Dynamics of Physicochemical Variables and Cultivable Bacteria in Vermicompost During Steady Food Waste Addition and Upon Feed Interruption

Louise Hénault-Ethier, Terrence H. Bell, Vincent J. J. Martin, and Yves Gélinas

Department of Biology, Concordia University, Montreal, QC, Canada; GEOTOP Research Center, Montreal, QC, Canada; Centre de Recherche sur la Biodiversité, Institut de Recherche en Biologie Végétale, Université de Montréal, Montréal, QC, Canada; Chemistry and Biochemistry Department, Concordia University, Montreal, QC, Canada

Abstract
Vermicomposting is a bio-oxidation process mediated by epigeic earthworms, which act synergistically with microbes to stabilize organic matter. Vermicompost bins are popular in homes and classrooms, but we know little about how physicochemical and bacteriological characteristics of vermicompost evolve with regular feed addition or upon feed interruption. Temperature, moisture, pH, organic carbon, total nitrogen, and labile and total carbohydrates were measured in twenty-four small-scale bins amended with fruit, vegetable, and coffee wastes for 266 days. Monitoring was maintained for 240 days following the last waste input. A canonical correspondence analysis with forward selection of explanatory parameters revealed that pH ($p < 0.0100$), organic C ($p < 0.0140$), and labile carbohydrates ($p < 0.0110$) significantly affected the composition of cultured bacteria through time. Through culture on different media for isolation and amplification (polymerase chain reaction) and sequencing of the 16S rRNA gene, several Bacillaceae, Enterobacteriaceae, and Pseudomonadaceae were observed in both active and maturing vermicompost, but Actinobacteria, Xanthomonadaceae, and Aeromonadaceae were most abundant after feeding interruption. Three phases of vermicomposting were observed in this semi-batch experiment: initiation, active degradation, and, following final feed addition, a phase akin to maturation. Our culture-based approach yields a subset of all microbes present in vermicompost, but provides further knowledge on which media are most useful for isolating vermicompost bacteria. This data will be helpful for low budget tracking (e.g., domestic or university operations), and cultivated vermicompost bacteria can also be characterized for their ability to efficiently metabolize organic waste.

Introduction
Composting is the bio-oxidation of organic matter catalyzed by microorganisms, which yields stabilized humified compounds and inorganic nutrients in a form that is readily available for plant uptake (Mustin 1987; Ndegwa and Thompson 2001). It is increasingly used to help manage global issues, such as food production, soil health, sustainable waste management, and mitigation of greenhouse gases (Lal et al. 2007; Bogner et al. 2008). In vermicomposting (VC), earthworms modify microbial activity and enhance decomposition by physically breaking down organic matter, which increases the surface area that is exposed to microorganisms (Domínguez 2004). Worm burrowing continuously mixes and aerates the substrate, and the release of antibacterial coelomic fluids can directly influence microbial community structure (Sinha et al. 2002, 2010). Relative to traditional composting, worms in VC can increase bacterial N$_2$ fixation (Ozawa, Risal, and Yanagimoto 2005; Tereshchenko and Napleková 2002); improve physicochemistry to further stimulate plant growth (Arancon et al. 2005), and may promote a more diverse microbial community that can suppress plant pathogens (Mustin 1987; Ndegwa and Thompson 2001). Vermicomposting output tends to be higher in nutrient content, humidity, and microbial density and activity than traditional composting with similar input material (Tognetti et al. 2005; Barrena et al. 2014).
Fluctuations in physicochemical parameters during vermicomposting reveal advancement of biodegradation. Rising temperatures indicate augmenting microbial metabolic activity (Mustin 1987). Acidification coincides with CO₂ and organic acid release, pH increase indicates ammonification and protein degradation, and the return to a near neutral pH is owed to the buffering capacity of humified materials (Peigné and Girardin 2004; Mustin 1987). The C/N ratio will decrease and stabilize as more C is lost through aerobic respiration than N is lost through denitrification (Mustin 1987). Decomposition decreases lignin, cellulose, and hemicellulose contents (Abouelwafa et al. 2008; Jouraiphy et al. 2005), while water-soluble carbohydrates may be rapidly produced and recycled by the resident microbiota for energetic purposes (Sánchez-Monedero et al. 1999).

The composting process is highly variable and is influenced by source materials, scale, and physicochemical conditions, leading to poor reproducibility in microbial community analyses (Ishii and Takii 2003). The microbial communities produced by traditional composting have been studied extensively (Ryckeboer et al. 2003), but those from VC have not been, even though they are known to diverge from traditional composting communities following the maturation stage (Fracchia et al. 2006; Gopal et al. 2009). Microbial communities also play a role in the suppression of human pathogens from source materials, but not only because of a thermophilic phase, on which the legislation focuses (Eastman et al. 2001), but also to pre-emption of resources (Eastman et al. 2001) and direct antagonism from antimicrobials and cell-wall degrading enzymes (El-Tarabily and Sivasithamparam 2006; Ingham 1998). Earthworms can also play a direct role in pathogen suppression by releasing antibacterial factors along with digestive enzymes, or through their coelomic fluids (Lassegues, Roch, and Valemois 1989; Li, Wang, and Sun 2011; Pedersen and Hendriksen 1993). Earthworm effects on microfloral and faunal communities have long been a topic of interest for researchers (Brown 1995). The worm gut may support more microbes than the surrounding soil, and worm gut bacteria may be more favorable to anaerobic bacteria (Karsten and Drake 1995). Furthermore, worms have their own autochtonous gut microbiota, and feed interruption may change in situ worm gut bacterial communities (Vinceslas-Akpa and Loquet 1995). Also, microbial inoculation of vermicompost may lead to differences in the rate of humification and enzymatic activity (Pramanik, Ghosh, and Banik 2009), and upon passage into their gut lumen, worms may activate bacteria, which are present in soil, such as denitrifying bacteria or nitrate dissimilating bacteria that emit N₂O (Ihssen et al. 2003).

To date, most composting research is not representative of typical domestic operations. For instance, the C/N ratio used to assess compost maturity may be higher in domestic systems, while respirometric measurements tend to be lower than those obtained from industrial composts (Barrena et al. 2014). In domestic settings, organic waste is added sporadically in drained and passively aerated plastic boxes, and balancing feed characteristics (such as C/N ratio) is not typically prioritized. Most research has focused on more easily studied batch operations or industrial-like continuous operations. Of ninety-one published papers on vermicomposting using Eisenia fetida since 2005, only two used typical domestic/institutional vermicomposting feed and feeding frequency and focused on the importance of initial litter composition (Kristiana et al. 2005) or on capacity, gaseous emissions, and end-product quality (Lléo et al. 2013), rather than on the ongoing physicochemical or microbial changes that were characterized in this study. The physicochemical conditions characteristic of semi-continuous VC systems likely differ from those of batch-operated VC systems fed pre-composted organic waste rather than raw materials (i.e., Neher et al. 2013; Sangwan, Kaushik, and Garg 2008; Suthar 2009), and from flow-through reactors in which fresh feed is layered directly on the litter (Gajalakshmi, Ganesh, and Abbasi 2005) or added sequentially in modules to allow vertical worm migration (Aira, Monroy, and Domínguez 2007).

In the province of Quebec (Canada), 5,776,000 tonnes of waste are eliminated annually, and organic material represents 27% of residential waste and 20% of industrial, commercial, and institutional waste (Recyc-Québec 2014). In 2012, only 25% of residential organic waste and 29% of industrial, commercial, and institutional organic waste were recycled, primarily in centralized composting facilities. For this reason, on-site small-scale composting is integrated into the government’s strategy to divert 60% of all organic waste by the end of 2015 and to ban organic waste landfills by 2020 (MDDEP 2011, 2012b). This study is aligned
with the waste diversion goals of Concordia University (Montreal, Quebec, Canada) for which the campus daycare and cafe waste need to be characterized to correctly design and operate large-scale on-site VC and composting systems with 16 and 100 tonnes of annual capacity, respectively (Hénault-Ethier and Fortin 2011). The aim of this study was to assess natural heterogeneity in the dynamics of physicochemical and biological characteristics in a VC system, as typically operated in a domestic or classroom setting with frequent additions of fruits, vegetables, and coffee wastes, interspersed with interruptions in feed addition. Total mass, worm density, temperature, pH, moisture content, organic carbon (OC), total nitrogen (TN), atomic C/N ratio, total and labile sugar concentrations, and cultivable bacteria were monitored by sampling among twenty-four controlled bins over 506 days. We hypothesized that feeding interruption (during academic breaks where organic waste production is low, or upon final storage of the product before use) would disrupt the equilibria of vermicomposting parameters. To our knowledge, the current study is the longest running semi-continuous VC experiment monitoring physicochemical and bacterial parameters. We use canonical correspondence analysis (CCA) to relate bacterial composition to the physicochemical characteristics of the VC material, which has not previously been done for these systems. The current study was intentionally designed as a long-term monitoring experiment to survey changes in bacterial and physicochemical parameters upon constant feeding and feeding interruption. The exploratory statistical approach used responds to this goal. An improved understanding of domestic VC composting is critical, as it will help to maximize nutrient retention from organic waste, while minimizing atmospheric and aquatic emissions (Peigné and Girardin 2004).

**Materials and Methods**

**Experimental set-up: bins and worms**

Continuous vermicomposting was conducted in twenty-four domestic-type units made of opaque polyethylene bins (40 cm x 60 cm x 35 cm high) fitted with a draining tube and lined with a geotextile pouch containing 7 kg of clean gravel to promote aeration and drainage (figure S1). The lids were pierced with sixteen aeration holes covered by mesh to prevent circulation of flies and earthworm escape. Exactly 2.8 kg of compost made from food and yard waste, wood chips, and bark (Ferti-MixMC, Ferti-Val®) was placed on the geotextile pouch and served as the initial litter. The bins were kept in a controlled room ($T^0 = 25.3 \pm 0.2^\circ C$ and ambient humidity = $40.3 \pm 3.2\%$). Any collected leachate was immediately redistributed on top of the VC, such that no nutrients could be lost through leaching. Five kilograms of *Eisenia fetida* worms were purchased from La Ferme Eugénia (Le Bic, Quebec), and were adapted to their new diet by feeding ad libitum for 2 weeks. Worms were distributed equally into the experimental bins by homogeneous mixing with the litter and balancing the weight. The worms of three bins were counted, cleaned by crawling on moist paper towels, and weighed at the beginning of the experiment (day 0), and only once during the experiment (day 194) to minimize disturbance to the system.

**Composition and physicochemical characteristics of the organic waste**

Organic waste consisting of fruits, vegetables, and used coffee grounds was collected daily at the Loyola Campus of Concordia University (Montréal, Quebec, Canada). The mixed fruits and vegetables were homogenized manually (<1 cm$^3$). Food waste and coffee grounds were added in a 1:1 wet mass ratio, and supplemented with two coffee filter papers as a source of carbon (C). Worms were fed once a week at a rate of 80–300 mg food/g worm/day wet mass (30% of body weight as per Kristiana et al. (2005) with feeding rate increasing over time). Food was buried just below the surface of the VC, as is commonly recommended for domestic vermicomposting practices, to reduce odors and pests in and around the boxes (Adi and Noor 2009). Ten samples of food and coffee inputs (200 g each) were collected for further analysis (pH, % H$_2$O, OC, N).

During the experiment, a total of 822 kg of food waste and 1434 kg of coffee waste were collected on campus. Of these, 18% of the fruits and vegetables and 9% of the coffee waste were added to the VC system used in this experiment. Excess organic waste was diverted to other campus composting facilities (figure S1). Even though large quantities of organic waste were collected each semester, much smaller quantities were available during mid-term and end of semester breaks (figure 1a). Since it was impossible to
Figure 1. A, OW feed to the VC bins per feeding event and average feeding rate over bi-weekly periods. The time in months allows to situate feeding rate variability induced by academic calendar breaks. The experimental time in days best illustrates physicochemical variability during the semi-continuous operations, as environmental conditions (in controlled environmental chambers) is not affected by the actual dates. Feeding was interrupted after day 266 (dashed line). Variations in physicochemical parameters during continuous vermicomposting: B, Vermicompost temperature compared to ambient room temperature; C, moisture content; D, pH; E, concentrations of total and labile carbohydrates along with cellulose (displayed as diamonds, squares, and triangles, respectively). As cellulose is calculated by subtracting labile sugars from total sugars, its error is roughly equal to the sum of their respective standard deviations (it is not illustrated here for clarity); F, concentration of organic carbon; G, concentration of total nitrogen; and H, C/N ratio. Feeding was interrupted after day 266 (dashed line). Physicochemical points are fitted with spline regressions with the 95% confidence interval to better illustrate the different phases of the VC process. Refer to text for details.
store organic waste over these periods, there were huge fluctuations in organic waste feeding rate, which is typical of both domestic and institutional VC operations.

To facilitate comparisons of our results with data in the literature, and in order to understand the composition of organic waste at Concordia University, we characterized the typical composition of the feed used in this work (table 1). Briefly, based on wet weight (dry weight given in parentheses) feed was composed of 45.6% (65.2%) spent coffee grounds, 26.3% (16.3%) fruits, 25.3% (15.5%) vegetables, 2.0% (2.8%) coffee filters, and 0.8% (0.1%) egg shells. Besides coffee, the most abundant food by wet weight was melon (>20%), followed by strawberry, lettuce and cabbage, banana, potato, carrot, celery, and broccoli (5–10% each), then cucumber, cauliflower, sweet potato, pineapple, orange, brown paper, beet, apricot, egg shell, lemon, and onion (1–5% each), and finally grape, avocado, apple, prune, pepper, tomato, pear, leaf, and bean (<1%). The pH and moisture content of our mixed feed additions was relatively constant across samples. C/N ratios of coffee grounds and filter papers were also similar throughout the experiment; however, C/N ratios of the mixed fruits and vegetables were highly variable (nearly 50% variability across the thirty samples analyzed), reflecting the normal variability of domestic fruit and vegetable waste.

**Vermicompost physicochemical characterization**

The physicochemical parameters of the VC were monitored weekly to observe global changes in the VC material over time (as opposed to the short-term effects of each feeding event). Close monitoring of the twenty-four bins was conducted for 100 days to acknowledge variability and ascertain the similarity of trajectories in the initiation phase. Following this, a reduced number of bins were sampled for ongoing monitoring. Bin weight, air temperature, and compost temperature were recorded at four random sites per bin. The difference between room and compost temperature is reported as $\Delta T^\circ$. Moisture content was assessed in situ with a moisture meter (Mantis Tiller®). The VC was sprinkled with distilled water when the average moisture content ($n = 4$) was below 60%. Five grab samples free of earthworms, hatchlings, and cocoons were collected weekly with a spoon from random regions of the bins, and the ~25 g collected were pooled for further analysis (see below). Earthworms, cleaned on moist paper towels and purged of their gut contents, were also analyzed for organic carbon (OC) and N content for mass balance calculations.

After 266 days, feeding was interrupted, which encourages earthworm migration into deeper layers, facilitating VC harvest from the surface (Gajalakshmi and Abbasi 2008). Extended feeding interruption is detrimental to the decomposer community, but here it was done intentionally to understand the physicochemical (and eventually microbial) changes in the system once equilibrium conditions were disrupted (feed interruption) as occurs when VC is stored before use. VC sampling was extended until pH and $\Delta T^\circ$ had stabilized.

Depending on the heterogeneity of the substrates, physicochemical analyses were conducted on different sample sizes of the fruit and vegetable waste ($n = 30$), coffee grounds ($n = 5$), coffee filters ($n = 3$), and initial compost litter ($n = 3$). pH was determined from samples of 10 g for food waste and 3 g for VC (10% wet matter weight/volume homogenized for 1 min in an aqueous suspension of distilled water at pH 7.00, measured with a Symphony probe, VWR®, Mississauga, Ontario, Canada). Moisture content was determined on samples of 100 g for food waste and 10 g for VC (weight loss after freeze-drying, expressed as a percentage on a wet mass basis). The remainder of the sample was frozen at −80°C, freeze-dried (−50°C and 500 mbar, ModulyoD, Termo Savant®, Waltham, MA, USA), and ground manually (mortar and pestle) prior to elemental and carbohydrate analysis. OC and TN contents

| Organic C (wt% ± SD) | Total N (wt% ± SD) | C/N (± SD) | Moisture content (% ± SD) | pH |
|-----------------------|-------------------|------------|---------------------------|----|
| Food                  | 37.9 ± 11.2       | 1.92 ± 0.69| 22.8 ± 10.5               | 85.6 ± 1.6 | 5.13 ± 0.39 |
| Coffee                | 53.7 ± 1.6        | 2.27 ± 0.10| 23.7 ± 0.7               | 66.3 ± 2.2 | 6.10 ± 0.12 |
| Filters               | 42.4 ± 0.1        | 0.10 ± 0.003| 413 ± 13                  | 66.3 ± 2.2 | nd          |
| Average feed          | 51.2 ± 0.8        | 2.20 ± 0.02| 23.2 ± 0.2               | 71.8 ± 1.6 | nd          |
| Initial litter        | 49.9 ± 0.8        | 1.87 ± 0.04| 26.7 ± 0.9               | 75.0 ± 0.0 | nd          |

Note. (nd: not determined)
were measured in carbonate-free samples (12 h exposure to vapor-phase HCl) of 10.0 mg and food waste samples of 4.0 mg with an elemental analyzer (Perkin-Elmer, model 2400, Waltham, MA, USA) calibrated using a range of acetalnilide and β-alanine masses.

Total and labile sugars were analyzed on 100-mg samples (n = 24) following the modified protocols of Lowe (1993). Briefly, 0.1 g of homogenized dried compost was reacted with 10 mL 0.5 M H2SO4 (J.T. Baker) for 2 h at 95°C. The solution was filtered (GF-B, Whatman®) and adjusted to 25 mL. For total sugars, the hydrolysis was preceded by cellulose digestion using 0.4 mL of 12 M H2SO4 at room temperature for 4 h. Sugars were quantified using a 1-mL aliquot of the suspension mixed with 2 mL of a 2 g L⁻¹ solution of anthrone’s reagent (Acros Organics) and incubated at 85°C for 15 min. The solution was then cooled for 30 min, and the absorbance was read at 660 nm (WinCary UV/VIS spectrophotometer, Varian®). The concentration of cellulose + hemicellulose was obtained by subtracting the concentration measured for labile sugars from that obtained for total sugars. Results were transformed into mg sugars g⁻¹ dry VC.

**Bacterial sampling and isolation**

On experimental days 217, 266, 328, and 378, the VC was screened for cultivable bacteria using six different selective and differential growth media (MacConkey Agar, Levine EMB Agar, LB Miller Agar, m-Enterococcus Agar, m-Staphylococcus Agar, and Yersinia Agar, all by Difco™, BD®, USA). Briefly, 10 g of VC was harvested from four randomly selected bins (matching physicochemical monitoring) and suspended in 100 mL of sterile phosphate buffered saline (PBS, pH 7.4) using a Waring blender. The solution was stirred at maximum intensity three times for 60 s, separated by 60 s pauses to avoid heating the solution. The VC suspension was then serially diluted and, based on a preliminary screening, 100 µL of the 10⁻¹ to 10⁻³ dilutions were plated on four growth media, 10⁻³ to 10⁻⁶ dilutions plated on LB and 10⁻¹ plated on M-Enterococcus. Plates were incubated for 18 to 48 h at 25 or 35°C according to the manufacturer’s recommendations. The bacterial colonies isolated on each medium were characterized morphologically (size, shape, elevation, contour, color, optical characteristics, consistency, and texture) and then counted. Samples representing each of the different observed morphologies were re-streaked for isolation on the same media, and the selected colonies were separated into two aliquots. The first aliquot was stored in 30% glycerol at −80°C for re-growth when needed. The second aliquot was suspended, vortexed, and centrifuged (16.1 g) twice in 500 µL of sterile PBS prior to suspension in 250 µL of sterile distilled water and three cycles of freeze-thaw using liquid N.

**Bacterial identification by Sanger sequencing of 16S rDNA sequencing**

The 16S rDNA of the unknown bacterial samples (one of each isolated morphotype per sampling event) was amplified directly from cell suspensions using the universal bacterial primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGgyTACCTGGTACGACTT-3'). The polymerase chain reaction (PCR) amplifications were conducted in a total volume of 50 µL (5 µL of 10X PCR buffer, 1.5 mM MgCl₂, 200 mM dNTPs, 2.5u Taq from Fermentas International Inc., Burlington, Canada, 10 pM of each primer, and 4 µL of aqueous cell suspension and completed with distilled H₂O). PCR cycling conditions were as follows: 5 min at 95°C; thirty-five cycles of 1 min at 95°C, 1 min at 50°C, and 2 min at 72°C; and a final extension of 7 min at 72°C. The PCR products were visualized on a 0.8% agarose gel stained with ethidium bromide under ultraviolet light. Samples with the expected 1.5-Kb band were directly sent to Genome Quebec Innovation Center (McGill University, Montreal, Quebec, Canada) for Sanger sequencing using the same bacterial universal primers (8F and 1492R) and another set of inner primers (331F (5'-TCCTACGGAGGCAGCAGT-3') and 1194R (5'-ACGTCRTCCMAACCTTCTC-3'), in order to have a near complete coverage of the 16S rRNA gene. Sequences were aligned using the Ribosomal Database Project II (Release 9.53) alignment tool (Wang et al. 2007). A consensus sequence was obtained with the BioEdit Sequence Alignment Editor (Hall 1999) using the CAP program (Huang 1992). Sequences were classified by their closest match in GenBank. Misassembled sequences and chimeras identified by NCBI Chimera Check were removed. Final sequences were deposited in GenBank, and are publicly available under the accession numbers JX093111–JX093195.
**Statistical analysis**

Data is reported in the form of average ± standard error. Individual composting phases based on the definitions used in traditional composting were assigned using visual changes in the physicochemical variables fitted with spline regressions using the Proc mixed package of SAS (v.9.4, SAS Institute, Inc., Cary, NC, USA). Time of sampling was set as a random effect and the quadratic regressions were set on five equal intervals (three for sugars, which had fewer sampling points). Heterotrophic bacterial counts on the different days and growth media were compared using ANOVA (JMP 10, SAS Institute Inc.). Descriptive statistics of physicochemical parameters on bacterial sampling days were tested using Wilcoxon tests on ranked values when the data did not conform to the normality and homoscedasticity assumptions. CCA was used to explore multivariate relationships between cultivable bacteria and physicochemical parameters across time using the statistical software CANOCO (v.4.5) (Lepš and Šmilauer 2003). Several types of multivariate analysis have previously been used in waste management studies, as reviewed in Böhm, Smidt, and Tinter (2013), including principal component analysis on physicochemical and/or microbiological characteristics of compost (Abouelwafa et al. 2008; Campitelli and Ceppi 2008; LaMontagne et al. 2002); however, because of the multinormality requirements and use of qualitative parameters, CCA was best suited for our analyses (Legendre and Legendre 2012). First, we tested whether cultivable bacterial composition (species in CANOCO) changed with time (environmental variable in CANOCO) using a mathematical approximation of a non-linear discriminant analysis. In the matrix used, time was coded as a binary variable to emphasize inter-group differences and the significance of this analysis was tested with the Monte-Carlo permutation test (999 permutations) (Lepš and Šmilauer 2003). As both the first axis and all axes combined were significant, we used forward selection to determine the days in which cultivable bacterial composition days was significantly different from the others. We repeated the same analysis for physicochemical parameters. Finally, we conducted a CCA analysis to relate the bacterial data to the physicochemical parameters, emphasizing intra-species differences, using automatic forward selection, and tested the significance with Monte-Carlo permutation test (999 permutations).

**Results and Discussion**

**Worm population size, growth, and feeding rate**

On day zero, the bins were inoculated with 79.5 ± 6.0 g wet mass of worms (510 ± 18 worms \[n = 3\] with an average individual weight of 0.16 ± 0.10 g wet mass). On day 194, the bins contained on average 238 ± 24 g wet mass of worms (647 ± 73 worms \[n = 3\] that weighed on average 0.37 ± 0.50 g wet mass). The total mass and individual body weights of the worms increased by ~300% and ~240%, respectively, while worm number increased by ~25%.

The average feeding rate (>185 mg feed/g worm/day wet mass) was within the range of that reported in previous studies (0.75–300 mg feed/g worm/day; Ndegwa and Thompson 2001). However, the average worm population growth rate of 1 mg earthworm/day is much lower than the 5.8–29.1 mg earthworm/day range that has been reported for manure or sludge mixtures (pig manure and maple leaves, cow manure, horse manure, or activated sludge) (Domínguez and Edwards 1997; Neuhauser, Hartenstein, and Kaplan 1980). The density of *E. fetida* (0.3 to 1.0 kg earthworm/m²), was also below that reported by Ndegwa and Thompson (2001) (1.60 kg worms/m²) with activated sewage sludge and paper-mulch feed. Nevertheless, the worms appeared healthy as suggested by the early appearance of juveniles. Feed type influences *E. fetida* weight gain (Neuhauser, Hartenstein, and Kaplan 1980). Coffee grounds added to food waste improves texture, aeration, and moisture retention capabilities, making this feed suitable for vermicomposting (Adi and Noor 2009). Feeding interruptions due to breaks in the academic calendar (figure 1A) could also have affected worm population growth rates. Finally, the physicochemical characteristics of the VC remained within the vital range of *E. fetida* at all times, though the slightly more alkaline pH than common growth conditions could have also influenced growth and reproduction (Tripathi and Bhardwaj 2004).

**Physicochemical parameters**

Temporal fluctuations of all physicochemical parameters discussed below are presented in figure 1, while inferred rates of C and N volatilization based on mass balance calculations are presented in figure 2.
Temperature: Vermicomposting is strictly mesophilic. *E. fetida* tolerates 0°C–35°C (Alidadi et al. 2005) but grows optimally around 25°C (Tripathi and Bhardwaj 2004). Semi-continuous feed input prevented the onset of a sharp thermophilic stage, as evidenced by $\Delta T^\circ$ (between VC and ambient temperatures remained between 1.4–3.5°C, with an initial increase followed by a decrease after a climax around day 200; figure 1B). This is consistent with the $\Delta T^\circ = \pm 2°C$ reported by Abbasi, Gajalakshmi, and Abbasi (2009) during vermicomposting. Mesophilic temperatures favor the onset of a more diverse microbial community, as most non-spore-forming mesophilic microbes are killed during the thermophilic phase of batch composting (Mustin 1987).

Humidity: Throughout the experiment, moisture levels remained within the vital range of 60%–90% (Alidadi et al. 2005), and though weekly watering prior to day 194 was necessary, food thereafter supplied sufficient moisture to keep the substrate within the optimal 80%–90% range (Domínguez and Edwards 1997; figure 1C). Despite moisture contents ≥70%, which would lead to anoxia (waterlogging), slower degradation rates, and the generation of foul-smelling compounds in traditional composting (Mustin 1987), no odor was detected in the current experiment owing to the constant burrowing, mixing, and aerating performed by the earthworms.

pH: The initial pH of the VC was close to that of the feed material (figure 1D), but then led to a bimodal distribution with a peak of ~7.0 around day 100, and ~7.5 around day 266 perhaps due to ammonification reactions driven by protein degradation. The subsequent pH drop to ≤6.0 likely resulted from the release

**Figure 2.** Variations in the average cumulative organic carbon (A) and total nitrogen (B) contents of the bins. Cumulative nutrient inputs (black circles) include feed and initial litter. The total nutrient mass accounted for in the vermicompost (black squares) is based on total compost weight and measured nutrient concentration. The difference between the total inputs and nutrients present in the vermicompost is assumed to have escaped from the system as gas (empty triangles) and is represented as an estimated daily volatilization rate of C (C) and N (D), normalized to total vermicompost dry mass. Feeding was interrupted on day 266 (dashed line).
of organic acids during the die-off of the decomposer community, and eventually, the earthworms. While continuously decreasing pH values are common in vermicomposting (Atiyeh et al. 2000; Ndegwa, Thompson, and Das 2000), bimodal trends have previously been reported with peaks as high as pH 8 from vermicomposting human feces (Yadav, Tare, and Ahammed 2010). Low weekly feed input rate relative to the total VC mass (average of 9.87 ± 4.07% of the total dry VC mass), with less food added at the beginning, and slightly more later in the experiment during worm colony growth, likely prevented sharp acidogenesis or ammonification peaks. A gradual pH decrease in the VC could be due to organic nitrogen and phosphorus mineralization into nitrites/nitrates and orthophosphates, as well as conversion into organic acid intermediates whose subsequent processing would reverse the pH shift (Ndegwa, Thompson, and Das 2000).

Organic carbon (OC), total nitrogen (TN), and C/N ratio: The initial feeding rate was higher than the mineralization rate, leading to OC increases, followed by net mineralization evidenced by a slow decrease of OC when feeding was stopped, with overall concentration fluctuations remaining at <5% throughout the experiment (figure 1E). Total OC decreases during batch vermicomposting (Elvira et al. 1998), or continuous vermicomposting (Aira, Monroy, and Domínguez 2007; Yadav, Tare, and Ahammed 2010) due to the aerobic release of CO₂. This is supported by important atmospheric losses of OC, based on a mass balance calculation including initial litter (compost), feed inputs, nutrients in the VC and earthworm biomass (figure 2).

Important atmospheric losses of TN were also inferred from the mass balance (figure 2). The TN content of the VC nearly doubled before day 266 and then decreased slightly when feeding was interrupted (figure 1F). We originally intended to monitor the OC and TN content of the leachate, but leachate production was non-existent before day 194, rare and in negligible amounts (drops) between days 194 and 266, and again non-existent after day 266 (feeding interruption). Recirculating the few drops of leachate into the compost prevented N losses, and is akin to several domestic systems that do not produce leachate. Because no leachate escaped the system (the drops collected between days 194 and 266 were spread on the VC), the only output of OC and TN would have been through atmospheric losses (even when accounting for the negligible sample removal). Earthworms contained 9.82 ± 0.83% TN and 47.4 ± 0.7% OC (n = 6), which represents <1% of the OC or TN inputs on day 195 for example. Variations in OC and TN differed broadly over the course of the experiment.

Carbon volatilization is likely composed of CO₂ and, to a lesser extent, CH₄ (Majumdar et al. 2006). The daily C volatilization rates inferred from the mass balance increased steadily from the start of the experiment, peaked around days 150–200, and started to decrease to a constant intermediate level before the end of feeding, suggesting a steady degradation rate. OC volatilization rates close to zero were calculated beyond day 400, reflecting high VC stability. In a batch VC system, total N should increase over time due to the mineralization of organic matter containing proteins, conversion of ammonium into nitrate, excretion of nitrogenous compounds by earthworms, and N fixation by bacteria present in the VC (Atiyeh et al. 2000; Pramanik et al. 2007; Suthar 2007). However, N loss has been observed here and in another continuous VC experiment, probably due to a low initial carbon-to-nitrogen (C/N) ratio (Yadav, Tare, and Ahammed 2010). Nitrogen losses occur through ammonia volatilization (NH₃), denitrification by microorganisms (N₂, NO₂, and N₂O), or leaching of nitrate (NO₃⁻) (Peigné and Girardin 2004), the latter of which can be ruled out here because no leachate escaped the system. In the current experiment, the TN content in the VC first increased steadily and nearly 75% of the TN input was present in the VC shortly after day 266. Feeding interruption seemed to trigger N losses, most likely occurring via volatilization and denitrification. Within 100 days, more than 40% of the TN remaining in the VC was released to the atmosphere, while the calculated C volatilization remained <15%. Denitrifying bacteria thriving in anaerobic microsites and consuming organic N (likely from the die-off of the decomposer community) as a source of energy may be responsible for this N volatilization, as labile C sources were becoming scarce (C/N ratio <15 beyond day 100) (Domínguez and Edwards 1997; Yadav, Tare, and Ahammed 2010). Earthworms exert a major influence on N cycling in soil (Bityutskii et al. 2002; Ozawa, Risal, and Yanagimoto 2005) and in VC (Atiyeh et al. 2000). Non-assimilated ingested material is excreted in granular aggregates called vermicastings, which contain mucus, nitrogenous
excretory substances, body fluids, growth-stimulating hormones, enzymes, and decaying dead tissue of earthworms (Suthar 2007; Tripathi and Bhardwaj 2004). Earthworms stimulate non-symbiotic N fixation in the substrate by modulating the microbial community in a way that favors N-fixing bacteria (Tereshchenko and Naplekov 2002). However, they also stimulate N2O-producing soil bacteria during gut transit due to anoxia, high osmolarity, and the high nitrite and nitrate concentrations prevailing in their gut (Horn et al. 2006). Concomitantly with N2O emissions (greenhouse gas), earthworms also emit N2 (non-greenhouse gas), which is the end-product of complete denitrification (Horn et al. 2006). A discussion of observed trends in N content in relation to the abundance of putative microbial denitrifiers is detailed below.

The decreasing C/N ratio is an expected characteristic of both batch composting and continuous vermicomposting, and is affected by the initial C/N ratio of the feed mixture (Gupta and Garg 2009). The C/N ratios of the litter (∼27) and feed (∼23) were close to that of the VC at the beginning of the experiment (table 1), and close to the optimal ratio of 25 to sustain bacterial growth in composting systems (Alidadi et al. 2005; Ndegwa, Thompson, and Das 2000). The rapid initial decrease in C/N ratio likely corresponds to N immobilization in the biomass as the bacterial and worm community develops, eventually reaching a value that reflects a mixture of N-enriched bacterial biomass with N-depleted recalcitrant materials (lignin, waxes, etc.) during the maturation stage (figure 1G). In a continuous system, the rate of decrease in C/N ratio might become higher as the worm population increases (Ndegwa, Thompson, and Das 2000). The inflection point around day 231 may indicate that, at this point, the bacterial community was efficiently turning over new feed additions, which accounted for only a small fraction of the total compost mass at this time (10.1 ± 1.4% on a dry mass basis).

Carbohydrates: Because complex sugars were continuously added through weekly feedings, the abundance of total sugars, including cellulose, increased until day 61 (figure 1H). Between days 61 and 231, the concentrations of these sugars remained relatively stable, as inputs were balanced by the high turnover rate (dynamic equilibrium) before eventually decreasing when food input was interrupted on day 266. The concentration of labile sugars did not vary dramatically throughout the experiment (27.9 ± 5.0 mg sugar/g VC dry mass), most likely because the degradation of complex sugars continuously replenished the pool of labile carbohydrates, i.e., labile sugars are both mineralized and synthesized during the composting process (Mustin 1987), and this may originate from a rapid use of the labile carbohydrates, resulting from the degradation of cellulose and hemi-cellulose by the decomposer community (Sánchez-Monedero et al. 1999). Thus, water-soluble carbohydrates would mainly be used as an energy source for the decomposer community, rather than as precursors for the synthesis of humic substances in the compost.

**Cultivable bacterial composition and abundance**

The total abundance of cultivable bacteria did not vary much before and after feeding ended (remaining close to 10⁶ colony forming units (CFU)/g dry VC) (figure 3), but the number of cultured bacterial classes and genera increased gradually through the experiment (figure 4). When considering all growth media separately across each sampling point, we observed strongly significant variations in bacterial heterotrophic counts with time (p < 0.0001) and between growth media (p < 0.0001). The time effect was also different on different media types (interaction,
lower than cow manure VC stored for 1 year, sieved fresh cow manure VC, or tree-leaves and organic waste VC (10^6–10^7 CFU/g dry mass), and is much lower than non-sieved cow VC, leaves and cow manure VC, starchless potato pulp and composted grass fresh or granulated VC, immature and mature sawdust and tree leaves VC (10^7–10^8 CFU/g dry mass; cultured on TSA agar; Grantina-Ievina et al. 2013). Selective culture media allowed us to culture and isolate a subset of bacteria that can metabolize the substrate and tolerate potentially growth-inhibiting compounds, while general media may support the growth of a wider range of microorganisms. The use of several media formulations allowed us to capture some of the more rare bacteria, which may have been excluded in the least selective formulations by the growth of certain rapidly growing isolates. Although the sum of all heterotrophic counts of each selective media is nearly identical to the heterotrophic plate counts on LB agar, using an array of different media allowed us to capture a greater proportion of the bacterial diversity in our vermicompost samples.

**Relationship between cultivated bacteria and physicochemical parameters**

The correspondence analysis revealed that the composition of cultured bacteria varied with time (1st axis and all axis \( p = 0.001 \)), with day 328 being the most distinct. Physicochemical parameters also varied significantly with time (1st axis \( p = 0.002 \) and all axis \( p = 0.001 \)), with day 217 being the most distinct. The CCA with forward selection of explanatory parameters explained 91.8% of the global bacterial and physicochemical variability with four canonical axes (figure 4). It revealed that pH (\( p = 0.0100 \)), OC (\( p = 0.0140 \)), and labile carbohydrates (\( p = 0.0110 \)) significantly affected bacterial community changes through time, while total carbohydrates were nearly significant (\( p = 0.0630 \)). We can easily distinguish sampling day 217 with the samples 1–4 in the upper left corner, characterized by higher OC, lower labile carbohydrates, and intermediate pH values. Members of the Enterobacteriaceae (unclassified and *Citrobacter*) and Bacillaceae (*Halobacillus*) were most abundant under these conditions. Samples 5–8 from day 266 were characterized by a lower OC content, a low pH, and low labile carbohydrate content. However, sample 7 seems to diverge from this grouping and appears most likely to
contain *Pseudomonas* (*Pseudomonadaceae*). The third sampling event (day 328) is hardly distinguishable from the preceding one, at least for samples 9 and 10. Samples 11 and 12 grouped together at a higher pH value, but lower labile carbohydrate content. *Stenotrophomonas* (*Xanthomonadaceae*) and *Aeromonas* (*Aeromonadaceae*) are the most characteristic bacteria of this sampling event. Finally, on the last sampling day, samples 13 to 16 grouped together in the lower left quadrant, though environmental parameters appeared to fluctuate widely at that date. Finally, *Bosea* (*Bradyrhizobiaceae*); *Oceanobacillus* (*Bacillaceae*); and unclassified genera of the *Micrococcineae*, *Xanthomonadaceae*, *Gammaproteobacteria*, and *Neisseriaceae* appear characteristic of this last sampling event. Note that *Cupriavidus* (*Burkholderiaceae*) and an unclassified genus of *Streptomycineae*, observed in later VC samples, were also grouped in that region when included in the analysis. However, because of their low counts on the sampling plates (growth only on the least diluted plates, which were overcrowded by other bacteria), they could only estimate their abundance (1 CFU g\(^{-1}\) dry mass) and excluded them from the above discussion. Labile carbohydrates and feeding (binary variable) were the only statistically significant variables when they were included in the CCA. Multivariate statistical analyses, such as CCA, allow us to take an integrated look at how several environmental parameters affect several biological response variables. Therefore, this analysis helped to identify the most important parameters affecting the semi-continuous vermicomposting system.

Grantina-Ievina et al. (2013) recommend long-term dry storage of VC as a means to reduce undesired organisms, as microbial propagules (except spores) are no longer present in air-dried VC. They observed significantly lower heterotrophic counts in dry VC (5%–6% moisture) than in wet VC (64%), and maximal counts in VC containing an intermediate amount of moisture (22%). The humidity content on the four sampling days of the current experiment did not vary significantly (Wilcoxon test, \(p = 0.7788\)), and ranged from 77.3 ± 4.5% to 71.2 ± 15.4%. There was no correlation between moisture content and the total number of bacteria (based on CFU/g dry or wet mass), except for the wet weight CFU for one media type (m-Staphylococcus, \(r^2 = 0.44, p = 0.0003\)). In contrast, Grantina-Ievina et al. (2013) observed a weak correlation between heterotrophic counts and humidity \((r^2 = 0.27)\), and a strong correlation between total coliforms \((r^2 = 0.76)\) and humidity content. Our heterotrophic counts on MacConkey agar, a media selective for Gram-negative and enteric bacilli, had a very weak and insignificant correlation with humidity content \((r^2 = 0.12, p = 0.1224)\).

Our results also suggest community variations with time (figure 5B), as the *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* were only detected at the last sampling event, approximately 100 days after weekly feeding was interrupted. Although *Actinobacteria* may be observed in the active thermophilic phase of composting (Xiao et al. 2011), most are killed in the thermophilic phase (above 60°C) (Ryckeboer et al. 2003) and they become more abundant in the mesophilic curing phase (Xiao et al. 2011). As high temperature regulation of Actinobacteria may be ruled out from our vermicomposting experiment, our results suggest a role of nutrient abundance in the control of *Actinobacteria*, which becomes more abundant when feeding is interrupted. As others have suggested, it is also likely that in more active microbial communities promoted by earthworms in vermicompost, *Actinobacteria* are likely to be less abundant (Lazcano, Gomez-Brandon, and Domínguez 2008). Overall, the *Bacilli* and *Gammaproteobacteria* remained relatively abundant throughout the experiment, and perhaps became slightly more abundant with time. The abundance of the *Bacilli* increased slightly after the first sampling event and the diversity of the *Gammaproteobacteria* culminated 50 days after feeding ended. The *Gammaproteobacteria* are present and their abundance is relatively high and stable throughout the experiment (with a peak on day 329 for the *Pseudomonadales*), except for the *Xanthomonadales*, which appear in very high abundance (but low number of genera) only after feeding had stopped. These results suggest that the active degradation stage is dominated by a few taxa, whereas the maturation stage allows a diverse array of bacteria to cohabit, all of this under a constant abundance of bacteria. Indeed, a succession of microbial communities through time, already demonstrated for thermophilic composting (Haruta et al. 2004), is also observed during vermicomposting.

A close examination of the species present in our bins reveals a similarity with bacteria isolated from traditional composters. These include *Klebsiella pneumoniae* (Tiago et al. 2004) and several species of *Bacillus*: *B. cereus* (Ryckeboer et al. 2003; Tiago et al. 2004), *B. licheniformis* (Ryckeboer et al. 2003; Tiago et al. 2004).
B. pumilus (Ryckeboer et al. 2003), and B. megaterium (Ishii and Takii 2003). B. licheniformis is common during all phases of thermophilic composting (Ryckeboer et al. 2003). Here, there is an apparent decrease in B. licheniformis abundance after the interruption of feeding, but this may be due to incomplete identification, as the abundance of Bacillus sp. increased dramatically during the two last sampling events (figure 5A). Bacillus are thought to dominate thermophilic composting microbial communities (Dees and Ghiorse 2001; Schloss et al. 2003), and we show here that Bacillus are also very abundant and diverse (five species identified) in continuous vermicomposting.
Occurrence of regulated bacterial pathogens

Fruits and vegetables are important bacterial vectors (Leff and Fierer 2013) and may sometimes harbor pathogens (Beuchat 2002) that are then introduced in VC systems. Nevertheless, we did not expect high abundances of potentially pathogenic bacteria, such as coliforms, Salmonella, and Enterococcus, as the VC was produced from food destined for human consumption. Salmonella was never detected (even though its growth is supported on MacConkey and Levine EMB media) on 10-g wet mass samples, as per Quebec guidelines (MDDEP 2012a), a guideline similar to the Canadian standards on 4-g dry mass samples (CCME 2005). Enterococcus were only observed in one out of the four bins sampled, on a single day (328) and in low abundance (<25 CFU/g dry mass), conforming to legislations in other countries (Latvia’s requirements [<10³ CFU/g dry mass]; Grantina-levina et al. 2013) in absenta of a specific requirement in Quebec (MDDEP 2012a) or Canada (CCME 2005). Among the common coliform bacteria, no Escherichia coli was cultured from the VC and only Citrobacter was positively identified, at 2.1 × 10⁵ ± 3.0 × 10⁵, 2.2 × 10⁶ ± 3.5 × 10⁴, 7.1 × 10⁴ ± 1.4 × 10⁵ and 4.8 × 10⁵ ± 3.6 × 10⁴ CFU/g dry mass on days 218, 266, 329, and 379, respectively; and an unidentified Enterobacteriacea was present on the first day of sampling, but not later, at a concentration of 8.7 × 10⁵ ± 5.2 × 10⁵ CFU/g dry mass. In Quebec, it is not necessary to test source-separated organic material that is uncontaminated with human or animal fecal matter for E. coli prior to direct agricultural uses. However, composted organic waste must have fewer than 2 × 10⁶ CFU g dry mass⁻¹ (MDDEP 2012a). Canadian Guidelines require fewer than 10³ CFU g dry mass⁻¹ of fecal coliforms, recognizing that direct E. coli counts may actually be more representative of sanitation standards (CCME 2005).

Bacterial community composition using culture-based and culture-independent methods

Bacterial culturing has been used successfully in the past to understand the composting and VC process (Anastasi, Varese, and Marchisio 2005; Franke-Whittle et al. 2009; Gopal et al. 2009; Grantina-Ievina et al. 2013; Haruta et al. 2004; Ryckeboer et al. 2003). Culturing allows quantification of absolute abundance and its variation in time. Though only a subset of the culturable bacterial communities present (10⁶ CFU/g with the least selective LB agar) may have been captured, both media selectivity and composting process may explain the counts >10¹⁰ CFU/g (using tryptic soy agar) for semi-continuous fruit, vegetable, and meat compost (Haruta et al. 2004).

We have contributed to an expanding knowledge on cultivable VC bacteria by demonstrating cultures grown on media that have previously been unreported for VC (Levine EMB, m-Enterococcus, m-Staphylococcus, and Yersinia agar). Evidently, because of the selectivity or richness of different growth media, and because of the presence of viable but non-cultivable bacteria, culturing techniques may only provide a restricted and biased portrait of microbial communities (Vartoukian, Palmer, and Wade 2010). Hence, care should be taken to avoid overgeneralization in community descriptions. Nevertheless, culture and isolation remain important for subsequent characterization of the metabolism and physiology of the bacteria, which may play a role in inhibiting pathogens or promoting plant growth (Gopalakrishnan et al. 2014; Vartoukian, Palmer, and Wade 2010).

High-throughput sequencing of commercial-scale processes has revealed differences in the bacterial and fungal communities of compost and vermicompost of pre-composted recipes of manure/silage and hardwood (Neher et al. 2013). However, this technique may still bias community descriptions, as some microbes may be harder to lyse than others (Tringe and Rubin 2005), genomic DNA may be sheared from detergents or bead beating during extraction, or because of co-extraction of PCR inhibitors like humic acids (LaMontagne et al. 2002). Furthermore, high throughput sequencing may cover a smaller portion of the 16S rDNA gene than the method used here, hence providing lower confidence in taxonomic classifications at the genus level.

Current knowledge on the composition of microbes in vermicompost

GenBank, the primary repository for sequences from cultured isolates, contains 39,470 nucleotide sequences corresponding to the keyword compost, but vermicompost sequences represent only 2% of those. The Sequence Read Archive from NCBI (a repository for high-throughput sequencing data) contains 97 hits, while the metagenomic analysis server MG-RAST contains none. Most of the GenBank VC sequences
characterized bacteria using the 16S rRNA gene. The current study is the first Canadian VC study to submit bacterial sequences to GenBank, and the first worldwide to document bacteria from typical domestic wastes of fruits, vegetables, and coffee. Though GenBank submissions may not represent the true bacterial diversity of VC (as some studies have genera specific focus, i.e., VC with antifungal properties targeting *Actinobacteria*; Yasir et al. 2009), it is interesting to compare our data with the dominant groups of bacteria studied in VC. Figure 5C shows differences in the relative abundance of the major phyla/classes and genera identified in the GenBank 16S rRNA vermicompost sequences (excluding those from the current study) with those obtained in this study (mean of the four time points). Sequences were reclassified using the 16S rRNA GreenGenes database in Mothur (Schloss et al. 2009) so that the two datasets would be directly comparable, since the source of annotation for GenBank sequences is variable. We used the ‘classify.seqs’ command with a bootstrap cutoff of 90. This method identified 87 genera in the GenBank dataset, compared with 16 genera in ours. Relative to the GenBank sequences, our study has a higher relative abundance of the *Firmicutes* and *Gammaproteobacteria*. *Bacillus* and *Pseudomonas* were abundant in both datasets, but more markedly in this study. Certain genera, like *Klebsiella* and *Oceanobacillus*, were present in our sequences, but were not observed among the other sequences in GenBank. In contrast, *Acinetobacter*, *Cellulomonas*, and *Rhodococcus* were all over 1% in GenBank sequences, but absent in ours.

**Denitrifying bacteria in vermicompost**

Bacteria that are closely related to the denitrifying *P. aeruginosa* and *P. nitroreducens* were isolated from the VC at densities of $10^2$–$10^4$ CFU/g on day 266 and $>10^6$ on day 329, but were not detected on day 379, >100 days after the last feed. The abundance of unidentified *Pseudomonas* precluded a correlation between estimated atmospheric N losses and the abundance of putative denitrifiers. However, mass balance calculations revealed that under the active vermicomposting, few gaseous TN losses occurred, although feeding interruptions, which starve the decomposer community, led to greater losses. Due to extended feed interruption, nearly half of the TN was lost through volatilization during the course of this experiment. A focus on denitrifying bacteria and N gas specification (N$_2$, NO$_2$, or N$_2$O) could be interesting in future long-term semi-continuous VC experiments, with extended feed interruption or storage. Such further studies appear pertinent in light of the work by Vinceslas-Akpa and Loquet (1995), which suggested that feed interruption changes worm gut microbiome, and based on the work of Ihssen et al. (2003), which showed that worms can activate denitrifiers naturally present in the soil. Since N$_2$O is a powerful greenhouse gas, a better characterization of the N-cycle in vermicomposting is essential, especially if vermicomposting facilities are operated at high earthworm densities and waste-processing rates (Frederickson and Howell 2003), which is not the case for domestic systems.

**Vermicompost maturity**

After a brief phase akin to initiation, followed by active degradation, vermicompost maturation was apparent. C/N ratios alone cannot be considered a satisfying index of maturity, but they provide useful information on the biodegradation process. In this experiment, the C/N ratio decreased steeply and stabilized at a value near 10 just before feeding was stopped (figure 1G). After the last feed, despite ongoing changes in OC content and pH, the C/N ratio remained stable around 10. The feeding interruption markedly influenced the observed trends with an abrupt decline in pH and an elevation in N volatilization rate, which correlated with a decrease in survival of the decomposer community. After the feeding interruption, the inferred C volatilization rate rapidly decreased below the compost maturity threshold of 4 mg C-CO$_2$ g$^{-1}$ compost day$^{-1}$ (under the Canadian and Quebec bureau of normalization norm #CAN/BNQ 0413-200; equivalent to). Others have observed that domestic VC may stabilize at a higher C/N ratio (~15) than industrial compost (~11), despite reaching lower dynamic respiration index (~330 versus ~1510 mg O$_2$ kg$^{-1}$ organic matter h$^{-1}$; Barrena et al. 2014); the Canadian norm recommends a consumption index $\leq$400 mg O$_2$ kg$^{-1}$ organic matter h$^{-1}$ (#CAN/BNQ 0413-200). One hundred days after feeding interruption, the VC seemed stable, although the total sugar levels may still have been declining, suggesting an ongoing humification process and/or apparent but low ongoing C and N volatilization.
Vermicomposting is considered a sustainable waste management practice. However, we still do not fully understand the interactions between the microbial community and the physicochemistry of the VC. Continuous and batch operations do not behave similarly, the former exhibiting transient and gradual changes, and the latter characterized in the literature by more abrupt and generally unidirectional changes in physicochemical parameters. This study reveals that under semi-continuous operations typical of domestic operations (with regular feed additions and occasional harvest) there is a lag phase during which the decomposer community adapts and physicochemical parameters change, but we observed no equilibrium point where all physicochemical parameters remain stable over an extended period of time. The C/N ratio of the VC may reach a low value suggesting biological stability, despite regular additions of food in the VC bin, most likely because when a critical mass is reached, small food inputs (with optimal C/N ratio 25) only negligibly affect the global VC mass (with an advanced biodegradation C/N ratio 10). However, feeding interruption causes other changes in the physicochemical parameters. The TN trapped in the decomposer biomass during regular feeding may volatilize rapidly from the VC if it is stored before use or if feeding is interrupted, as is recommended for sanitation purposes or as happens when a VC bin operator goes on vacation. Hence, if N-rich VC is desired, the worms should be fed regularly and the VC should be used fresh (not stored for extended periods). Based on culturing followed by 16S rRNA gene sequencing, we identified members of the Bacillaceae, Enterobacteriaceae, and Pseudomonadaceae in both active and maturing vermicompost, but Actinobacteria, Xanthomonadaceae, and Aeromonadaceae were most abundant after feeding interruption. The resulting food waste complied with regulatory requirements for pathogenic bacteria, despite the absence of a thermophilic phase. Our culture-based approach may have yielded only a subset of all microbes present in vermicompost compared to culture-independent community descriptions, but we observed genera not previously present among vermicompost-labeled sequences in GenBank (Klebsiella and Oceanobacillus), and we increased the number of bacterial sequences from vermicompost in GenBank by 13%. We demonstrated that bacterial community fluctuations in VC are affected by pH, OC, and labile carbohydrate content. The knowledge gained on the natural phases and intrinsic heterogeneity of physicochemical parameters in vermicomposting systems during active feeding and upon feed interruption is useful for understanding the behavior of further manipulative experiments focusing on the role of feed, worms, and vermicomposting microflora on the survival of the potential human pathogen, *E. coli*, in mesophilic composting operations. Our experiment provides further knowledge of the media supporting VC bacteria growth, which can be useful in lower budget bacterial monitoring experiments, for bacterial cultures intended for metabolic characterization, or for producing process inoculants. Finally, this study also sets a foundation for a much-needed assessment of VC microbial community dynamics and warrants further research on domestic vermicomposting, considering the gaseous emissions of C and especially N, to quantify the emissions of greenhouse gases.

**Acknowledgments**

The authors wish to thank Alexis Fortin and Ilenna Vuong for their help. Thanks also go to Serge Paquet for helping with the statistical analyses and to Jill Vandermeerschen at the Assistance Center on Statistical Analyses from the Mathematics department (UQAM).

**Funding**

The authors gratefully acknowledge the support of the Concordia University, the Concordia Center for Functional and Structural Genomics, as well as the NSERC of Canada, and the FQRNT-Quebec for scholarships (LHE) and grants (VJJM, YG).

**References**

Abbasi, T., S. Gajalakshmi, and S. A. Abbasi. 2009. Towards modeling and design of vermicomposting systems: Mechanisms of composting/vermicomposting and their implications. *Indian Journal of Biotechnology* 8 (2):177–82.

Abouelwafa, R., G. A. Baddi, S. Souabi, P. Winterton, J. Cegarra, and M. Hafidi. 2008. Aerobic biodegradation of sludge from the effluent of a vegetable oil processing plant mixed with household waste: Physical chemical, microbiological, and spectroscopic analysis. *Bioresource Technology* 99 (18):8571–77.

Adi, A. J., and Z. M. Noor. 2009. Waste recycling: Utilization of coffee grounds and kitchen waste in vermicomposting. *Bioresource Technology* 100 (2):1027–30.

Aira, M., F. Monroy, and J. Dominguez. 2007. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities...
improving carbon mineralization during vermicomposting of pig manure. Microbial Ecology 54 (4):662–71.
Alidadi, H., A. R. Parvaresh, M. R. Shahmansouri, and H. Pourmoghadas. 2005. Combined compost and vermicomposting process in the treatment and bioconversion of sludge. Journal of Environmental Health Science and Engineering 2 (4):251–54.

Anastasi, A., G. C. Varese, and V. F. Marchisio. 2005. Isolation and identification of fungal communities in compost and vermicompost. Mycologia 97 (1):33–44.

Arancon, N. Q., P. A. Galvis, and C. A. Edwards. 2005. Suppression of insect pest populations and damage to plants by vermicomposts. Bioresource Technology 96:1137–1142.

Atiyeh, R. M., J. Dominguez, S. Subler, and C. A. Edwards. 2000. Changes in biochemical properties of cow manure during processing by earthworms (Eisenia andrei, Bouche) and the effects on seedling growth. Pedobiology 44 (6):709–24.

Barrena, R., X. Font, X. Gabarrell, and A. Sánchez. 2014. Home composting versus industrial composting: Influence of composting system on compost quality with focus on compost stability. Waste Management 34 (7):1199–16.

Beuchat, L. R. 2002. Ecological factors in microbial diversity in soil. Eurasian Soil Science 35 (10):1100–07.

Bogner, J., R. Pipatti, S. Hashimoto, C. Diaz, K. Mareckova, L. Diaz, P. Kjeldsen, S. Monni, A. Faaij, G. Qingxian, Z. Tianzhu, A. M. Abdelrafié, R. T. M. Sutamihardja, and R. Gregory. 2008. Mitigation of global greenhouse gas emissions from waste: conclusions and strategies from the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report. Working Group III (Mitigation). Waste Management & Research 26 (1):11–32. doi: 10.1177/0734242x07088433.

Bohm, K., E. Smidt, and J. Tintner. 2013. Application of multivariate data analyses in waste management. In Multivariate analysis in management, engineering and the sciences, eds. L. Freitas and A. P. Freitas, 15–38. rijeka, Croatia: InTech. http://dx.doi.org/10.5772/53975.

Brown, G. G. 1995. How do earthworms affect microfloral and faunal community diversity. Plant and Soil 170 (1):209–31.

Campitelli, P., and S. Ceppi. 2008. Chemical, physical and biological compost and vermicompost characterization: A chemometric study. Chemometrics and Intelligent Laboratory Systems 90 (1):64–71.

CCME (Canadian Council of Ministers of the Environment). 2005. Guidelines for compost quality. http://www.ccme.ca/files/Resources/waste/compostdlns_1340_e.pdf (accessed January 28, 2015).

Dees, P. M., and W. C. Ghiorse. 2001. Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiology Ecology 35 (2):207–16. doi: 10.1111/j.1574-6941.2001.tb00805.x.

Dominguez, J. 2004. State of the art and new perspectives in vermicomposting research. In Earthworm ecology, ed. C. A. Edwards, 401–24. Boca Raton, FL: CRC Press.

Dominguez, J., and C. A. Edwards. 1997. Effects of stocking rate and moisture content on the growth and maturation of Eisenia fetida in pig manure. Soil Biology & Biochemistry 29 (3–4):743–46.

Eastman, B. R., P. N. Kane, C. A. Edwards, L. Trytek, B. Gunadi, A. L. Stermer, and J. R. Mobley. 2001. The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. Compost Science & Utilization 9 (1):38–49.

El-Tarabily, K. A., and K. Sivasitharaman. 2006. Non-streptomyces actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Soil Biology & Biochemistry 38 (7):1505–20.

Elvira, C., L. Sampedro, E. Benítez, and R. Nogales. 1998. Vermicomposting of sludges from paper mill and dairy industries with Eisenia andrei: A pilot-scale study. Bioresource Technology 63 (3):205–11.

Fracchia, L., A. B. Dohrmann, M. G. Martinotti, and C. C. Tebbe. 2006. Bacterial diversity in a finished compost and vermicompost: Differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. Applied Microbiology and Biotechnology 71 (6):942–52.

Franke-Whittle, I. H., B. A. Knapp, J. Fuchs, R. Kaufmann, and H. Insam. 2009. Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microbial Ecology 57 (3):510–21.

Frederickson, J., and G. Howell. 2003. Large-scale vermicomposting: Emission of nitrous oxide and effects of temperature on earthworm populations. Pedobiology 47:724–30.

Gajalakshmi, S., and S. A. Abbasi. 2008. Solid waste management by composting: State of the art. Critical Reviews in Environmental Science and Technology 38 (5):311–400.

Gajalakshmi, S., P. S. Ganesh, and S. A. Abbasi. 2005. A highly cost-effective simplification in the design of fast-paced vermireactors based on epigeic earthworms. Biochemical Engineering Journal 22 (2):111–16.

Gopal, M., A. Gupta, E. Sunil, and G. Thomas. 2009. Amplification of plant beneficial microbial communities during conversion of coconut leaf substrate to vermicompost by Eudrilus sp. Current Microbiology 59 (1):15–20.

Gopalakrishnan, S., S. Vadlamudi, P. Bandikinda, A. Sathya, R. Vijayabhargathi, O. Rupela, H. Kudapa, K. Katta, and R. K. Varshney. 2014. Evaluation of Streptomyces strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiological Research 169 (1):40–48.

Grantina-Ievina, L., U. Andersone, D. Berkolde-Pire, V. Nikolaijeva, and G. Ievinsh. 2013. Critical tests for determination of microbiological quality and biological activity in commercial vermicompost samples of different origins. Applied Microbiology and Biotechnology 97 (24):10541–54.

Gupta, R., and V. K. Garg. 2009. Vermi remediation and nutrient recovery of non-recyclable paper waste employing Eise-

nia fetida. Journal of Hazardous Materials 162 (1):430–39.
Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Haruta, S., M. Kondo, K. Nakamura, C. Chanchitpricha, H. Aiba, M. Ishii, and Y. Igarashi. 2004. Succession of a microbial community during stable operation of a semi-continuous garbage-decomposing system. Journal of Bioscience and Bioengineering 98 (1):20–27.

Hénault-Ethier, L., and A. Fortin. 2011. Guide technique pour le compostage sur site en ICI. Montréal: Concordia University, Recyc-Québec, Canadian Compost Council and Association.

Horn, M. A., R. Mertel, M. Gehre, M. Kastner, and H. L. Drake. 2006. In vivo emission of dinitrogen by earthworms via denitrifying bacteria in the gut. Applied and Environmental Microbiology 72 (2):1013–18.

Huang, X. 1992. A contig assembly program based on sensitive detection of fragment overlaps. Genomics 14 (1):18–25.

Ihssen, J., M. A. Horn, C. Matthies, A. Gossner, A. Schramm, and H. L. Drake. 2003. N$_2$O-producing microorganisms in the gut of the earthworm Aporrectodea caliginosa are indicative of ingested soil bacteria. Applied Environmental Microbiology 69 (3):1655–61.

Ingham, E. 1998. Without the heat, will weed seeds still be in vermicompost? What about plant disease-causing organisms? Biocycle 39 (11):109–119.

Ishii, K., and S. Takii. 2003. Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel electrophoresis analysis. Journal of Applied Microbiology 95 (1):109–19.

Jouraiphy, A., S. Amir, M. E. Gharous, J.-C. Revel, and M. Hafidi. 2005. Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. International Biodeterioration & Biodegradation 56 (2):101–08.

Karsten, G. R., and H. L. Drake. 1995. Comparative-assessment of the aerobic and anaerobic microflora of earthworm guts and forest soils. Applied and Environmental Microbiology 61 (3):1039–44.

Kristiana, R., J. Nair, M. Anda, and K. Mathew. 2005. Monitoring of the process of composting of kitchen waste in an institutional scale worm farm. Water Science and Technology 51 (10):171–77.

Lal, R., R. F. Follett, B. A. Stewart, and J. M. Kimble. 2007. Soil carbon sequestration to mitigate global warming and advance food security. Soil Science 172 (12):943–56. doi: 10.1097/ss.0b013e31815cc498.

LaMontagne, M. G., F. C. Michel Jr., P. A. Holden, and C. A. Reddy. 2002. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. Journal of Microbiological Methods 49 (3):255–64.

Lassegues, M., P. Roch, and P. Valenbois. 1989. Antibacterial activity of Eisenia fetida andrei coelomic fluid: Evidence, induction, and animal protection. Journal of Invertebrate Pathology 53 (1):1–6.

Lazcano, C., M. Gomez-Brandon, and J. Domínguez. 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 72 (7):1013–19.

Leff, J. W., and N. Fierer. 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS One 8 (3):e59310.

Legendre, P., and L. F. J. Legendre. 2012. Numerical ecology. Vol. 20. Berlin, Germany: Elsevier.

Lep, J., and J. Šmilauer. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge, UK: Cambridge University Press.

Li, W., C. Wang, and Z. Sun. 2011. Vermipharmaecuticals and active proteins isolated from earthworms. Pedobiologia 54:549–56.

Lleó, T., E. Albacete, R. Barrena, X. Font, A. Artola, and A. Sánchez. 2013. Home and vermicomposting as sustainable options for biowaste management. Journal of Cleaner Production 47:70–76.

Lowe, L. E. 1993. Total and labile polysaccharide analysis of soils. In Soil sampling methods analysis, ed. M. R. Carter. Boca Raton, FL: Lewis Publishers.

Majumdar, D., J. Patel, N. Bhatt, and P. Desai. 2006. Emission of methane and carbon dioxide and earthworm survival during composting of pharmaceutical sludge and spent mycelia. Bioresource Technology 97 (4):648–58.

MDDEP (Ministère du développement durable de l’environnement et des parcs). 2011. Politique québécoise de gestion des matières résiduelles—Plan d’action 2011–2015. Quebec, Canada: Government of Quebec.

MDDEP (Ministère du développement durable de l’environnement et des parcs). 2012a. Guide sur le recyclage des matières résiduelles fertilisantes—Critères de référence et normes réglementaires. Quebec, Canada: Government of Quebec.

MDDEP (Ministère du développement durable de l’environnement et des parcs). 2012b. Lignes directrices pour l’encadrement des activités de compostage. Quebec, Canada: Government of Quebec.

Mustin, M. 1987. Le compost, gestion de la matière organique. Paris: Éditions François Dubuc.

Ndegwa, P. M., and S. A. Thompson. 2001. Integrating composting and vermicomposting in the treatment and biocconversion of biosolids. Bioresource Technology 76 (2):107–12.

Ndegwa, P. M., S. A. Thompson, and K. C. Das. 2000. Effects of stocking density and feeding rate on vermicomposting of biosolids. Bioresource Technology 71 (1):5–12.

Neher, D. A., T. R. Weicht, S. T. Bates, J. W. Leff, and N. Fierer. 2013. Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times. PLoS One 8 (11):e79512.

Neuhauser, E. F., R. Harterstein, and D. I. Kaplan. 1980. Growth of the earthworm Eisenia fetida in relation to population density and food rationing. Oikos 35:93–98.

Ozawa, T., C. P. Risal, and R. Yanaigimoto. 2005. Increase in the nitrogen content of soil by the introduction of earthworms into soil. Soil Science and Plant Nutrition 51 (6):917–20.
Pedersen, J. C., and N. B. Hendriksen. 1993. Effect of passage through the intestinal tract of detritivore earthworms (Lumbricus spp.) on the number of selected Gram-negative and total bacteria. Biology and Fertility of Soils V16 (3):227–32.

Peigné, J., and P. Girardin. 2004. Environmental impacts of farm-scale composting practices. Water Air and Soil Pollution 153:45–68.

Pramanik, P., G. K. Ghosh, and P. Banik. 2009. Effect of microbial inoculation during vermicomposting of different organic substrates on microbial status and quantification and documentation of acid phosphatase. Waste Management 29 (2):574–78.

Pramanik, P., G. K. Ghosh, P. K. Ghosal, and P. Banik. 2007. Changes in organic—C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresource Technology 98 (13):2485–94.

Ryckeboer, J., J. Mergaert, J. Coosemans, K. Deprins, and J. Swings. 2003. A survey of biotical aspects of biomass during composting in a monitored compost bin. Journal of Applied Microbiology 94 (1):127–37.

Ryckeboer, J., J. Mergaert, K. Vaes, S. Klammer, D. De Clercq, J. Coosemans, H. Insam, and J. Swings. 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Annals of Microbiology 53 (4):349–410.

Sánchez-Monedero, M. A., A. Roig, J. Cegarra, and M. P. Bernal. 1999. Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. Bioresource Technology 70 (2):193–201.

Sangwan, P., C. P. Kaushik, and V. K. Garg. 2008. Vermiconversion of industrial sludge for recycling the nutrients. Bioresource Technology 99 (18):8699–704.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, and C. J. Robinson. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75 (23):7537–41.

Schloss, P. D., A. G. Hay, D. B. Wilson, and L. P. Walker. 2003. Tracking temporal changes of bacterial community fingerprints during the initial stages of composting. FEMS Microbiology Ecology 46 (1):1–9. doi:10.1016/S0168-6496(03)00153-3.

Sinha, R. K., S. Herat, S. Agarwal, R. Asadi and E. Carretero. 2002. Vermiculture and waste management: Study of action of earthworms Elsinea foetida, Eudrilus eugeniae and Perionyx excavatus on biodegradation of some community wastes in India and Australia. The Environmentalist 22:261–268.

Sinha, R. K., S. Herat, G. Bharambe, and A. Brahembhatt. 2010. Vermistabilization of sewage sludge (biosolids) by earthworms: Converting a potential biohazard destined for landfill disposal into a pathogen-free, nutritive and safe biofertilizer for farms. Waste Management & Research 28 (10):872–881.

Suthar, S. 2007. Nutrient changes and biodynamics of epigeic earthworm Perionyx excavatus (Perrier) during recycling of some agriculture wastes. Bioresource Technology 98 (8):1608–14.

Suthar, S. 2009. Vermicomposting of vegetable-market solid waste using Eisenia fetida: Impact of bulking material on earthworm growth and decomposition rate. Ecological Engineering 35 (5):914–20.

Tereshchenko, N. N., and N. N. Naplekov. 2002. Influence of different ecological groups of earthworms on the intensity of nitrogen fixation. Biological Bulletin 29 (6):628–32.

Tiago, I., I. Teixeira, S. Silva, P. Chung, A. Verissimo, and C. M. Manaia. 2004. Metabolic and genetic diversity of mesophilic and thermophilic bacteria isolated from composted municipal sludge on poly-epsilon-caprolactones. Current Microbiology 49 (6):407–14.

Tognetti, C., F. Laos, M. J. Mazzarino, and M. T. Hernández. 2005. Composting vs. vermicomposting: A comparison of end product quality. Compost Science and Utilization 13 (1):6–13.

Tringe, S. G., and E. M. Rubin. 2005. Metagenomics: DNA sequencing of environmental samples. Nature Reviews Genetics 6 (11):805–14.

Tripathi, G., and P. Bhardwaj. 2004. Comparative studies on biomass production, life cycles and composting efficiency of Eisenia fetida (Savigny) and Lampit mauritii (Kinberg). Bioresource Technology 92 (3):275–83.

Vartoukian, S. R., R. M. Palmer, and W. G. Wade. 2010. Strategies for culture of ‘unculturable’ bacteria. FEMS Microbiology Letters 309 (1):1–7. doi:10.1111/j.1574-6968.2010.02000.x.

Vaz-Moreira, I., C. Faria, A. R. Lopes, L. Svensson, E. Falsen, E. R. Moore, A. C. Ferreira, O. C. Nunes, and C. M. Manaia. 2009. Sphingobium vermicomposito sp. nov., isolated from vermicompost. International Journal of Systematic and Evolutionary Microbiology 59 (Pt 12):3145–49.

Vincelas-Akpa, M., and M. Loquet. 1995. Observation in-situ of the microflora in the gut of Eisenia-Fetida-Andrei (Lumbricidae). European Journal of Soil Biology 31 (2):101–10.

Wang, G., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73 (16):5261–67.

Xiao, Y., G.-M. Zeng, Z.-H. Yang, Y.-H. Ma, C. Huang, W.-J. Shi, Z.-Y. Xu, J. Huang, and C.-Z. Fan. 2011. Effects of continuous thermophilic composting (CTC) on bacterial community in the active composting process. Microbial Ecology 62 (3):599–608.

Yadav, K. D., V. Tare, and M. M. Ahammed. 2010. Vermicomposting of source-separated human faeces for nutrient recycling. Waste Management 30 (1):50–56.

Yasir, M., Z. Aslam, S. W. Kim, S. W. Lee, C. O. Jeon, and Y. R. Chung. 2009. Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. Bioresource Technology 100 (19):4396–403.