Vermiproductivity, maturation and microbiological changes derived from the use of liquid anaerobic digestate during the vermicomposting of market waste

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ABSTRACT

Recently, it has been suggested that the liquid fraction of anaerobic digestate, derived from the treatment of wastewater and solid wastes, could be used in vermicomposting as a solution to its disposal, and even for its valorization. Nevertheless, the literature does not provide enough information about its impact on the process of vermicomposting itself and on the final quality of the end-product. In this study, the effect of different doses of digestate in the vermicomposting process treating market waste is assessed measuring earthworm population dynamics, the bacterial community succession present in the vermibeds, as well as maturation and the end-quality of the vermicompost. Our results show that the addition of liquid digestate to the vermibeds increased the earthworms biomass, i.e. 71%, 94% and 168% in control, and vermibeds with 30% and 60% digestate, respectively. Further, the increase in the amount of N in the vermicompost decreased as the digestate addition increased, i.e. 75%, 8%, 3%. The maturity achieved was high in all treatments as shown by the C/N ratio, 7.98, 7.40 and 10.20, and the high seed germination rate, above 90%. Finally, the succession of the microbial community was not disturbed and compositional stabilization was reached after 92 days.

Key words | anaerobic digestion, digestate, market waste, microbial community, residues valorization, vermicomposting

HIGHLIGHTS

- The addition of liquid digestate to the vermibeds increased the presence of young earthworms.
- The addition of different amounts of digestate did not affect the time to maturity.
- The addition of different amounts of digestate did not affect the composition and succession of the bacterial community.
- The analysis of pH, moisture content, phytotoxicity and C/N ratio indicated maturation and good quality of the final humus produced.
- The addition of liquid digestate to vermicomposting treatments is found to be safe and recommended as end-disposal route.

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INTRODUCTION

Anaerobic digestion is a mature and valuable technology to treat wastewater and solid wastes due to its capacity for energy and nutrient recovery. While the carbon is mostly recovered in the form of methane and carbon dioxide in the biogas, most of the nutrients present in the raw waste are still present in the digestate, which provides a high value as fertilizer and soil conditioner (Arthurson 2009).

Despite its positive characteristics, the management of digestate presents serious challenges related to its composition and large volumes (Golkowska et al. 2014; Dahlin et al. 2015; Zeng et al. 2016). In many countries, digestate is considered as a potentially hazardous waste due to the possible presence of pathogens and heavy metals (Koszel & Lorencowicz 2015). Further, costs of its handling can be quite high, particularly when dealing with raw digestate with high water content; 1.94 €/m³ have been reported for direct application (Drosg et al. 2015), and they can go as high as 13–32 €/ton input without including transport costs (Golkowska et al. 2014). Transport costs, on the other hand, have also been found to play a dominant role in defining the profitability of a biogas operation (Bojensen et al. 2014; Dahlin et al. 2015). Options that reduce the volume and increase the transportability and fertilizing value of the digestate are therefore desired. Ammonia stripping, ion exchange, and struvite precipitation can recover nutrients, and evaporation and membrane processes allow for nutrient concentration and water purification; costs in this case go from 5.45 up to 10 € per cubic meter (Dahlin et al. 2015; Drosg et al. 2015).

Due to the mentioned challenges, in order to take full advantage of anaerobic digestion technology for organic residues valorization at urban level, handling and transport of digestate needs to be carefully designed not to counteract its economic, energy and greenhouse gas (GHG) abatement advantages.

Biological processes treating urban solid wastes, such as vermicomposting or composting, could be regarded as potentially complementary processes to anaerobic digestion as they can provide a final disposal method for digestate as they demand water and nutrients.

Indeed, vermicomposting is one of the best-known environmentally appropriate technology for the valorization of different types of organic wastes, such as kitchen waste, agricultural residues, food and animal waste, sewage sludge or paper industry waste (Ali et al. 2015; Biruntha et al. 2019). This process is based on the biotransformation of organic wastes into a vermicompost-like material under the joint action of microorganisms and earthworms (Ndegwa & Thompson 2001). Microorganisms play a key role in organic waste degradation and its transformation into a safe organic fertilizer (Dominguez et al. 2014; Leow et al. 2016). Earthworms are essential drivers for this process because they promote aeration conditions and organic substrate fragmentation, which increase the microorganism’s activity (Dominguez et al. 2011).

In this context, the use of the liquid fraction of the untreated anaerobic digestate for vermicomposting could be a sustainable and cost-effective alternative for its final disposal. Water contained in the liquid digestate, i.e. 83–97% for wet substrates (Drosg et al. 2015), can be used to maintain desirable moisture content in vermicomposting beds (vermibeds), avoiding the use of freshwater. Whereas nutrients available, i.e. 2–9 kg N/ton FM 0.9–2.3 kg/ton FM (Drosg et al. 2015) can find a valuable end use in vermicompost. Thus, liquid digestate post-treatment via vermicomposting not only gives a solution for digestate management but can also partially close the loop of water and nutrients contained in digestate.

In recent years, a few studies have investigated the use of the solid fraction of anaerobic digestate as substrate for vermicomposting (Lin et al. 2015; Krishnasamy et al. 2014; Hanc & Vasak 2015) but a significant lack of knowledge concerns the vermicomposting using liquid digestate as substrate as well as hydrating agent to maintain a desirable moisture in
vermibeds. Recent findings point at vermicomposting as a useful technology for the valorization and stabilization of liquid anaerobic digestate (Lin et al. 2013; Rajpal et al. 2014); nevertheless, the literature does not provide enough information about its impact to the vermicomposting process.

In this regard, the microbiological changes within the vermicomposting process derived from the use of liquid anaerobic digestate are still unknown, as well as the impact in the final quality and maturation of the vermicompost. Diverse microbial communities are involved in the vermicomposting process, such as free-living nitrogen fixers bacteria, ammonifying bacteria, phosphate solubilizers, silicate solubilizers, fungi, and spore formers, which were identified in a vermicompost from coconut leaves and cow manure (Gopal et al. 2009). Recent literature has reported differences in abundance per phylum and family for different substrates mixtures and earthworms densities (Chen et al. 2018; Budroni et al. 2020). Others have reported the presence of different types of bacterial species such as antifungal, plant growth-promoting and enzyme-producing during the vermicomposting process (Pathma & Sakthivel 2013). These microbial communities may also have the potential to improve soil fertility, promote crop production and suppress soil borne pathogens (Huang et al. 2013; Domínguez et al. 2019). Hence, we can formulate the hypothesis that the use of the untreated liquid digestate for the vermicomposting process may enhance the earthworm’s activity as well as vermicomposting microbial biodiversity.

In this study, the effect of different doses of liquid digestate in the vermicomposting process treating market waste is assessed by means of measuring maturation time, earthworm population dynamics, and the bacterial community succession present in the vermibeds.

**MATERIALS AND METHODS**

**Earthworms and substrates**

The earthworm species *Eisenia fetida* was used in this study due to its widespread use, tolerance to environmental factors (pH, moisture content, temperature, etc.) and its high rate of organic matter degradation (Gajalakshmi & Abbasi 2004). The ability of *E. fetida* to consume and breakdown a wide variety of degradable organic wastes have been studied, including crop residues, vegetables and fruits waste, cow dung, horse and sheep manure, kitchen waste, wheat straw, biogas slurry, spent mushroom substrate and different industrial wastes (Ali et al. 2015; Gong et al. 2019; Karmegam et al. 2019; Esmaeili et al. 2020; Mondal et al. 2020). In this study, the earthworms were obtained from the Ecoparque Peñalolén-UAI (Peñalolén, Santiago de Chile), where they were already adapted to treat market waste.

The organic solid waste selected for this study, mainly vegetable and fruits waste, was obtained from the open markets in the commune of Peñalolén (Santiago, Chile). The market waste was mainly composed of lettuce, tomato, corn stover, chard, lemon and bell pepper wastes (see Supplementary Material). The main characteristics of this market waste material were: dry matter (DM): 140.0 ± 6.0 g kg FM⁻¹; volatile solids (VS) 112.0 ± 5.2 g VS kg FM⁻¹ market waste; carbon/nitrogen ratio (C/N) 66.7; total nitrogen (TN) 3.0 g kg FM⁻¹. No additional comminution was performed. The characterization of all other substrates is presented in the Supplementary Material (see Table S1).

The earthworms and digestate used in this study were obtained from the existing vermibeds and anaerobic digester at the Ecoparque Peñalolén-UAI (Peñalolén, Santiago de Chile) an experimental and educational project where market waste treatment is taking place.

The main characteristics of the digestate were as follows: total solids (TS) 6.3 ± 0.8 g TS L⁻¹, VS 4.0 ± 1.2 g VS L⁻¹, pH of 7.0 ± 0.4, soluble chemical oxygen demand (sCOD) 4.2 ± 1.1 g L⁻¹ and ammonium (NH₄⁻N) 1.12 ± 0.04 g N L⁻¹.

**Experimental set-up**

Three vermibeds, managed as static piles with a volume of 1.94 m³ were studied. The starting material consisted of 640 kg FM of vermicompost, 70 kg FM of market waste, 170 kg FM straw and 450 kg FM of local soil (clay-loam type) as bulking agent for each vermibed (see Supplementary Material for bed configuration). The initial vermicompost was harvested from existing vermibeds treating market waste and its addition was meant to ensure that a community of microorganisms adapted to the used substrate were present in the experiment.

The vermibeds were inoculated with *E. fetida* collected from the same existing vermibeds working with market waste. A representative sample was collected and divided into two groups, based on their body weight. Young earthworms were classified having a mean weight of 0.22 ± 0.03 g, whereas adults had a mean weight of 0.46 ± 0.07 g. This is in accordance to average weights reported elsewhere (Monroy et al. 2006). Following the composition found in
the working vermibeds, at the beginning of the experiment 24,000 young and 5,600 adult earthworms were added to each of the experimental vermibeds.

During the vermicomposting period, 70 kg of market waste were fed to each vermibed every three weeks for five consecutive periods. The feeding was stopped 25 days before harvesting the beds. The total duration of experiment was 130 days. Vermibeds were hydrated using two different mixtures of digestate and untreated local water (v/v) for the experimental period: 30% digestate + 70% water (T1) and 60% digestate + 40% water (T2). In addition, a control vermicibed was introduced using only water for hydration (T3). The hydrating mixtures were applied once a week for the whole experimental period. The initial moisture content of vermibeds was adjusted to 65% (w/w) and moisture content was maintained between 55% and 60% (w/w) by sprinkling, when required, additional quantity amounts of the hydrating mixture. The experiments were conducted at environmental temperature which ranged between 7.3 and 23.8 °C.

Chemical analysis

Physico-chemical analyses were conducted in duplicate. pH was measured on samples mixed with distilled water (1:5, w/v, dry basis) using an electrode connected to a pH meter (Jenko, 6010M). To assess the maturity of the final products, carbon/nitrogen ratio (C/N), total nitrogen percentage (%N), pH, moisture content and seed germination rate were determined. Moisture content of the samples was determined by drying the sample at 105 °C for 24 h. Total Kjeldahl nitrogen and C/N ratio were determined by the methods described by Bremner et al. (1996) and TMECC (2002), respectively.

Biological analysis

Seed germination test

A seed germination test was conducted at the end of the experiment, carried out in triplicate and according to the specifications given in the Chilean Compost Standard (INN Chile 2015). Radish (Raphinus sativus L.) seeds were used and sown by hand in expanded polystyrene seedbeds (4 × 4 × 5 cm of each slot). Twenty-six slots were used for vermicompost obtained from T1, T2 and T3, and a set of 26 slots was used as control treatment. These tests were carried out in a greenhouse at ambient temperature ranging between 16 and 25 °C for 14 days. The seed germination percentage was determined using the following equation (Zucconi et al. 1981):

\[
\text{Seed germination} \% = \frac{\text{Number of seeds germinated}}{\text{Number of total seeds tested}} \times 100
\]

Earthworm population analysis

The changes in earthworm populations were studied during the experimental period by sampling the vermibeds and counting the earthworms, both young and adult specimens. Three samples of immature vermicompost were taken at three different points of each vermibed after 0, 16, 60, 109 and 150 days of experimentation, using PVC cones of 1.2 L capacity. Each vermicompost sample was sized to calculate the volume, and weighed. Adults and young earthworms were separated from the vermicompost by hand sorting, after which they were counted and weighed after washing with water and drying them with tissue paper. Thereafter, all earthworms were returned to their respective units.

Bacterial community succession analysis

In order to observe the changes in bacterial community composition and analyze bacterial community stability, six groups of samples were taken to perform a molecular analysis: terminal restriction fragment length polymorphisms (T-RFLP). Two groups were obtained by sampling between days 25 to 33 (cycles 1 and 2) of the vermicomposting process, other two groups were sampled between days 91 to 99 (cycles 3 and 4) and the final two groups were sampled between days 109 to 117 (cycles 5 and 6). Each group of samples corresponds to three consecutive samples (a cycle), one taken before digestate addition and two samples (at 6 and 24 h) after digestate addition. In each sampling time, two samples of 15 g of each were taken, frozen and stored for no more than seven days, before DNA extraction. While the sample was still frozen, 1 g of homogenized soil sample was taken to obtain metagenomic DNA using the FastDNA spin kit for soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer’s instructions. This protocol yielded DNA concentrations ranging from 0.1 to 0.9 μg μL⁻¹ in a final volume of 50 μL, as quantified by spectrophotometry (Infinite® 200 PRO NanoQuant, Tecan Group Ltd, Männedorf, Switzerland). Metagenomic DNA was used as a template for polymerase chain reaction (PCR) with primer pairs 27F (5’-AGA GTT TGA TCC
TGG CTC AG-3’ labeled with the fluorochrome 6-FAM at the 5’ end, and 1492R (5’-ACG GGC GGT GTG TAC-3’) without fluorescent label (Weisburg et al. 1991). Each PCR amplification contained 5 μL of 10× PCR buffer (200 mM Tris-HCl, pH 8, 500 mM KCl), 3 mM MgCl2, 0.2 μM of each primer, 0.2 mM dNTP, 0.2 mg mL−1 bovine serum albumin, 10–50 ng of soil DNA, and 1 U of Taq polymerase, in a total reaction volume of 50 μL. PCR reaction conditions were as follows: 94 °C for 5 min; 50 cycles at 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 2 min; and a final extension at 72 °C for 7 min. PCR products were digested with 20 U MspI or HaeIII restriction enzymes in appropriate buffers for 3 h at 37 °C in a final volume of 20 μL. Each PCR product was digested separately with each enzyme to assess T-RFLP profiles consistency. As the profiles indicated the same trends, only the MspI profiles are reported here. After desalting, the DNA fragments were separated and detected through capillary electrophoresis (Macrogen, Korea) and analyzed using Peak Scanner software (v1.0, AB Applied Biosystems, USA). The fragment sizes were estimated using the internal standard LIZ 1200 as reference. Raw data were terminal restriction fragments (T-RFs) sizes, measured in base pairs, and individual peak areas measured in fluorescence units. Only T-RFs from 60 to 600 bp were included in the analysis. T-RFs representing less than 0.5% of the total area were not considered and the data were standardized by calculating the area of each peak as a percentage of the total area (Morán et al. 2008). Relative abundances of bacterial phylogenotypes were determined from relative intensities of T-RF signals. Then, the composition of each community was described in terms of operational taxonomic units (OTUs), represented by the T-RFs signals. Alpha diversity was analyzed to compare this change over time, through calculation of diversity index (H) using the Shannon-Weaver formula \[ H = -\sum_{i=1}^{S} p_i \log_2 p_i \] (Shannon 1948; Blackwood et al. 2007), where \( p_i \) is the proportion of an individual T-RF area relative to the sum of all T-RFs areas and \( S \) is the number of species.

Quality control

Quality control was assured by using analytical grade chemicals, standard protocols and operating procedures, and equipment calibration with standards. The protocol for collecting worm samples consisted in harvesting samples from all the three established vermicomposting units in triplicate at different parts of the bed. The samples were taken at different time intervals for their analysis, before and after the addition of the digestate for a total of six cycles, i.e. days 0, 16, 32, 60, 109 and 130. The experimental temperature was measured three times per week at different points in the vermicbeds to follow vermicompost maturation. Further, pH was measured in duplicate once every week to ensure process stability.

To statistically test differences in microbial diversity, one-way analysis of variance (ANOVA) and a posteriori Tukey test were performed. Beta diversity was analyzed using Bray-Curtis distance matrices based on the square root-transformed abundance of each T-RF, to perform non-metric multidimensional scaling (NMDS) analyses using grouping data according to their similarity (Clarke 1993) and PERMANOVA was performed to determine statistical differences between treatments and through the time.

RESULTS AND DISCUSSION

Temperature profiles and maturity degree of final vermicomposting products

The evolution of vermicomposting temperature for the three treatments was followed during vermicomposting process (Figure 1). The ambient temperature fluctuated between 7.3 and 23.8 °C. The patterns of temperature change in experiments T1, T2 and control were similar: after mixing the different proportions of vermicompost, neutral ground and market waste, temperature decreased from 19 °C to 14 °C during the first three weeks of the experiment. Afterwards, it increased to 22 °C at day 28, when the vermicbeds were fed with fresh market waste, and subsequently decreased to around 18.5 °C, keeping this temperature until day 65. Thereafter, from day 66 until the end of the experiment, vermicbeds temperature dropped slowly to around 14 °C (ambient temperature). Similar patterns have been observed in vermicompost experiments where the temperature of the pile mostly follows the ambient temperature (Borah et al. 2007).

Assessing the stability and maturity of vermicompost is important to ensure a suitable vermicompost quality for safe soil application (Majlessi et al. 2012). In this study, when the vermicomposting process was completed, the stability and maturity of obtained vermicompost were assessed considering as indicators the total N content, C/N ratio, moisture, pH and seed germination test. The C/N ratio and total N content provide an indication of the level of organic matter degradation for vermicomposting process.
In order to start the experiments at the same conditions, the initial total N and C/N values were 0.80% DM and 55.1, respectively, for all vermibeds.

The final C/N ratio of the vermicompost beds decreased considerably, 7.98, 7.40 and 10.20 was found for vermibeds hydrated with 30% of digestate, 60% of digestate and control, respectively. These means higher amounts of carbon were lost as compared to Nitrogen in the treatments. Important reductions in C/N ratio have been reported by other authors (Chen et al. 2018; Karmegam et al. 2019; Srivastava et al. 2020a, 2020b). Carbon content is reduced during vermicomposting process by joint action of microorganisms and earthworms through respiration, such degradation and mineralization of organic matter, including the humification and decomposition of complex substrates, plus the loss of carbon in respiratory activity is hence explanatory. In addition, N enrichment in the vermicompost can be the case, as was found in our experiments. As C/N ratio below 20 is a good indicator of vermicompost maturity (Morais & Queda 2003), therefore vermicompost produced in the present study could be considered as mature and stable, its quality being also desirable for agricultural use where a C/N ratio lower than 15 is recommended (Senesi 1989; Suthar 2007).

Moreover, the moisture content (%) and pH in the obtained vermicompost from the three treatments ranged between 65–70% (w/w) and 7.6–7.8, respectively, showing no important differences among them; similar values of pH and moisture content have been reported by other authors (Krishnasamy et al. 2014; Rajpal et al. 2014; Hanc & Vasak 2015).

Immature vermicompost may possess toxic substances that suppress plant growth (Bhat et al. 2017). Thus, one of the key methods to assess vermicompost quality are plant growth tests. The germination rate is a good index for the determination of vermicompost phytotoxicity and maturity (Majlessi et al. 2012; Karmegam et al. 2019). Therefore, in our research, a seed germination test was carried out (Zucconi et al. 1981; Raj & Antil 2011). A germination percentage higher than 70% for radish seeds indicate good vermicompost maturity (Raj & Antil 2011), ensuring the absence of phytotoxicity. Our results showed that the germination rate of radish seeds was higher than 90% in all vermicompost containing slots. The treatment with vermicompost from the vermibed hydrated with 60% of digestate exhibited the highest germination ratio, i.e. 96.2%. These results confirm that the obtained vermicompost were mature enough and free from phytotoxins, at least for radish plants. Similar results were obtained for other substrates where the treatment led to similar C/N ratios (Karmegam et al. 2019).

The analysis of pH, moisture content, phytotoxicity and C/N ratio indicated maturation and good quality of final vermicompost. Therefore, the products obtained from vermicomposting treatments could be used as organic fertilizers. Furthermore, by comparing the germination test, final temperature and C/N ratio results, the vermibed hydrated with 60% digestate gave an indication of higher maturity and final product stabilization.

Effect of anaerobic digestate on growth and reproduction of earthworms

Even when microorganisms cause most of the biochemical degradation of organic waste in vermicomposting, earthworm activities strongly influence the degradation process...
as they enhance degradation by modifying physical and biochemical properties of the organic matter. Hence, the activity of earthworms during the vermicomposting process is considered a key factor for organic waste degradation (Domínguez et al. 2011). In our study, the dynamic of earthworm’s growth and reproduction was studied for the whole experimental period.

Earthworm growth was assessed by comparing the number of adult specimens in time whereas reproduction success was measured by means of counting the worms below 4 mm in size having in average weight 0.22 ± 0.03 g. Earthworms showed growth and reproduction in all vermibeds; however, the rate of growth and reproduction were dissimilar in the different vermicomposting treatments (Figure 2).

The total number of earthworms added in each vermibed at the beginning of the vermicomposting process was 29,600 earthworms m⁻³ (24,000 and 5,600 young and adults m⁻³). Initially the vermibeds showed a very slow increase in the number of individuals by day 60 the total amount of earthworms ranged between 30,600 and 36,100 earthworms m⁻³. After day 60, the amount of earthworms rapidly increased in all treatments until the end of the vermicomposting process experiment (Figure 2). At day 109, the increase in the number of earthworms was higher in vermibed hydrated with 30% digestate (Figure 2). This increase in the amount of earthworms was a consequence of a higher young production, as from days 60 to 109, a 3.6-, 2.4- and 2.5-fold increase in young earthworms was witnessed in vermibeds hydrated with 30% of digestate, 60% of digestate and control, respectively (Figure 2). However, differences in earthworm growth were observed by the end of vermicomposting process, as the amount of earthworms in vermibeds hydrated with 30% of digestate, 60% of digestate and control, were 155,000; 191,500; and 168,000 earthworms·m⁻³, respectively (Figure 2). That means 5.2-, 6.5-, and 5.7-fold increases, respectively as compared with the total initial earthworms present in vermibeds (Figure 2), implying a positive influence of digestate in the reproduction of individuals.

These results are in accordance with previous studies in which the total amount of earthworms in vermibeds reached a maximum value after 11–16 weeks. Hanc & Vasak (2015) found that the maximum earthworm biomass was reached after 16 weeks (112 days) of dewatered digestate vermicomposting, achieving an increase of 9-fold at the end compared with the beginning of the experiment. Suthar (2007, 2008, 2009) has reported that the maximum earthworms biomass was achieved after 16 weeks, whereas the maximum number of earthworms was achieved after 11–14 weeks for vegetable waste vermicomposting. Here, we report that most earthworms corresponded to young earthworms and that their presence was significantly increased over time in all vermibeds from day 60 onwards (Figure 2). The differences between the results obtained in the previous studies and in this work may be related to the type of bulking material, earthworm species and/or experimental conditions.

It is important to note that the amount of young earthworms at the end of the vermicomposting process, represented 97%, 99% and 98% of the total earthworms in the vermibeds hydrated with 30% of digestate, 60% of
digestate and vermibed control, respectively. These results are in agreement with findings by Tripathi & Bhardwaj (2004), who reported that the cocoon of *E. fetida* takes around 54 days to hatch and then young earthworms take around 67 days to reach sexual maturity.

In accordance, young *E. fetida* individuals showed great adaptability, as young earthworms adapted to the presence of digestate with additions of $191 \pm 50$ g COD m$^{-3}$ and $510 \pm 81$ g COD m$^{-3}$ and $3,965 \pm 1,058$ g N m$^{-3}$ and $6,440 \pm 1,690$ g N m$^{-3}$ for vermibed hydrated with 30% and 60% of digestate, respectively. Still, the amount of adult earthworms decreased from 5,600 to 4,730 earthworms m$^{-3}$ in vermibed hydrated with 30% of digestate, to 2,500 earthworms m$^{-3}$ in vermibed hydrated with 60% of digestate and to 3,300 earthworms m$^{-3}$ in control vermibed (Figure 2). The highest reduction of the adult earthworms was detected in the vermibed hydrated with 60% of digestate but also is there where the highest amount of young individuals was also visible in terms of biomass (Figure 3); this is in line with previous findings pointing at good conditions for earthworms’ reproduction (Suthar 2008). Considering worms can live more than 500 days (Venter & Reinecke 1988) and they cannot leave the vermibeds due to its concrete floor, further studies are needed to identify other possible factors interfering in the study such as feed amount or composition.

Further insights into this aspect can also be found when analyzing in the N content in the final vermicompost. In our experiment, increase in the N content per kg FM was observed in the final product of all vermibeds.

Figure 3 | Changes in population density and mass density of *Eisenia fetida* during vermicomposting in vermibed hydrated with 30% digestate, 60% digestate and water (control).
Several authors have found significant Nitrogen increase in the vermicompost as compared to the original materials, they have exposed reasons such as mineralization of N-rich materials, the excretory substances of earthworms and the action of N-fixing bacteria which are known to be present in vermi-beds (Rajpal et al. 2014; Budroni et al. 2020; Srivastava et al. 2020a, 2020b). The rapid reduction of volume or weight of fresh flow velocity waveform (FVW) could also result in the relative larger nitrogen content as reported by Huang et al. (2014). These authors found an initial increase followed by a reduction in N content over time, they hypothesized leachate losses as explanatory for the reduction in N content. In our case, these losses do not occur as our experiment is carried out in impermeable concrete beds.

Further, in our research we found higher increase in N content of the vermicompost in the treatment without the addition of nitrogen, i.e. in the control vermi-bed 75% as found as compared to the vermi-bed irrigated with 30% digestate and 60% digestate, i.e. 18% and 3%, respectively. We hypothesize that this phenomenon has to do with the concomitant observed phenomena shown above (see Figure 3) in which the greatest number of worms and the highest amount of worm biomass is found in the treatments having digestate addition. Indeed by the end of the experiment there was 168% more worm biomass in the beds irrigated with 60% digestate as compared with the one irrigated with 30% digestate, presenting only 94% increase in worm biomass and in the control, showing only 74% increase. Therefore, the extra N added via digestate appears to be used for worm growth, hence it is not found in the vermicompost itself. Arora & Kaur (2019) have mentioned that an increase in nitrogen addition gave maximum population buildup of E. fetida (cocoons, hatchlings and worm biomass). Previously, Albanell et al. (1988) have mentioned the increase in worm biomass as possible justification for N decrease in vermicompost. Other possibility is that the higher amount of worms influenced major N losses in the vermi-beds showing increased worm abundance. This means a higher activity in the digestate containing vermi-beds would lead to higher N losses but also to higher carbon mineralization coming from a higher microbial and earthworms activity in the vermi-bed. Indeed, Rajpal et al. (2014) mentioned that N enrichment pattern and mineralization activities mainly depend upon the total amount of N in the initial waste material and the excretory behaviour of the earthworms. A full mass balance would allow to test these hypotheses, so further research is needed to elucidate this aspect.

**Effect of digestate on bacterial community composition**

As has been proposed, the composition of the bacterial community is an excellent indicator of soil fertility and health (Hermans et al. 2016). The study of changes in the structure of microbial communities provides the opportunity to establish causal relationships between the observed patterns in the efficiency of the vermicomposting process and the changes in worm populations; considering that microorganisms can quickly react to changes in the environment, like the exposure of the vermi-bed to digestate, as microbial communities are quite sensitive (Blaud et al. 2015), and even small doses of pollutants can cause a sharp decline in particular populations or even the eradication of some species. The reaction of microbial communities to environmental stress can be reflected in changes in the number of species not only in particular, but even in complete functional groups within the population, and in general, the loss of biodiversity of the microbial community (Gorovtsov et al. 2017).

In this study we analyzed bacterial diversity by means of T-RFLP, a culture independent, molecular technique (Weisburg et al. 1991; Osborne et al. 2006). Alpha and beta diversity were estimated to follow the changes in local alpha diversity (over time) and analyze the differences between communities over time and between treatments (beta diversity). Results indicated that, in each cycle, the addition of digestate, independently of its concentration, had no significant effects on alpha diversity (Figure 4). As non-significant differences were found between treatments (Figure 4), a mean of diversity was calculated, averaging the diversity values of the different treatments in each cycle, to detect differences across the time factor (between cycles). A posteriori Tukey test showed significant differences in alpha diversity as a function of the cycle, particularly between cycle 2 and 3 (Figure 4). A stabilization of the diversity index average was observed from cycle 4 onwards (Figure 4).

In agreement, PERMANOVA results of beta-diversity analysis also showed that bacterial community composition depends mainly on time factor (cycle), but treatment also affects bacterial composition (Table 1), however no significant differences were found concerning the interaction of both factors (Table 1).

The time associated differences in bacterial community composition are represented by a shift in the bacterial community composition between cycles 2 and 3 (between days...
33 and 92), grouping samples from cycles 1 and 2 (orange and red symbols in Figure 5), and samples from cycles 3 to 6 into a big cluster (green, cyan, violet and black symbols in Figure 5). This indicates that around cycle 3 (92 days) bacterial communities reach compositional stabilization, and also that the digestate addition have no mayor effects in bacterial community compositional changes compared to the time factor (Figure 5). An initial decline in microbial population in vermicompost beds due to the presence of earthworms has been reported, followed by renewed abundance and diversity (Chen et al. 2014). On the other hand, bacterial community that composes digestate (asterisk in Figure 5) is very different to vermibeds’ bacterial communities, which implies that there is no significant contribution of microorganisms from the digestate to the vermibeds bacterial community (Figure 5).

As has been stated, in the presence of earthworms, microbial communities can change as a function of earthworm density (Chen et al. 2018), thereby utilizing the available energy source efficiently (Vasanthy et al. 2013). Therefore, the vermibed systems functions much better and increases the rate of decomposition and mineralization (Schönholzer et al. 1999) when due to the decomposition of decaying substrate by earthworm, the availability of nutrients for the microorganisms decreases (Brown 1995). Our results indicate that large changes are observed in the structure of bacterial communities during cycles 1 and 2 (before day 34) and between cycles 2 and 3 (between 33 and 90 days), and compositional changes are minimized at the end of the vermicomposting process (Figure 5). The stabilization of the levels of diversity (Figure 4) and the low compositional changes can be related to the stabilization of the bacterial community, indicating that after an initial adaptation, the process operates in a robust manner, i.e. the entry of new organic matter, either in the form of fresh waste or digestate, and then there are no further alterations in the structure of the bacterial community (Figure 5). Such stability appears in our experiment to stimulate the development of earthworms (see Figures 2 and 3). These findings correspond with those of Gómez-Brandón et al. (2016), Zhao et al. (2018) and Chen et al. (2018) who have found the influence of vermicomposting in the presence of certain
microorganisms, such as fungi, protozoa, and several phylum of bacteria, as a function of time and worm density. Therefore, it is considered important to relate population dynamics, physiology and ontology of earthworm during vermicomposting to the changes in microbial communities (Vasanthy et al. 2017), since there may be a relationship between the intestinal process of earthworms and the microbial population (Gómez-Brandón et al. 2011; Ingrid et al. 2014); however, the mechanisms of action are remain unclear.

CONCLUSIONS

The addition of liquid digestate amendments coming from market waste to the vermicomposting process of the same residue is concluded to be a safe end-disposal method. The treatments applied indicated maturation and good quality of final vermicompost, as judging from the analysis of pH, moisture content, phytotoxicity and C/N ratio. Seed germination rate is high using vermicompost as substrate, regardless of the addition of digestate during the process showing that digestate amendments do not add phytotoxins and could even exert a positive effect on the germination ratio.

Young earthworms flourish in the treatments with digestate addition. Changes on adult/young populations of E. fetida are interesting aspects to explore in future research where the effect of digestate over the physiology and ontology of these earthworms can be addressed.

The addition of digestate to vermicomposting process do not significantly affect bacterial communities. During the early stages of vermicomposting, bacterial communities present high changes in their composition, but these changes are independent of the digestate amendments. Community changing stabilization is achieved at later stages of the vermicomposting.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.
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