellia karroo ((Hayne) Banfi & Galasso), Moringa oleifera (Lam.) and C. tagasaste could be used as feedstock in the process of vermicomposting. Vermicomposting is an environmentally friendly practice of using organic wastes and incorporating them with selected earthworms in order to form a humus-like byproduct that is used as a source of nutrients for the plant growth [10]. Vermicomposting involves the use of earthworms to mix, fragment and aerate organic waste material making it more conducive to microbial activity [10]. It reduces the harmful effects of waste material through biodegradation and releases nutrients that can be used for soil fertility improvement [11]. The mutual action of digestion in guts of earthworms and microbes fragment the waste into fine homogeneous humus manure, which is odor-free and rich in nutrients. Vermicomposting reduces the C:N ratio and retains more N than the traditional methods of preparing comports [12].

Vermicomposting could also be used as a management strategy where encroachment of the invasive N-fixing Acacia species on farms is uncontrollable. The Vachellia spp., in particular are indiscriminate aggressive invasive species which generate large quantities of biomass and are widespread in the savanna and grassland biomes of Limpopo Province [13]. M. oleifera, which is widely cultivated around farms in Limpopo Province has the potential to be a dominant agroforestry species due to its fast growth rate, exceptionally high nutritional value of its leaves, fruit, flowers, and immature pods compared with other food crops and a range of medicinal uses [14–17]. Vermicomposting has the ability to biodegrade the toxic metabolites such as polyphenols, tannins, phytotoxins and saponin produced by M. oleifera [18,19] into harmless nutrients that could be used for plant nutrition. C. tagasaste is a legume plant that nodulates freely and can fix atmospheric N in association with the rhizobium bacteria, thereby reducing the requirements for additional soil N fertilisation [8]. The three species provide high quality biomass and residue feedstock for vermicomposting and for the improvement of soil fertility.

This study sought to (i) characterise the chemical composition of tree lucerne, Acacia and Moringa vermicomposts and (ii) determine their potential to improve soil fertility. It was hypothesised that vermicomposting could increases the nutrient content of the compostable feedstock of three agroforestry tree pruning residues and could potentially be used for soil fertility improvements.

2. Materials and Methods

2.1. Collection and Processing of Feedstock Materials

The V. karroo, which is vastly distributed in the province was collected from the University of Limpopo farm. M. oleifera and C. tagasaste were outsourced from neighbouring farms within a 5 km distance of the University farm. The species were all collected in summer 2015, dried and then chipped to leaf-size pieces using a portable wood chipper (Portable Chipper Machine, HR 40, China) in preparation for vermicomposting. Only leaves and small branches were collected for use as
feedstock material. The earthworm species *E. fetida* (Marie.) Savigny) used were obtained from the Bertie van Zyl (Pty) farms in the Limpopo Province, South Africa.

### 2.2. Study Site and Experimental Design

The study was conducted under ambient conditions with a recommended moisture content ranging from 75–80%, with perforations on the lid and at the bottom of the vermicomposting boxes. The vermicomposting experiment was arranged as a Completely Randomised Design with three treatments *V. karroo*, *C. tagasaste* and *M. oleifera*, replicated three times. Vermicomposting was carried out in dark coloured ultraviolet resistant polyvinyl plastic boxes measuring 0.50 m × 0.40 m × 0.30 m (length × width × depth), which provided a volume of 0.06 m³. Mature earthworms (*E. fetida*) were introduced at the recommended stocking rate of 1.6 kg earthworms m⁻² into each box [20].

### 2.3. Sampling and Analyses of Vermicomposts

Sampling of the vermicomposts was done randomly in each box at two-week intervals. From each box, 200 g of the vermicompost were collected and then carefully turned for ensuring sample homogeneity and to preserve the existing earthworms. During sampling of the vermicompost, care was taken not to include any earthworms and where earthworms were sampled, they were counted and removed from the samples and then added to the final count. The samples collected during the vermicomposting were air-dried to constant weight and crushed into fine powder in preparation for analyses.

The vermicompost samples were analysed for total N using a block digester (SC154 Hot Block digester, Environmental Express, UK) [21]. Soil organic carbon by was estimated by the loss on ignition method [22]. Soil pH and EC were estimated using a pH meter (HI2002-01 edge, UK) and EC meter (HI-2030 edge hybrid multi parameter, UK), respectively, in a mixture of compost to water ratio of 1:5 w/v. The UV/VIS spectrophotometer (Thermo Scientific Spectronic, Helide Gamma, NY, USA) was used to measure P after extraction from compost with a 0.5 M NaHCO₃ solution at pH 8.5 [23]. Potassium, Mg, Ca, Zn, Na, Fe, Mn, and Cu were extracted from 2 g of air-dried ground vermicompost using 2 mL of Mehlich 3 extracting solution [24] and quantified with UV/VIS spectrophotometer.

The humic and fulvic acid fractions were extracted in 0.1 M NaOH at ratio of 1: 20 w/v [25]. The supernatant was then divided into portions, with one half being analysed for total extractable C (TEC) fraction. The other half was acidified to pH 2 with H₂SO₄ and then centrifuged at 8000 rpm after coagulation, with the supernatant being used for analysis of the fulvic acid (FA) portion. The C contents in the supernatants were determined using the dichromate oxidation method, with the content of the humic acid (HA) fraction being calculated as the difference between TEC and FA. Humification index (HI), humification ratio (HR) and polymerisation index (PI) which are indices used for evaluation of humification level in the vermicompost were calculated using the formula:

\[ HI = \frac{HA}{C} \times 100 \]  
\[ PI = \frac{HA}{FA} \times 100 \]

Phytochemicals testing of flavonoids was done following the method by Borokini and Omotayo [26], alkaloids using Dragendorff’s reagent [27], tannins following the method by Trease and Evans (1989); and saponins test using the method by Odebiyi and Sofowora [28]. Phytotoxicity tests were done at the end of the composting cycle using lettuce and onion seeds because of their high sensitivity to toxic materials and rapid germination [29].

Manual earthworm counts were done at the start and final stages of the experiment. Microbiological analyses of total bacteria, total fungi, total *E. coli* and total phosphate solubilising bacteria was done every two weeks since the beginning of the vermicomposting using the serial dilution and spread plate counting [30]. For total bacteria, nutrient agar was used with a serial dilution of 10⁵ and the plates were incubated at 37 °C for 24 h before counting the number of colony forming units (CFU). The
m-FC agar was used for total *E. coli* counts with a serial dilution of $10^3$. The plates were incubated at 44 °C for 24 h following which counts were made to determine the number of CFU. The Pikovskaya’s Agar was used for total phosphate solubilising bacteria (PSB) with a serial dilution of $10^4$ being used. The plates were incubated for a period of 3–5 days at 30 °C and counts were made to determine the number of CFU. The Rose Bengal Chloramphenicol agar was used for the determination of total fungi with a serial dilution of $10^3$ being used. The plates were incubated at 30 °C for a period of 3–5 days, following which counts were made to determine the number of CFU.

### 2.4. Statistical Analyses

The collected data were subjected to repeated measures analysis of variance (ANOVAR) using the Statistix software version 10.0 (Tallahassee, FL, USA). All assumptions, such as the equality of variances and the normality of residuals were satisfied before running the ANOVA. Significant differences between mean values were assessed using the Fisher’s least significant difference (LSD) test at a probability level of 95% ($p \leq 0.05$) [31]. Pearson correlations between the chemical and microbiological parameters were used to analyse the relationships between the analysed parameters. The Principal Components Analysis was performed to create a minimum dataset for interpretation of results and only highly weighted Principal Components (>10%) and the measured soil properties with a loading matrix >0.6 were retained in the dataset [32].

### 3. Results and Discussion

#### 3.1. Nutrient Content of *V. karroo*, *M. oleifera* and *C. tagasaste* Vermicomposts

Table 1 shows the nutrient content of *V. karroo*, *M. oleifera* and *C. tagasaste* vermicomposts at the final week of sampling compared with soil critical limits adopted by various researchers [33–35]. The results show that nutrient contents in vermicomposts depended on the feedstock i.e., the material being vermicomposted. These results were observed for all the nutrients determined except for Cu, Mn and C, which did not vary among the vermicomposts. Of the three feedstock materials, *M. oleifera* had the highest content of most nutrients compared to *V. karroo* and *C. tagasaste*. For instance, Ca content in *M. oleifera* vermicompost was 154 mg kg$^{-1}$, which was more than 170% higher than the other two vermicomposts. Similarly, such large differences in contents among *M. oleifera* and the other vermicomposts were also observed with other macronutrients (K, Mg, and P). Primary macronutrients (N, P and K) differed between the vermicomposts and followed the order *M. oleifera* > *V. karroo* > *C. tagasaste* for N. For P and K the trend was *M. oleifera* > *C. tagasaste* > *V. Karroo*. Micronutrients such as Fe and Zn were lower in *C. tagasaste* compared to the other two vermicomposts. The pH of the vermicomposts were slightly alkaline.

When compared over time, the macronutrients (Ca, Mg, K, P), with the exception of N, were higher at the later stage of vermicomposting during week 6. (Table 1) while no differences were observed after zero-, two- and four-weeks. The general increase in nutrients particularly basic cations at the later stages of vermicomposting could be a result of earthworm digestion. Several studies have also reported high levels of exchangeable bases in vermicompost, agreeing with the findings of this study [36,37]. Higher quantities of basic cations explain the slightly alkaline pH observed at week six.

Nitrogen content was higher at the initial vermicomposting stage and lower at the later stage, a similar trend observed also for some micronutrients such as Mn, Zn, and B. The low N content at later stages of vermicomposting could be a result of earthworm digestion. Several studies have also reported higher nitrogen content at later stages of vermicomposting compared with the initial stage [38]. The decrease in the micronutrient as the vermicompost matures are contrary to the findings of some studies [39]. The contradiction observed in micronutrients may be attributed to the differences in the vermicomposted material where Sharma and Garg [39] used rice straw. The feedstocks used in this study are of relatively low C:N ratio compared to the rice straw. *M. oleifera*, *V. karroo* and *C. tagasaste* are all leguminous trees and hence have lower C:N ratio than wheat straw. The observed results may partially be attributed to the slightly higher pH in *M. oleifera*
and *C*. tagasaste. In soils, for example, it is well established that micronutrients become less available under alkaline conditions.

Table 1. Nutrient content of *V*. karroo, *M*. oleifera and *C*. tagasaste vermicomposts at week six (maturity) and at different times during vermicomposting.

| Vermicompost | Ca (mg kg⁻¹) | K (KCl) | Mg (KCl) | Na (KCl) | P (mg kg⁻¹) | Cu (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Mo (mg kg⁻¹) | Zn (mg kg⁻¹) | B (mg kg⁻¹) | N (%) | C (%) | pH |
|--------------|-------------|---------|----------|---------|------------|--------------|--------------|-------------|-------------|-------------|-------------|-------|-------|----|
| *V*. karroo  | 55b         | 91c     | 39b      | 81c     | 54c        | 0.85         | 0.29b        | 0.06        | 1.10b       | 0.31b       | 0.33b       | 12 ± b | 66.12 | 7.43b |
| *M*. oleifera| 154a        | 186a    | 257a     | 419a    | 107a       | 0.79         | 0.49a        | 0.13        | 1.85a       | 0.67a       | 0.61a       | 22 ± a | 60.04 | 7.59a |
| *C*. tagasaste| 56b        | 102b    | 57b      | 153b    | 76b        | 0.86         | 0.53a        | 0.10        | 0.65c       | 0.53a       | 0.36b       | 10 ± c | 68.19 | 7.61a |

Critical soil test values adapted from FSSA-MVSA (2007); Landon (1991); Havlin et al. (2013). Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, ns means not significant. Means separated by different letters in each column are significantly different.

After six weeks of vermicomposting, *M*. oleifera generally had higher nutrient content compared to the other vermicomposts (Table 1). However, the pH remained relatively similar. These results suggest *M*. oleifera provides high quality vermicompost when mature compared to the other two feedstocks. The low N content for all vermicomposts at week six (Table 1) may be due to changes in conditions such as the duration of the process which is known to drastically affect the N content [40]. Other researchers stated that phenolic compounds such as tannins and flavonoids found in plant tissue stimulate microbial activity which subsequently reduces plant available N through assimilation by microorganisms as a source of energy and growth [38]. In this study, *V*. karroo did not contain any tannins nor flavonoids. These phenolic compounds were only consistently present in *M*. oleifera (See Section 3.5). However, the stimulation of microbial activity was not evident as the microbial numbers (CFUss) were rather constant throughout the vermicomposting period (See Section 3.4). Therefore, a plausible reason for the decrease in N with time can be attributed to the utilisation of N by earthworms themselves for growth. This is indeed supported by the five-fold increase in earthworm numbers as discussed under Section 3.4.

Phosphorus content in all vermicomposts were above the critical soil values, particularly of in *M*. oleifera with a content of 107 mg kg⁻¹ of P which was seven times higher than the critical soil limit value of 15 mg kg⁻¹ [35]. Norman, Arancon [41] found that P was 64% higher in vermicomposts compared to other organic materials such as animal manure or human wastes.

Molybdenum and B contents are normally very low in most soils [42]. In this study, Mo and B were relatively high therefore suggesting that vermicomposts could also be used as a source of micronutrients and can act as slow release fertiliser, in agreement with Atiyeh, Domínguez [43] who reported that plants grown on a vermicompost medium outgrew plants that were grown in other media due to the slow release of the nutrients. The presence of humic and fulvic acids in vermicompost could be one of the reasons for the relatively high content of the, Mo and B which were above critical soil test values. Khaled and Fawy [44], reported that humic and fulvic acids form chelates with micronutrients increasing their bio-availability for plant use.

Basic cations such as K, Ca, Mg and some micronutrients such Mn, Cu and Zn were below the soil critical values in all vermicomposts. It is unclear why basic cations were so low because according to Elvira, Sampedro [45] and Parthasararathi, Ranganathan [46], earthworm cast have enhanced content of such nutrients due to the mineralisation process taking place in their guts. A possible reason for this
could be that the earthworms are normally found in soils and they ingest soil material which generally have higher Ca, Mg, and K. In this study the earthworms mineralised nutrients that were in the feedstocks. Therefore, the quantities of these nutrients should be a reflection of the amount of nutrients in the feedstocks. However, the contents of basic cations were still rather too low than expected. A study by Mabapa, Ayisi [17] showed that *M. oleifera* dry biomass contained significantly higher quantities of basic cations than those found in the present study. The lower contents of micronutrients in some of the vermicomposts could be attributed to the high pH which is known to reduce their availability [47].

The pH value of all the three vermicompost was found to be alkaline at 7.35. A good soil pH is essential in order to ensure the availability of nutrients because some nutrients are found to become unavailable at pH that is extremely alkaline or acidic [48]. The pH also controls enzyme activity as well as biotic decomposition [49], hence the need for optimal soil pH. High pH levels in vermicompost may result from the release of excess organic N released as ammonia that quickly dissolved in water producing ammonium and consequently increasing the pH [50]. It has already been observed that vermicompost tend to have higher N levels [51]. So, considering the leguminous nature of the feedstocks and the relatively high N content associated with them, ammonification could have occurred. Thus, vermicomposts have the potential to ameliorate soil acidity. However, some studies have shown that in some instances pH may decrease during vermicomposting especially towards maturity [52,53].

### 3.2. Correlation Relationships and Principal Components Analysis

Bivariate correlation analyses for the different vermicomposts shown in Tables 2–4 for *V. karroo*, *M. oleifera*, and *C. tagasaste*, respectively, show that *M. oleifera*, and *C. tagasaste* had positive correlations between pH and basic cations (Ca, Mg, K, and Na) and negative relationship with micronutrients (Cu, Fe, Mn, Zn, B). However, Mo was the only micronutrient positively related to the vermicompost pH. Such relationships confirm earlier discussions where pH had positive and negative relationships with bases and micronutrients, respectively. In contrast to the correlations shown by *M. oleifera*, and *C. tagasaste*, *V. karroo* showed a significant negative relationship between pH and Ca while the other basic cations had non-significant positive relationships with pH.

Principal components analysis for *M. oleifera* vermicompost showed that PC1 and PC2 exhibited a variance of >10% and were therefore retained (Figure 1 and Table S1). The cumulative variance for the two PCs was 84%. In the first PC, all the bases, P and HR showed a strong positive loading. In contrast, micronutrients Mn, Fe and B showed a strong negative loading. In PC2, only HI and N loaded significantly.

Meanwhile, the principal components analysis for *C. tagasaste* vermicompost produced three significant PCs which accounted for 92% of variance (Figure 2 and Table S2). In PC1, Na, K, P, Mg, and pH exhibited significant positive loadings while micronutrients, Mn, Fe and Zn showed significant negative loadings. Humification indices, N and HA showed positive loadings in PC2 with carbon and FA showing significant loadings. Bases, P, and HR showed a strong positive loading. Parameters Cu, Ca and Mo loaded significantly in PC3. The strong loading of the above nutrients serves to confirm their dominant occurrence in *C. tagasaste*. The negative loading of most micronutrients also confirms the negative interactive effects between micronutrients and the PCA. In the PCA analysis negative values of loadings of variable in the components of the PCA means the existence of an inverse correlation between the factor PCA and the variables.
### Table 2. Bivariate correlations of the chemical properties of V. karroo.

| K (mg kg⁻¹) | Mg (mg kg⁻¹) | Na (mg kg⁻¹) | P (mg kg⁻¹) | Ca (mg kg⁻¹) | Cu (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Mo (mg kg⁻¹) | Zn (mg kg⁻¹) | B (mg kg⁻¹) | pH (KCl) | Carbon% |
|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|----------|---------|
| 0.546       | 0.893 **     | 0.493 **     | 0.984 **     | 0.889 **     | 0.924 **     | 0.830 **     | 0.868 **     | 0.896 **     | 0.856 **     | 0.841 **     | 0.914 **  | 0.848 ** |
| 0.595 *     | 0.770 **     | 0.931 **     | 0.946 **     | 0.904 **     | 0.830 **     | 0.868 **     | 0.896 **     | 0.856 **     | 0.841 **     | 0.914 **     | 0.848 **  | 0.830 ** |
| 0.527       | 0.474        | 0.876 **     | 0.889 **     | 0.850 **     | 0.807 **     | 0.780 **     | 0.876 **     | 0.916 **     | 0.951 **     | 0.914 **     | 0.848 **  | 0.889 ** |
| 0.987 **    | −0.223       | −0.908 **    | −0.877 **    | −0.910 **    | −0.782 **    | −0.795 **    | −0.896 **    | −0.856 **    | −0.841 **    | −0.780 **    | −0.795 ** | −0.795 **|
| −0.531      | −0.508 **    | −0.958 **    | −0.967 **    | −0.916 **    | −0.856 **    | −0.841 **    | −0.780 **    | −0.795 **    | −0.780 **    | −0.795 **    | −0.795 ** | −0.795 **|
| 0.287       | 0.287        | 0.873 **     | 0.866 **     | 0.8071 **   | 0.727 **     | 0.841 **     | −0.896 **    | −0.841 **    | −0.780 **    | −0.795 **    | −0.795 ** | −0.795 **|
| −0.486      | −0.861 **    | −0.866 **    | −0.880 **    | −0.859 **    | −0.710 **    | 0.813 **     | 0.838 **     | 0.742 **     | 0.813 **     | 0.838 **     | 0.742 **  | 0.742 ** |
| −0.239      | −0.903 **    | −0.866 **    | −0.896 **    | −0.768 **    | −0.743 **    | 0.954 **     | 0.914 **     | 0.810 **     | 0.745 **     | 0.810 **     | 0.745 **  | 0.745 ** |
| −0.623 *    | 0.233        | 0.170        | 0.232        | −0.109      | 0.222        | −0.482       | −0.224       | 0.351        | −0.023       | −0.502       | −0.023   | −0.502   |
| 0.947 **    | −0.446       | −0.489       | −0.428       | −0.674 **   | −0.366       | 0.130        | 0.416        | −0.189       | 0.299        | 0.179        | 0.502    | 0.502    |
| −0.848 **   | −0.876 **    | −0.895 **    | −0.856 **    | −0.952 **   | −0.801 **    | 0.643 *      | 0.874 **     | −0.695 *     | 0.723 **     | 0.665 *      | 0.197    | 0.761 ** |

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns means not significant.

### Table 3. Bivariate correlation of the chemical properties of M. oleifera.

| K (mg kg⁻¹) | Mg (mg kg⁻¹) | Na (mg kg⁻¹) | P (mg kg⁻¹) | Ca (mg kg⁻¹) | Cu (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Mo (mg kg⁻¹) | Zn (mg kg⁻¹) | B (mg kg⁻¹) | pH (KCl) | Carbon% |
|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|----------|---------|
| 0.996 **    | 0.978 **     | 0.999 **     | 0.989 **     | 0.994 **     | 0.994 **     | 0.998 **     | 0.998 **     | 0.981 **     | 0.968 **     | 0.981 **    | 0.934 **  | 0.945 ** |
| 0.978 **    | 0.999 **     | 0.989 **     | 0.994 **     | 0.994 **     | 0.998 **     | 0.998 **     | 0.998 **     | 0.981 **     | 0.968 **     | 0.981 **    | 0.934 **  | 0.945 ** |
| 0.997 **    | 0.999 **     | 0.989 **     | 0.994 **     | 0.994 **     | 0.998 **     | 0.998 **     | 0.998 **     | 0.981 **     | 0.968 **     | 0.981 **    | 0.934 **  | 0.945 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |
| 0.996 **    | 0.991 **     | 0.973 **     | 0.986 **     | 0.986 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **     | 0.981 **    | 0.981 **  | 0.981 ** |

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns means not significant.
Table 4. Bivariate correlation of the chemical properties of *C. tagasaste*.

|                | Ca (mg kg\(^{-1}\)) | K (mg kg\(^{-1}\)) | Mg (mg kg\(^{-1}\)) | Na (mg kg\(^{-1}\)) | P (mg kg\(^{-1}\)) | Cu (mg kg\(^{-1}\)) | Fe (mg kg\(^{-1}\)) | Mn (mg kg\(^{-1}\)) | Mo (mg kg\(^{-1}\)) | Zn (mg kg\(^{-1}\)) | B (mg kg\(^{-1}\)) | pH (KCl) | Carbon% |
|----------------|----------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------|----------|---------|
| Ca (mg kg\(^{-1}\))               | 0.375                |                     |                      |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| K (mg kg\(^{-1}\))                 |                      | 0.988 **            |                      |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| Mg (mg kg\(^{-1}\))               |                      |                      | 0.971 **             |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| Na (mg kg\(^{-1}\))               | 0.228                | 0.987 **            |                      |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| P (mg kg\(^{-1}\))                | 0.396                | 0.992 **            | 0.967 **             |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| Cu (mg kg\(^{-1}\))               | 0.386                | 0.033               | 0.155                |                      |                      |                      |                      |                      |                      |                      |                     |          |         |
| Fe (mg kg\(^{-1}\))               | -0.724 **            | -0.818 **          | -0.850 **            | -0.737 **            | -0.823 **            | -0.324               |                      |                      |                      |                      |                     |          |         |
| Mn (mg kg\(^{-1}\))               | 0.051                | -0.871 **          | -0.865 **            | -0.923 **            | -0.856 **            | -0.015               |                      |                      |                      |                      |                     |          |         |
| Mo (mg kg\(^{-1}\))               | 0.617 *              | -0.371             | -0.327               | -0.508               | -0.363               | 0.416                | -0.053               | 0.673 *              |                      |                      |                     |          |         |
| Zn (mg kg\(^{-1}\))               | -0.740 **            | -0.776 **          | -0.806 **            | -0.699 **            | -0.762 **            | -0.491               | 0.827 **             | 0.514                | -0.119               |                      |                     |          |         |
| B (mg kg\(^{-1}\))                | 0.317                | -0.541             | -0.570               | -0.633 *             | -0.506               | -0.228               | 0.243                | 0.816 **             | 0.742 **             | 0.345                |                     |          |         |
| pH                          | 0.257                | 0.686 *            | 0.598 *              | 0.672 *              | 0.718 **             | -0.630 *             | -0.460               | -0.479               | -0.313               | -0.325               | -0.028               |          |         |
| C%                           | -0.497               | -0.002             | 0.052                | 0.077                | -0.057               | 0.325                | 0.197                | -0.309               | -0.332               | 0.099                | -0.616 *             | -0.418   |         |

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns means not significant.
Figure 1. Principal components. Analysis of significant factors of vermicompost *M. oleifera*. PI = Polymerisation index; HI = Humification index; HA = Humic acid; FA = Fulvic acid; HR = Humification ratio. More information is presented in the supplementary Table S1.

Figure 2. Principal components. Analysis of significant factors of *C. tagasaste* vermicompost. PI = Polymerisation index; HI = Humification index; HA = Humic acid; FA = Fulvic acid; HR = Humification ratio. More information is presented in the supplementary Table S2.
Also, three PCs were retained for *V. karroo* accounting for 95% variance (Figure 3 and Table S3). The high loading parameters in PC1 with loading values >0.90 were P, K, Na and Mg. Zn, B and Fe showed a strong negative loading of >−0.88. In PC 2 carbon exhibited a strong negative loading (−0.829). Meanwhile, the humification parameters loaded showed a significant positive loaded with soil pH loading negatively. The strong loading shows the importance of nutrients such as P, K and micronutrients in *V. karroo* vermicompost while the negative loading of C indicates the depletion of C sources with vermicomposting. The P loaded strongly in all the three species showing its strong influence in the vermicomposts.

**Figure 3.** Principal Components Analysis of significant factors *V. karroo*. PI = Polymerisation index; HI = Humification index; HA = Humic acid; FA = Fulvic acid; HR = Humification ratio. More information is presented in the supplementary Table S3.

### 3.3. Humic and Fulvic Acids of *V. karroo*, *C. tagasaste* and *M. oleifera* Vermicomposts

The C content in the FA fraction (FA) was highest in *M. oleifera* (1.30%) followed by *V. karroo* with 0.63% and *C. tagasaste* had the lowest with 0.23% (Table 5). The carbon in HA was highest in *C. tagasaste* (1.72%) but did not differ between *M. oleifera* (1.25%) and *V. karroo* (1.27%). The TEC which is the sum of FA and HA was higher in *M. oleifera* compared to the other two vermicomposts.

The indices calculated, showed that the polymerisation index (PI) was highest in *C. tagasaste* with an index value of 786.93 while the humification ratio (HR) was highest in *M. oleifera* with an index value of 4.25. On the other hand, the humification index (HI) did not differ between the vermicomposts. Both PI and HI can be used to determine maturity of a compost. According to Raj and Antil [54], a mature compost should have a PI ratio of >1.9 and an HI of >30%. The mean values of PI ratio observed in this study were found to be higher than 1.9 thus indicating the maturity of the compost [54,55]. However, the HI index of the vermicomposts were too low at week 6 (1.92 to 2.53), well below the 30% proposed by Raj and Antil [54] which suggest that the vermicomposts were probably not fully mature according to this index. Although the HI of the vermicomposts were very low, the PI ratios suggests that the vermicomposts were mature. The low HI could be the result of slow vermicomposting which...
consequently reflects low humification rate as reported by [56]. It is worth noting that no individual parameter can be used to determine maturity of the vermicompost [54]. The mean values for the parameters differ based on the type of feedstock used, which explains the low or high mean values recorded for the week 6 parameters in this study.

Table 5. Mean maturity indices (FA, HA, TEC, PI, HI and HR) at week 6 and their variation with time.

| Vermicompost | FA  | HA  | TEC | PI   | HI  | HR  |
|--------------|-----|-----|-----|------|-----|-----|
| *V. karroo*  | 0.63b | 1.27b | 1.90b | 203b | 1.92 | 2.88b |
| *M. oleifera* | 1.30a | 1.25b | 2.55a | 99b  | 2.08 | 4.25a |
| *C. tagasaste* | 0.23c | 1.72a | 1.95b | 787a | 2.53 | 2.87b |

| Time (weeks) | FA  | HA  | TEC | PI   | HI  | HR  |
|-------------|-----|-----|-----|------|-----|-----|
| 0           | 1.40a | 0.59b | 1.99b | 57b  | 0.91b | 3.05b |
| 2           | 1.08ab | 1.23a | 2.31a | 1539a | 2.11a | 3.85a |
| 4           | 0.44c | 1.51a | 1.95b | 848ab | 2.46a | 3.13b |
| 6           | 0.72bc | 1.41a | 2.13ab | 363ab | 2.18a | 3.33ab |

Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, ns means not significant. Different letters in the same column represent significant differences.

Table 5 also shows the mean variation of FA, HA and maturity indices (PI, HR and HI) with time (combined for all vermicomposts) while Figure 1 shows the relationship of FA and HA at different times. The results in Table 5 show that the mean FA generally decreased with time with higher values being observed at the beginning of the vermicompost while mean HA generally increased with time. Humification index (HI) behaved similarly to HA with a lower value at the beginning (0.91) which significantly increased at week two and then remained relatively constant throughout the vermicomposting period. As vermicomposting progresses, the percentage of humic substances is expected to increase [57]. This is because during the initial stage of vermicomposting, the organic matter is progressively mineralized. However, during the later (maturing) stage of vermicomposting, humic substances particularly humic acids increases and most of the organic matter stabilises [58]. The other indices (PI and HR) fluctuated throughout the vermicomposting period.

It was observed that *M. oleifera* and *C. tagasaste* all started with relatively high contents of FA compared to HA (Figure 4A) while *V. karroo* had similar levels of HA and FA (Figure 4B). Notable was the mirror reflection of the contents of HA and FA whereby when HA was high FA was lower. There was also a significant negative relationship (−0.90; p < 0.001) between FA and HA. The alternating decrease and increase of the humic and fulvic acids may result from the transformation of humic substances through degradation [59] to the more soluble fulvic acid form at any given time. Hence when the fulvic acids in a certain period increase, the humic acids decrease.

The increase in fulvic acids in this study could have resulted from continued mineralization during the vermicomposting process. The increase in fulvic acids, results in low humic acid content because of the unstable organic matter. Labile forms of fulvic acids are associated with the part of organic carbon referred to as dissolved organic carbon while the less labile forms are humic acids bound to cations and humins [60,61]. Degradation of humic acids results in the accumulation of fulvic acids which contributes to their increase while decreasing the humic acids. The presence of humic acids in the vermicompost can enhance the nutrient uptake by plants and thus act as a soil conditioner [62,63]. By increasing the permeability of the root cell membrane and also the proliferation of the root hairs, the humic acids improve the nutrient uptake of plants [64]. They also form chelates with micronutrients thus increasing the bio-availability for plant use [44].
3.4. Microbial and Earthworm Populations in Vermicomposts

The bacterial populations were found to be lower in V. karroo compared to other two vermicompost (Table 6). V. karroo had 9.00 colony-forming units (cfu) compared to 9.34 in both M. oleifera and C. tagasaste. Phosphate Solubilising Bacteria differed between all vermicompost at week six. Phosphate solubilising bacteria followed the order M. oleifera > C. tagasaste > V. karroo. There were no E. coli in V. karroo and C. tagasaste at week six. Only M. oleifera had E. coli at week six with a cfu of 5.33. Fungi on the hand was higher in M. oleifera (5.75 cfu) compared to V. karroo and C. tagasaste, which both had 5.49 CFU. Vermicompost are known to enhance soil biodiversity by promoting the beneficial microbes which in turn enhances plant growth directly by production of plant growth-regulating hormones and enzymes and indirectly by controlling plant pathogens, nematodes and other pests, thereby enhancing plant health and minimizing the yield loss. In addition, earthworms also enhance the beneficial microflora and suppress harmful pathogenic microbes [65]. However, the existence of E. coli in M. oleifera was unexpected because the type of earthworms used in this study (E. fetida) are known to completely destroy E. coli in their guts [65,66]. Therefore, the non-existence of any E. coli in V. karroo and C. tagasaste is in agreement with findings of Pathma and Sakthivel [65] and Emperor and Kumar [66].

| Vermicompost | Bacteria | Phosphate Solubilising Bacteria | E. coli | Fungi |
|--------------|----------|--------------------------------|---------|-------|
| V. karroo    | 9.0b     | 6.4c                           | 0.0b    | 5.5   |
| M. oleifera  | 9.3a     | 7.1a                           | 5.3a    | 5.8a  |
| C. tagasaste | 9.3a     | 6.7b                           | 0.0b    | 5.5b  |

| Time in weeks | Bacteria | Phosphate Solubilising Bacteria | E. coli | Fungi |
|---------------|----------|--------------------------------|---------|-------|
| 0             | 9.2a     | 7.0a                           | 4.5     | 5.7   |
| 2             | 8.9b     | 6.8b                           | 4.0     | 5.5   |
| 4             | 8.9b     | 7.0ab                          | 4.2     | 5.6   |
| 6             | 9.2a     | 6.8b                           | 1.8     | 5.6   |

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, ns means not significant. Different letters in the same column represent significant differences.

Bacterial population were higher at the beginning, decreased at week two and four before increasing again at week six (Table 6) while PSB fluctuated with time. The mean cfu of E. coli and fungi did not vary with time.
The results also show that the vermicomposts increased earthworm population by more than 500% in all the composts (Table 7). The earthworm population count ranged from 584-761 at week six, with *M. oleifera* being the highest and *C. tagasaste* as the lowest. The increase in earthworm population for each treatment has resulted in improved microbial activity and nutrient availability as shown by the results in this study. Most of the nutrient contents especially for *M. oleifera* were found to be at their optimum levels when compared to critical soil values, which is attributed to the increase in earthworm populations.

Table 7. Earthworm population count for *M. oleifera*, *V. karroo* and *C. tagasaste* vermicompost, initially and at week 6.

| Treatments               | Initial Number of Earthworms | Number of Earthworms at Week 6 |
|--------------------------|------------------------------|-------------------------------|
| *M. oleifera*            | 100                          | 761                           |
| *V. karroo*              | 100                          | 658                           |
| *C. tagasaste*           | 100                          | 584                           |

3.5. Phytochemical Constituents of *C. tagasaste*, *V. karroo* and *M. oleifera* Vermicompost

Qualitative analysis of phytochemicals showed that at any given time during the vermicomposting process; saponins were not detected in the three vermicomposts (Table 8). *Moringa oleifera* contained tannins during the entire period of vermicomposting while no tannins were detected *C. tagasaste* and *V. karroo* during the period (Table 8).

Table 8. Phytochemical constituents of *C. tagasaste*, *V. karroo* and *M. oleifera* vermicompost.

| Time (Weeks) | Treatment         | Saponins | Tannins | Alkaloids | Flavonoids |
|--------------|-------------------|----------|---------|-----------|------------|
| 0            | M. oleifera       | Nil      | Present | Nil       | Present    |
|              | V. karroo         | Nil      | Nil     | Present   | Present    |
|              | C. tagasaste      | Nil      | Present | Nil       | Present    |
| 2            | M. oleifera       | Nil      | Present | Nil       | Present    |
|              | V. karroo         | Nil      | Nil     | Present   | Present    |
|              | C. tagasaste      | Nil      | Nil     | Present   | Present    |
| 4            | M. oleifera       | Nil      | Present | Nil       | Present    |
|              | V. karroo         | Nil      | Nil     | Nil       | Nil        |
|              | C. tagasaste      | Nil      | Nil     | Present   | Present    |
| 6            | M. oleifera       | Nil      | Present | Nil       | Present    |
|              | V. karroo         | Nil      | Nil     | Nil       | Nil        |
|              | C. tagasaste      | Nil      | Nil     | Present   | Present    |

*Moringa oleifera* is known to be rich in different forms of phytochemicals [67], which could have been the reason for the presence of tannins as well as flavonoids observed in this study. This is also supported by Rengarajan, Melanathuru [68] who indicated that methanol extracts of *M. oleifera* petals contained flavonoids, tannins, saponins and alkaloids. Kraus, Yu [69] reported that tannins may limit litter decomposition in a number of different ways such as by themselves being resistant to decomposition or by sequestering proteins in protein-tannin complexes that are resistant to decomposition. In this study, the presence of tannins in *M. oleifera* vermicompost may benefit growing plants by slowing down decomposition and supplying plants with nutrients for longer as opposed to when vermicomposting is faster and quickly depleting nutrients.

Alkaloids were not detected in *M. oleifera* and *V. karroo* but only in *C. tagasaste*. These results are in contrast with Rengarajan, Melanathuru [68] who found that *M. oleifera* contained alkaloids among other phytochemicals. It is not immediately clear why none of the analysed phytochemicals were not detected in *V. karroo* vermicompost in this study. However, previous studies have shown that *M. oleifera,*
V. karroo and C. tagasaste contain secondary metabolites [67,70,71]. The absence could be due to the fact that phytochemicals do not automatically accumulate at their site of synthesis and as a result of genetic variability or leaf age development [72] may have been translocated to other parts of the plant that were not analysed. Reports also indicate that external stimuli can modulate the synthesis and change the composition or quantities of the phytochemicals in plants [73,74]. The quantities are also affected by environmental factors, such as soil composition, temperature, rainfall and ultraviolet radiation incidence [75,76]. Research has certified that flavonoid contents in some leaf exudates can be enhanced by ultra-violet radiation induction or by drought [77]. These findings indicate that the phytochemical contents of the tree species in this study may have possibly been affected by the above-mentioned factors resulting in the absence of saponins and alkaloids.

Flavonoids were detected in M. oleifera and C. tagasaste vermicompost throughout the vermicomposting period (Table 8). These results are also in agreement with Rengarajan, Melanathuru [68] who noted that M. oleifera contains flavonoids among other phytochemicals. Legumes exude specific flavonoids that act as signalling molecules to attract N-fixing bacteria [78], which is essential in making N available in the soil for plant use. Flavonoids are produced when plants are infected or injured [79] or when there are low nutrients [80]. This implies that their presence in the vermicompost is essential to plant health. This is supported by the fact that among others, phytochemicals are a source of carbon or energy for microorganisms which are consequently beneficial for plant health and they also release hormone effectors of cell differentiation in plants [81]. Flavonoids give ultra violet protection to plant tissues, and their accumulation due to ultra-violet exposure is also well documented [82].

The absence of some phytochemicals such as saponins and tannins in some tree species indicates that they are naturally unavailable [83], or present in undetectable amounts. This supports the results obtained in this study, since the phytochemicals were not detected in some of the vermicompost treatments. The presence of phytochemicals in the vermicompost indicate possible defence against harmful bacteria and pests [29]. Therefore, this shows that the vermicomposts have potential to be used for soil fertility enhancement. Seed germination (phytotoxicity) was also above 70%, which indicates that the compost could not have been toxic to plants [29].

4. Conclusions

This Vermicomposting of the agroforestry tree species V. karroo, M. oleifera and C. tagasaste has proven to be very beneficial given the chemical and biological characteristics observed in this study. The chemical composition of the vermicomposts were characterised by a significantly high nutrients such as P, N, K, Mg and Ca that are essential for plant growth and soil fertility enhancement. Although the nutrient content of each vermicompost varied during the vermicomposting period, the quantities of the nutrients found in the vermicomposts indicated that they have potential to be used as for soil fertility improvements and thus reduce the use of synthetic fertilisers in crop production.

Further studies need to evaluate the potential of the vermicomposts as mediums for plant growth and its effect on soil properties and plant growth when applied as soil amendment.

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