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# A User-Friendly and Sustainable Toilet Based on Vermicomposting

**Fernando Alonso-Marroquin [1,*], Ghulam Qadir [1,*], Jad Nazha [1], Vanessa Pino [2] and Arianna Brambilla [3]**

[1] School of Civil Engineering, The University of Sydney, Camperdown, NSW 2050, Australia
[2] School of Life and Environmental Sciences, The University of Sydney, Camperdown, NSW 2050, Australia; vanessa.pino@sydney.edu.au
[3] School of Architecture, Design, and Planning, The University of Sydney, Camperdown, NSW 2050, Australia; arianna.brambilla@sydney.edu.au
* Correspondence: fernando.alonso@sydney.edu.au (F.A.-M.); ghulam.qadir@sydney.edu.au (G.Q.)

**Abstract:** Environmental awareness has sparked increasing interest in changing the way humans interact with their environment. This awareness includes the change in paradigm of considering human manure (humanure) not as a waste but as a valuable bioproduct instead. In this regard, composting is an age-old technique for nutrient recovery that has gained renewed interest, as it may be a sanitary and financially viable solution to closing the loop of human–nature interactions. This work investigates environmental solutions for toilet systems that are user-friendly and sustainable based on systems that filter nutrients via vermicomposting. The methodology is based on (1) reviewing several surveys across different continents to select the most appropriate interface of a targeted society, and (2) investigating the microbial dynamics of vermicomposting. The microbial activity was compared with the activity of the aerobic composting systems by measuring soil temperature, soil composition, decomposition rate, stabilization factor, and biological diversity. The microbial decomposition process in vermicomposting was faster due to the presence of earthworms, but the increase in temperature and volatile ammonia led to the earthworms burrowing into the soil. Overall, the flush toilet is still the most socially accepted toilet interface, and the connection of vermicomposting to this toilet interface poses challenges in managing high ammonia content and maintaining healthy conditions for the earthworm population.

**Keywords:** waste management; biophilia; vermicomposting; UN goals; climate change

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## 1. Introduction

Human waste management systems have made substantial advancements over time. Still, globally, 3.6 billion people lack access to safely managed sanitation services [1]. But in terms of percentage, 90–100% of the population in developed countries have access to safe sanitation compared with 50–70% in under-developed countries [2]. Also, the unhygienic practice of open defecation is very rare (1%) in developed countries [3] but due to a lack of toilet facilities, this percentage is around 15–20% in under-developed countries as the use of unimproved pit latrines and open shared latrines is quite common, contributing to this high percentage [4]. Many countries have well-established systems to control sewerage that have played a vital role in the minimization of contamination and disease spreading. Nonetheless, in cases where sewerage systems are accessible, the presence of such infrastructure still encompasses serious environmental and socio-economic implications. For example, the accumulation of nutrients in water resources (eutrophication) [5], the destruction of coral reefs [6], and greenhouse gas emissions by sewerage systems and treatment plants [7] provoke substantial costs and environmental threats. The composting of waste into nutrient-rich fertilizer represents a biophilic approach that may support a reduction in such risks. This process can be classified into two prevalent categories: aerobic composting (AC), which utilizes bacteria for decomposition, and vermicomposting (VC), which engages the use of both bacteria and earthworms for decomposition. Another method is anaerobic composting, which occurs in an airtight closed container and makes use of microorganisms that do not require oxygen to break down waste, generating methane ($CH_4$). This methane is a useful source of energy; however, it is powerful greenhouse gas when released into the atmosphere [8].

AC has gained a significant level of development and establishment, as demonstrated by the presence of commercial aerobic composting toilets and the regulations governing their usage. On the contrary, VC toilets represent a novel technology that has been garnering significant attention in the scientific community in recent times. But overall, there are no specific regulations on composting toilets (AC or VC), even in developing countries, e.g., in the US, there is a general lack of detailed guidelines [9]. As per regulations, composting toilets are permitted on site where soil conditions are unsuitable for onsite sewage treatment or water under pressure is not available from the municipality and in flood-prone areas [8].

Different research studies [10,11] have highlighted the advantages of VC over AC in various aspects such as sanitation, compost quality, and operational results. VC surfaced as a highly esteemed fertilizer in agriculture, asserting a premium price compared with conventional compost [12]. Contemporary research in the field of VC toilets has primarily focused on process optimization. For instance, a comparative study [13] was conducted to gauge the effectiveness of various filter media used in vermifilter toilets, ultimately concluding that river bed material yielded the most favorable results. Another study [14] focused on comparing different worm bedding materials, finally identifying coir and woodchips as the top-performing options. In recent times, a research study [15] conducted a comparison of various VC hydraulic loading rates and filter media compositions, intending to determine the most affirmative loading rates and layouts.

Although extensive research has been devoted to optimizing specific aspects of the vermicompost toilet, a comprehensive design that adopts these recommendations into a user-friendly and sustainable solution is yet to be established. There is also limited information available regarding the social acceptability and financial viability of such a toilet, as both are crucial factors in achieving the broader objective of utilizing composting designs to direct waste management challenges. Enhancing the understanding of VC toilets and promoting their large-scale adoption requires conducting an extensive analysis of vermicomposting's effectiveness in contrast to aerobic composting. Furthermore, improving the VC design and gaining insights into the solution's social acceptability and financial viability are crucial steps to advance the VC toilets' potential.

In this regard, this research proposes an innovative and optimized composting, micro-flush toilet design. This paper first discusses the composting reactor mechanism and the use of toilet user interfaces. This is followed by a review of the social perception of eco toilets across different continents by analyzing survey results. Based on that, a design for a vermicompost chamber is proposed, and a composting experiment is set-up. An eco-friendly aspect is added to the design by connecting the composting systems to native plants that re-absorb the nutrients and close the cycle of nutrient recovery with minimal human intervention. The experiment has three composting systems, which include soil compost, compost with manure, and earthworms with manure compost. A comparison between the systems is then drawn based on the analysis of the compost temperature, physicochemical properties, and micro fauna analysis (tea bag index tests and DNA sequencing). This gave a blueprint of the composting effect on the soil. In the end, suggestions are given for the best toilet user interface.

## 2. Composting Reactor Systems

Aerobic composting (AC) takes place in adequately ventilated environments, where oxygen-utilizing microorganisms disintegrate and decay organic matter. In this composting process, aerobic microorganisms, such as bacteria, actinomycetes, and fungi, facilitate the oxidation of organic materials, resulting in the production of carbon dioxide ($CO_2$), ammonia ($NH_3$), water ($H_2O$), and volatile compounds [8]. AC has different stages: mesophilic, thermophilic, and curing stages. Mesophilic composting takes place within a temperature range of 20–40 °C and generally lasts for a few days. In this stage, the mesophilic bacteria commence the decomposition of organic matter and generate substantial heat, which triggers the successive phase of thermophilic composting. Thermophilic composting takes place over several months. This stage is characterized by the dominance of thermophilic bacteria that thrive in higher temperatures, ranging between 45 and 65 °C. The bacteria in this stage metabolize proteins, fats, and carbohydrates, while the heat generated during this stage helps eliminate pathogens. Eventually, as the depletion of the energy sources occurs, the temperature steadily decreases, and this leads to the final curing phase where mesophilic organisms once again become dominant and decompose any remaining organic material. Efficient aerobic composting requires specific conditions to be met, e.g., an oxygen concentration of 15–20%, a moisture content between 50 and 65%, a temperature ranging from 50 to 65 °C, a carbon-to-nitrogen ratio of 25–30, pH levels between 6.7 and 9, and a porosity of 35–50% [8]. For optimal composting, it is essential to control these factors through measures such as employing ventilation fans, regular mixing, water sprinklers and drainage systems, heaters, and insulation, as well as an assortment of high-carbon materials in the form of sawdust, shredded paper, coffee grounds, straw, etc.

Vermicomposting (VC) employs earthworms and microorganisms to break down organic matter and generate nutrient-rich vermicompost, or worm compost. This process consists of two primary stages: the active stage, facilitated by earthworm digestion, and the maturation stage, driven by mesophilic bacteria and fungi. In the former, the earthworm's digestive system utilizes enzymes, intestinal mucus, and natural antibiotics to eliminate pathogens and foster a diverse and beneficial microbial community that enhances plant growth [16]. After traversing the earthworm's digestive tract, the organic matter is excreted as "casts" surrounded by microorganisms, enzymes, and fermenting substances. During the successive maturation phase, bacteria and fungi promptly act upon the partially digested casts, transforming them into mature vermicompost. Unlike AC, this entire process takes place within the mesophilic temperature range (15–28 °C) for optimal performance. Within 4–8 weeks, this process yields valuable vermicompost that enriches soil fertility, promotes plant growth, eliminates harmful pathogens, and aids in disease management [16]. For developing an environment for vermicomposting to grow and to create an optimal ecosystem, specific conditions distinct from aerobic composting are compulsory. These conditions include maintaining a higher moisture content of 60–75%, lower temperatures (preferably around 25 °C, but with survival possible within 0–35 °C), a reduced carbon to nitrogen ratio of 25:1, lower pH levels of 6.5–8, and moderate porosity to facilitate worm burrowing (35–50%) [17]. Through various means, these conditions can be achieved, e.g., incorporating high-carbon materials, utilizing ventilation fans, providing insulation, implementing water sprinklers, and establishing effective drainage systems.

The construction of a vermicomposting reactor requires the implementation of a worm filter, also known as a VC filter. This biological filtration system engages earthworms to break down organic matter and filter the resulting liquid as a by-product known as vermicast [18]. Within the VC, the vermicast passes through layers of organic materials, such as peat moss, coconut coir, or compost, serving as a substrate for the earthworm bed. The earthworms maintain the aeration of the compost through the tunneling system they create, and they increase the compost temperature through their metabolism. The worms consume the organic matter and convert it into worm cast (solid phase) and worm tea (liquid phase). Driven by gravity, the worm tea, along with the diluted cast, undergoes filtration through a series of granular layers with varying grading. The final liquid holds considerable value as a nutrient-rich soil amendment, suitable for gardening or agriculture usage [19].

## 3. User Interfaces

The "Toilet interface" envelopes different interactive elements that ease individuals to engage with a toilet, notably in accommodating diverse genders, ages, and abilities. An optimal interface encompasses a range of components and features that ensure maximum levels of hygiene, comfort, and satisfaction during toilet use. Therefore, it is important to understand how each toilet interface adapts to the idiosyncrasies and customs of a particular social group.

In ancient civilizations, the toilet systems consisted of holes or pits in the ground. A remarkable example is the communal spaces of ancient Rome [20], reflecting a collective acceptance of bodily functions as natural and integral parts of life. However, as societies evolved and notions of privacy and modesty emerged, toilets became enclosed and private spaces, often associated with shame or embarrassment. The flush toilet that we know today has roots back to the 16th century as Sir John Harington invented the first flush toilet [21]. This was followed by the tank-based flush toilets in the mid-19th century first presented as patent by Alexander Cummings, who had an idea of using a separate water tank for flushing [22]. By the mid-1980s the dual-flush toilets were in use [23]. In the late 20th century, sensor activated toilet technology was introduced and it gained progressive popularity [24]. In recent years, bidet toilets and smart toilets are mostly driven by Japanese innovations, but they are still not widely used [25].

There are several types of toilets. Therefore, a characterization was proposed based on (1) the position adopted by the user (standing [26], squatting [27], sitting [28], etc.) and (2) the evacuation method of humanure from the user interface was also taken into consideration, including options like dry/flush urine-diverting toilets, dry toilets, and flush toilets.

The three most common toilet positions are standing, squatting, and sitting. Standing is commonly used for male urination [26]. Squatting is an organic posture that helps to build the pressure required for easy evacuation [27]. Sitting is a comfortable position that requires physical contact with the toilet, which is widely adopted in Western societies [28]. In the classification of toilets that uses the method of evacuation of the humanure, there are three general methods, dry toilets, urine-diverted toilets, and flush toilets. The "dry toilet", also called the latrine, shown in Figure 1a, operates without flush water, and it is placed over pits. The humanure and the cleansing material are evacuated by gravity. Latrines do not have a water seal to stop odors. These toilets are designed usually for sitting rather than squatting. They allow the waste to decompose through natural processes. Typically, the waste is deposited into a chamber or container where it undergoes composting.

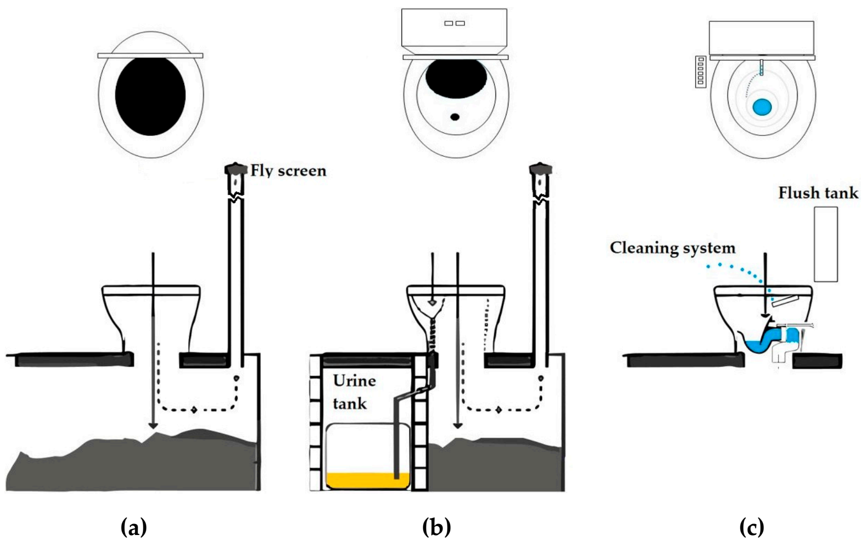


**Figure 1.** Top and cross-section view of typical toilet user interfaces, showing the place of urine and stool through arrows pointing downward: (**a**) Dry toilet latrine. The fly screen shown is used to keep insects, dirt, leaves, and debris away from the composting chamber. (**b**) Waterless urine-diverter toilet with a separate chamber for urine collection. (**c**) Flush toilet–bidet with a combination of micro flush technologies and a water jet cleansing system.

The urine-diverter toilet is shown in Figure 1b. The interface collects urine in the front of the toilet, and solids are collected in the back, using separate compartments. By separating humanure at the source, urine diverters enable greater nutrient recycling, offering the possibility to convert urine into a valuable fertilizer. This type of toilet can accommodate both sitting and squatting positions and also both dry and flush models. There is flexibility for catering to user preferences in the design. But due to social acceptance and high labor costs, there are certain limitations in the use of urine diverters.

Urine contains a relatively high concentration of nutrients, including 80% nitrogen, 50% potassium, and 50% phosphorus [29]. The urine-based fertilization can be performed through two low-cost methods. The first method for nutrient recovery is to dry the urine and keep the urea $(NH_2)_2CO$ as a solid material to be used as fertilizer [30]. In the second method, urine is collected and mixed with water creating a diluted solution that is directly applied to the soil [31]. The natural bacteria present in the soil convert the urea into ammonia ($NH_3$) and then into nitrates ($NO_3^-$) that are assimilated by plants to build amino acids. These are essential nutrients in the food chain. Potassium and phosphorous are essential elements in the food chain that, unlike nitrogen, are not present in the atmosphere. Although urine-diverting toilets are available for use, societal acceptance may not be immediate. It is prone to misuse and clogging. The capital and operating costs are high. A survey study of 17,499 households in South Africa revealed low satisfaction with such facilities and a perceived odor in the toilets, leading users to prefer flush toilets [32].

The centerpiece of today's modern user interface is the flush toilet. The first patent was granted to Alexander Cumming in 1977 and included the s-shaped pipe below the bowl that seals odor from entering the bathroom. The main drawback of the flush toilet is that it uses large amounts of water, very often drinkable water, for the flush operation. However, modern toilets have created new technologies to reduce the water used during the flush, such as double flush buttons, and micro-flush systems that combine non-stick technologies with tornado-like flush modes for efficient evacuation. Thanks to these technologies, the amount of water required to flush has been progressively reduced from 20 litres to 3.5 litres. Many companies in Japan, e.g., Toto, have established a reputation for producing high-quality toilets that prioritize performance, comfort, and sustainability [33]. The TOTO electronic toilet operates as a Smart Toilet, working with an integrated water-cleansing system for superior and comfortable personal hygiene [34]. The Toto-toilet is shown in Figure 1c.

## 4. Social Perception of Toilets

Toilets are fundamental facilities for every human being, yet the acceptance of society towards the new toilet system is influenced by cultural attitudes and social perceptions towards hygiene, privacy, and social norms. The choice of a suitable combination of user interface and the composting method for a sustainable toilet should consider different factors of the targeted community, such as hygiene practices, economic priorities, connectivity with the environment, and gender-related preferences. This paper focus on determining the acceptable levels of people regarding composting systems, or no-mix (urine-diverting) technologies, across various locations worldwide: the United States [35], Europe [36], Philippines [37], and Australia [38]. To achieve this, survey reports from the literature were analyzed.

Positive acceptance and a willingness to adopt sustainable technologies, e.g., eco-toilets (ET) and urine-diverting toilets (UDT), has been reported in four different continents. In these surveys, the ET was presented as a system that uses a small amount of water or no water and recovers nutrients from human waste, and the UDT as a system that separates urine and solid waste during the toilet-use process. Two locations in the US have been surveyed: Cape Cod Island [35] and Hawaii [35]. In Cape Cod Island [35], only 42% of the participants had positive attitudes about septic systems and many were willing to adopt eco-toilets. Most respondents would be completely willing to use an ET in a friend's home (63%), and many would be completely willing to stay at a hotel or other short-term lodging with an ET installed (46%). The percentage of respondents who agree that the risks of using an ET are acceptable to obtain the benefits was much larger than the percentage who reported complete willingness to install eco-toilets in their own homes. This difference suggests that more participants would accept the risk-versus-benefit tradeoffs than the group that may be eco-toilet advocates. In Hawaii, 84% would allow to have a UD toilet installed in their home and 65% would pay about 50 USD to have a UDT in their home [35]. A comprehensive survey in Europe across seven different countries revealed that participants considered UD toilets to be the same or better than conventional toilets in terms of hygiene (85%), smell (77%), comfort (86%), flushing (56%), and cleaning (52%) [36]. In the Philippines, the main factors to be considered by all respondents for installing an ET at their home/community were (1) opportunities for saving water (47%), a shouldered cost of the installation of an ET, and the reuse of the nutrients from humanure (22%) [37]. In Australia, 30% of the respondents were positive towards the UDT but only 15% had a positive attitude towards dry ET [38]. Overall, the survey reports across the mentioned locations revealed that most of the users have positive acceptance and a willingness to adopt eco-toilets and urine-diverting toilets. But still, there is a need for further developments and public education to address the risk and benefits related to these types of toilets.

The surveys also reported on the attitude of the community towards the recovery of nutrients from humanure. In Hawaii, 82% of the respondents conveyed that human waste can be safely recycled instead of being disposed of and 92% agreed that it can be safely treated to be converted into fertilizer [35]. In Europe, the survey on UDT showed that 85% of participants consider using urine as fertilizer as a good or very good idea [36]. A different perception was shown in the survey in the Philippines, where 56% and 76% of the participants thought that urine and stools can be used as fertilizer [37]. On the other hand, a moderate enthusiasm of the Australian community for the manual handling of the compost was surveyed; a fraction of the respondents would volunteer to mix compost (67%), add carbon-rich material to it (97%), and harvest it (39%). Interestingly, 37% of the Australian respondents approved of using earthworms as an alternative for manual compost mixing, while 36% disapproved of using them, and 37% were neutral to this idea. Overall, these worldwide surveys show a positive attitude towards composting humanure, especially if it does not compromise the users' time on handling compost. These survey results bring up the opportunity to capitalize on this attitude in the development of novel, user-friendly, and sustainable toilets that involve minimal interaction of the users with the composting process.

## 5. Methodology

According to the surveys, the possibility of using the aerobic composting (AC) system that requires the continuous handling of the compost heat to achieve optimal moisture, aeration, and a carbon–nitrogen ratio was less attractive than vermicomposting (VC). For this reason, to minimize human intervention in the composting process, a composting chamber was devised in this paper. The chamber controls temperature, oxygen content, and humidity in a way that mimics the natural processes involved in composting with no human intervention. The bioproducts of the worms (worm cast and worm tea) were applied directly to native vegetation adjacent to the composting chamber. This strategy reduced the manual handling of the compost to zero. The details of the design and setup of the composting chambers are presented in this section.

### *5.1. Vermicomposting Design*

Based on survey results, critical design principles, and best practices for composting, different composting chambers were designed, built, and tested to find the best choice of the composting chamber for a micro-flush toilet interface. The focus was placed on systems with an optimal composting rate and minimal human intervention in the composting process.

Due to safety regulations, humanure was not used in this experiment, instead, a recipe for Synthetic Humanure (SMN) was created by mixing 4 parts of sheep manure, 4 parts of liquid pig manure, and 3 parts of chicken manure. This selection was intended to mimic the variable diet of an average human, while the chicken manure provided a high content of urine capturing the contribution of both urine and stool in the humanure. Toilet paper was considered an important contribution to the composting chambers. Paper is a carbon-rich material, so it provides a balance between the carbon and nitrogen content in the compost chamber [39]. To add carbon to the SHM, cardboard samples were cut into 100 mm squared-shaped pieces and compost was sandwiched between the cardboard layers.

Based on the design, the three composting systems labeled as Control (C), Vermicompost (VC), and Aerobic compost (AC) were placed parallel to each other as shown in Figure 2 (plan view). Final design drawings were drafted using Autodesk Revit, shown in Figure 3. Three identical plywood timber frames, for three composting systems with dimensions of 1100 × 1200 × 660 mm, were constructed. The frames were placed on a concrete slab that had a 200 µm thick plastic sheet at the base, shown in Figure 3a. Plant trays were placed inside the frames and garden mix soil was poured into the trays to fill them up. The plant trays not only provided built-in pots for the plants to grow in but also transferred the load of the compost to the concrete floor. Four different plants in four corners of every tray were planted, as shown in Figure 2. The planted plants were Arum lily (*Zantedeschia Aethiopica*), Carex Evergold (*Carex oshimensis*), Carex Feather falls (*Carex oshimensis*), and Greater Brown Sedge (*Carex Brunnea*). The plants were in the initial stages of their growth.

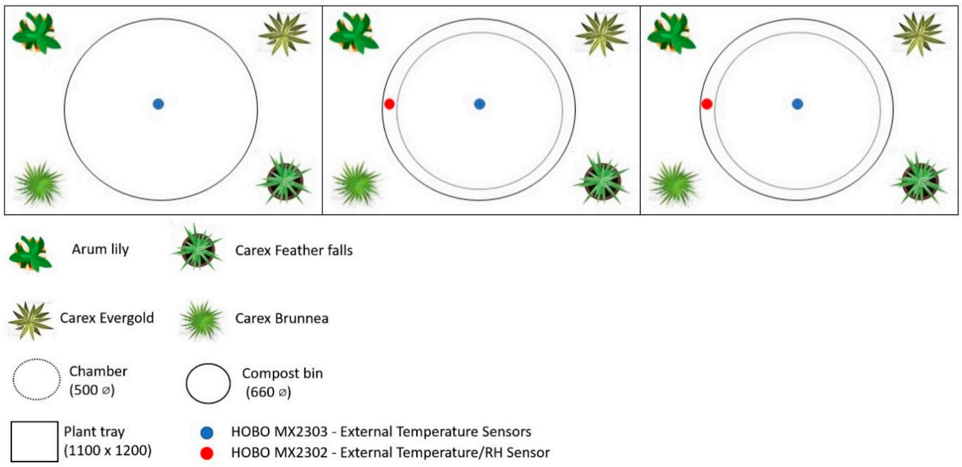


**Figure 2.** Arrangement of the plants in the corners around the composting systems (left to right): C, AC, and VC. The location points of the plants in each tray were the same, e.g., *Carex Feather falls* was in the bottom right of each plant tray, and *Arum Lilly* was in the top left. The composting chamber was placed only in AC and VC. The location of the sensors for compost temperature, air temperature, and relative humidity is also shown.

### *5.2. Experimental Set-Up*

Three compost systems (C, VC, and AC) were set up, one each in every tray. To protect the chambers from the external environment, they were surrounded by a black plastic bin [40]. The chamber in Figure 3a is a cylindrical steel frame with a steel mesh creating an air layer between the compost and the plastic bin. The air gap created by this three-millimeter wire mesh reduces heat exchange with the environment and improves aeration in the compost. This improved chamber results in more oxygen flowing into the composting chamber and higher temperatures. Inside the steel mesh cylinder, substrate layers were arranged. The layer from bottom to top were hydro corn clay pebbles (50 mm), black silica stone pebbles (50 mm), sand (700 mm), and soil (800 mm). A Geo-fabric sheet was used to avoid mixing the substrate layers. The only difference between VC and AC chambers was the incorporation of earthworms in the former. The VC chamber had 1000 earthworms poured into the soil layer. The Control compost chamber had only garden mix soil and no substrate layers. A wooden lid top with a fitting of a washbasin sink (mimicking a micro-flush cistern water supply) was installed for both VC and AC up to the manure addition period (up to Day 24). An automated water supply was discharging two liters of water (one-minute duration) to the chambers every day in three intervals (11 am, 2 pm, and 5 pm). The manure was added three times a week (600 mL of chicken, 600 mL of sheep, and 1800 mL of pig) for the first three weeks. Then, 2400 mL each of chicken and sheep were added all together. The composting and vermicomposting took place in AC and VC systems, respectively. The synthetic humanure (SHM) entered the chamber and was processed by earthworms in the topmost bedding layer while the excess liquid was filtered through the chamber geo-filter containing various sands and gravels before being drained out as a more pathogen-free effluent. The same process happened in the AC system but without earthworms.

The arrangement of all the components of the compost bin system is shown in Figure 3. The compost bins were checked from the inside from time to time. The purpose of checking was to make sure the dip in the height of the compost was noted, e.g., on Day 43, the compost height had reduced inside the chambers by 8 and 12 cm in the VC and the AC, respectively. Cross sections of different filtering layers inside the composting bin with manure addition at the top are shown in Figure 3c.

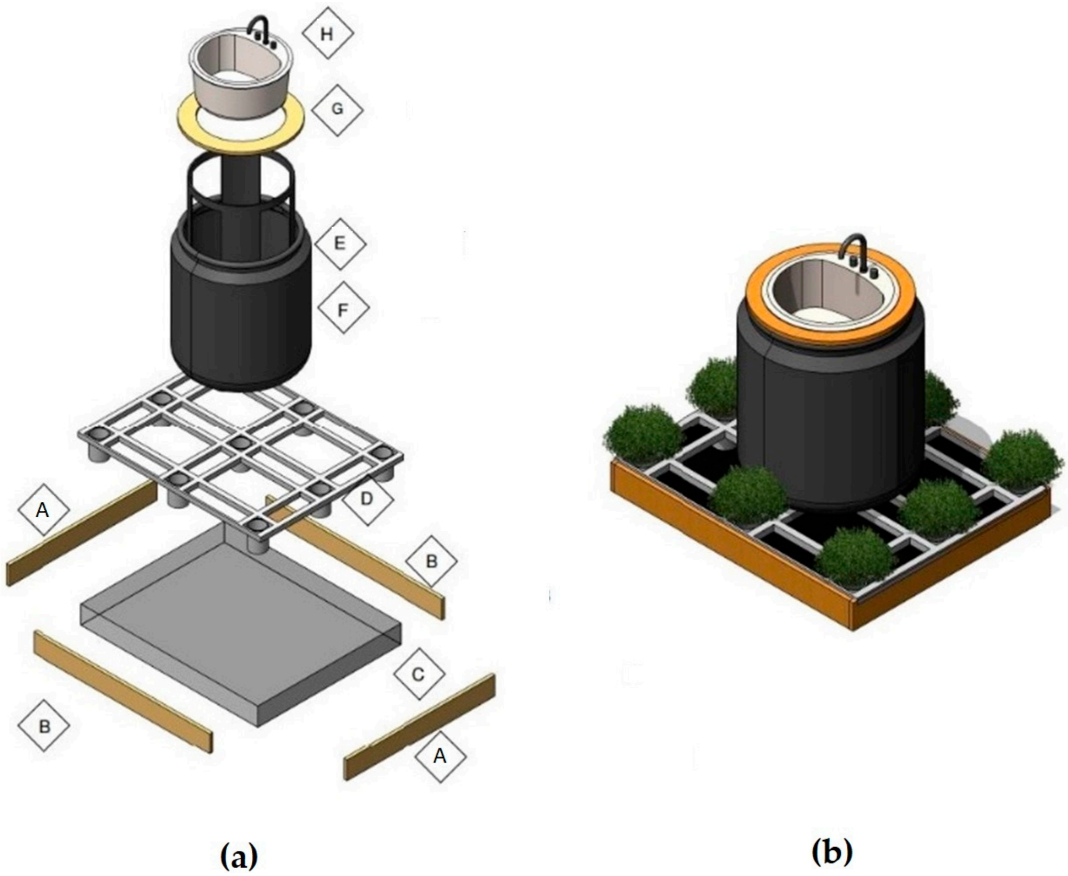


**Figure 3.** *Cont*.

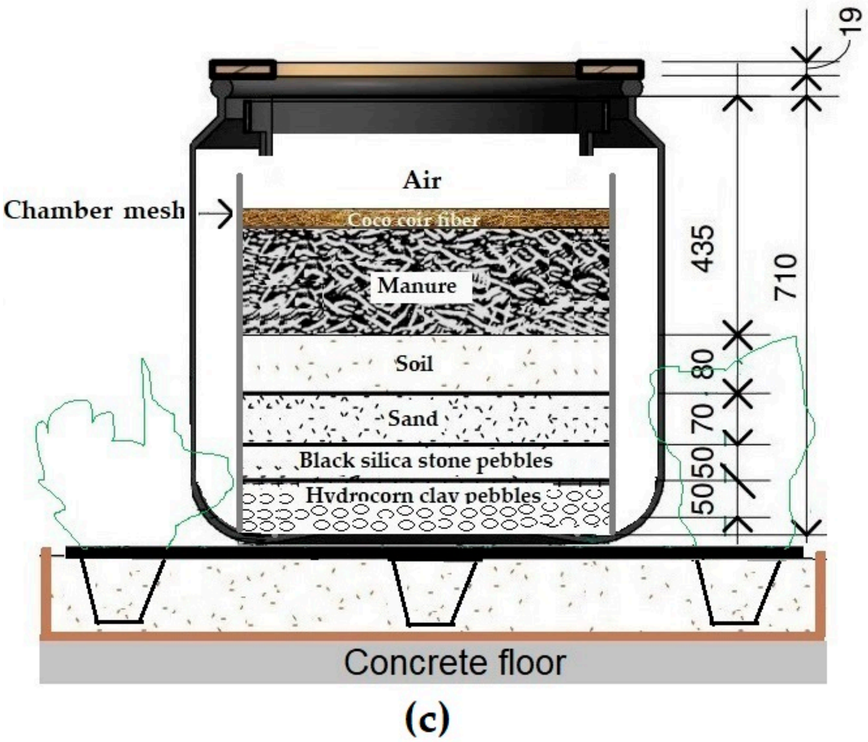


**Figure 3.** (**a**) Exploded of all the pieces involved in the construction of the composting system. (A) Plywood side walls—lengthwise, (B) Plywood side walls—Breadth wise, (C) Plastic sheet, (D) Plants base for plantation, (E) Steel mesh, (F) Compost bin, (G) Compost lid, (H) Vanity, (**b**) Three-dimensional view of the complete experimental set-up of a chamber. (**c**) Cross section of the composting system showing all the filtering layers (from hydro corn pebbles to soil) in the AC and VC systems. The manure is shown being added on top of the soil layer which merges with the soil. In VC, the earthworms were poured to mix with the soil. The thickness of manure changes with time depending on the addition and filtration. The top surface is covered with a coco-coir fiber layer to avoid excessive oxidation. All the thicknesses are in millimeters (mm).

It is important to understand the reasons behind what type of different tests were conducted for comparison of the composting samples, during, before, and after the experiment shown in Figure 4, as well as the methods used to perform these tests, e.g., compost pile temperature, physiochemical tests, tea bag index, and soil DNA sequencing. The compost pile temperature range indicates different stages of composting [41], e.g., the mesophilic stage between 20 and 40 °C, thermophilic from 45 to 65 °C, and so on. Based on that, the biological activity inside a compost can be determined.

Temperature monitoring helps the identification of the stage of the composting process [42] as it ensures that the composting process is reaching a stable product. The compost pile temperature was monitored through HOBO MX2303 data logger sensors [43]. The physiochemical tests of soil compost included pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (TN), ammonia ($NH_3$), and moisture content (MC). Monitoring the pH of a soil compost is important because it ensures nutrient balance inside the soil for optimal plant growth leading to improved agricultural and gardening outcomes [9]. EC measurements of the compost provide valuable information about the nutrient status and assess the salinity level of the compost [44]. Finding TOC and TN results in understanding the carbon–nitrogen ratio (C:N) of the compost [9] as C:N ratio is essential for finding compost quality, decomposition progress, and nutrient availability, which helps manage the compost in a better way. Finally, finding the ammonia ($NH_3$) content is significant because it provides insight into the maturity and stability of the compost [45]. Ammonia production is typically high in the early stages of the compost but with time it decreases because the decomposition process slows down. Also, high levels of ammonia emissions can contribute to air pollution and unpleasant smells. The metabolism of earthworms also produces ammonia [46]. The tea bag index (TBI) testing provides a standardized, economical, and practical approach to assess the decomposition and evaluate the stabilization rates inside a compost pile. Lastly, DNA sequencing technique used was important because it gives taxonomic classification of phylum. It provides valuable insights into the microbial community composition and diversity within the compost. The physiochemical tests, TBI, and DNA sequencing results were obtained through the same techniques mentioned in the greywater system filtering system [47]. The descriptions of the physiochemical test methods used are shown in Appendix A. The detailed method of the tea bag index studies is shown in Appendix B.

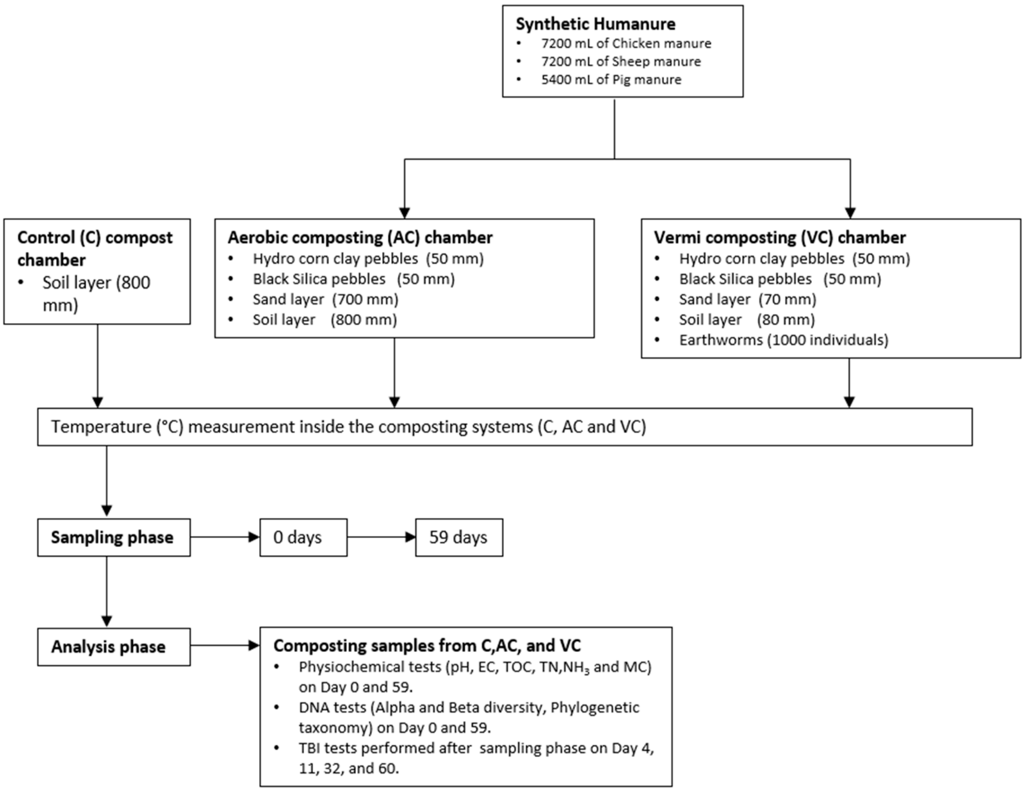


**Figure 4.** Methodology flowchart of the experimentation. The procedure to obtain the optimal composting chamber is shown in the flowchart and consists of the preparation of the recipe for Synthetic Humanure SHM. Design and building of three composting chambers: control (C), aerobic composting (AC), and vermicomposting (VC), and (the testing of the composting process in the chamber based on compost temperature, Tea Bag Index, physicochemical soil properties, and DNA analysis. The total manure quantity that was added during the experiment is shown in AC and VC composting systems. The layers of materials and their thicknesses are shown for each compost system. The compost temperature was measured inside each system. The sampling phase started on June 27 and ended on August 26, 2022 (59 days). The sampling phase corresponds to the days on which the composting soil samples were taken. The analysis phase included the type of tests conducted on the composting samples collected on specific days from the three composting systems (C, AC, and VC) shown.

In the DNA analysis of the soil, universal primers were used in DNA sequencing for diversity studies. This is because they target conserved regions of the genetic material [48]. Universal primers offer a standardized approach to DNA sequencing, which enables a comparison of diversity across different studies and environments. One commonly used universal primer targeted the 16S rRNA gene in bacteria. This approach is widely used in microbial ecology and environmental studies to assess microbial biodiversity and help in identifying specific bacterial taxonomy [49]. The technique used for DNA sequencing results is shown in Appendix C.

## 6. Results

The results are organized into four sections. The first section analyses the compost pile temperature inside each chamber. The second section provides a review of the physio-chemical properties of the composting soil samples. The third section investigates the TBI results, which is followed by the last section on soil DNA analysis.

### *6.1. Composting Temperature*

Figure 5 shows the temperature data inside the composting piles. The VC temperatures reached a maximum of 41.31 °C on Day 41, while the AC temperatures reached a maximum of 35.65 °C on Day 42 at 1:00 h. The mean temperature during the experiment for both VC and AC was 21 °C, which is in the recommended range for VC but not for AC. In VC, the continuous temperature rise was noted from Day 24, when 4810 mL of manure was added. After this addition, the compost pile temperature in VC went past the ideal range for earthworms from Day 39 to Day 45. This resulted in earthworms burrowing because they would not survive in this high temperature. The AC temperature did not achieve the ideal range (45–65 °C) throughout the experiment [50] as the thermophilic phase (>45 °C) [9] was not achieved. Compost requires the cultivation of aerobic, or oxygen-loving, bacteria to ensure thermophilic decomposition [51]. Also, to reach thermophilic phase decomposition, it takes a minimum of 1 $m^3$ (one cubic meter) of compost [52].

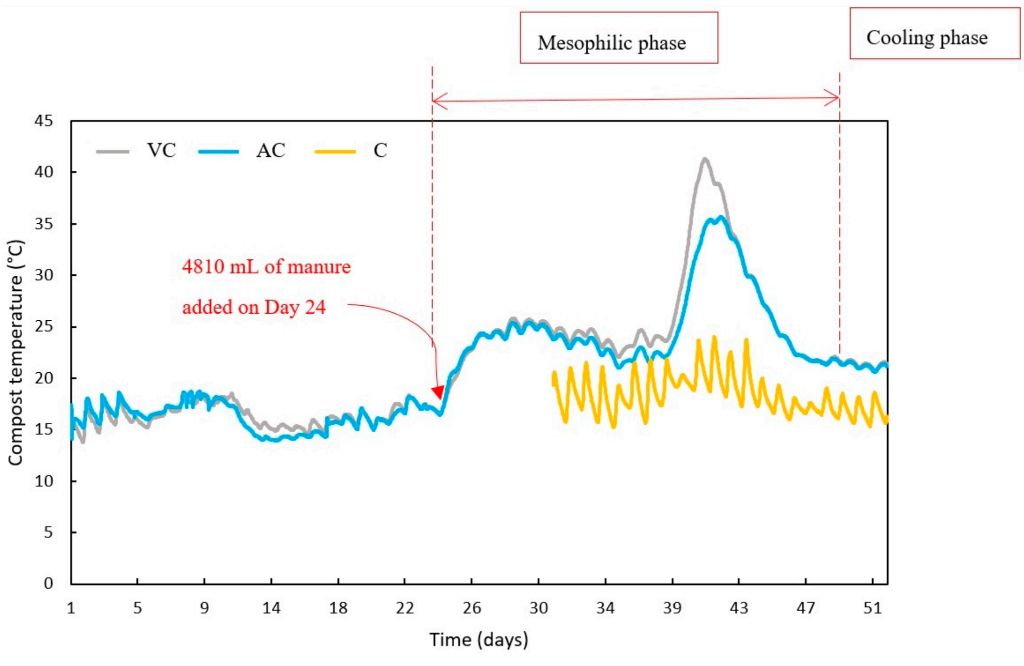


**Figure 5.** The temperature variation graph inside the composting piles of control (C), aerobic compost (AC), and vermicompost (VC) systems. The data of C compost were recorded from Day 30 to Day 51 (21-day period). From Day 1 to 23, mixed manure (600 mL of chicken, 600 mL of sheep, and 1800 mL of pig) was added with a 2-day frequency. On Day 24, 4810 mL of manure (2400 mL of chicken, 2400 mL of sheep, and 10 mL of pig) was added together, increasing the temperature of the compost to the mesophilic stage. The two phases (mesophilic, and cooling/curing) are highlighted. The mesophilic stage temperature range (20–45 °C) started from Day 24 because 35% of the manure quantity was added to the system altogether.

*6.2. Physiochemical Properties of Soil*

The before-testing soil (BTS) sample properties were compared with the soil composting samples from C, VC, and AC after the end of the irrigation period (Day 57).

From Figure 6, the effect on soil physicochemical properties of adding the manure into VC and AC composting systems is very clear. All the compared properties have shown higher values for both VC and AC. The experimental period of 59 days in the BTS in the control composting system increased the pH by 0.4 ± 0.08 (Figure 6a). This 5% increase in pH over 59 days has also been found in another study of 60 days [53]. The pH of both VC and AC was slightly alkaline (>7.5); this pH is ideal for the thermophilic phase of composting [54]. If the pH is too acidic or too alkaline, bacterial activity will be hindered or stopped completely. The pH turning into alkaline at the curing phase (Day 59 sample) [9] is normal. Overall, the pH values achieved by both VC and AC were in the desired range (5–8) [9].

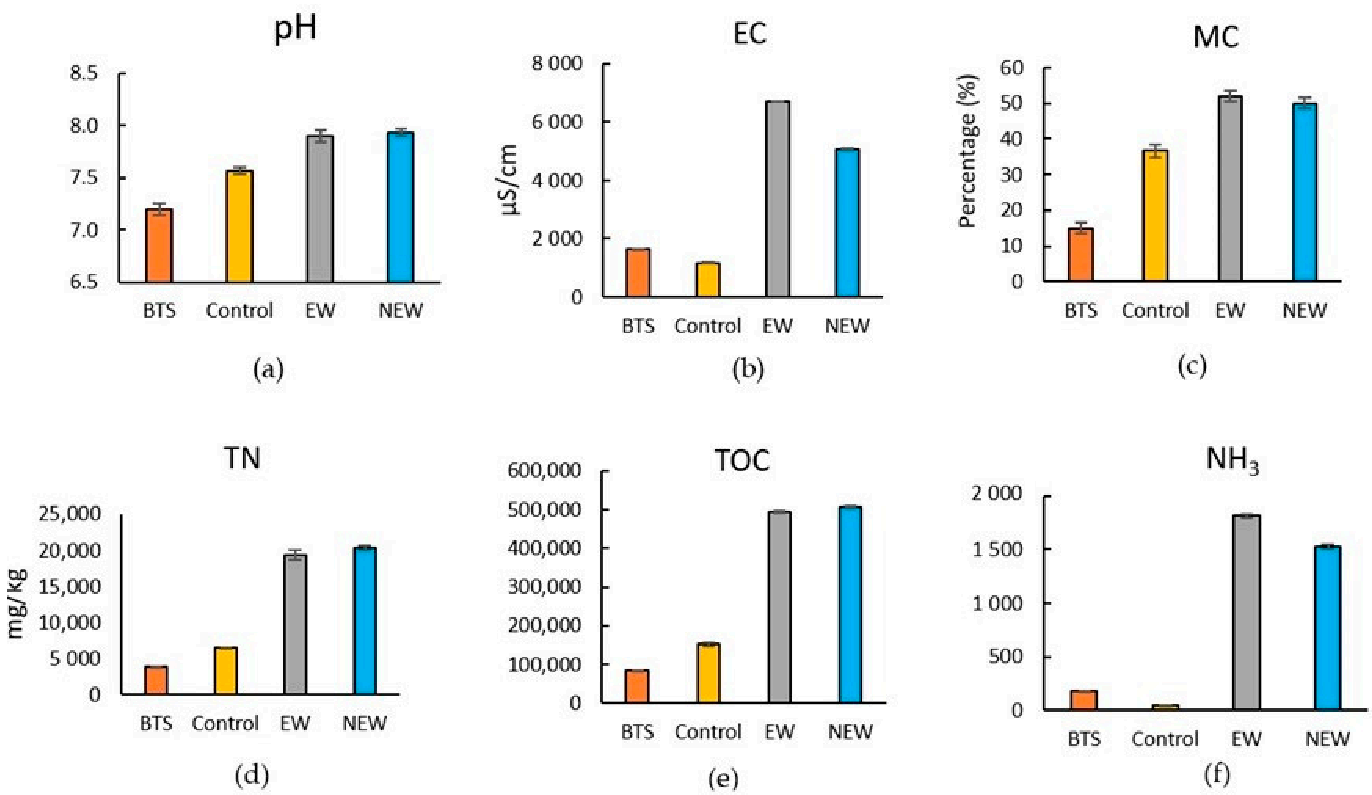


**Figure 6.** Physiochemical properties of the soil samples before treatment of soil (BTS), control (C), vermicomposting (VC), and aerobic composting (AC) composting systems. The measured quantities are (**a**) pH, (**b**) Electrical conductivity, (**c**) Moisture, (**d**) Total Nitrogen (TN), (**e**) Total organic carbon (TOC), and (**f**) Total ammonia ($NH_3$). The error bars encode the mean and standard deviation of three subsamples.

The 59 day experimental period of BTS inside the control compost bin decreased the EC by 28% (Figure 6b). The EC values for both AC and VC were above the desired range. AC was only 12% greater than the ideal value (5000 µS/cm) but the EC value in VC was 123% greater than the required value (3000 µS/cm). This high value is due to the nutrient content and other non-essential soluble salts [55] in VC, due to the digestion and mixing by earthworms. A difference of 1633 ± 6.7 µS/cm was noted between VC and AC. In dairy manure-based vermicompost, the EC readings range from 10,000 to 20,000 µS/cm [56]. This high EC value in VC can also be due to water occupying the larger pores and being connected, suggesting that the EC of soil increases when the water content and degree of saturation increase [57].

The moisture content (MC) level in the AC was suitable but below the typical range by 10% in the VC, though earthworms can still operate and decompose organic matter within a broader moisture range [58]. Also, the samples were taken on Day 59, i.e., the MC had decreased by then therefore, overall, the MC level was in the acceptable range (50–60%) [59], as shown in Figure 6c. This MC level was suitable for aerobic degradation [60,61].

The high total nitrogen TN and total organic carbon TOC (shown in Figure 6e,f) can immobilize the soil and degrade pesticides, nitrates, phosphorous, and other chemicals that can become pollutants [9]. The carbon/nitrogen ratio (C:N) calculated was found to be 23:1 for C and 25:1 each for VC and AC. The ratio is in the recommended range of the C:N ratio for a composting pile (20:1 to 35:1.16 [62]). The manure mix (pig, sheep, and chicken) added to the VC and AC systems had a mean C:N value of 15:1 [63]. This means the mixing of the manure with soil and cardboard fencing the composting chamber alongside separating the composting layers increased the C:N ratio of the VC and AC by 66%. A similar increase in the nitrogen cycle was also observed in another incubated composting study [64].

The recommended ammonia ($NH_3$) levels for both AC and VC should be less than 500 mg/kg [65] but they were found to be 1500 and 1800 mg/kg, respectively, as shown in Figure 6f. The difference was 293 $\pm$ 2 mg/kg. The difference can be attributed to the ammonia produced by the earthworms [66]. This high increase in the $NH_3$ content of VC and AC is mostly related to the chicken manure because it contains a combination of faeces and urine. The urea present in the urine can be converted into ammonia through a process called urea hydrolysis. A chemical reaction occurs when urea reacts with water, resulting in the breakdown of urea into ammonia ($NH_3$) and carbon dioxide ($CO_2$). This reaction is catalyzed by the enzyme urease, which is produced by certain microorganisms [67]. Part of the produced ammonia is further transformed into nitrates (essential soil fertilizers) by nitrifying bacteria. Another part of the ammonia became volatile and produced an unpleasant odor and air pollution. Earthworms are sensitive to high levels of ammonia as it is toxic for them [46]. Therefore, the increase in $NH_3$ levels caused the increase in the bad odor which resulted in earthworms leaving the VC chamber.

### *6.3. Teabag Index Studies*

The incubation times (t) inside the soil for the teabags were: $t1$ = 0 days, $t2$ = 4 days, $t3$ = 11 days, $t4$ = 25 days, and $t5$ = 60 days. The TBI analysis enabled 48 tea bag index in-field incubations (replicates included) in this project. Only one replicate was neglected because of getting damaged during the withdrawal process from the soil. The ratio of the final weight to the initial weight of green and rooibos tea bags in all the composting systems (C, AC, and VC) is shown in Figure 7. The green tea mass loss averaged 24% in C, and 15% and 20% in AC and VC, respectively. The rooibos tea mass loss averaged 12% in C and 9% each in AC and VC, respectively. This faster decomposition was expected in green tea because it is a leaf material and consists of a more easily degradable material compared with rooibos tea, which is made from wooden shrubs [68,69].

At the beginning of the TBI results ($t4$, $t11$, and $t32$), the decomposition was faster in the C compost compared with the VC and AC. The reason was that microbes in VC and AC have a diverse range of materials to decompose compared with C. But, as the TBI analysis reached $t60$, the decomposition in VC was approximately the same as C, and the fitting curve indicates a further increase in it beyond $t60$. Thus earthworms in the VC chamber fastened the decomposition process, resulting in higher metabolism rates compared with AC [70]. This is also beneficial in decreasing the pile volume [9]. The other reason behind the VC decomposition curve looking similar in shape to the C, in the long run, is that when the earthworms left the compost pile after reaching a plateau after a certain point, means that their rate of activity will slow down or stabilize [71] and their impact on the composting process decreased [72]. The efficiency of organic residue decomposition during VC is directly affected by the biomass and population structure of earthworms [73]. In the course of VC, earthworms ingest organic material, grind it with gizzard, and pass it through the gut [74]. This results in an increase in the active sites for microbial activity in the substrate. As the microbial activity increases, a portion of the carbon content is lost in the form of $CO_2$, and, subsequently, the mineral content in the substrate increases [75]. The mineralization of nitrogen occurs, resulting in increased concentrations of nitrate and ammonium. Also, C:N ratios between 20 and 30 are considered suitable for earthworm growth [76]. The results also show that rooibos tea ingredients are hard for worms to digest because the percentage loss is approximately the same. The burrowing of the earthworms in VC due to high temperature and excessive ammonia content with time caused similarity in the results between the VC and C systems.

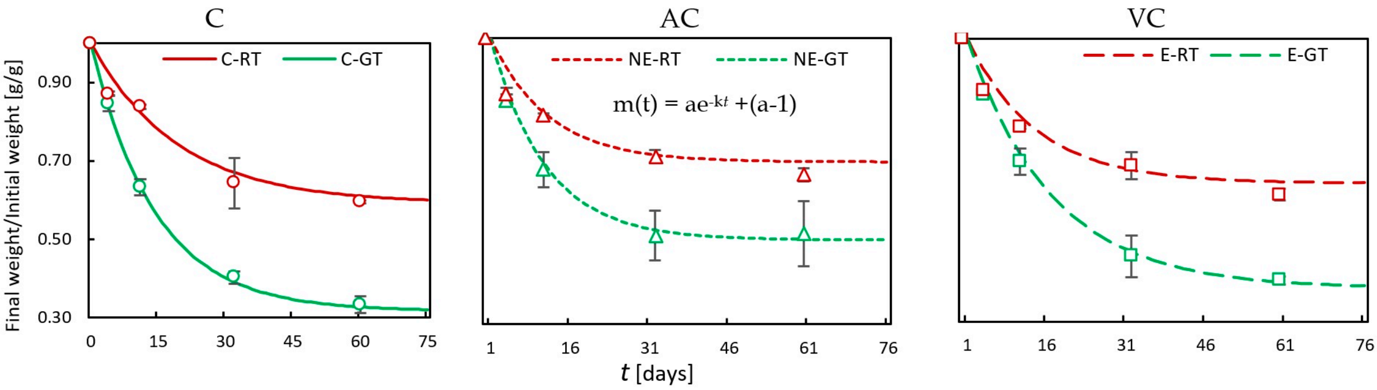


**Figure 7.** The relative mass of tea bags as measured in laboratory incubations for rooibos tea (RT) and green tea (GT) bags in C, AC, and VC systems. The green line is for GT and the red is for RT. The shapes with error bars are experimental data collected on those specific days and the curve is extrapolated up to 75 days. The equation $m(t) = ae^{-kt} + (a-1)$ is used for all the curves, here 't' is the incubation time, 'a' is the labile fraction, and 'k' is the decomposition fraction (with 95% confidence bounds). Values are the mean of three replicates and the $\pm$ error bars indicate standard error means (SEM).

The constant decomposition rate pattern in all the composting systems is shown in Figure 8. The mean decomposition for the measured incubation times ($t$) in C and VC was around 0.05 $\pm$ 0.01 and, in AC, it was 0.07 $\pm$ 0.01. In C and AC, 50% and 40% decreases were noted in '$k$' from t4 to t11, respectively but no decrease was noticed in VC from $t$4 to $t$11. This lack of change is related to the early settlement period of the vermicompost as vermicompost takes time (up to 2 months) to attain a marked transformation of organic matter to vermicast (work cast and worm tea) [77]. Also, the value of 'k' in VC then decreased steadily by 17% from t11 to $t$32 to $t$60. This decrease is related to the high-temperature rise inside the VC chamber because the fatal temperature range for earthworms is between 35 and 48 °C [78]. Therefore, the VC had already reached this temperature range before the tea bags were planted, i.e., the earthworm had left or burrowed by then.

The high stabilization factor in AC indicates that the organic matter of teabags decomposed slowly [68], as microbial breakdown did not easily happen compared with VC. The findings of this study were compared with other studies undertaken in different locations and climates, as shown in Figure 9. This revealed how realistic these findings are. It was noticed that the C and VC stabilization was in the domain of mangroves and mixed forest conditions. Also, the effect of manure makes the compost more stable and suitable to be used as fertile soil. It was noticed that decomposition was high for the composting systems compared with the other studies. The rich microbial diversity increased the decomposition in manure composting systems that were more efficient in increasing the metabolism and promoting soil ecosystem functioning [79]. The stabilization of the C compost and VC were approximately like mangrove forests and mixed forests, respectively. This is because mangrove forests have a high moisture content like the C compost and the mixed forest is rich in soil microbial diversity.

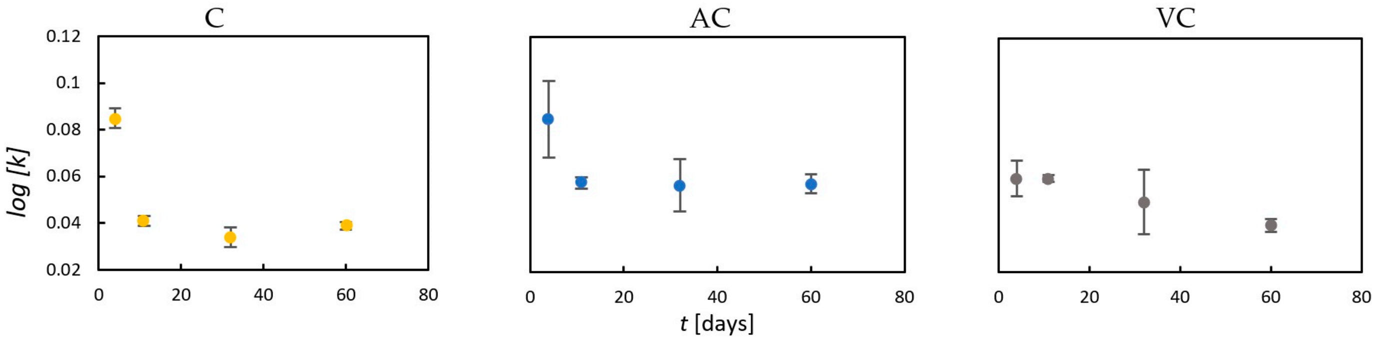


**Figure 8.** Comparing the constant decomposition rate (k) values for C, AC, and VC composting systems. The *k* was calculated using Equation (A3) shown in Appendix B. The dot points (Yellow, Blue, and Grey) are the mean of three replicates at *t*, and error bars indicate ± SEM.

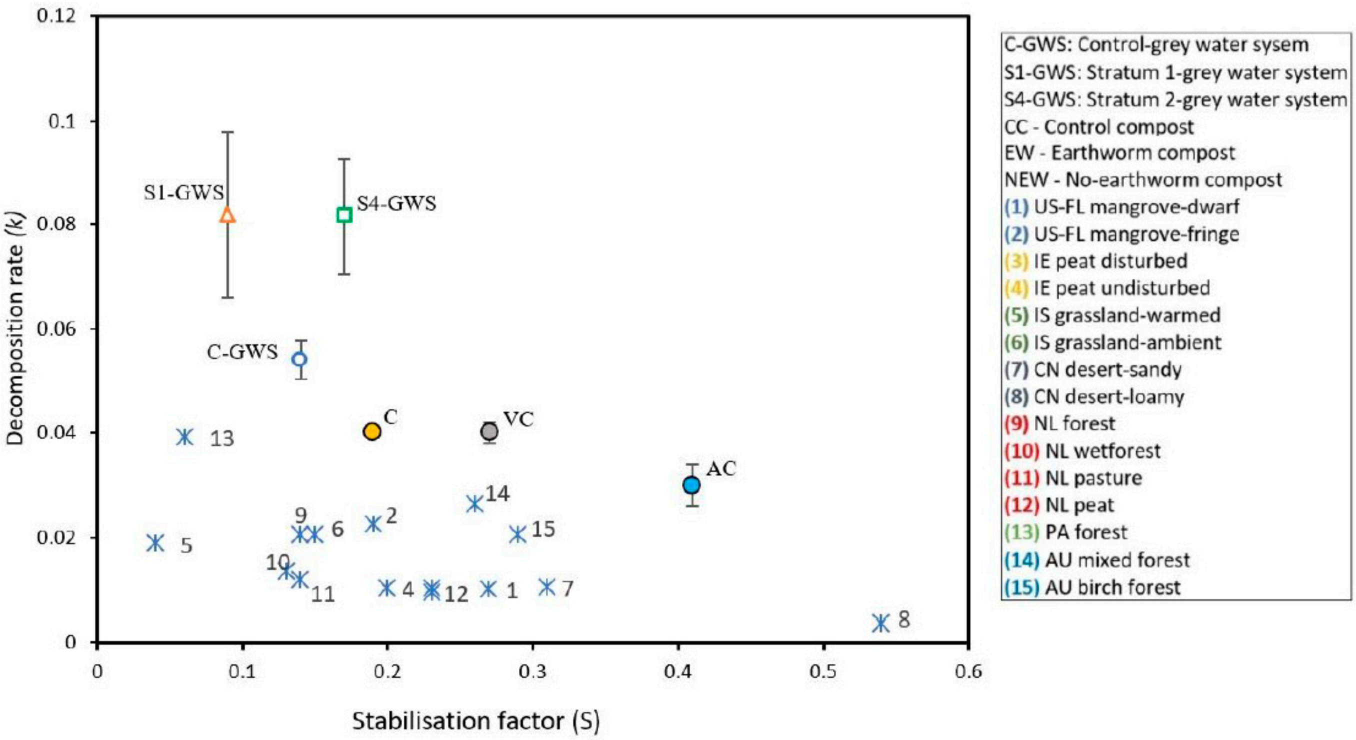


**Figure 9.** The Decomposition (*k*) and Stabilisation factor (*S*) for experimental data of the compost chambers C, VC, and AC were compared with different case studies from different countries found in the literature. The data points of strata of the greywater-treated system (S1-GWS, C-GWS, S4-GWS) of a wetland staircase research study are also shown [47]. The first step S1 had the maximum greywater concentration and the last step S4 the minimum, compared with the control stratum C, which was fed with tap water only. The incubation time (*t*) was extrapolated to 66 days for all the strata (C, AC, and VC composting systems). Blue asterisks (*) are references to tea bag index (TBI) parameters from different environments, as shown by Keuskamp et al. [68], where the numbers of labels indicate country and ecosystem (United States–Florida = US-FL; China = CN; Panama = PA, the Netherlands = NL; Austria = AU; Ireland = IE; and Iceland = IS). The *t* value for all the other environments varied between 66 and 90 days.

It was also noticed that composts are more stabilized and lower in decomposition compared with the wetland biofiltration systems, shown in Figure 9. There are different factors behind the faster decomposition process in wetland systems compared with compost soil; the wetland had 9–12% of phylum (Acidobacteria, Actinobacteria, Verrucomicrobia, Gemmatimonadetes, etc.) which were less than 2% present in soil manure composts of

AC and VC. Therefore, the rich microbial diversity increased the decomposition in the wetland system and was more efficient in increasing the metabolism and promoting soil ecosystem functioning [79]. Also, moist environments with good aeration can have the fastest decomposition [80], as in wetland plants the soil was moist. Lastly, the presence of certain compounds in the greywater soap may have stimulated the growth of bacteria that were able to decompose organic matter more rapidly, leading to a higher soil decomposition rate in greywater-absorbed soil compared with compost soil.

### *6.4. DNA Sequencing*

The DNA sequencing results were found from a single soil sample taken from BTS, C, AC, and VC, and its α-diversity was examined. A T-test/ANOVA statistical method was used, and the taxonomic level was Feature-level. The Chao 1 diversity index as a measure of species diversity took into account the abundance of individual species in the tested samples [81]. The main difference in specie diversities was the manure because the 59 days' time by BTS in control compost did not increase the species shown in Figure 10a, whereas the manure increased this percentage by approximately 57% in both VC and AC as the estimated richness of species within the two composting systems was similar. This also means the diversity was not affected by earthworms or the addition of manure in VC, and AC was the only reason for the increase in specie richness reaching up to 123 on the index shown in Figure 10a.

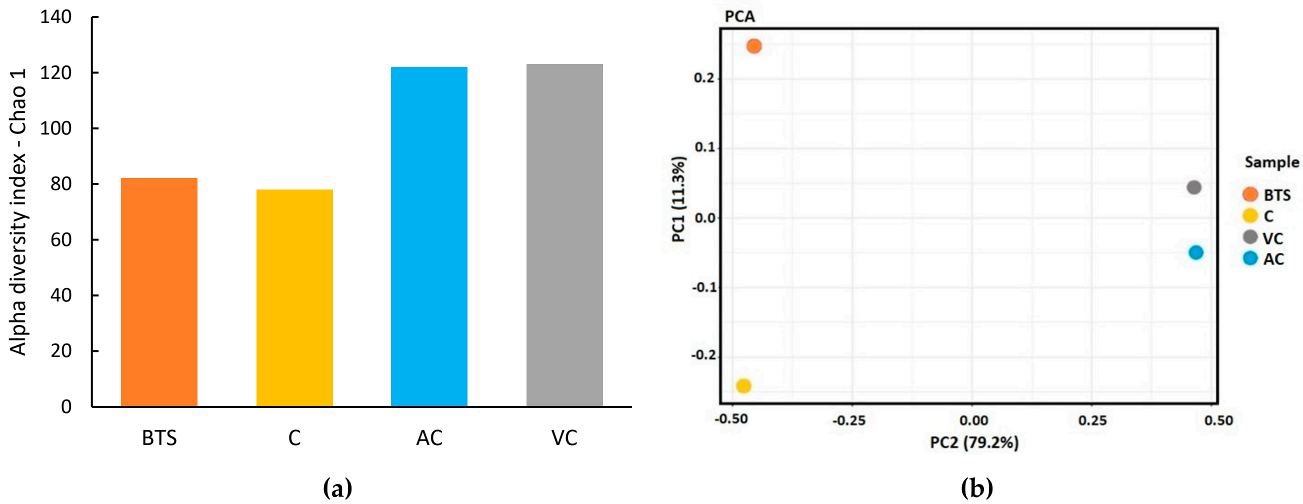


**Figure 10.** (**a**) Comparing the α-diversity of the before treatment soil (BTS), C, AC, and VC systems using Chao 1 diversity index. (**b**) Comparing the β-diversity using Principal Coordinate Analysis (PCA) shows the variance axis of BTS and composting systems (C, AC, and VC). PC1 reveals the most variation, while PC2 reveals the second most variation. Differences among data points along the PC1 axis are larger than the similar-looking distances along the PC2 axis. Positive loadings indicate a variable and a principal component are positively correlated: an increase in one results in an increase in the other. Negative loadings indicate a negative correlation. In both (**a**,**b**) plots, the AC and VC showed similarities, which reflects the effect of the manure.

Beta diversity differences between all the samples were examined, measuring the diversity between the samples. The principal coordinates analysis (PCA) technique was used to visualize this. PCA is a simple way of showing complex data as it reduces the number of variables while maintaining important information from the data [82]. In Figure 10b, the two main components of the data are represented as Axis 1 (79.2%) and Axis 2 (11.3%) on a 2D graph. The graphs show that BTS and C form a cluster on PC1, whereas VC and AC are also in a cluster form but on PC2. This indicates a clear effect of the manure, while the impact of earthworms was negligible because VC and AC are very close to each other and far from BTS and C.

The actual abundance of different types of phyla in BTS, C, AC, and VC is shown in Figure 11. A total of 105,866 Operational Taxonomic Units [OTU] were retrieved. AC and VC had 87% (43 and 44%) of the total OTUs. The total number of identified phyla was 11. In the taxonomical phylum analysis of VC and AC, the most dominant phyla were Bacteroidetes and Proteobacteria, showing the clear effect of manure. The percentage of Bacteroidetes in the AC and VC was 62% each. Proteobacteria was the second most abundant phylum and was found to be 30% and 32% in AC and VC, respectively. The presence of these phyla also indirectly influences the nitrogen cycle and nitrification processes in compost [83], as they support the growth and activity of nitrifying bacteria in the composting systems in the form of urease-producing bacteria. This bacteria can be found within these phyla, e.g., some species of Proteobacteria, such as Klebsiella and Pseudomonas, are known to produce urease (the enzyme that converts the urea into ammonia) [84]. Similarly, certain species within the phylum Firmicutes, like Bacillus, are also capable of urea hydrolysis [85]. The high nitrification due to ammonia is then fixed back into the soil by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) [86]. Both AOB and NOB are commonly found within the classes of Beta and Gamma proteobacteria, while the latter is typically found within the class Nitrospira [87], which is also within the phylum Proteobacteria.

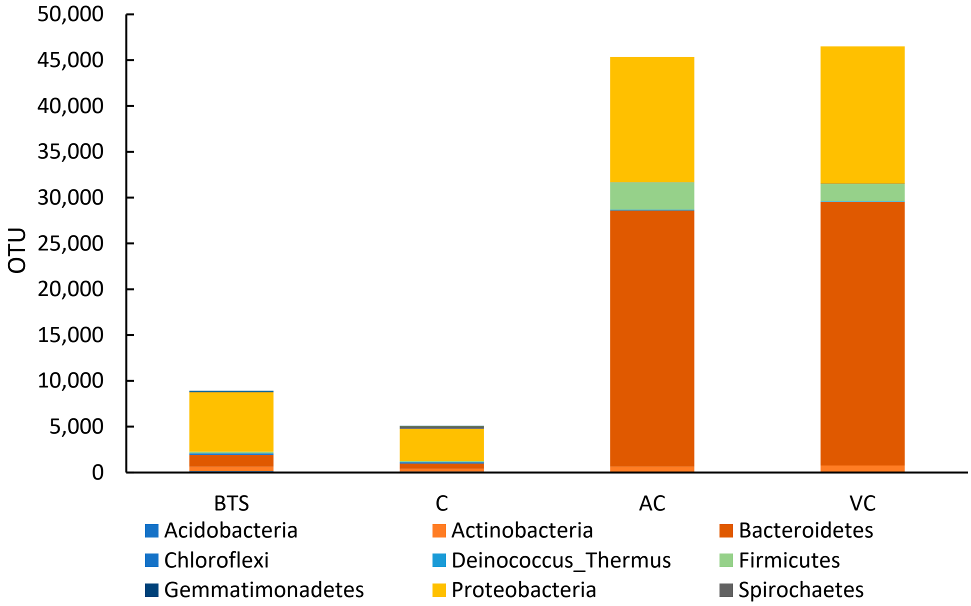


**Figure 11.** Vertical bar plot displaying Operational Taxonomic Units (OTUs) on the *y*-axis and the phylum-level taxonomy distribution of BTS, C, AC, and VC samples on the *x*-axis. The sampling time difference is 59 days between BTS and the composting systems (C, AC, and VC). The manure addition to AC and VC resulted in high numbers.

Overall, the presence of Bacteroidetes, Proteobacteria, and Firmicutes found in samples is generally considered beneficial for plant growth in a garden mix. However, to detect specific pathogens related to the nitrification and denitrification of bacteria, e.g., fecal coliforms, Salmonella, Campylobacter, Salmonella, etc., additional targeted techniques are required that can identify and quantify each pathogen, e.g., culturing methods, immunological assays, and molecular methods [88]. These methods have distinct approaches with different principles and techniques. Also, to a certain degree, phylogenetic tree analysis can be used [89] to find the ratio between harmful and useful pathogens. This sequencing data can be used for the phylogenetic tree analysis.

A simplified structure of the phylogenetic tree up to the class level is shown in Figure 12. The tree was the same in all samples and only the percentages shown in Figure 11 varied among them. The three were constructed using the national center for biotechnology information (NCBI) database [90]. Figure 12 shows the categories of different levels of classification being represented hierarchically, starting with the kingdom "Bacteria". From the kingdom Bacteria, three major phyla, Bacteroidetes, Proteobacteria, and Firmicutes, are shown. Each phylum then branches out into more specific groups in the form of class and order. Proteobacteria and Firmicutes have a diverse range of classes, but Bacteroidetes have only one order and no class. All the remaining phyla accounted for only 1–2% of the total taxonomical percentage. Due to their relatively minor representation, these phyla were considered negligible for the scope of this study and were not shown.

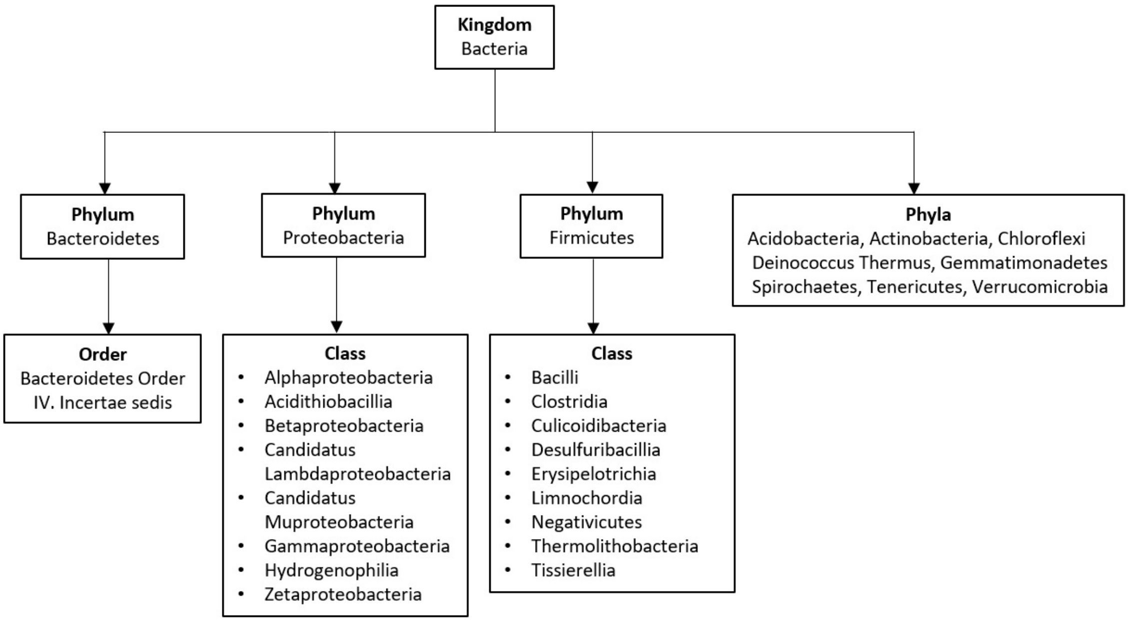


**Figure 12.** Phylogenetic tree showing taxonomic relationships of the major Bacterial phyla: Bacteroidetes, Proteobacteria, and Firmicutes. All the phyla shown belong to the Bacteria kingdom. Each phylum branches out into respective order and class. The combined Phyla frame accounts for 1–2% of the total taxonomical percentage and is not shown as it is a minor representation.

The compost bin filtering system comprised of different layers was producing filtered liquid fertilized in the end. This fertilizer had a positive impact on the growth of the plants around the composting chambers. The effect of liquid fertilizer on the Carex Feather fall plant was more prominent compared with Carex Gold, as the latter showed very slow progress during the experiment. The maximum plant growth was seen in Arum lily as its leaf numbers increased from one to four. It was evident that the liquid fertilizer can be safely used for ornamental plant irrigation providing a biophilic environment.

## 7. Discussion

In this paper, we investigated several possibilities to design user-friendly and sustainable toilets by (1) reviewing several surveys on different continents to identify the perception of the societies to the different toilet systems, and (2) building an experimental protocol to investigate the performance of two different composting systems: aerobic composting (AC) and vermicomposting (VC).

The surveys' research aimed to gauge the social acceptance and financial viability of composting toilets. The recommended design revolves around a VC toilet, capable, in theory, of continuously processing waste into valuable compost while conserving water through a micro-flush interface and operating without manual handling. The results of the conducted surveys across different continents regarding the acceptance of composting toilets or urine-diverting toilets in homes or outside their homes revealed that most of the users are willing to adopt these toilet technologies. Based on the targeted questions related to willingness, this percentage was quite high in Europe and it was relatively low in Australia. In the United States, the percentages were high when the users were given a choice of free installations, if not then the percentages dipped considerably. In the Philippines, the order of priorities was water saving, economic costs, and the recovery of fertilizers.

An experimental investigation into the performance of AC and VC chambers was conducted and compared with the performance of a control C chamber. The results summarized in Table 1 revealed that composting significantly increased the temperature of the chambers, and due to earthworms' metabolism in VC during the mesophilic stage, the mean compost temperature was 2 °C higher compared with AC compost. Also, in VC, the temperature exceeded the ideal range for the worms, and it created unfavorable conditions for the earthworms. As shown in Table 1, the physicochemical properties of the soil were approximately the same for both VC and AC. It held a remarkably large amount of ammonia in the compost bin due to the activity of both microbes and earthworms, creating an inhospitable condition for the earthworms.

**Table 1.** Aerobic Composting versus Vermicomposting Analysis.

| Metrics | Aerobic Composting | | Vermi Composting | |
|---|---|---|---|---|
| | **(Ideal) Range** | **Experimental** | **(Ideal) Range** | **Experimental** |
| Temperature | 49–71 °C | 14–36 °C (21 °C) | 13–25 °C | 14–41 °C (21 °C) |
| Electrical conductivity | 1000–5000 µS/cm | 5600 µS/cm | 1000–3000 µS/cm | 6700 µS/cm |
| Moisture content | 40–60% | 50% | 60–80% | 52% |
| Carbon–Nitrogen ratio | 25:1–30:1 | 25:1 | 20:1–30:1 | 25:1 |
| pH | 6–8 | 7.9 | 5–8 | 7.9 |
| $NH_3$ | <500 mg/kg | 1523 mg/kg | <500 mg/kg | 1817 mg/kg |

The TBI studies revealed that VC compost decomposition and stabilization were closer to the C compost in the longer run. In the beginning, the decomposition in the control chamber was faster but, with time, as the earthworms settled and slowly burrowed in the compost bin, the decomposition became the same as the C. The AC achieved maximum stabilization by day 60. The microbial activity was different than in wetland systems. The composting chamber showed a lower decomposition rate than in wetlands. A clear distinction in the stabilization index was observed from the TBI analysis; the level stabilization was ranked from smallest to largest in wetlands, control, vermicompost, and aerobic compost.

The results clearly showed that the diversities of species ($\alpha$ and $\beta$) were influenced by the manure usage because the period of 59 days without manure in the compost bin did not have a significant impact on the diversities, as the BTS and C samples had the same findings. In VC and AC, the most prevalent microbes were Bacteroidetes and Proteobacteria. This highlights the importance of manure in shaping the microbes' percentages in compost, which are interconnected with the potential effect of the nitrogen cycle. The data obtained from this current research will serve as a solid foundation for future investigations.

The use of earthworms in the vermicomposting process to reduce manual handling possess several challenges. This is because the composting activity produces high temperatures and a high level of ammonia content that creates an unfavorable environment for the earthworms. There are several alternatives to alleviate these issues, such as using larger compost chambers with high levels of ventilation to allow sufficient temperature zones for thermophilic and mesophilic bacteria and earthworms to thrive. The use of geotextiles to separate the different layers in the VC creates difficulties for the worms to migrate to the different levels of the compost. To overcome this issue, it is recommendable to design special *worm elevators*, which are vertical channels connecting the substrates. These elevators would allow the worms to surface and burrow according to the temperature and ammonia levels of the compost. Last but not least, it is imperative to design systems to separate urine and stool without user intervention. Both byproducts should be treated by two separate composting chambers: *VC* treating the stools and *urea-filters* treating the urine.

Two candidates for future toilet systems are shown in Figure 13. The urine-diverted flush toilets should capitalize on the recent advancement in Swedish technologies, such as the urine-separated flush toilet interface [36], the aquatron separator of stool and water [91], and the sealing membrane and blue traps for waterless urinals [92]. The blue traps have a density lower than urine so they can effectively seal its unpleasant odors. The urine passes through the sealing membrane, which then closes when the urine flow stops and effectively seals the urine outlet, preventing smell, as shown in Figure 13a. Stools have low levels of nutrients and high contents of bacteria that may be more favorable for the earthworm activity in the VC. The aquatron separates stools from water using centrifugal forces. The stools fall into a compost pile and the water can be directed either to the sewage or to a wetland system, see Figure 13a. On the other hand, the urine contains urea as a valuable fertilizer and a low level of bacteria making it almost sterile, so it should be treated separately by a urea-filter. When distilled by passive strategies such as solar radiation, urea can be stabilized and straightforwardly converted into fertilizer.

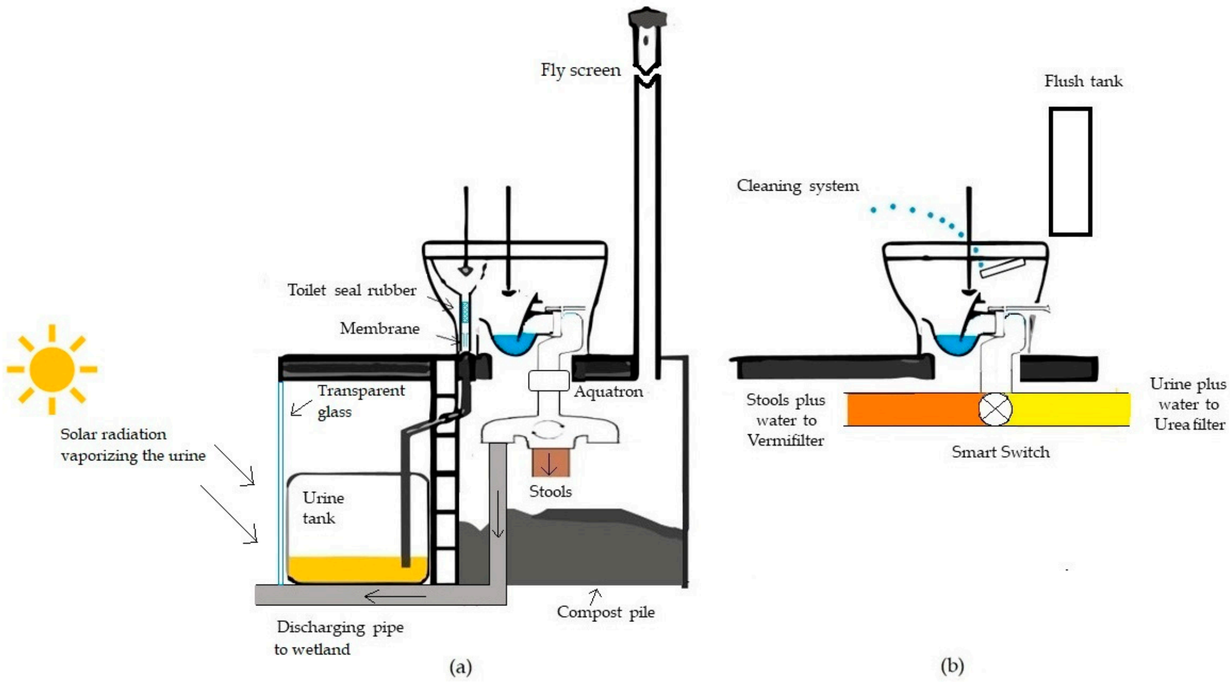


**Figure 13.** (**a**) Swedish toilet interface showing separate urine collection. The water in the urine evaporates and leaves the phosphorous, nitrogen, and potassium content. The aquatron operates to direct the stool to the compost pile and the greywater is discharged through a pipe toward the sewer or wetland filtering system. (**b**) The Japanese toilet concept design and the future version of the vermi/urea filters. These should operate from a Smart Switch separating the urine from the stools and it directs the urine and stools to the ureafilter and the vermifilter.

A second candidate for toilet systems is shown in Figure 13b. This is a design inspired by the Japanese systems that embrace the technology of Smart Toilets and focus on the comfort and positive experience of the user [34]. The advanced and commercially available TOTO micro-flush toilets can be connected to a VC and urea-filter by a Smart Switch, shown in Figure 13b. Based on the optical analysis, the Smart Switch should detect whether the flushed material contains stool and consequently divert the flush either to the VC or the urea-filter. Different from the Swedish system, the urine in this system will be mixed with water so that it is more reasonable to directly apply this mixture to a bed of nitrogen-hungry plants for recovering nutrients in the food chain.

This study does not provide a final answer on the suitability of a sustainable and user-friendly toilet system. However, it does provide future directions on the design of the sustainable toilet, aiming to minimize human intervention and create a biophilic environment around the urban environment. In this environment, valuable nutrients can be safely returned to the ecosystem through efficient biological filtration. These insights pave the way for potential advancements in sustainable toilet technology.

## 8. Conclusions

This paper examined surveys conducted across different continents found in the literature. The surveys aimed to gauge the acceptance of composting toilets or urine-diverting toilets. It was found that most users had positive acceptance and willingness to adopt the mentioned toilets but the percentage varied, e.g., in the US, two locations had percentages of 63% and 46% but in Australia, it was 30%. The composting chamber was then designed and the experimental methodology and investigations shed light on the composting processes, demonstrating a difference of 6 °C between VC and AC chambers during the mesophilic stage. The physicochemical properties of both AC and VC samples exhibited similarities. However, both were noticeably distinct from the properties observed in C compost due to the addition of manure.

The other noteworthy findings indicate that the VC decomposition rate closely resembled the C compost in the longer run as, by day 60 for both VC and C, the decomposition rate was approximately 0.04. This underscores the potential effectiveness of vermicomposting for organic waste management. Also, the DNA sequencing results revealed the same type of phylogenetic taxonomy for both AC and VC with the domination of Bacteroidetes (62%) and Proteobacteria (32%). The presence of these phyla influences the nitrogen cycle and nitrification processes in compost.

Based on these valuable insights, this study suggests two promising candidates for future toilet systems. One approach could leverage recent Swedish technological advancements in urine-diverted flush toilets, while another design inspired by Japanese Smart Toilet technology holds promise. These innovative designs pave the way for sustainable toilets, where essential nutrients can be safely returned to the ecosystem through efficient biological filtration.

**Author Contributions:** The authors' contributions are as follows: conceptualization, F.A.-M. and J.N.; methodology, G.Q., J.N. and F.A.-M.; validation, G.Q., V.P. and F.A.-M.; formal analysis, G.Q.; investigation, G.Q.; data curation, G.Q.; writing—reviewing and editing, G.Q. and F.A.-M.; visualization, G.Q. and F.A.-M., supervision, F.A.-M. and A.B.; project administration, G.Q., J.N. and F.A.-M. All authors have read and agreed to the published version of the manuscript.

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## Appendix A. Physiochemical Test Methods Used to Find the Compost Properties

| | |
|---|---|
| pH | Measured using pH meter and electrode in accordance with American Public Health Association (APHA) latest edition, 4500-H+. |
| Electrical conductivity (EC) | Measured using a conductivity cell at 25 °C in accordance with APHA latest edition 2510 and Rayment and Lyons. |
| Moisture content (MC) | Determined by heating at 105 ± 5 °C for a minimum of 12 h. |
| Total organic carbon (TOC) | A titrimetric method that measures the oxidizable organic content of soils. |
| Total Nitrogen (TN) | Calculated as the sum of TKN (Total Kjeldahl Nitrogen) and oxidized nitrogen. Alternatively analyzed using combustion and chemiluminescence. |
| Ammonia ($NH_3$) | Ammonia—determined colourimetrically, based on APHA latest edition 4500-$NH_3$ F. Waters samples are filtered on receipt prior to analysis. Soils are analyzed following a potassium chloride (KCl) extraction |

## Appendix B. Tea Bag Index (TBI) Method

The TBI method assumes that any litter incorporated into the soil consists of a labile (decomposable) and a recalcitrant (stable) fraction. Let $M_0$ be the initial mass of the litter and $M_t$ its mass at time t to define the mass fraction as $m(t) = M_0/M_t$. The decomposition is assumed to obey an exponential law with two reaction rates [93]:

$$m = a\, e^{-kt} + (1-a)e^{-k't} \tag{A1}$$

where $a$ is the labile fraction, $k$ is the decomposition rate of the labile fraction, $(1 - \mathrm{a})$ is the recalcitrant fraction, and $k'$ is the decomposition rate of the recalcitrant fraction. The reaction rate of the recalcitrant fraction $k'$ is considered to be small in comparison with the labile fraction k, so that for small times $(k't << 1)$ Equation (A1) can be reduced to

$$m(t) = ae^{-kt} + (1-a) \tag{A2}$$

The TBI method used two different litters: the green tea, a labile litter, and the rooibos, a more recalcitrant litter. They show contrasting decomposition rates. We use subindexes "g" and "r" to encode the parameters of the green tea and the rooibos tea.

The parameter of the exponential model of Equation (A2) was obtained using non-linear regression. The range of values for variables 'a' and 'k' of Equation (A2) was generated. Based on that range, the best possible fit was plotted. The generated curve touched most of the experimentally plotted points and inferred values of a function where no experimental data were available.

If k is assumed constant, it can be obtained by isolating it from Equation (A2):

$$k = \frac{1}{t}\ \ln\left[\frac{a}{m(t)-(1-a)}\right] \tag{A3}$$

During this decomposition, some parts of the labile compounds stabilize and become recalcitrant tea [94]. Environmental factors play an important role in this stabilization [95] resulting in a deviation of the actual decomposed fraction (i.e., limit value) '$a$' from the hydrolysable (i.e., chemically labile) fraction H. This aberration can be interpreted as the suppressing effect of the environmental conditions on the decomposition of the labile fraction and will be referred to as stabilisation factor S:

The stabilization factor ($S$) was calculated as follows [68]:

$$S = 1 - \frac{a_g}{H_g} \tag{A4}$$

where $H_g$ is the hydrolysable fraction of the green tea equal to 0.842. This constant value of $H_g$ for green tea was quantified using the method proposed by Van Soest [96], in which the use of two detergents divides the plant cells into less digestible cell walls and mostly digestible cell contents (contains starch and sugars).

The decomposable fraction of rooibos tea was $a_r$ predicted as follows:

$$a_r = H_r\ (1 - S) \tag{A5}$$

where $H_r$ hydrolysable fraction is constant of rooibos tea.

## Appendix C. DNA Extraction Technique Used in the Metagen Lab Queensland

The 16S rRNA gene sequencing technique used for DNA extraction in this soil study was used for the identification, classification, and quantitation of microbes [97]. The DNA of each stratum was extracted from 10 g subsamples of soil using a modification of the modular universal DNA extraction protocol [98]. Briefly, this involved 10 g soil samples being mixed with sterile garnet sand and lysis buffer before being processed in a SPEX 2010 Geno Grinder homogenizer (SPEX SamplePrep, Metuchen, NJ, USA) at 1700 strokes per minute for 10 min. After centrifugation to remove soil particles, 9 mL of the supernatant was treated with a flocculant solution designed to remove humic acid contaminants. Samples were again centrifuged, and DNA was recovered from 10 mL of the supernatant using SPRI beads [99]. The purified DNA was then eluted in 200 μL of Tris-HCl pH 8.0 and was assessed for yield and quality using the Quantifluor dsDNA system (Promega, MI, Madison, WI, USA) and agarose gel electrophoresis.

The metabarcoding of eukaryotic and bacterial/archaeal communities was conducted using the primer sets NF1/18S2rB [100] and Pro341F/Pro805R [101], respectively. A two-step PCR protocol was used to generate dual-indexed amplicons adapted from the Illumina protocol for 16S Metagenomic Sequencing Library Preparation. The naïve Bayesian Classifier was used to assign taxonomy to the genus level for the 16S amplicon with version 128 of the Silva reference database [102].

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