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Appraisal of lignocellusoic biomass degrading potential of three earthworm species using vermireactor mediated with spent mushroom substrate: Compost quality, crystallinity, and microbial community structural analysis

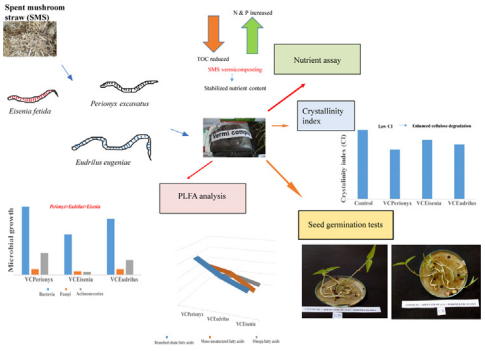
Jinnashri Devi [a], Utsab Deb [b], Soma Barman [a], Subhasish Das [a,c], Satya Sundar Bhattacharya [a,*], Yiu Fai Tsang [d], Jin-Hong Lee [e], Ki-Hyun Kim [f,*]

[a] Soil and Agro Bio-engineering Lab, Department of Environmental Science, Tezpur University, Tezpur 784 028, India
[b] Defence Research Laboratory (DRDO), Tezpur, Assam, India
[c] Department of Environmental Science, Pachhunga University College, Mizoram University, Aizawl 796001, India
[d] Dept. of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, New Territories, Hong Kong
[e] Department of Environmental Engineering, Chungnam National University, Daejon 34148, Republic of Korea
[f] Dept. of Civil & Environmental Engineering, Hanyang University, 222 Wangsimni-Ro, Seoul 04763, South Korea

HIGHLIGHTS

- SMS degradation greatly varies depending on earthworm species during vermicomposting.
- Biomass degradability of earthworms was first assessed via XRD-crystallinity index.
- PLFA assay revealed greater microbial diversity in *Perionyx* vermibeds than others.
- Vermicomposted SMS was effective enough to greatly boost seed germination and vigor.

GRAPHICAL ABSTRACT



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ABSTRACT

Spent mushroom substrate (SMS) is a recalcitrant lignocellulosic waste. Recycling of SMS through composting has been reported; however, the process is lengthy due to its complex biochemical composition. Although vermitechnology is known for its high efficiency, it has rarely been applied to recycle SMS. In this study, the qualitative value of vermicomposted SMS mediated by three earthworm species (i.e., *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*) was evaluated on the basis of nutrient availability, microbial activity, phospholipid fatty acid (PLFA) profiles, and seed germination assays. Degradation profiles of the lignocellulosic substrate in the vermireactors were assessed by monitoring the changes in crystallinity and distribution of functional groups using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy, respectively. Total organic carbon decreased by 1.4–3.5 folds with approximately 2.1–2.4 folds increase in nitrogen and phosphorus availability in all vermibeds. Interestingly, pH declined in the *Eisenia* and *Eudrilus* systems but increased in the *Perionyx*-vermibeds. XRD-derived crystallinity index was reduced significantly by 1.37 folds in *Perionyx*-vermicompost with concurrent microbial enrichment. Further, profuse abundance of vital functional groups (C=O, NH, and OH) was clearly

* Corresponding authors at: Department of Environmental Science, Tezpur University, Assam 784028, India and Department of Civil & Environmental Engineering, Hanyang University, 222 Wangsimni-Ro, Seoul 04763, South Korea.
E-mail addresses: satya72@tezu.ernet.in (S. Sundar Bhattacharya), kkim61@hanyang.ac.kr (K.-H. Kim).

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observed in the vermicompost with *Perionyx* followed by that with *Eisenia*. Moreover, PLFA illustrated significant variations in fatty acid distributions and microbial communities of the three vermicomposting systems. The seed germination assay showed that the germination index and relative root-shoot vigor of *Perionyx*-vermicompost treated seeds were 1.05–1.30 times greater than those of *Eisenia* and *Eudrilus* vermicompost treated ones. The results suggest that SMS degradability was affected by the growth of a healthy microbial community through vermicomposting.

1. Introduction

Commercial cultivation of mushrooms generates a substantial amount of revenue in many countries (Mleczek et al., 2018). The mushroom industry is growing at a good rate, and approximately 25 million tons of mushrooms are produced globally each year (Dygico et al., 2019). Rice or cereal straws are mostly used as a substrate for cultivation of mushrooms because they have a significant impact on the growth and functional properties of the fungus. Approximately 5 kg of spent mushroom substrate (SMS) is generated during the production of 1 kg of mushrooms (Grimm and Wösten, 2018). Generally, the spent substrates are mostly disposed of in landfills or partly reutilized by composting after mushroom harvest (Carrasco et al., 2018). In many countries, open burning is another major disposal pathway of SMS; this practice, however, is well known to result in airborne hazards (Kim Oanh et al., 2018).

Environmental legislation and vigilance activities in India have compelled mushroom growers to search for sustainable disposal pathways for SMS (Ravindra et al., 2019). Besides cereal straws, wood saw dust and cotton waste are also frequently found in SMS (Nasehi et al., 2017). The accumulation of cellulose-rich materials in large proportions gives rise to the recalcitrant nature of the material. Landfilled SMS causes large-scale eutrophication of surface water bodies through leaching of nutrients (nitrogen (N) and phosphorus (P)) (Grimm and Wösten, 2018). Thus, it remains a challenging task to establish a sustainable avenue for SMS disposal.

Recycling the spent substrate through composting approaches has been proposed by several researchers (Rinker, 2017; Tran, 2016). To date, vermicomposting is one of the most efficient solid waste stabilization routes currently available. However, its application to SMS recycling has rarely been reported in the literature. The decay process of SMS under traditional composting systems is very slow due to the strong crystalline structure of the polymer, which greatly retards microbial activity. X-ray diffraction (XRD)-based assessments of crystallinity in lignocellulosic material has already been reported in many previous works (Bansal et al., 2010; Sasmal et al., 2012). To the best of our knowledge, no studies have yet been applied to understand the conversion efficiency of lignocellulosic biomass by earthworm species commonly used in vermitechnology. Moreover, how interactions between earthworms and microbial communities vary in SMS-mediated vermibeds is an interesting and novel topic of study. In fact, the synergy between earthworms and microorganisms is known to be largely responsible for the benefits achieved during vermicomposting with respect to nutrient availability and enzyme activity (Hussain et al., 2016; Negi and Suthar, 2018). Recently, Zhu et al. (2019) reported the utility of multivariate analysis tools (like structural equation models and residual/network analyses) in determining the key environmental factors which influence the composting process. They opined that microbial proliferation in composting systems can be controlled by the combined effects of several variables (e.g., pH, C/N ratio, and moisture content).

The phospholipid fatty acid (PLFA) profile is a dependable biological index used to describe microbial diversity in different environmental situations (Luo et al., 2016). PLFAs are produced by microorganisms for maintaining cell membrane stability and cellular functions under varying conditions such as dehydration and changes in the ionic strength of the microenvironment (Quideau et al., 2016). Although this tool has seldom been applied to assess the microbial community in vermicomposting systems, we anticipated that PLFA will aid in detecting any possible variation in the microbial population of the end product based on the vermicomposting pathway. The following research questions were identified on the basis of the studied literature: (a) How do different earthworm species respond to SM-based feedstock? (b) Does the presence of earthworms affect the microbial community structure in such feedstock? (c) Does the change in biomass crystallinity vary according to the earthworm species used for vermicomposting? The third question is important because the crystalline arrangement in lignocellulosic materials largely inhibits enzymatic degradation (Hirano et al., 2016). In this study, the changes in SMS feedstock during vermicomposting with three earthworm species, namely, *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*, were evaluated on the basis of an XRD-derived crystallinity index (CI), Fourier transform infrared (FTIR) spectroscopy, nutrient availability (N and P), microbial growth, metabolism, and microbial community structure. Seed germination assays were also conducted to evaluate the effect of vermicomposted SMS on plant growth.

2. Materials and methods

2.1. Collection of SMS, earthworm species, and cow dung (CD)

The SMS samples were procured from the mushroom production unit of the Defense Research and Development Organization (DRDO), Defence Research Laboratory (DRL), Tezpur, Assam, India. The SMS is the by-product of the standardized cultivation system of oyster mushroom (i.e., *Pleurotus ostreatus*). Clitelleted specimens of three epigeic and well-documented earthworm species, namely, *E. fetida*, *Eu. eugeniae*, and *P. excavatus*, weighing about 300–400 mg each, were collected from a designated stock maintained in the vermiculture unit of the Department of Environmental Science, Tezpur University. Two three-day-old urine-free CD samples were also used in this experiment.

2.2. Experimental set up and techniques

Three earthen vermireactors were prepared on a parallel basis for the three different earthworm species. The vermireactors were designed according to standard sizes and dimensions [size: 3 L; dimensions: 45 cm (height) × 15 cm (base radius) × 30 cm (top radius)] with one leachate hole at the base (Hussain et al., 2018). The vermireactors were placed on a concrete-floored vermi-yard with a roof made of corrugated sheet and open sides. The collected SMS materials were thoroughly mixed with CD at a ratio of 3:1 after determination of their inherent physico-chemical characteristics (Table 1). This substrate mixture recipe has been adopted from our previous study (Paul et al., 2018). Generally, the ratio values (e.g., 1:1, 2:1, and 3:1) determined in our earlier studies were used

Table 1
Physico-chemical characteristics of the spent mushroom substrate (SMS) and cow dung (CD) used for the study. Values represent mean ± standard deviation.

	SMS	CD
pH	6.27 ± 0.02	6.84 ± 0.01
Moisture content	54.8 ± 3.66	37.2 ± 5.01
Bulk density	0.87 ± 0.06	1.09 ± 0.32
Total organic carbon (%)	2.73 ± 0.35	3.90 ± 0.00
Total nitrogen (%)	0.66 ± 0.04	1.23 ± 0.251
Available phosphorus (mg kg^{-1})	110.5 ± 0.02	100.38 ± 0.04
Microbial biomass carbon (μg g^{-1})	1128.4 ± 2.98	1470.40 ± 0.47
Compost respiration (μg $g^{-1}h^{-1}$)	3423.61 ± 1310.74	2760.42 ± 2604.17
Crystallinity index (%)	18.15 ± 0.01	6.80 ± 0.06

by considering the relative dryness of the substrate. As the proportion of CD was raised slowly, the results derived at 3:1 ratio were seen as the most congenial milieu for the insipient earthworms. Correspondingly, 3 kg of the homogenized mixture of SMS and CD were poured into the vermireactors, and the selected earthworm species were separately introduced into the reactors at a density of 10 worms per kg of vermicompost. A series of aerobic composting reactors with identical feedstocks was maintained under the same conditions for comparison. Details of the treatment combinations are given below:

Control – Aerobic composting with SMS + CD (3 : 1) feedstock (as composting control)

$VC_{Eisenia}$ – Vermicomposting of SMS + CD (3 : 1) with *E. fetida*

$VC_{Eudrilus}$ – Vermicomposting of SMS + CD(3 : 1) with *Eu. eugeniae*

$VC_{Perionyx}$ – Vermicomposting of SMS + CD (3 : 1) with *P. excavatus*

The experiment was conducted for 60 days with temperatures of 27–31 °C and moisture contents of 40%-50%. These conditions were maintained by sprinkling water on the reactors and turning the pile twice daily at 9 am and 4 pm. The rate of leachate generation was negligible due to adequate aeration provided by the daily mixing operation. The generated leachates were collected at the bottom of the reactors in earthen trays and were recycled for watering. The moisture and temperature were regularly recorded by a wet bulb thermometer. After the incubation period of 60 days, the earthworms and their cocoons were carefully sieved out from the vermicomposted materials and counted. Then, the prepared vermicomposts were kept in a drying shade for 6–7 days until a stable weight was achieved. The average temperature under the drying shade was about 32 ± 2 °C. Afterwards, the samples were ground into smaller pieces and passed through a 2-mm mesh sieve. Parts of the samples were used for analysis and other parts were stored in plastic containers in a freezer at −20 °C.

2.3. Chemical analysis

The temporal changes in the chemical properties of the bioprocessed SMS were analyzed based on the variations in pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and available P according to standard protocols (Page et al., 1994). High purity analytical grade chemicals and deionized water were used to prepare the reagents and other analytical solutions. All glassware was cleaned with deionized water before use to ensure the removal of adherent compounds and was dried at 42 °C (USEPA, 1989).

2.4. XRD and FTIR spectroscopy: Crystallinity and functional groups of the vermicomposted SMS

The air-dried samples were kept in a glass sample holder and were analyzed under plateau conditions using an X-ray diffractometer. Subsequently, the CI was computed from the XRD intensity recorded at ~2θ = 18° (amorphous plane) and ~22.8° (crystalline plane) using the Eq. (1) (Sasmal et al., 2012).

$$CI = \frac{(I_{002} - I_{amor})}{I_{002}} \times 100 \quad (1)$$

where

CI = crystallinity index

$I_{002(crystalline)}$ = intensity at 22.8°

I_{amor} = intensity at 18^o

The CI was used to assess the extent of degradation of the SMS under different vermicomposting systems.

FTIR spectroscopy was performed for determining the abundance of various functional groups and chemical compositions of the vermicomposted samples (Sasmal et al., 2012). The samples were dried at 50 °C for 24 h, after which 2 mg of each powdered sample were dispersed on IR-grade potassium bromide (200 mg). The formed pellets were then used for analysis. FTIR spectra were obtained at a resolution of 1 cm^{-1} in a NICOLET spectrophotometer (Model IMPACT 410).

2.5. Microbial growth and microbial community analyses

Total bacterial and fungal counts in the vermicomposted samples were assessed according to the method of Pramer and Schmidt (1964) using 10 g of sample suspended in 90 ml of sterile distilled water at the very outset followed by serial dilutions following a log scale. Bacterial and fungal colonies were incubated using nutrient agar and potato dextrose agar, respectively.

The microbial biomass C (MBC) was enumerated by following Jenkinson (1988). Briefly, 10 g of each sample were processed via two parallel routes; one was subjected to chloroform fumigation while the other was un-fumigated. Both fumigated and un-fumigated samples were extracted in 2 M KCl solution, and the filtrate was used to measure ninhydrin-N in a UV–visible spectrophotometer at 570 nm. The equation (2) was used to convert ninhydrin-N into MBC.

$$MBC(\mu g g^{-1}) = \left[(A_f \times A_{uf}) \times \frac{40}{10} 10 \times (W_m \times W_d)\right] \times 31 \quad (2)$$

Where

A_f = Absorbance (at 570 nm) of fumigated sample

A_{uf} = Absorbance (at 570 nm) of un-fumigated sample

W_m = Moist weight of sample in gram

W_d = Difference of moist weight and dry weight of sample in gram

We followed the phenolphthalein titration method for enumerating the CO_2 evolution rate of the compost, i.e., compost respiration (CR) (Epstein, 1997). About 10 g of sample was incubated at 25°C inside cork-stoppered flasks containing 5 ml of 0.5 N NaOH for 24 h. In the succeeding stage, the NaOH was taken out and titrated with 0.5 N HCl (in the presence of $BaCl_2$ and phenolphthalein indicator) untill the pink solution turned colorless. The microbial quotient (Mq) was derived from the ratio of MBC (C_{mic}) to TOC (C_{org}) (Tripathy et al., 2014). The microbial metabolic quotient (qCO_2) was then measured from the ratio of MBC to CR (Anderson and Domsch, 1990).

The PLFA analysis was performed to study the microbial community structure in the selected vermicomposted samples. The selection of vermicomposted samples for PLFA assay was done on the basis of the results obtained from microbial counts, Mq, and qCO_2. The detailed procedure of PLFA assay has been described in our previous paper (Hussain et al., 2018). In short, vermicompost samples were first dried in centrifugal evaporator and then extracted with Bligh-Dyer extractant. The Bligh-Dyer extractant was prepared by mixing K_2HPO_4 (50 mM), methanol, and chloroform in 4:10:5 ratio. The extracts were again subjected to solid-phase extraction to separate the lipids, then dissolved in 100 μl hexane, transferred in gas chromatography vials, and analyzed in a gas chromatograph equipped with flame ionization detector (Agilent 6850). The microbial communities were identified based on PLFA profiles using Sherlock software (Luo et al., 2016).

2.6. Seed germination assay

The efficiency of the various vermicomposted SMS samples as plant growth promoting agents was evaluated on the basis of their effects on plant growth potential with respect to seed germination index (GI), relative root (RRG), and shoot (RShG) growth. The assay was performed using fresh, robust, and disease-free seeds of green gram (i.e., *Vigna radiata*) according to standard methods (Das et al., 2016). Specifically, 10 g of the composted and vermicomposted SMS samples were dissolved in Milli-Q water at a ratio of 1:10 in sealed plastic containers and were shaken at 140 rpm by a mechanical shaker for 1 hr. The containers were allowed to stand until the stabilization of the precipitates, and the supernatants were collected eventually. Then, the green gram seeds (n = 10) were kept in filter papers placed on a series of sterilized glass petri plates. They were then inoculated with the previously collected supernatants of the SMS-vermicompost solutions. The plates were incubated at 25 °C under the dark condition. The germinated seeds and sprouted roots and shoots were counted and recorded accordingly. The equations used for determination of relative seed germination (RSG), RRG, RShG, and GI are given as follows:

$$\mathrm{RSG}\% = \frac{\text{Number of seeds germinated with the extracts of treated vermicomposts amples}}{\text{Number of seeds germinated in distilled water}} \times 100 \quad (3)$$

$$\mathrm{RRG}\% = \frac{\text{Mean root length of seeds treated with vermicompost samples}}{\text{Mean root length of seeds in distilled water}} \times 100 \quad (4)$$

$$\mathrm{RShG}\% = \frac{\text{Mean shoot length ofseeds treated with vermicompost samples}}{\text{Mean shoot length of seeds in distilled water}} \times 100 \quad (5)$$

$$\mathrm{GI}\% = \frac{\mathrm{RSG}}{\mathrm{RRG}} \times 100 \quad (6)$$

2.7. Statistical analysis of vermicomposting systems

All data obtained from the vermicomposting experiment were subjected to two-way ANOVA by considering the types of vermicomposting systems and duration for completion of vermicomposting. The least significant difference (LSD) test and Duncan's Multiple Range Test (DMRT) were then performed to determine the statistical significance in variations among the different treatments.

3. Results and discussion

3.1. Characteristics of raw materials

The inherent properties of the SMS and CD are summarized in Table 1. Rice straw was the main ingredient of the SMS under study. In fact, rice straw is one of the most widely used materials for edible mushroom cultivation (Carrasco et al., 2018). The physical natures of the SMS and CD materials were determined on the basis of CI. The CI of the SMS was about three times higher than that of CD, indicating a stronger crystalline arrangement in the former due to the lignocellulosic composition of the material (Zhu et al., 2013). Compared with CD, the SMS was more alkaline due to its high salt content with a slightly lower TOC content (Table 1). Cereal waste contains less fermentable sugar (organic C) unlike vegetable waste which reciprocates to the reduced formation of organic acids during bio-deterioration process (Kim et al., 2018). Edible mushrooms are generally cultivated in various plant litters; therefore, their physicochemical characteristics considerably fluctuated from place to place. High contents of N (~1.90%) and P (>100 ppm) have been found in SMS by some researchers (Gupta et al., 2004; Izyan et al., 2009). This finding may be due to the recalcitrant nature of the SMS. That is, the SMS greatly retards the transformation of organic-end to inorganic-end. Interestingly, MBC was lower in the SMS than in the CD. This indicated that the wasteful expenditure of microbial energy is greater in SMS than in CD (Vos et al., 2017).

3.2. XRD- and FTIR-based physico-chemical analyses

The crystallinity index (CI) was determined by using XRD spectra as presented in Fig. 1. A significant reduction in CI of the SMS feedstocks was observed after 60 days of incubation in all the three vermicomposting systems. Such a reduction was the greatest in the *Perionyx*-mediated vermibeds (1.37-fold) followed by the *Eudrilus* (1.34-fold), and *Eisenia* (1.31-fold) vermibeds after 60 days of incubation ($p < 0.01$; LSD = 0.28). Cellulose-rich SMS materials are largely resistant to enzymatic hydrolysis due to their crystalline structure (Hirano et al., 2016). The hydrogen bonds linking glucose molecules impart crystalline characteristics in cellulosic substances (Bansal et al., 2010). However, the crystalline arrangements of biomolecules in SMS (e.g., cellulose, hemicelluloses, and lignin) have been hypothesized to undergo degradation during vermicomposting. Conceptually, lower CI values indicate lesser crystalline (i.e., more amorphous) arrangement of macromolecules in the biomass (Sasmal et al., 2012). Hence, the results revealed that the matrix of lignocellulosic compounds in the feedstocks were efficiently degraded by the earthworms in combination with the microorganisms and their enzymes in the earthworm intestines.

The results of FTIR spectral analysis are presented in Table 2. The typical stretching frequencies of vital functional groups (e.g., C=O, NH, and OH) occurred between 4000 and 1300 cm^{-1} (Segneanu, 2012). The transmittance peaks in the range of 3400–3500 cm^{-1} indicated the presence of OH groups of alcohols, phenols, and aldehydes in all samples collected on Day 0 and Day 60. Evidence of the C–H (i.e., 2850–3000 cm^{-1}) and C=C (i.e., 1640–1650 cm^{-1}) stretching vibrations of hydrocarbons, alkanes,

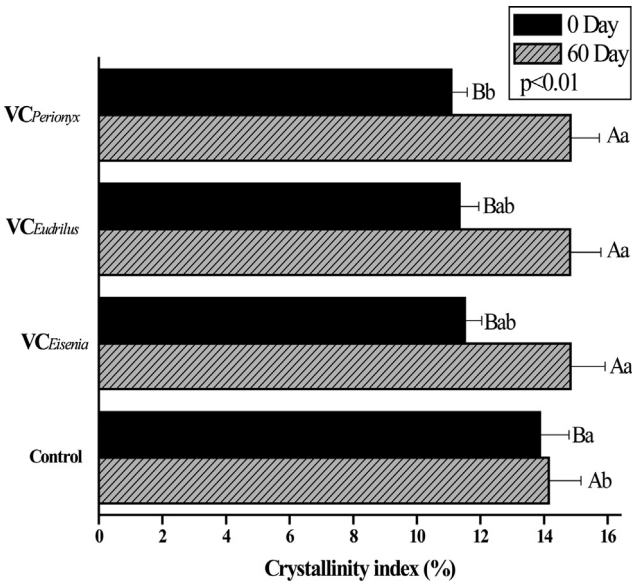

Fig. 1. Structural deformation in the spent mushroom substrate (SMS) under the vermicomposting and composting treatments as verified from the crystallinity index. [Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida;* $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae;* $VC_{Perionyx}$ - Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation. Similar letters represent no significant differences as per DRMT at $p < 0.05$. (Capital letters represent significant temporal variation while small letters represent the treatment-wise variation.)

and alkenes were also found in both composted and vermicomposted samples. N compounds in the form of amines (NH_2 and NH) and esters (S-OR) were found in the feedstocks (Table 2). In addition, the typical bending vibrations of Si-O-Si and O-Si-O in the spectral region of 465–470 cm^{-1} were conspicuous in all vermibeds and compost beds. The signatures of sulfur compounds, such as sulfates (S═O), were initially detected in all feedstocks in the IR spectral region of 1350–1390 cm^{-1}; these peaks, however, were absent from the spectra of the vermicomposted feedstocks collected on Day 60 (Dores-Silva et al., 2015). Interestingly, despite the overall similarity of the spectral distributions of the vermicomposted SMS samples, peaks in the spectral region of 1420–1425 cm^{-1} assigned to aromatic ring structures were only detected in the $VC_{Eudrilus}$ and $VC_{Perionyx}$ samples after 60 days of incubation. These variations indicate that earthworm-mediated biodegradation encourages the formation of stable humified substances with the concomitant destruction of sulfur compounds. A noticeable reduction in the transmittance energy (%T) was also recorded in the composted and vermicomposted feedstocks after 60 days. Generally, a decrease in the peak intensity vis-à-vis transmittance at any frequency in the FTIR spectrum indicates the predominance of bonds with vibrational energies identical to that of the incident light (Maurya et al., 2018). As a result, penetration of light through the given substance is hindered. This phenomenon is the eventual outcome of the transformation of lignocellulosic crystals into amorphous constituents (Traoré et al., 2018). These findings strongly confirm the results of the XRD-based crystallinity analysis.

3.3. Changes in pH, TOC, TKN, and P levels under different conditions

It was imperative to assess the state of organic matter decomposition with regard to certain indicative chemical parameters such as pH, TOC, TKN, and available P. The temporal changes in these attributes during vermicomposting of the SMS feedstock with the three different earthworm species are presented in Fig. 2. In general, the substrate pH decreased over time in the *Eisenia*- and *Eudrilus*-mediated vermicomposting systems ($VC_{Eisenia}$ and $VC_{Eudrilus}$) when compared with the initial values. In contrast, pH slightly increased in the *Perionyx*-mediated vermibeds (i.e., $VC_{Perionyx}$). *Perionyx excavatus* has been reported to reduce pH in different feedstocks (Tran, 2016). The increase in pH of the $VC_{Perionyx}$ substrates may be attributed to the activity of the calciferous glands in the esophageal epithelium of earthworms. These glands contain carbonic anhydrase, which fixes CO_2 as calcium carbonate and prevents reductions in pH (Mubeen and Hatti, 2018). The shift in pH toward acidity in the *Eisenia* and *Eudrilus* systems has been reported in previous work (Das et al., 2016; Paul et al., 2018). Such reductions in pH may be accounted for by the production of organic acid, CO_2, and nitrate during mineralization of organic matter.

The TOC of the vermibeds was reduced significantly (e.g., by 1.4- to 2.5-fold) as compared to composting systems (Fig. 2). However, the reductions in TOC were most prominent in the $VC_{Eisenia}$ system. *Eisenia fetida* is a voracious feeder, and their excretion rate is also greater than other worm species (Domínguez, 2018). In general, a reduction in TOC signifies the efficiency of vermitechnology for nutrient mineralization due to accelerated microbial activity (Hussain et al., 2018). Correspondingly, TKN levels significantly increased in the vermibeds as compared to composting (Fig. 2). TKN levels were slightly higher in $VC_{Eudrilus}$ than in $VC_{Perionyx}$ and $VC_{Eisenia}$ after 60 days of incubation, but the variation observed was not statistically significant (Fig. 2). The efficiency of nutrient solubilization from recalcitrant feedstock is greater in *Eu. eugeniae* than in *E. fetida* and *P. excavatus* (Domínguez, 2018). The increase in nitrogen availability in vermicomposts may be due to N-fixing

Table 2
Transmittance values (T%) and the main absorbance bands in FTIR spectra of the composted and vermicomposted samples along with their assignments.

Wave number (cm^{-1})	Functional Groups	Control				$VC_{Eisenia}$				$VC_{Eudrilus}$				$VC_{Perionyx}$			
		0 d	T %	60 d	T %	0 d	T %	60 d	T %	0 d	T %	60 d	T %	0 d	T %	60 d	T %
3400–3500	O–H groups of alcohols, phenols, aldehydes	Yes	53	Yes	41	Yes	54	Yes	52	Yes	53	Yes	50	Yes	50	Yes	43
2850–3000	C–H stretching of hydrocarbon alkanes	Yes	60	Yes	57	Yes	58	Yes	57	Yes	55	Yes	59	Yes	62	Yes	53
1640–1650	C═C of hydrocarbon alkene; C═O of amides	Yes	59	Yes	59	Yes	58	Yes	56	Yes	54	Yes	53	Yes	55	Yes	48
1420–1425	Aromatic ring structure	No	–	No	–	No	–	No	–	No	–	Yes	58	No	–	Yes	58
1350–1390	S═O of sulfates of other S compounds	Yes	49	Yes	47	Yes	48	No	–	No	–	No	–	Yes	51	No	–
1090–1100	Silicon compounds e.g., silane (S-OR)	Yes	40	Yes	30	Yes	43	No	40	Yes	46	No	43	Yes	40	No	38
660–850	Amines (NH_2 and N–H), esters (S-OR)	Yes	62	Yes	56	Yes	63	Yes	63	Yes	62	Yes	65	Yes	63	Yes	57
500–540	S-S disulfide bonds	Yes	59	Yes	57	Yes	58	Yes	58	Yes	62	Yes	60	Yes	60	Yes	59
465–470	Bending vibration of Si-O-Si and O-Si-O, Si-aliphatic, alkyl, and alkenes	Yes	56	Yes	46	Yes	58	Yes	55	Yes	55	Yes	55	Yes	40	Yes	38

Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida*; $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae*; $VC_{Perionyx}$ – Vermicomposting of SMS + CD (3:1) with *Perionyx excavates*.

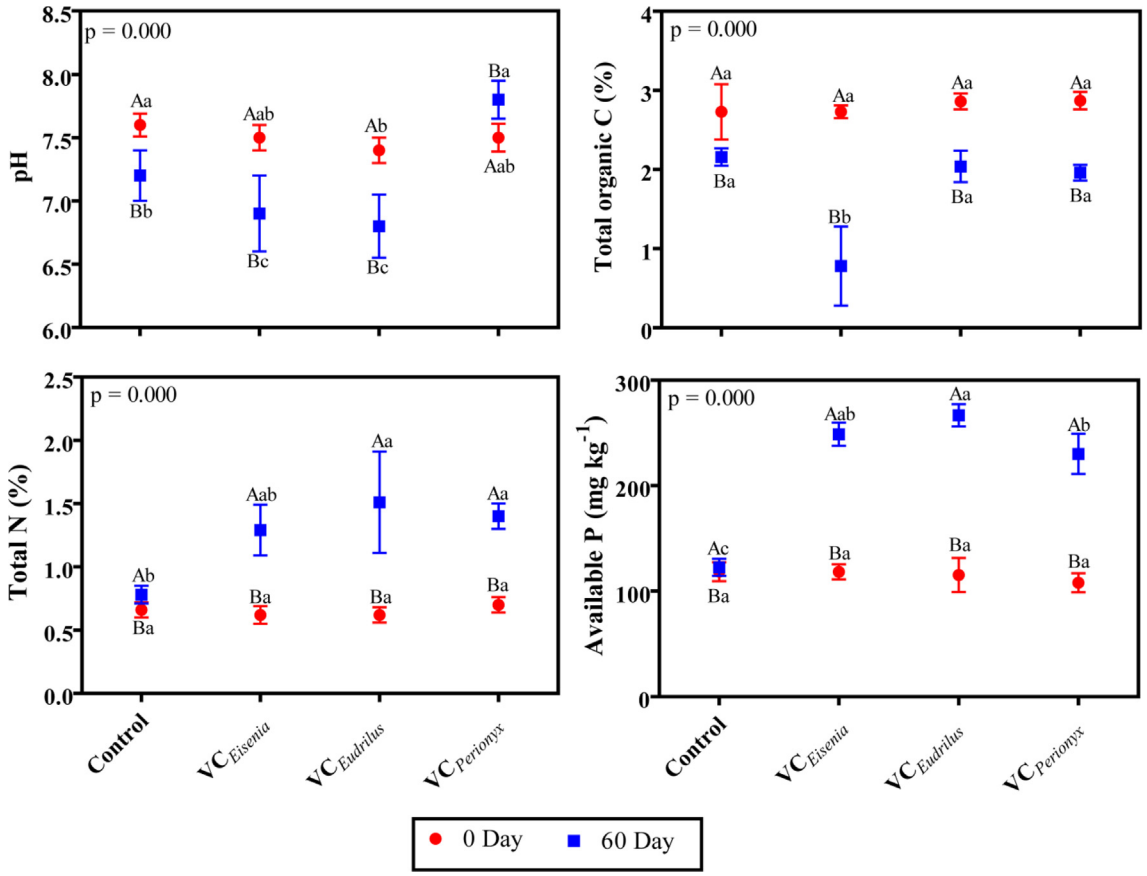

Fig. 2. Temporal variation in pH, total organic C, total Kjeldahl N, and available P of spent mushroom straw based feedstocks under the composting and vermicomposting system [Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida;* $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae;* $VC_{Perionyx}$ - Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation. Similar letters represent no significant differences as per DRMT at $p < 0.05$. (Capital letters represent significant temporal variation while small letters represent the treatment-wise variation.)

microorganisms released via earthworm excreta and the breakdown of small- to medium-length polysaccharides (Hussain et al., 2016). Moreover, recently, it has been reported that many types of bacterial communities can greatly enhance N availability by degrading the high molecular weight nitrogenous compounds into low molecular weight soluble substances (Zhu et al., 2019). Overall, the bioavailability of phosphorus was significantly higher in the vermicompost than in the compost at the end of the incubation period. The availability of phosphorus was significantly higher in the $VC_{Eudrilus}$ vermibeds than in the $VC_{Eisenia}$ and $VC_{Perionyx}$ ($p = 0.000$; LSD = 10.30). Recently, *E. eugeniae* was reported to have high efficiency in solubilizing large amounts of phosphorus (Paul et al., 2018). Phosphorus bioavailability is greatest at neutral pH (i.e., from 6.5 to 7.1) (Adhikari et al., 2017). Interestingly, the pH of *Eisenia*- and *Eudrilus*-mediated vermibeds was nearly neutral, while that of the *Perionyx*-mediated feedstock was slightly alkaline after 60 days; the observed difference in pH trends may be explained at least partly by the fact that calciferous substances in the feedstock are released by the worm species (Mubeen and Hatti, 2018). Hence, alkalinity may restrict P solubility despite the considerable proliferation of P-solubilizing microorganisms in such feedstocks.

3.4. Changes in microbial growth, metabolism, and diversity: Microbial biomass, respiration, metabolic quotient, and PLFA based investigation

The changes in MBC, CR, Mq, and qCO_2 are plotted in Fig. 3. The MBC and CR increased by 1.6–4.8 times after 60 days in all feedstocks, but increases were the greatest in the *Perionyx*-treated feedstock followed by the *E. eugeniae*-treated feedstock (p value of MBC & CR = 0.000; LSD: MBC = 73.1, CR = 392.1). The increases in MBC imply improvements in microbial health and proliferation due to vermicomposting. Earthworm intestines can harbor diverse microbial genera by providing favorable growing conditions (Hussain et al., 2016), which are eventually released to the vermibeds through the earthworm excreta (Dvorak et al., 2016). However, microbial respiration (i.e., CR) was the lowest in $VC_{Eisenia}$ despite the significant increase in MBC after 60 days. The enhancements in MBC resulted in high Mq (i.e., the ratio of MBC to TOC) in the $VC_{Perionyx}$ reactor followed by the $VC_{Eisenia}$ and $VC_{Eudrilus}$ reactors (Fig. 3). The qCO_2 (i.e., ratio of CR to MBC) in the feedstocks followed the order: C > $VC_{Eisenia}$ = $VC_{Eudrilus}$ > $VC_{Perionyx}$ ($p = 0.000$; LSD = 0.80) (Fig. 3). CR is often considered to be a wasteful expenditure of acquired energy for maintenance rather than growth in challenging environments (Tripathy et al., 2014). Therefore, a low qCO_2 in the vermicomposted feedstock signifies a quicker decline in microbial respiration and stabilization of the biodegradation process compared to aerobic composting (Vos et al., 2017; Hussain et al., 2018).

Microbial counts were considerably greater in the vermicomposted SMS than in the composted substrates (Table 3). The total bacterial count was significantly higher in the $VC_{Perionyx}$ than in the others, while the fungal growth was the highest in the $VC_{Eudrilus}$ ($p = 0.000$; LSD = 10.6). However, proliferation of P-solubilizing and N-fixing bacteria was remarkably greater in $VC_{Perionyx}$ compared with the $VC_{Eisenia}$ and $VC_{Eudrilus}$ ($p = 0.000$; LSD = 108.02). Earth-

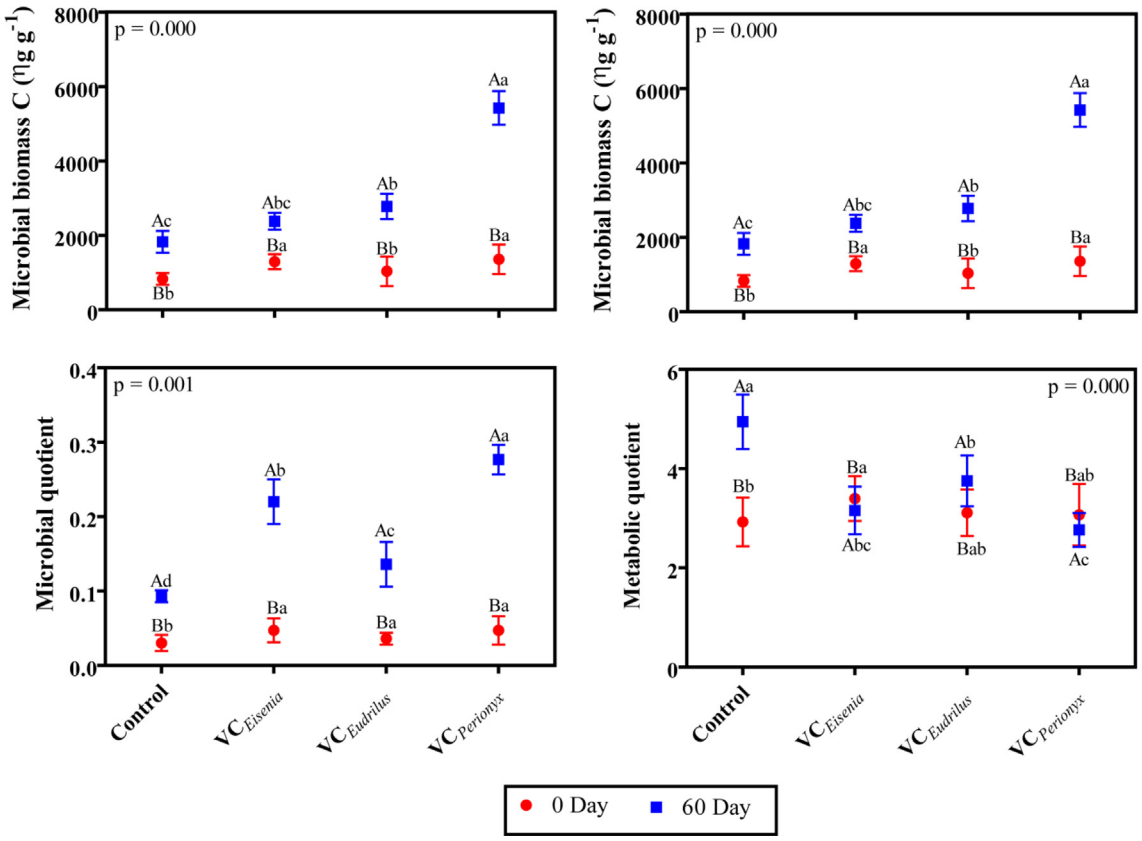

Fig. 3. Changes in microbial biomass C, compost respiration, microbial quotient, and microbial metabolic quotient under various bio-composting treatments. [Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida;* $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae;* $VC_{Perionyx}$ - Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation. Similar letters represent no significant differences as per DRMT at $p < 0.05$ (Capital letters represent significant temporal variation while small letters represent the treatment-wise variation).

Table 3
Count of total bacteria (TBC), total fungi (TFC), P solubilizing bacteria (PSB), and N fixing bacteria (NFB) in the composted and vermicomposted SMS at 60 days of incubation. Values represent mean ± standard deviation.

	TBC ($\times 10^5$ CFU g^{-1})	NFB ($\times 10^4$ CFU g^{-1})	PSB ($\times 10^5$ CFU g^{-1})	TFC ($\times 10^2$ CFU g^{-1})
Control	230 ± 5	73 ± 6	18 ± 2	80 ± 4
$VC_{Eisenia}$	1000 ± 9	400 ± 17	280 ± 7	120 ± 9
$VC_{Eudrilus}$	900 ± 16	210 ± 6	350 ± 11	1900 ± 17
$VC_{Perionyx}$	3600 ± 11	2000 ± 19	1440 ± 9	1300 ± 14
P	0.001	0.000	0.000	0.000
LSD	116.7	83.97	108.02	10.6

Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida*; $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae*; $VC_{Perionyx}$ – Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus; CFU = Colony forming unit; LSD = least significant difference*.

worms efficiently pulverized feed materials into minute pieces with the help of their gizzard, thereby exposing larger surface areas for microbial activity (Hussain et al., 2018). In fact, the levels of compatibility between earthworm species and feed composition greatly dictated the microbial enrichment in the processed materials (Vos et al., 2017; Hussain et al., 2016).

The PLFA assay was performed to understand the structural adjustments in microbial communities in vermibeds in response to SMS treatment. Long- and short-chain PLFAs provided strength and stability to the microbial cell membrane (Quideau et al., 2016). The structural compositions of PLFAs greatly varied among microbial communities depending on their immediate environment (Luo et al., 2016). In this study, the total microbial biomass was remarkably higher in the $VC_{Perionyx}$ than in $VC_{Eisenia}$ and $VC_{Eudrilus}$ (Fig. 4). The Gram-negative bacterial PLFAs were greater in $VC_{Eudrilus}$ than in the other two systems, whereas Gram-positive bacterial PLFAs were most abundant in $VC_{Perionyx}$ followed by $VC_{Eisenia}$. The occurrence of actinomycete groups was also significantly higher in the $VC_{Perionyx}$ followed by $VC_{Eudrilus}$ and $VC_{Eisenia}$ (Fig. 4). The abundance of Gram-positive bacterial communities in the $VC_{Perionyx}$ and $VC_{Eisenia}$ signifies favorable earthworm-feedstock compatibility in these systems. The dominance of Gram-negative bacterial communities in $VC_{Eudrilus}$ indicates that the SMS feedstock is probably stressful for the *Eudrilus*-mediated system because Gram-negative organisms tend to tolerate aberrant environments (Eberlein et al., 2018). Moreover, many arbuscular mycorrhizal fungi (AMF) were observed in the $VC_{Perionyx}$ and $VC_{Eisenia}$ feedstocks. AMFs are natural biofertilizers that normally

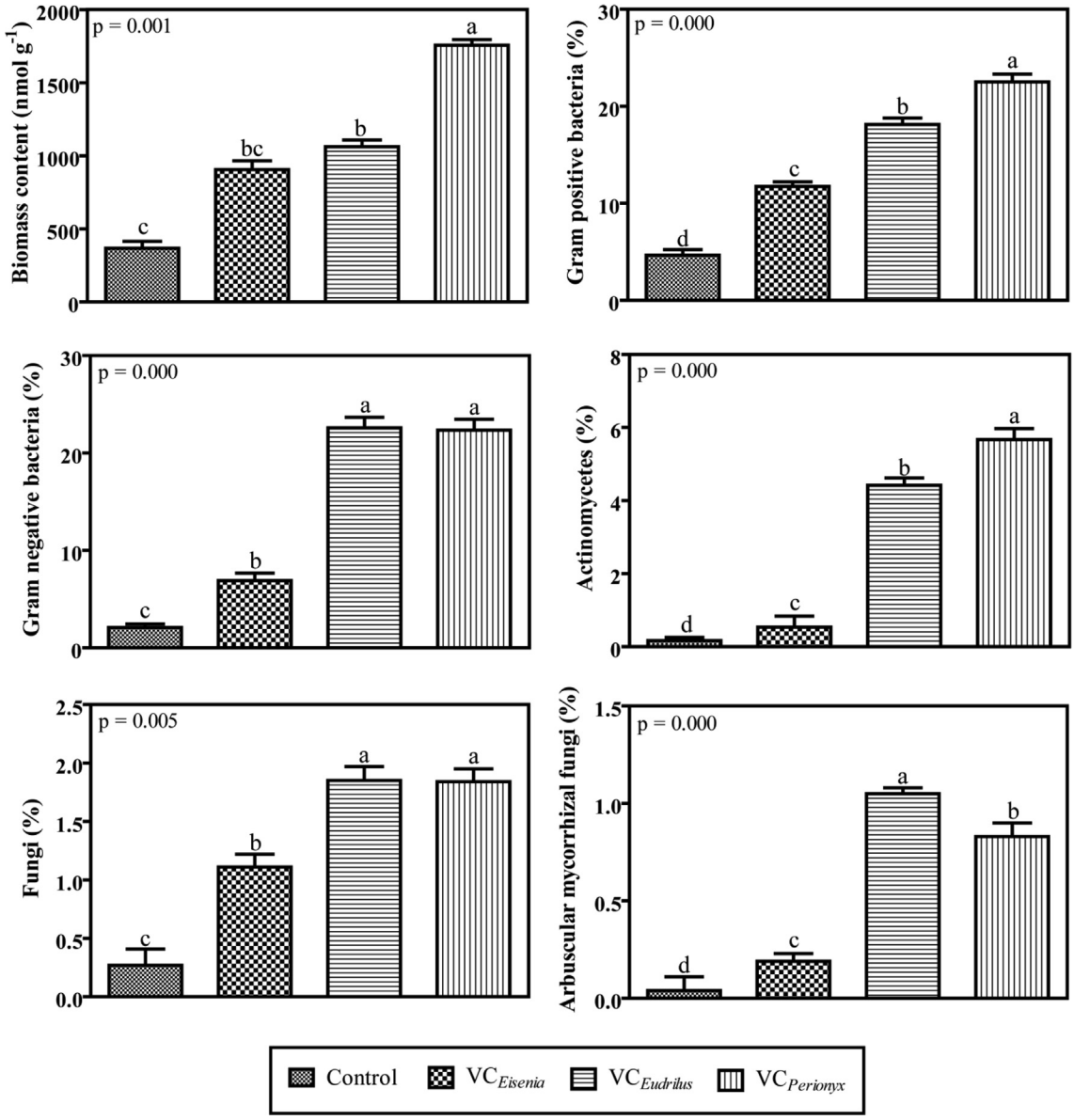

Fig. 4. Phospholipid fatty acid (PLFA) identified microbial groups in the composted and vermicomposted SMS-based feedstocks at 60 days of incubation. [Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida;* $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae;* $VC_{Perionyx}$ - Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation. Similar letters represent no significant differences as per DRMT at $p < 0.05$ (Capital letters represent significant temporal variation while small letters represent the treatment-wise variation).

reside in the root zone soil in agricultural or forest lands (Berruti et al., 2015). Hence, the occurrence of AMFs in these SMS vermicomposts adds greater value to the sustainable recycling of agricultural waste.

Interestingly, the proportions of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the total PLFA were the highest in the $VC_{Perionyx}$ and $VC_{Eisenia}$, respectively (Fig. 5). MUFA and PUFA are known to maintain membrane fluidity in many bacterial species, thereby ensuring membrane stability under unfavorable conditions (de Carvalho and Caramujo, 2018). The signatures of some typical PUFA (e.g., 18:1ω9c and 18:2ω6,9c) in the PLFA profiles of the vermicomposted SMS samples revealed the presence of eukaryotes and ectomycorrhizal fungal communities in the processed materials (Quideau et al., 2016). In a similar work, Zhu et al. (2019) reported the presence of 52 bacterial genera that were involved in degrading amino acid-N, amine-N, and amino sugar-N into simpler products during chicken dropping-garden waste composting.

3.5. Effects of composted and vermicomposted SMS on seed vigor

The results of the germination assay are shown in Table 4. This assay is a reliable means of evaluating the response of plant species to a growth stimulant (Hussain et al., 2018). The germination index and relative seed germination were significantly higher in the $VC_{Perionyx}$-treated seeds than in the $VC_{Eisenia}$- and $VC_{Eudrilus}$-treated seeds ($p = 0.000$; LSD = 3.18). Correspondingly, the relative root and shoot vigor (RRG and RShG) of seeds treated with $VC_{Perionyx}$ were greater than all other inoculants (RRG: $p = 0.000$, LSD = 6.85; RShG: $p = 0.000$, LSD = 5.76). In general, vermicomposts are known to energize the hormonal and enzymatic activity in the growth medium, which in turn remarkably improves the viability

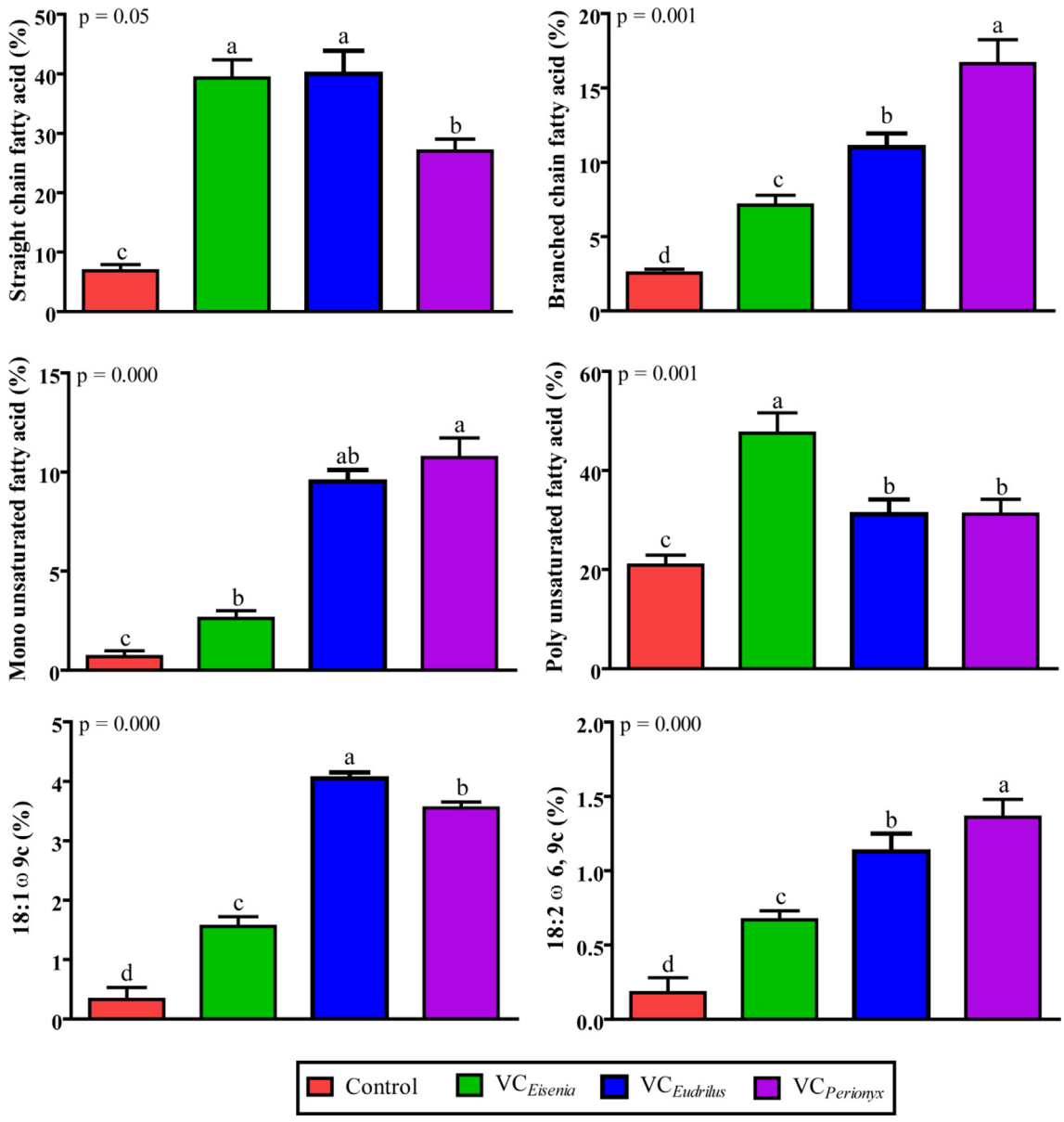

Fig. 5. Percentage composition of different types of fatty acids in the vermicomposted and composted feedstocks detected through Phospholipid fatty acid (PLFA) analysis. [Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida;* $VC_{Eudrilus}$ - Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae;* $VC_{Perionyx}$ - Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation. Similar letters represent no significant differences as per DRMT at $p < 0.05$ (Capital letters represent significant temporal variation while small letters represent the treatment-wise variation).

Table 4
Comparison between the composted and vermicomposted SMS extracts on relative root (RRG), shoot growth (RShG), relative seed germination (RSG), and germination index (GI) of green gram (*Vigna radiata*).

	RSG	RRG	RShG	GI
Control	70.0 ± 8.97	67.3 ± 6.50	64.7 ± 6.09	47.1 ± 3.22
$VC_{Eisenia}$	90.0 ± 10.0	96.5 ± 10.3	69.8 ± 4.99	107.4 ± 10.2
$VC_{Eudrilus}$	80.0 ± 9.22	88.2 ± 12.9	66.3 ± 5.49	86.8 ± 5.87
$VC_{Perionyx}$	121.7 ± 16.1	141.4 ± 11.8	96.5 ± 5.67	113.1 ± 11.9
p	0.000	0.000	0.000	0.000
LSD	3.18	6.85	5.76	8.08

Control – Aerobic composting with SMS + CD (3:1) feedstock (as composting control); $VC_{Eisenia}$ – Vermicomposting of SMS + CD (3:1) with *Eisenia fetida*; $VC_{Eudrilus}$ – Vermicomposting of SMS + CD (3:1) with *Eudrilus eugeniae*; $VC_{Perionyx}$ – Vermicomposting of SMS + CD (3:1) with *Perionyx excavatus; LSD = least significant difference.*

of crop seeds (Das et al., 2016). In the present study, microbial enrichment in the $VC_{Perionyx}$ system might have promoted qualitative improvement in the SMS vermicompost that could induce significant seed vigor. These results also suggest that vermicomposting can be a useful way for SMS to meet the demands of mushroom growers.

4. Conclusions

We studied the lignocellulosic biomass (i.e., SMS) degradation mechanism and efficiency of three earthworm species. The XRD and FTIR spectra suggest that cellulosic crystallinity of SMS is more efficiently broken in the $VC_{Perionyx}$ vermireactors than in the $VC_{Eudrilus}$ and $VC_{Eisenia}$ systems. About 31% reduction in XRD derived crystallinity index was observed from *Perionyx* based vermicomposting systems, while those of *Eudrilus* and *Eisenia* mediated systems exhibited 25% and 22%, respectively. Such results strongly affirm the remarkable lignocellulosic biomass degradation efficiency of *P. excavatus*. Such comminuted biomass particles also aided in higher microbial colonization and proliferation (e.g., bacteria, fungi, N-fixers, and P-solubilizers). Correspondingly, augmentation of microbial communities and their metabolic activities were the greatest in the $VC_{Perionyx}$ system. PLFA profiles noticeably varied among the vermibeds. On the other hand, about 2.0 to 2.4 folds increment in N and P availability in the $VC_{Eudrilus}$ and $VC_{Eisenia}$ vermibeds was remarkable as compared to the $VC_{Perionyx}$ system. Significant vigor and growth of green-gram seeds were observed in the $VC_{Perionyx}$ treatment. Overall, the study revealed that nutrient enrichment potential of all the three species were equally significant, although *P. excavatus* efficiently degraded SMS by rapid disintegration of the crystal framework of lignocellulosic materials. However, the major benefit of breakdown of crystallinity by *Perionyx* excavatus was reflected in imparting profuse germination of healthy seedlings of *Vigna radiata*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Adhikari, D., Jiang, T., Kawagoe, T., Kai, T., Kubota, K., Araki, K.S., Kubo, M., 2017. Relationship among Phosphorus circulation activity, bacterial Biomass, pH, and mineral concentration in agricultural soil. Microorganisms 5, 79. https://doi.org/10.3390/microorganisms5040079.

Anderson, T.-H., Domsch, K.H., 1990. Application of eco-physiological quotients (qCO2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem. 22, 251–255. https://doi.org/10.1016/0038-0717(90)90094-G.

Bansal, P., Hall, M., Realff, M.J., Lee, J.H., Bommarius, A.S., 2010. Multivariate statistical analysis of X-ray data from cellulose: A new method to determine degree of crystallinity and predict hydrolysis rates. Bioresour. Technol. 101, 4461–4471. https://doi.org/10.1016/j.biortech.2010.01.068.

Berruti, A., Lumini, E., Balestrini, R., Bianciotto, V., 2015. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front. Microbiol. 6, 1559. https://doi.org/10.3389/fmicb.2015.01559.

Carrasco, J., Zied, D.C., Pardo, J.E., Preston, G.M., Pardo-Giménez, A., 2018. Supplementation in mushroom crops and its impact on yield and quality. AMB Express 8, 146. https://doi.org/10.1186/s13568-018-0678-0.

Das, S., Deka, P., Goswami, L., Sahariah, B., Hussain, N., Bhattacharya, S.S., 2016. Vermiremediation of toxic jute mill waste employing *Metaphire posthuma*. Environ. Sci. Pollut. Res. 23, 15418–15431. https://doi.org/10.1007/s11356-016-6718-x.

de Carvalho, C.C.C.R., Caramujo, M.J., 2018. The Various Roles of Fatty Acids. Molecules 23, 2583. https://doi.org/10.3390/molecules23102583.

Domínguez, J., 2018. Earthworms and Vermicomposting. IntechOpen, Rijeka, p. Ch. 5. https://doi.org/10.5772/intechopen.76088

Dores-Silva, P.R., Landgraf, M.D., Rezende, M.O.O., 2015. Chemical Differentiation of Domestic Sewage Sludge and Cattle Manure Stabilized by Microbioreators: Study by Pyrolysis Coupled to Gas Chromatography Coupled to Mass Spectroscopy . J. Brazilian Chem. Soc. .

Dvorak, J., Roubalova, R., Prochazkova, P., Rossmann, P., Skanta, F., Bilej, M., 2016. Sensing microorganisms in the gut triggers the immune response in Eisenia andrei earthworms. Dev. Comp. Immunol. 57, 67–74. https://doi.org/10.1016/j.dci.2015.12.001.

Dygico, L.K., O'Connor, P.M., Hayes, M., Gahan, C.G.M., Grogan, H., Burgess, C.M., 2019. Lactococcus lactis subsp. lactis as a natural anti-listerial agent in the mushroom industry. Food Microbiol. 82, 30–35. https://doi.org/10.1016/j.fm.2019.01.015.

Eberlein, C., Baumgarten, T., Starke, S., Heipieper, H.J., 2018. Immediate response mechanisms of Gram-negative solvent-tolerant bacteria to cope with environmental stress: cis-trans isomerization of unsaturated fatty acids and outer membrane vesicle secretion. Appl. Microbiol. Biotechnol. 102, 2583–2593. https://doi.org/10.1007/s00253-018-8832-9.

Epstein, E., 1997. The Science of Composting. Technomic Publishing Co. Ltd., Lancaster, USA.

Grimm, D., Wösten, H.A.B., 2018. Mushroom cultivation in the circular economy. Appl. Microbiol. Biotechnol. 102, 7795–7803. https://doi.org/10.1007/s00253-018-9226-8.

Gupta, P., Indurani, C., Ahlawat, O.P., Vijay, B., Mediratta, V., 2004. Physicochemical properties of spent mushroom substrates of *Agaricus bisporus*. Mushroom Res. 13, 84–94.

Hirano, K., Kurosaki, M., Nihei, S., Hasegawa, H., Shinoda, S., Haruki, M., Hirano, N., 2016. Enzymatic diversity of the *Clostridium thermocellum* cellulosome is crucial for the degradation of crystalline cellulose and plant biomass. Sci. Rep. 6, 35709.

Hussain, N., Das, S., Goswami, L., Das, P., Sahariah, B., Bhattacharya, S.S., 2018. Intensification of vermitechnology for kitchen vegetable waste and paddy straw employing earthworm consortium: Assessment of maturity time, microbial community structure, and economic benefit. J. Clean. Prod. 182, 414–426. https://doi.org/10.1016/j.jclepro.2018.01.241.

Hussain, N., Singh, A., Saha, S., Venkata Satish Kumar, M., Bhattacharyya, P., Bhattacharya, S.S., 2016. Excellent N-fixing and P-solubilizing traits in earthworm gut-isolated bacteria: A vermicompost based assessment with vegetable market waste and rice straw feed mixtures. Bioresour. Technol. 222, 165–174. https://doi.org/10.1016/j.biortech.2016.09.115

Izyan, N.N., Jamaludin, A.A., Mahmood, N., 2009. Potential of Spent Mushroom Substrate in Vermicomposting, Dynamic Soil, Dynamic Plant.

Jenkinson, D., 1988. Determination of microbial biomass carbon and nitrogen in soil, in: Wilson, J. (Ed.), Advances in Nitrogen Cycling in Agricultural Ecosystems. CAB International, pp. 368–386.

Kim, H.M., Park, J.H., Choi, I.S., Wi, S.G., Ha, S., Chun, H.H., Hwang, I.M., Chang, J.Y., Choi, H.-J., Kim, J.-C., Park, H.W., 2018. Effective approach to organic acid production from agricultural kimchi cabbage waste and its potential application. PLoS One 13, e0207801.

Kim Oanh, N.T., Permadi, D.A., Hopke, P.K., Smith, K.R., Dong, N.P., Dang, A.N., 2018. Annual emissions of air toxics emitted from crop residue open burning in Southeast Asia over the period of 2010–2015. Atmos. Environ. 187, 163–173. https://doi.org/10.1016/j.atmosenv.2018.05.061.

Luo, X., Fu, X., Yang, Y., Cai, P., Peng, S., Chen, W., Huang, Q., 2016. Microbial communities play important roles in modulating paddy soil fertility. Sci. Rep. 6, 20326.

Maurya, S.K., Nakajima, T., Mizobata, K., Zen, H., Kii, T., Ohgaki, H., 2018. Real-time observation of structural change in semi-crystalline polymer films through mid-IR transmission spectroscopy: determination of instantaneous film temperature during recrystallization. Opt. Express 26, 21615–21625. https://doi.org/10.1364/OE.26.021615.

Mleczek, M., Siwulski, M., Rzymski, P., Budka, A., Kalač, P., Jasińska, A., Gąsecka, M., Budzyńska, S., Niedzielski, P., 2018. Comparison of elemental composition of mushroom Hypsizygus marmoreus originating from commercial production and experimental cultivation. Sci. Hortic. (Amsterdam) 236, 30–35. https://doi.org/10.1016/j.scienta.2018.03.029.

Mubeen, H., Hatti, S.S., 2018. Earthworms diversity of Koppal district with the updated information on genus Thatonia of Hyderabad-Karnataka region, Karnataka. India. J. Asia-Pacific Biodivers. 11, 482–493. https://doi.org/10.1016/j.japb.2018.08.002.

Nasehi, M., Torbatinejad, N.M., Zerehdaran, S., Safaie, A.R., 2017. Effect of solid-state fermentation by oyster mushroom (*Pleurotus florida*) on nutritive value of some agro by-products. J. Appl. Anim. Res. 45, 221–226. https://doi.org/10.1080/09712119.2016.1150850.

Negi, R., Suthar, S., 2018. Degradation of paper mill wastewater sludge and cow dung by brown-rot fungi Oligoporus placenta and earthworm (*Eisenia fetida*) during vermicomposting. J. Clean. Prod. 201, 842–852. https://doi.org/10.1016/j.jclepro.2018.08.068.

Page, A.L., Miller, R.H., and Keeney, D.R., 1994. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, Agronomy Monograph. American Society of Agronomy, Soil Science Society of America, Madison, WI. https://doi.org/10.2134/agronmonogr9.2.2ed

Paul, S., Das, S., Raul, P., Bhattacharya, S.S., 2018. Vermi-sanitization of toxic silk industry waste employing *Eisenia fetida* and *Eudrilus eugeniae*: Substrate compatibility, nutrient enrichment and metal accumulation dynamics. Bioresour. Technol. 266, 267–274. https://doi.org/10.1016/j.biortech.2018.06.092.

Pramer, D., Schmidt, E.L., 1964. Experimental soil microbiology. Soil Sci. 98, 211.
Quideau, S.A., McIntosh, A.C.S., Norris, C.E., Lloret, E., Swallow, M.J.B., Hannam, K., 2016. Extraction and analysis of microbial phospholipid fatty acids in soils. J. Vis. Exp. 1–9. https://doi.org/10.3791/54360.
Ravindra, K., Singh, T., Mor, S., 2019. Emissions of air pollutants from primary crop residue burning in India and their mitigation strategies for cleaner emissions. J. Clean. Prod. 208, 261–273. https://doi.org/10.1016/j.jclepro.2018.10.031.
Rinker, D., 2017. Spent Mushroom Substrate Uses: Technology and Applications, in: Zied, DC, Pardo-Giménez, A. (Ed.), Edible and Medicinal Mushrooms: Technology and Applications. John Wiley & Sons, Inc., New Jersey, USA, pp. 427–454. https://doi.org/10.1002/9781119149446.ch20
Sasmal, S., Goud, V.V., Mohanty, K., 2012. Characterization of biomasses available in the region of North-East India for production of biofuels. Biomass and Bioenergy 45, 212–220. https://doi.org/10.1016/j.biombioe.2012.06.008.
Segneanu, A.E., 2012. Organic Compounds FT-IR Spectroscopy, in: Gozescu, I. (Ed.), Macro To Nano Spectroscopy. IntechOpen, Rijeka, pp. 145–164. https://doi.org/10.5772/50183
Tran, H., 2016. Vermicomposting of Spent Mushroom Compost Using Perionyxexkavatus and Artificial Nutrient Compound. Int. J. Environ. Agric. Res. 2, 101–109.
Traoré, M., Kaal, J., Martínez Cortizas, A., 2018. Differentiation between pine woods according to species and growing location using FTIR-ATR. Wood Sci. Technol. 52, 487–504. https://doi.org/10.1007/s00226-017-0967-9.
Tripathy, S., Bhattacharyya, P., Mohapatra, R., Som, A., Chowdhury, D., 2014. Influence of different fractions of heavy metals on microbial ecophysiological indicators and enzyme activities in century old municipal solid waste amended soil. Ecol. Eng. 70, 25–34. https://doi.org/10.1016/j.ecoleng.2014.04.013.
USEPA, 1989. Soil Sampling Quality Assurance User's Guide (Second Edition). Las Vegas.
Vos, A.M., Heijboer, A., Boschker, H.T.S., Bonnet, B., Lugones, L.G., Wösten, H.A.B., 2017. Microbial biomass in compost during colonization of Agaricus bisporus. AMB Express 7, 12. https://doi.org/10.1186/s13568-016-0304-y.
Zhu, H.-J., Liu, J.-H., Sun, L.-F., Hu, Z.-F., Qiao, J.-J., 2013. Combined alkali and acid pretreatment of spent mushroom substrate for reducing sugar and biofertilizer production. Bioresour. Technol. 136, 257–266. https://doi.org/10.1016/j.biortech.2013.02.121.
Zhu, L., Zhao, Y., Zhang, W., Zhou, H., Chen, X., Li, Y., Wei, D., Wei, Z., 2019. Roles of bacterial community in the transformation of organic nitrogen toward enhanced bioavailability during composting with different wastes. Bioresour. Technol. 285,. https://doi.org/10.1016/j.biortech.2019.121326 121326.