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Research Article

Survival Rates of Microbial Communities from Livestock Waste to Soils: A Comparison between Compost and Digestate

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Livestock waste-based products, such as composted manure, are often used in crop production systems. The products' microbial characteristics differ depending on animal waste treatment methods used (e.g., biogas production/composting). The question remains whether different livestock waste-based products differently impact soil microbiota. A pot experiment with five treatments (control, chemical fertilizer, digestate + chemical fertilizer, wheat straw compost + chemical fertilizer, and woodchip compost + chemical fertilizer) was conducted to compare the survival rates of microbial communities from digestate and composted manure, after their application to agricultural soil. Potatoes were planted in each pot. The changes in soil pH, the concentration of ammonium and nitrate, and the microbial community properties were monitored after 1, 6, 10, and 14 weeks of the application of livestock waste-based products. The application of composted manure, especially woodchip compost, showed a relatively more extensive impact on the soil microbial community structure than the other treatments. Woodchip compost contained a relatively more abundant and diverse bacterial community than digestate, and its family-level bacterial community structure was similar to that of the soil. These characteristics might determine the extent of the impact of livestock waste-based products on soil microbial communities. Digestate markedly influenced the inorganic nitrogen concentrations in soils but did not affect the soil microbial community. In conclusion, the survival rate of microbes of livestock waste-based products varies depending on the product type. Further investigation is needed to fully understand their impact on soils' microbial functions.

1. Introduction

The sustainable management of livestock waste is becoming more and more crucial with the increasing consumption of livestock products such as meat and milk on a global scale. Livestock waste is often applied to agricultural soils as livestock waste-based products such as composted manure and anaerobic digestate, the byproduct of biogas systems. Biogas systems are widely known as a cost-effective alternative to conventional fertilization (chemical fertilizer and composted manure), as the systems allow livestock farms to become self-sustaining in electrical energy supply [1, 2].

The chemical and physical benefits of the use of digestate and composted manure have been widely studied. The use of digestate as a fertilizer is considered beneficial, as it provides nutrients (nitrogen (N), phosphorus (P), and potassium (K)) and improves the soil structure with the addition of organic matter [3]. For example, the application of digestate has been shown to have limited effects on the soil organic carbon (C) dynamics [4, 5] but increases the soil nitrate (NO_3^--N) concentration [6] and reduces N_2O emissions [7, 8]. Similarly, the use of composted manure is recognized as beneficial because it increases soil organic matter content and decreases soil bulk density to mitigate the soil compaction issue [9]. The application of composted manure also results in an increase in soil pH, electrical conductivity, and plant-available P and NO_3^--N concentrations [10–12]. Thus, similar to chemical fertilizers, livestock waste-based products have some merits on the soil chemical properties, but the most important difference between chemical fertilizers

and livestock waste-based products is that the latter contains diverse microbial communities [13, 14].

The effects of microbes in livestock waste-based products to soils have to be evaluated as soil microbial properties are the indicators of soil quality due to their quick and sensitive responses to environmental changes and ecological relevance [15–19]. Microbial communities play important roles in soil processes (e.g., nutrient cycling and organic matter decomposition). Within studies on the soil microbiological properties after the application of livestock waste-based products, many focused on functional genes, such as antibiotic resistance genes [20–22], N-cycle-related genes [23–25], enzyme activities (dehydrogenase, nitrate reductase, β-glucosidase, and hydrolase; [26–28]), and microbial growth [29]. However, a few studies compared microbial communities between livestock waste-based products and soils (after their application) to have some idea on the survival of microbes from livestock waste in soils. Thus, to grasp the whole picture of the interaction between the two microbial communities, further studies are needed based on the evaluation of microbial communities in both the sources and the target.

More information is needed regarding microbes in livestock waste and soils, particularly in crop production systems, as most previous studies were performed for grassland systems [20, 29–31]. Few studies focused on the time-course changes in soil microbial communities with the growth of crops in soils after the application of livestock waste-based products. Also, the use of composted manure on crop farms has traditionally been performed in many regions, but the use of biogas digestate is still uncommon; thus, more information is needed to understand how crop soil microbial communities are affected when different livestock waste-based products are used.

Therefore, the aim of the present work was to study the impact of the application of digestate and two kinds of composted manure (wheat straw compost and woodchip compost) (different bedding materials) on agricultural soil microbial communities during the growth of potatoes (*Solanum tuberosum*). We conducted a pot experiment and monitored the chemical and microbiological parameters using molecular approaches (16S rRNA amplicon analysis) that provide information on the diversity and composition of soil microbial communities. Also, we examined the extent of the survival of microbes from livestock waste-based products in soils and the changes in microbial abundance in the soil after the addition of livestock waste-based products. We hypothesized that the magnitudes of the impact of livestock waste-based products on soil microbial communities could be explained by (1) the microbial properties of livestock waste-based products and (2) the changes in the chemical properties of the soil.

2. Materials and Methods

2.1. Soil, Livestock Waste-Based Products, and Chemical Fertilizer. For the experimental setup, agricultural volcanic ash soil was collected from Nakajimacho, Obihiro, Hokkaido, Japan (42°45′01.9″N, 143°08′20.4″E), in June 2019, from the edge of an experimental field, to minimize the impact of former applications of chemical fertilizers. The soil was brown Andosol and slightly acidic. To understand the soil's chemical properties, water content, pH, P_2O_5, and K_2O contents were measured by the Tokachi Federation of Agricultural Cooperatives. Also, the total C and N contents in the soil were measured within the Environmental Biochemistry laboratory at Hokkaido University, using an elemental analyzer (EA 2400 Series; Perkin-Elmer, Foster City, CA, USA). The determination of the inorganic-N concentrations (NO_3^--N and NH_4^+-N) in the soils was also performed in the same lab. Five grams of fresh soil was extracted with 15 mL of 10% KCl with 1 h shaking treatment, and the extractant was filtered through 1 μm filter paper (No. 5C filter paper; Toyo Roshi Kaisha Ltd., Tokyo, Japan) prior to the measurement. The inorganic-N concentrations in the soil were measured using colorimetric methods (Saltzman method for NO_3^--N [32] and indophenol method for NH_4^+-N [33]) with a flow injection analyzer (AQLA-700; Aqualab Co., Ltd., Tokyo, Japan) Table 1.

Anaerobic digestate and two kinds of composted manure (wheat straw compost and woodchip compost) were selected for experimental treatments. The digestate was obtained from a commercially operating biogas plant at Kamishihoro city, Tokachi region, Hokkaido (43°13′57.6″N 143°17′46.2″E). The source of the digestate was solely dairy cow manure, but it contained bedding materials from cowsheds such as wheat straw and woodchip as feedstock. The wheat straw compost was produced from beef cattle manure, produced by a farm in the same region where the digestate was produced. The wheat straw compost was not turned during the storage. The woodchip compost was produced by a dairy farm in the same region, but the woodchip compost was actively turned during the storage. Thus, when compared to the woodchip compost, the wheat straw compost was relatively more similar to raw manure. The characteristics of the livestock waste-based products were first analyzed by the Tokachi Federation of Agricultural Cooperatives (water content, pH, total C, total N, C/N ratio, P_2O_5, and K_2O). We further measured the inorganic-N concentrations (NO_3^--N and NH_4^+-N) in the livestock waste-based products using the same method for the soil, as described in the previous paragraph (Table 1).

The chemical fertilizer was obtained from Summit Agri-Business Corporation (Hokkaido, Japan). N/P/K fertilizer was applied by a fast-release fertilizer as NH_4NO_3 (guarantee of 21% as NH_4^+-N), Ca $(H_2PO_4)_2 \bullet H_2O$ (guarantee of 43% as P_2O_5), and K_2SO_4 (guarantee of 50% as K_2O), respectively.

2.2. Experimental Design and Sampling. Fresh soil (11.6 kg·pot^{-1}) was placed in 20 pots (18 L, diameter of 33.8 cm, depth of 35.1 cm). The pot was maintained at 15% wt. of gravimetric water content at the beginning of the experiment. Five types of treatments were applied with four replicates: (1) control without fertilizer (Con), (2) chemical fertilizer (N/P/K = 80/200/120 kg·ha^{-1}) (Che), (3) 40 t·ha^{-1} digestate + chemical fertilizer (P/K = 180/15 kg·ha^{-1}) (Dig), (4) 60 t·ha^{-1} wheat straw compost + chemical fertilizer (N/P/

K = 30/100/60 kg·ha^{-1}) (Whe), and (5) 60 t·ha^{-1} woodchip compost + chemical fertilizer (N/P/K = 30/100/60 kg·ha^{-1}) (Woo). The amount of chemical fertilizer was varied across different treatments to meet the crop's demands. For example, the chemical fertilizer used with the digestate did not contain N. The chemical fertilizer used with composts was approximately half of that in the chemical fertilizer-only treatment. We note that these variable chemical fertilizer application rates could be a factor controlling the survival rates of microbes from animal wastes to soils; however, unifying the chemical fertilizer application rates could cause nutrient toxicity effects for crops under some of our treatments; thus, it was not possible. The plant stresses could also be an independent factor influencing soil microbiota. Also, the adjustment of chemical fertilizer application rates based on the types of livestock waste-based products was commonly performed by local farmers where the soil was sampled (Hokkaido region, Japan). Thus, we believe that the approaches used in the current study were the best to evaluate the survival rates of microbes in the livestock waste-based products in the soil, after their application. The detailed application rates of the nutrients are listed in Table S1.

The time of application of livestock waste-based products was regarded as week 0 (Figure S1). On week 2, the chemical fertilizer was applied to each pot at 5 cm depth, and the potato seed was placed on top of the chemical fertilizer after the fertilizer was covered with a 1 cm layer of soil. The variety of the potato used was "Norin 1st." From each treatment, bulk soil was sampled at 0 (just before the application), 1, 6, 10, and 14 weeks after the application of livestock waste-based products. For sampling bulk soil, a soil sampler (ISIS Co., Osaka, Japan) was used. On week 14, rhizospheric soil was meticulously sampled from the root surfaces of the harvested potatoes. Also, on week 14, the above-ground plant and root biomass in each pot was measured after drying (65°C, 50 h), and the fresh potato biomass in each pot was measured. For soil chemical properties, bulk soil was used during the experiment. For soil microbial properties, bulk soil was used on weeks 0, 1, 6, and 10 and rhizospheric soil was used on week 14.

2.3. Measurement of the Soil Chemical Properties. The moisture content of the soil samples was measured by oven-drying the fresh soil at 100°C for >24 h and reweighing the dry soil. The pH of the soil samples was determined by mixing 5 g of the fresh soil with 25 mL deionized water, shaking for 1 h, and measuring the pH of the former solution using a pH sensor (AS800; As One Co., Osaka, Japan). The inorganic-N concentrations (NO_3^--N and NH_4^+-N) of the soil samples were measured using the same approach described in Section 2.1.

2.4. Soil DNA Extraction and Quantification by Quantitative Real-Time Polymerase Chain Reaction (PCR). Soil DNA was extracted from 0.35 g of the fresh soil sample or 0.5 g of the three kinds of livestock waste-based products (digestate, wheat straw compost, and woodchip compost) using NucleoSpin® Soil (Takara Bio, Inc., Shiga, Japan) according to the manufacturer's instructions (with SL2 buffer). For soil DNA extraction, 0.2 g skim milk was used to relieve the strong attachment of DNA to Andosol [34]. The extracts were stored in a freezer (−20°C) until further analysis.

To quantify the bacterial abundance in the soils and livestock waste-based products, quantitative real-time PCR was performed using the CFX96 Touch Real-time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The V4 region of the 16S rRNA gene was targeted to estimate the population size of each soil microbial group using the primer set (amplicon size ≈ 250 bp; forward primer 515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and reverse primer 806R: 5′-GGACTACHVGGGTWTCTAAT-3′). The PCR sample contained 10.4 μL Kapa SYBR® Fast Master Mix (2X) from the Kapa SYBR Fast qPCR Kit (Agilent Technologies, Palo Alto, CA, USA), 0.8 μL each of the forward and reverse primers, and 1 μL DNA extract and was made up to a final volume of 20 μL using nuclease-free water. The PCR cycling conditions were 600 s at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, and 60 s at 72°C. The annealing temperature for the qPCR was optimized to achieve the amplification efficiency that was the closest to 100%.

2.5. 16S rRNA Gene Amplicon Sequencing Analysis. For analyses of the bacterial community structures within the soils and livestock waste-based products, the extracted DNA was amplified targeting the V4 region of the 16S rRNA gene using the primer set (amplicon size ≈ 250 bp; forward primer 515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and reverse primer 806R: 5′-GGACTACHVGGGTWTCTAAT-3′). The PCR sample contained 10 μL AmpliTaq Gold® 360 Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 0.4 μL each of the forward and reverse primers, and 2 μL DNA extract and was made up to a final volume of 20 μL using nuclease-free water. The PCR cycling conditions were 600 s at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 57°C, and 60 s at 72°C. The annealing temperature was determined from the T_m values of the primers used. The amplification of the targeted region was confirmed by agarose gel electrophoresis. Then, the PCR products were quantified using the Quantus™ fluorometer (Promega Corporation, Madison, WI, USA). Purification was conducted using the Agencourt AMPure XP Kit (Beckman Coulter, Inc., Brea, CA, USA) according to the manufacturer's protocol. The DNA sample was then attached to the Ion Xpress™ P1 Adaptor and each Ion Xpress™ Barcode (Thermo Fisher Scientific) to make the Ion Torrent sequencing samples specific. The PCR sample contained 10 μL AmpliTaq Gold® 360 Master Mix (Thermo Fisher Scientific), 0.4 μL Ion Xpress™ P1 Adaptor, 0.4 μL Ion Xpress™ Barcode, and 2 μL DNA sample and was made up to 20 μL using nuclease-free water. The PCR conditions were 600 s at 95°C followed by five cycles of 30 s at 95°C, 30 s at 57°C, and 60 s at 72°C. The libraries were purified using the Agencourt AMPure XP Kit (Beckman Coulter). The final length and concentrations of the amplicons were checked using a Bioanalyzer High Sensitivity DNA Kit (Agilent Technologies). The library was then diluted to 50 pM, and

25 μL of the targeted dilution library was loaded into the Ion 318 Chip (Thermo Fisher Scientific). Emulsion PCR was conducted using the Ion Personal Genome Machine (PGM™) Hi-Q™ View Chef Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. After the recovery of the ion spheres and enrichment, sequencing was performed using the Ion PGM™ Hi-Q™ View Sequencing Kit and Ion PGM™ (Thermo Fisher Scientific). Sequence data were deposited in the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) under accession number PRJNA663956.

The analyses for the obtained sequence data were conducted using the Quantitative Insights into Microbial Ecology 2 (QIIME2) software package version 2019.7 [35]. Sequence quality control was completed using the DADA2 pipeline incorporated into QIIME2. The clustering of operational taxonomic units (OTUs) at 97% sequence similarity was conducted using the SILVA database (release 132). To equalize the sampling effort, all samples were rarefied to 4,898 sequences (defined herein as the rarefied OTU table) based on the retained OTU table. For all microbial analyses, except for the volcano plot, as described in the next section, the rarefied OTU table was used. The Shannon diversity index was calculated by QIIME2.

2.6. Statistical Analyses. All statistical analyses were performed using R software version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). In all tests, $P < 0.05$ was considered statistically significant.

For the soil chemical and biological properties, the data were tested for the normality using Shapiro–Wilk tests with the P-value threshold of 0.05. The data were not normally distributed according to the Shapiro–Wilk tests; thus, we performed a nonparametric Kruskal–Wallis one-way analysis of variance for the data for each sampling week. When the significance based on the Kruskal–Wallis test was significant ($P < 0.05$), we performed a post hoc test using the pairwise test for multiple comparisons of mean rank sums (Nemenyi tests). This approach was taken for the data of NH_4^+-N concentration, NO_3^--N concentration, pH, bacterial gene abundance in soil, and Shannon diversity indices. For the plant biomass, the effects of the treatments were examined using one-way ANOVA followed by the Tukey–Kramer test.

For microbial community structures, nonmetric multidimensional scaling (NMDS) analysis of the community structure dissimilarity based on the Bray–Curtis index at the OTU level was performed to visually understand the differences of the soil microbial community structures among treatments at each sampling time via the "metaMDS" function in the vegan package of R. Permutational multivariate ANOVA was used to test the significance of the treatments using the "adonis" function of the R vegan package. Also, to illustrate the significant correlations among experimental soil properties with the NMDS values of points as vectors on NMDS ordination, the "envfit" function in the vegan package was used.

To compare the similarity and dissimilarity in OTUs among Che, livestock waste-based product treatments (Dig, Whe, or Woo), and each livestock waste-based product sources (digestate, wheat straw compost, and woodchip compost) on weeks 1, 6, and 10, Venn diagrams with unique and shared OTUs were generated using in "venn.diagram" function in VennDiagram package of R. The OTUs with the relative abundance of 0% in one of the treatments were removed before the analysis. Also, to investigate significantly increased OTUs in livestock waste-based product treatments (Dig, Whe, or Woo) compared to Che on weeks 1, 6, and 10, univariate statistical analysis was performed with the row OTU table using the "DESeq" function in R. Additionally, the microbiota in the original soil and those in each livestock waste-based product were compared at the family level, using Venn diagrams. The families with the relative abundance of less than 1% in one of the treatments were removed before the analysis.

3. Results

3.1. Soil Chemical Properties. NH_4^+-N concentrations significantly differed among treatments on week 1 ($P < 0.01$), week 6 ($P < 0.05$), week 10 ($P < 0.05$), and week 14 ($P < 0.01$; Figure 1(a)). On week 1, Che and Dig (9.15 and 74.8 mg N·kg^{-1} dry soil, respectively) had significantly higher values compared to Con (4.11 mg N·kg^{-1} dry soil; $P < 0.05, 0.01$, respectively). Also, on week 6, Che and Dig (14.8 and 14.7 mg N·kg^{-1} dry soil, respectively) showed significantly higher value compared to Con (8.65 mg N·kg^{-1} dry soil; $P < 0.05$). On week 10, Whe and Che showed significantly higher NH_4^+-N values compared to Woo. On week 14, Con and Woo showed significantly higher NH_4^+-N values when compared to Che.

NO_3^--N concentrations differed significantly among treatments on week 10 ($P < 0.05$) and week 14 ($P < 0.01$; Figure 1(b)). The NO_3^--N concentration decreased with plant growth period in all treatments, except Dig. In Dig, the NO_3^--N concentration reached a peak on week 6 (145 ± 79.8 mg N·kg^{-1} dry soil) and decreased to 8.02 ± 1.36 mg N·kg^{-1} dry soil on week 14.

In all treatments, soil pH increased with plant growth period, although it differed significantly among treatments on week 1 ($P < 0.01$), week 6 ($P < 0.01$), week 10 ($P < 0.01$), and week 14 ($P < 0.05$; Figure 1(c)). On week 1, soil pH in Dig was significantly higher than that in Con and Che ($P < 0.05$). However, on week 6, soil pH in Dig showed a significantly lower value compared to that in Whe and Woo ($P < 0.05$). Also, on week 10, soil pH of Dig and Woo was significantly different ($P < 0.01$). On week 14, soil pH in Che was significantly higher compared to that in Con ($P < 0.05$).

3.2. Bacterial Gene Abundance. In all treatments, the bacterial gene abundance increased along with plant growth; however, there was no significant difference in the bacterial gene abundance among treatments on each week (Figure 1(d)).

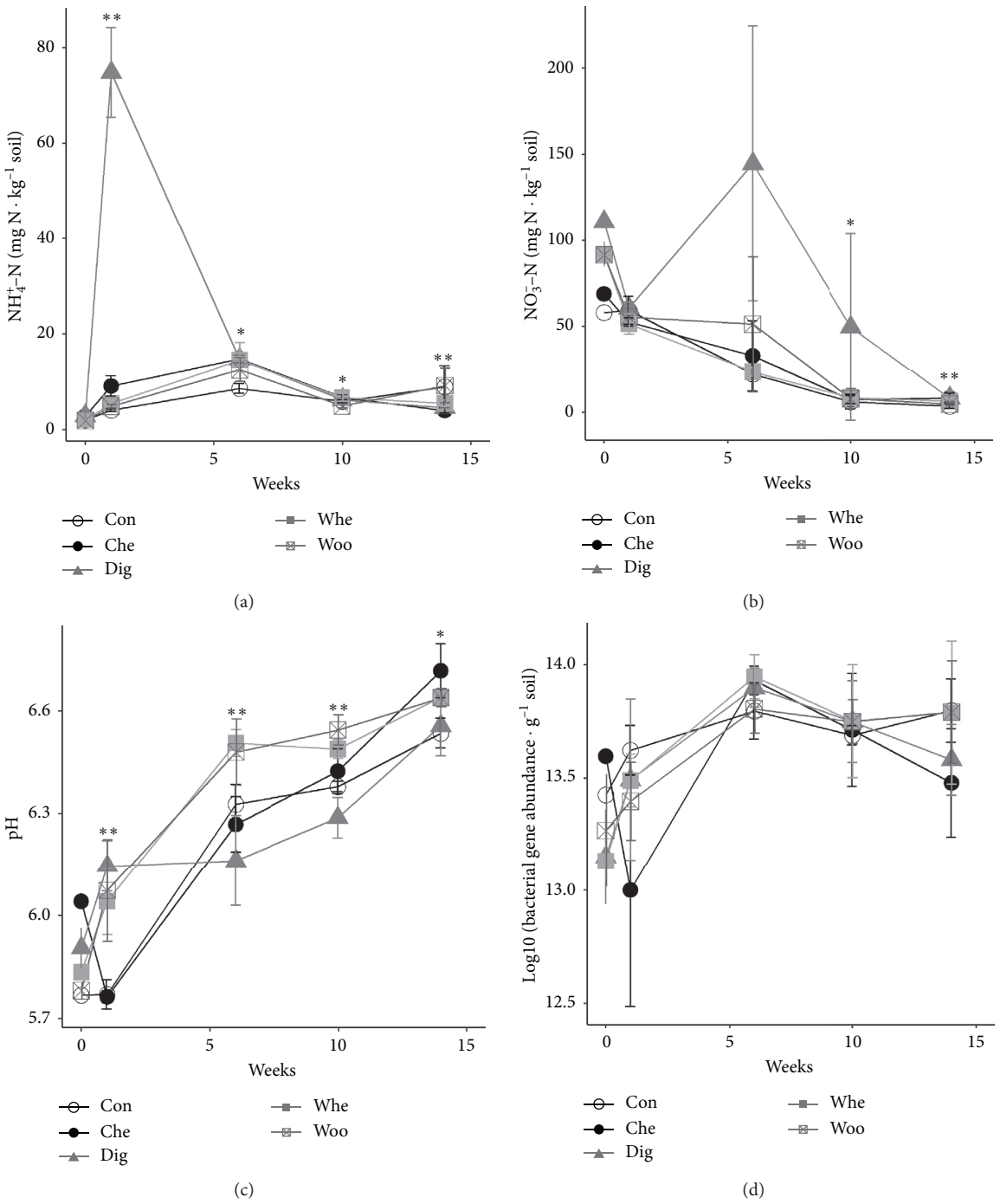

Figure 1: Changes in chemical and microbial properties in soils among treatments: control without fertilizer (Con), chemical fertilizer (Che), digestate + reduced chemical fertilizer (Dig), wheat straw compost + reduced chemical fertilizer (Whe), and woodchip compost + reduced chemical fertilizer (Woo). (a) NH_4^+-N concentrations, (b) NO_3^--N concentrations, (c) pH, and (d) log10 (bacterial gene abundance·g^{-1} dry soil) during the 14 weeks pot experiment. Data are shown as mean ± SD (n= 4). The results from the Kruskal–Wallis test were shown above values on each week of each figure. "∗∗" and "∗" stand for the significance level of $P < 0.01$ and $P < 0.05$, respectively.

The bacterial gene abundance in each livestock waste-based product source (digestate, wheat straw compost, and woodchip compost) applied to soils was 8.35×10^{14}, 7.75×10^{16}, and 5.04×10^{16} copies·pot^{-1}, respectively, and that in original soils before the application of livestock waste-based products was 2.40×10^{17} copies·pot^{-1} (Table 2). This result showed that the bacterial gene abundance applied from digestate was about one-hundredth of that from wheat straw compost and woodchip compost and one-thousandth of that from soils.

Table 1: Chemical properties in soils ($n = 20$) and livestock waste-based products (digestate, wheat straw compost, and woodchip compost) ($n = 3$) used in the pot experiment (mean ± SD).

	Soil	Digestate	Wheat straw compost	Woodchip compost
Water content (%)	12.08 ± 3.38	98.4 ± 0.06	81.5 ± 0.37	57.3 ± 0.27
pH	6.3 ± 0.0	7.85 ± 0.01	9.04 ± 0.03	7.35 ± 0.01
Total C (%)	4.00 ± 0.39	—	6.81 ± 0.20	16.9 ± 0.71
Total N (%)	0.38 ± 0.02	0.22 ± 0.00	0.54 ± 0.01	0.80 ± 0.06
C/N ratio	10.6 ± 1.71	—	12.6 ± 0.20	21.2 ± 2.26
P_2O_5 (%)	—	0.03 ± 0.00	0.41 ± 0.00	0.42 ± 0.04
K_2O (%)	—	0.27 ± 0.00	0.99 ± 0.03	0.69 ± 0.01
NH_4^+-N (mg·kg^{-1} wet soil)	1.93 ± 0.48	1,200 ± 302	100 ± 57.8	21.8 ± 2.80
NO_3–N (mg·kg^{-1} wet soil)	68.0 ± 16.3	2.50 ± 0.427	8.48 ± 2.89	354 ± 31.0

3.3. Characteristics of the Microbial Community Structure at the Phylum-Level. A total of 3,760,224 16S rRNA sequences were generated from the 100 soil samples (5 sampling times × 5 treatments × 4 replicates) and 9 livestock waste-based product samples (3 kinds × 3 replicates) in this study (sequence range, 4,898–143,956) and clustered into 7,528 OTUs at 97% sequence similarity. After equalizing the sampling effort, 533,882 sequences were retained for the analysis. These sequences were clustered into 6,384 OTUs at 97% sequence similarity, with 173 to 1,018 OTUs per sample.

For the soil microbial community structures, phyla Proteobacteria and Actinobacteria, of which relative abundance was 25.0% and 19.7% (averaging across the treatments), respectively, dominated within the total soil samples (Figure S2A). On week 1, Dig, Whe, and Woo had a relatively larger abundance of Chloroflexi (16.6%, 20.2% and 25.3%, respectively) than that of Che (8.56%). A similar fact was observed for the relative abundance of Thaumarchaeota (3.35%, 4.35%, 5.08%, and 1.29% for Dig, Whe, Woo, and Che, respectively). Contrastingly, the relative abundance of Acidobacteria in the livestock waste-based products (12.4%, 8.65%, and 9.80% for Dig, Whe, and Woo, respectively) was suppressed when compared to Che (16.2%). The relative abundance of Planctomycetes in the livestock waste-based products (4.78%, 2.52%, and 2.95% for Dig, Whe, and Woo, respectively) was also lower than that of Che (6.86%). As a difference among the livestock waste-based products, Whe showed a larger abundance of Bacteroidetes (3.60%) compared to other treatments (1.60–2.00%) on week 1. However, after week 6, the microbial community structures among different treatments became similar.

Based on the observation of the bacterial communities within livestock waste-based product sources, woodchip compost had a similar microbial community structure to the soils, with relatively higher proportions of Proteobacteria (25.0%) and Actinobacteria (12.4%) and lower percentages of Acidobacteria (8.98%; Figure S2B). Digestate and wheat straw compost had similar bacterial communities composed of the three most abundant phyla, Firmicutes (39.0% and 12.3%, respectively), Proteobacteria (28.0% and 29.4%, respectively), and Bacteroidetes (17.4% and 26.7%, respectively). Also, based on the family-level analyses, we found that the microbiota in the woodchip compost was the most similar to that in the original soil (Figure S3). Within the total bacterial abundance in the woodchip compost, more than 65% belonged to the families observed also in the soil. The same percentage values were 21.9% and 26.4% for the wheat straw compost and the digestate, respectively. The list of the family names is stated in Table S2–S4.

3.4. OTU-Level Analysis of the Microbial Community Structures. NMDS results revealed that the soil microbial community structures were significantly influenced by the treatments on week 1 ($P < 0.05$), week 6 ($P < 0.05$), and week 10 ($P < 0.01$; Figure 2). Soil pH was selected as a significant explanatory variable on week 1 ($P < 0.05$) and week 10 ($P < 0.001$). Woo always had a unique cluster apart from other treatments during the experiment, whereas Whe showed a unique cluster only on week 1. Dig always had a similar cluster to Che. On week 14, all samples, except Woo, were clustered closely together.

Che was also compared to one of the livestock waste-based product treatments. On week 1, the OTU numbers shared between one of livestock waste-based product treatments (i.e., Dig, Whe or Woo) and Che were 923 (896 + 27), 825 (809 + 16), and 807 (770 + 37), respectively (Figures S4A–S4C). These OTUs' relative abundance within the total bacterial abundances in Dig, Whe, and Woo was 87.9% (83.1 + 4.79%), 77.7% (74.7 + 3.00%), and 82.6% (78.6 + 4.01%), respectively (Figures S4A–S4C). At week 10, the same values (the relative abundance of the shared bacterial community between "Che and Dig," "Che and Whe," and "Che and Woo") increased to 93.8% (84.8 + 8.97%), 91.4% (90.6 + 0.842%), and 87.1% (83.8 + 3.31%) (Figures S4G–S4I). Also, based on the comparisons between the soils, after the application of livestock waste-based products and the products themselves, Woo and woodchip compost shared a relatively larger number of OTUs (Figures S4C, S4F, and S4I) compared to other pairs (Whe with wheat straw compost and Dig with digestate) when averaged across the sampling time. Whe and wheat straw compost shared 198 OTUs immediately after the application, but this number decreased to 22 on week 10 (Figures S4B, S4E, and S4H). For Dig with digestate, the number and abundance of the shared OTUs were the lowest among the pairs (14–44 OTUs; Figures S4A, S4D, and S4G). The community structures of the unique OTUs appeared

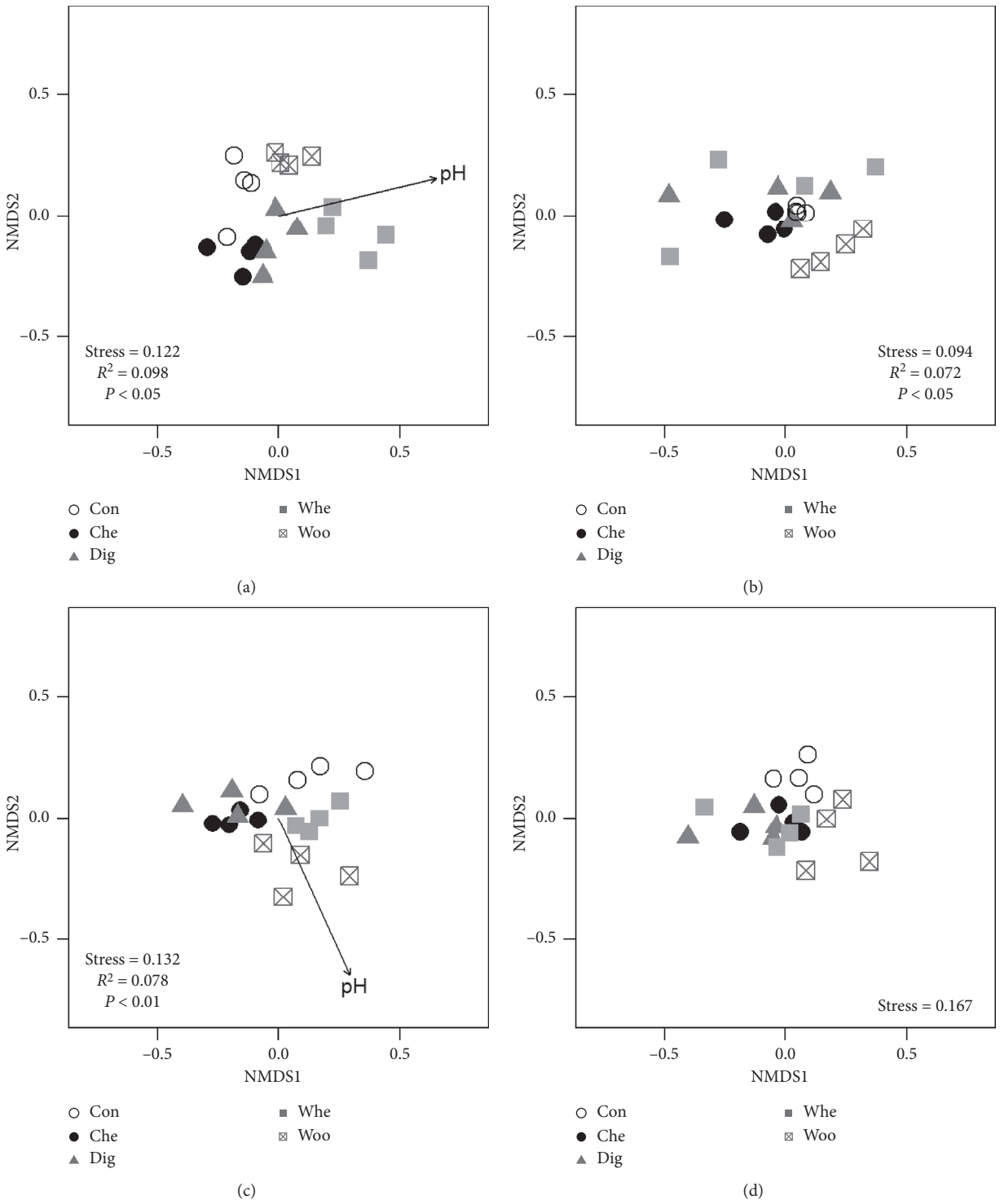

Figure 2: Nonmetric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities of microbial community structures in soils ($n = 4$) among treatments at four sampling times: (a) week 1, (b) week 6, (c) week 10, and (d) week 14. Analysis of similarity (permutational multivariate ANOVA) statistic R and its significance level are indicated. The arrows in (a) and (c) show the direction of the significant environmental factors ($P < 0.05$ and $P < 0.01$, respectively) obtained by fitting environmental factors in the ordination space of samples.

both in the soils with livestock waste-based products and the products themselves are shown in Figure S5. These suggested that unique OTUs maintained similar phylum-level balances to the products themselves (Figure S2B).

For OTUs that showed significant changes in their abundance based on the comparisons between Che and one of the livestock waste-based products treatments (Dig, Whe, or Woo), the pair of Che and Woo showed extended

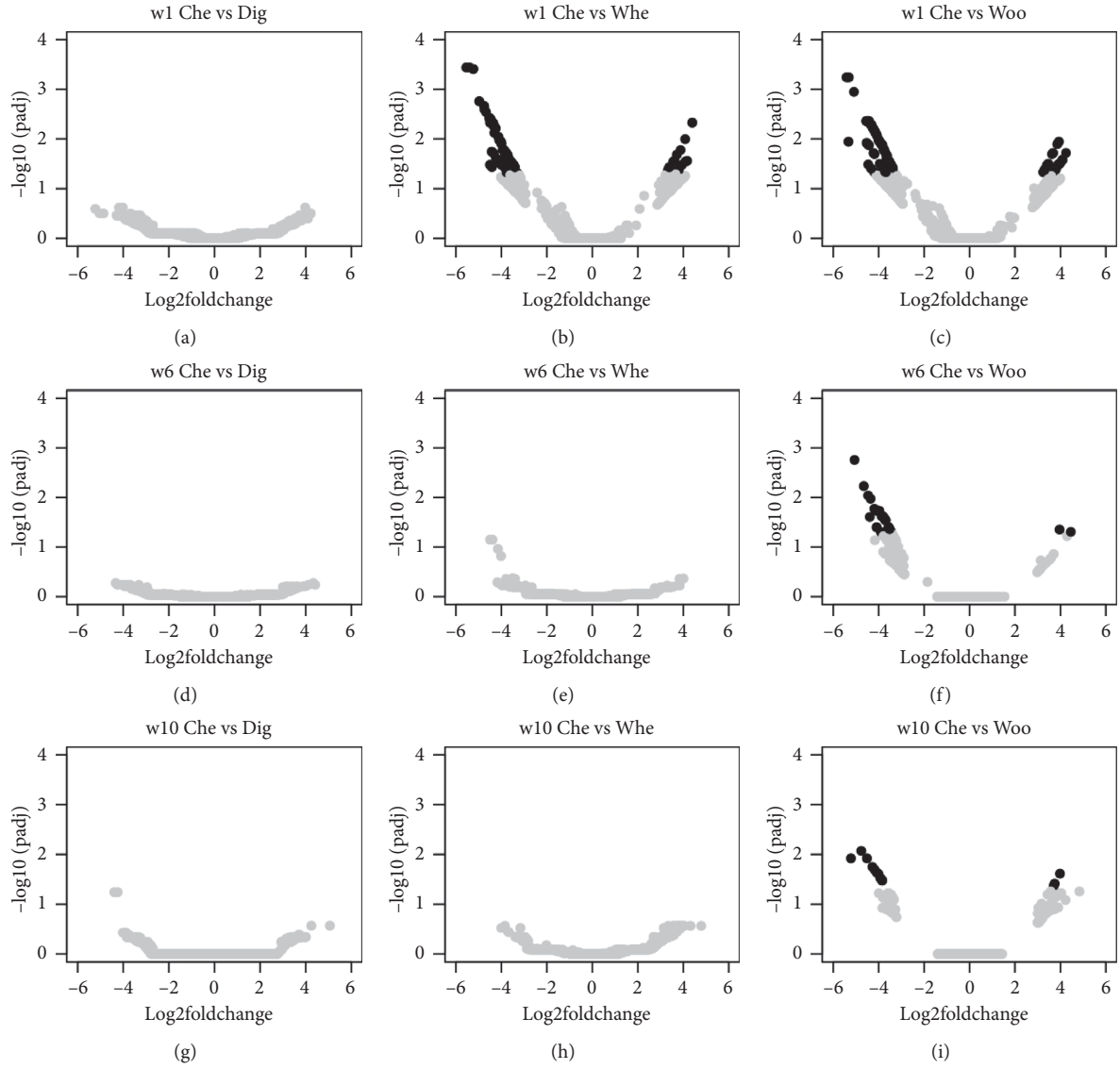

Figure 3: Volcano plots of differences in OTUs abundance between (a) Che and Dig, (b) Che and Whe, and (c) Che and Woo on week 1. Same pairs of the treatments on different timings are shown in (d), (e), and (f) (week 6) and in (g), (h), and (i) (week 10). Volcano plots showing the distribution of OTUs abundance according to the adjusted P value (−log10 scale) and the log twofold change between two treatments ($n = 4$). Black nodes in the right side represent OTUs significantly more abundant in the treatments with livestock waste-based products, while the black one in the left side corresponded to OTUs more abundant in Che.

differences (13–90 OTUs) throughout the experimental period (twofold change >2; $P < 0.05$; Figures 3(c), 3(f), and 3(i); Tables S5–S7). In contrast, 90 OTUs showed significant differences in Whe only on week 1 (twofold change >2; $P < 0.05$; Figure 3(b); Table S8), and no significant changes were observed in Dig (Figures 3(a), 3(d), and 3(g)) compared to Che.

The taxonomy of the significantly affected OTUs found in the previous paragraph was further analyzed. On week 1, phyla Planctomycetes (5/13 and 5/21 OTUs, respectively) and Proteobacteria (3/13 and 4/21 OTUs, respectively) were increased in Whe and Woo compared to Che, whereas Chloroflexi (18/77 and 22/69 OTUs, respectively) and Proteobacteria (14/77 and 18/69 OTUs, respectively) were decreased in Whe and Woo compared to Che (Table S5 and S8). At the family level, four families were significantly increased in Whe and Woo (i.e., Planctomycetaceae, Anaerolineaceae, Hyphomicrobiaceae, and Oxalobacteraceae) and seven families were decreased in Whe and Woo (i.e., Oxalobacteraceae, Roseiflexaceae, Marine Benthic Group D and DHVEG-1, Hyphomicrobiaceae, Gemmatimonadaceae, Chitinophagaceae, and Planctomycetaceae) compared to Che. Exceptionally, Whe increased the family Cystobacteraceae and decreased Marinilabiaceae and Methanobacteriaceae, and Woo increased Nitrosomonadaceae and decreased Trueperaceae and Xanthomonadales Incertae Sedis compared to Che.

On week 6 in Woo, two OTUs were significantly more abundant (phyla Cyanobacteria and Proteobacteria), and the families Xanthomonadaceae and Anaerolineaceae were less abundant compared to Che (Table S6). On week 10 in Woo,

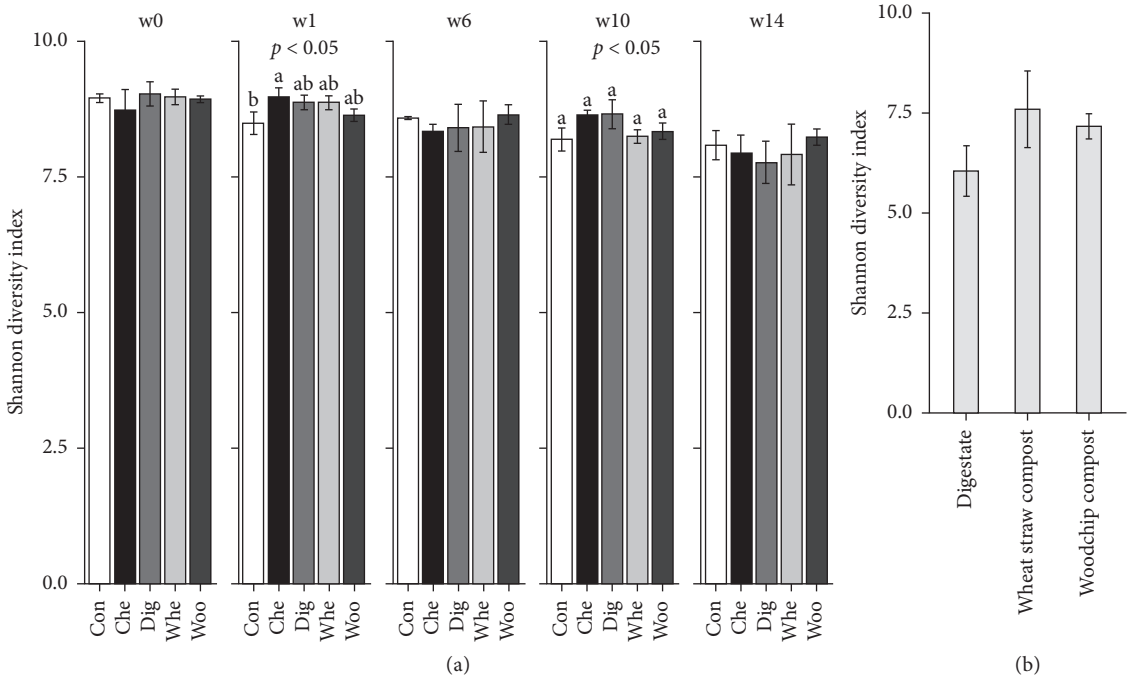

Figure 4: Shannon diversity index detected in (a) sampled soils ($n = 4$) and (b) livestock waste-based product sources (digestate, wheat straw compost, and woodchip compost) ($n = 3$). In (a), data are divided by sampling time: w0, week 0; w1, week 1; w6, week 6; w10, week 10; and w14, week 14. The Kruskal–Wallis test resulted in the significant differences among the treatments on weeks 1 and 10; thus, the results based on the pairwise comparison tests were shown in different alphabets. Data are shown as mean ± SD.

three OTUs were significantly more abundant (phyla Acidobacteria, Armatimonadetes, and Proteobacteria), and the families Anaerolineaceae and Caldilineaceae were less abundant compared to Che (Table S7).

In all treatments, the Shannon diversity index decreased with plant growth period. The significant differences among treatments were observed on week 1 ($P < 0.05$) and week 10 ($P < 0.05$; Figure 4(a)). On week 1, Che showed a significantly higher value of diversity than Con ($P < 0.05$). On week 10, there was no significant difference among treatments, despite the significant effect of the treatments by the Kruskal–Wallis test. Also, livestock waste-based products themselves had a relatively lower value than soil (on week 0; Figure 4(b)). In livestock waste-based product sources, digestate showed a relatively lower value than wheat straw compost and woodchip compost.

3.5. Plant Biomass. The above-ground plant dry weight and potato fresh weight significantly differed among treatments ($P < 0.001$; Figures 5(a) and 5(c)). The above-ground plant dry weight and potato fresh weight in Che, Dig, Whe, and Woo were significantly higher than those in Con. In contrast, there was no significant change in the root dry weight among treatments (Figure 5(b)).

4. Discussion

4.1. Woodchip Compost Had a Stronger Impact on Soil Microbes Compared to Other Livestock Waste-Based Products. The survival of microbes in woodchip compost in Woo occurred relatively more extensively compared to the other treatments, as suggested by the larger number of OTUs shared between Woo and the original livestock waste-based product source (woodchip compost) than Whe and Dig (Figures S4C, S4F, and S4I). These results suggested that the microbes in woodchip compost had the strongest impact on the soil microbial community. This fact was also supported by the appearance of unique clusters apart from other treatments in the NMDS analysis (Figure 2) and stronger changes in the abundance of OTUs compared to Che (Figures 3(c), 3(f), and 3(i)). One of the reasons for the stronger impact of woodchip compost on the soil bacterial communities is that a relatively larger number of microbes are applied to the soil as compost (Table 2). Also, the compost holds relatively more diverse bacterial communities than digestate (Figure 4(b)). These facts were common for both woodchip and wheat straw compost; however, the family-level similarity between the original soil bacterial community and the bacterial community in woodchip compost was the strongest among the livestock waste-based products used in this study (Figure S3), and this might be a reason for better survival of woodchip compost-derived microbiota in the soil, compared to wheat straw compost. Previous reports observed that the higher the diversity of microbial communities is, the greater the resilience against exposure to different microorganisms they have [36, 37]. Our study also highlighted that the family-level similarity of the two microbiota could be a factor controlling the survival of livestock waste-based products' microbiota in soil.

The environmental parameters of woodchip compost (e.g., pH and water content) were similar to the soil compared to the other livestock waste-based products (Table 1). This fact might

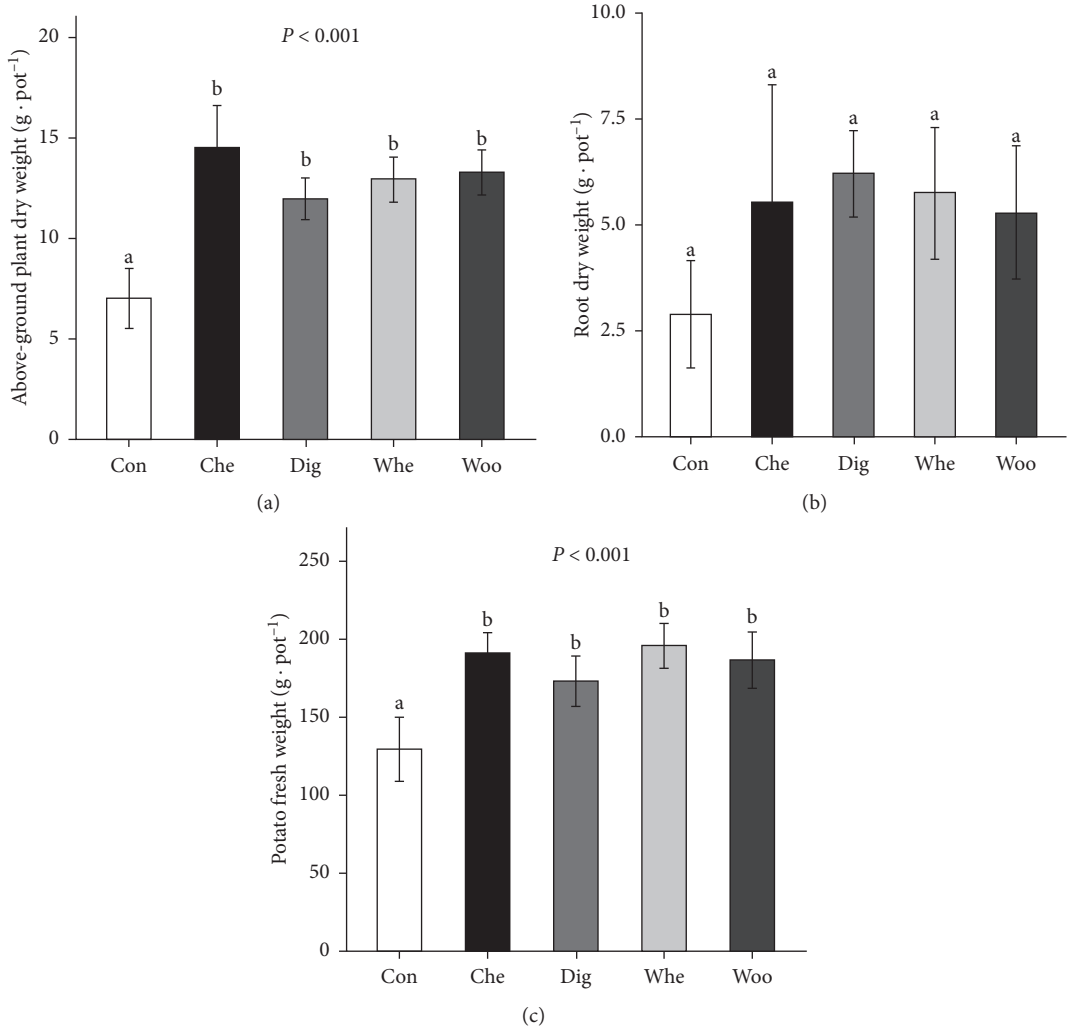

Figure 5: Effects of different treatments on potato plants traits at harvest (week 14). (a) Above-ground plant dry weight, (b) root dry weight, and (c) potato fresh weight. The significant results of one-way ANOVA are shown in the upper right. Lowercase letters indicate a statistically significant difference between tested average values by the Tukey–Kramer test. Data are shown as mean ± SD ($n = 4$).

Table 2: Bacterial gene abundance included in original soils in each pot and livestock waste-based products applied to each pot.

	Soil	Digestate	Wheat straw compost	Woodchip compost
Bacterial gene abundance (copies·g^{-1})	2.07×10^{13}	4.26×10^{12}	2.64×10^{14}	1.71×10^{14}
Applied weight (g·pot^{-1})	11,600	196	294	294
Applied bacterial gene abundance (copies·pot^{-1})	2.40×10^{17}	8.35×10^{14}	7.75×10^{16}	5.04×10^{16}

be a reason for the similar phylum-level balances between the soil and woodchip compost. As a previous study suggested, environmental parameters, such as soil pH and water content, influence the microbial community structure and composition [38, 39]. The survival of microbes in compost and digestate has been widely studied in relation to the survival of pathogenic bacteria during composting and anaerobic digestion processes [40, 41].

4.2. Similar Changes in Microbial Abundance between Compost Treatments against Chemical Fertilizer Treatment for a Short Period. The details of the changes in each OTU by the application of livestock waste-based products (compared to Che) were shown by the volcano plots. Interestingly, common families were significantly increased in Whe and Woo for a short period compared to Che (Tables S5 and S8). Within those families, members of Anaerolineaceae, organic matter degraders under anoxic conditions and coexisting microbes with methane producers [42], and members of Hyphomicrobiaceae, capable of NO_3^--N denitrification through N_2 and utilize N_2, NO_3^-, or NH_3 [43–45], were detected. Moreover, Whe increased the members of genus Anaeromyxobacter, which can fix atmospheric-N [46]. Woo increased the members of Nitrosomonadaceae, lithoautotrophic NH_3 oxidizers to nitrite, which is subsequently

oxidized by bacterial nitrite oxidizers to NO_3^- [47]. These results suggested that N-cycle-related microorganisms were activated by the addition of compost, possibly through the degradation of organic matters. Further studies are needed to know whether these microbial changes influenced the N-use efficiency of the crops and the N loss processes (NO_3^- leaching and gaseous N losses) in soils.

4.3. Differences between Digestate and Compost regarding Their Impact on Soil Biology and Chemistry. Significant fluctuations in inorganic-N concentrations were observed in Dig (Figures 1(a) and 1(b)) compared to compost treatments (i.e., Whe and Woo). NH_4^+-N concentrations dramatically peaked on week 1 in Dig and remarkably decreased with a high elevation of NO_3^--N concentrations along with the experimental period compared to the other treatments. The large variability of NO_3^--N in Dig might have been partly due to nonuniform distribution of the digestate in the soils. Although we mixed the soil thoroughly following the application of the digestate, the high moisture level of the digestate made it difficult to uniformly mix the soil and the digestate. Further studies are needed to test whether the nonuniform distribution of NO_3^--N is happening at the field level or not. It was reported that the addition of NH_4^+-N negatively influenced the diversity of soil bacterial communities [48], but this was not the case in the current study. Regarding the microbial diversity, previous reports showed digestate induced a slight increase after 60 days of pot experiments with wheat crop [49] or digestate could promote the microbial diversity compared to the treatment with a chemical fertilizer during a 128-day glasshouse experiment with wheat crop [50]. In contrast, Johansen et al. [6] showed minor microbial community changes after 9 days of incubation with digestate. Coelho et al. [31] showed no detectable impact after 2 years in a ryegrass-dominated grassland after the application of digestate. These results suggested that digestate's impact on soil microbial diversity varied among previous studies. As stated, the abundance of microbes added to soils from digestate was markedly smaller than those of compost in the current study. This can be one of the main reasons for a relatively minor impact of digestate on the soil microbial communities, although further studies are needed.

Another reason for the relatively lower survival rate of the digestate's microbiota in soils could be the water contents of the digestate and anaerobic conditions when the digestate was produced. Unlike the composts, the digestate was processed as a high moisture material and under an anaerobic condition for an extensive period. Thus, anaerobic bacteria dominated in our digestate, with agreement to a previous study [51], such as Peptostreptococcaceae and Ruminococcaceae. Also, families found mainly in gastrointestinal tracts (e.g., Rikenellaceae) dominated in the digestate. As soon as the digestate was applied to soils, the microbiota within the digestate faced the aerobic conditions in soils. Thus, the physical status of the organic materials (e.g., moisture) could be a factor controlling the survival rates of the organic material-derived microbiota in soils after their application.

For the woodchip compost, the survival rates of its microbiota in the soil, after the application, was the highest among the three materials used in the current study. Regarding the survival rates of compost-derived microbes in soils, most of the previous studies focused on the survival of specific pathogenic bacteria (e.g., *E. coli*), but a previous study noted that having the similar levels of nutrient levels between the source and host is an important factor controlling the survival of *E. coli* in the compost in soils, after the application with livestock waste-based materials [52]. The woodchip compost's pH was the closest to that of the soil, and the inorganic-N was dominated by NO_3^--N, similar to the soil (Table 1), suggesting that the woodchip compost was produced under an aerobic condition. These similarities of the physicochemical conditions between the woodchip compost and the soil might have promoted the survival rates of the microbiota in the woodchip compost in the soil.

We also note that the magnitude of the impact of digestate and compost on soil biology and chemistry depends on soil characteristics, such as pH buffering capacity of the soil. The soil used in the current study (Andosol) is characterized by a large pH buffering capacity [53], and this might be related to the relatively smaller survival rate of microbes from digestate in the soil. This was because the pH of the digestate was the highest among the livestock waste-based materials used in the current study (Table 1), whereas the soil pH maintained within the range of 5.5 to 7.0 (Figure 1(c)). If the soil pH was largely influenced by the digestate and increased from an acidic to alkali level, the survival rates of digestate-derived microbiota could be larger, although this needs to be confirmed with further studies.

4.4. General Impact of Livestock Waste-Based Products on Soils and Crop Production. In the experimental setup, livestock waste-based products did not increase the microbial populations compared to the use of chemical fertilizer (Figure 1(d)), although the microbial community structure was changed by the application of livestock waste-based products. This result was consistent with previous reports that showed digestate application had a low influence on soil microbial numbers [30, 31], although not in line with a report that showed livestock waste-based products treatments had a positive effect on the abundance of soil organisms [54]. Another previous study concluded that digestates and cow manures temporarily activated soil microbes, after their application, by supplying readily available C, but the soil respiration rates peaked within 50 hours after the application and then quickly decreased for both materials [55]. The same study also stated that the utilization rate of C (the proportion of added livestock waste-derived C to respired-C from soils during an incubation experiment for 12 days) did not differ between digestates and composts. Thus, the soil sampling frequency used in the current study (weekly to monthly) might not highlight the short-term increase in microbial abundance, even if it occurred. Thus,

due to the rapid utilization and depletion of readily available C from livestock waste-based products, they cannot be used to increase soil microbes for this soil, for the experimental period used in the current study (14 weeks). However, we note that many previous studies reported the long-term (e.g., decades) positive impacts of the use of livestock waste-based products on soil microbial biomass [26, 56]; thus, further research is necessary to evaluate the long-term impacts of the different livestock waste-based products on soil microbial biomass, although the impact was not clear in the current experiment, conducted only for one crop growth season.

All livestock waste-based products used here showed relatively lower microbial diversity values than the original soil (Figure 4). This result was consistent with a previous report that compared the differences between the horse manure-derived or chicken manure-derived amendments and the amended soils [57], which means that the soil ecosystem has extremely high biodiversity. With the growth of potatoes, a gradual decrease in bacterial communities' diversity was observed in all treatments (Figure 4). Thus, for the soil used in the current study (a C-rich volcanic soil), livestock waste-based products could not be used to increase its microbial diversity. This might be because the plant growth stage was the strongest controlling factor for soil microbiota in our study, rather than the added livestock waste-based products. This agrees to a previous study that observed the time course changes in soil microbiota during the growth of tobacco crops [58]. However, the impact of the plant growth stage on the soil microbiota depends on the crop types, according to a previous study comparing pea, wheat, and sugar beet [59]. Thus, to fully evaluate the impact of livestock waste-based products on soil microbial diversity, factors such as crop types and seasonal fluctuations of the diversity indices need to be further considered.

Regarding plant growth, all livestock waste-based product treatments showed the same level of growth compared to the chemical fertilizer treatment (Figures 5(a) and 5(c)). This result is consistent with previous reports by Alburquerque et al. [60], who showed that digestate application to soil led to yields comparable to the inorganic fertilization for the summer watermelon crop, and Eghball and Power [61], who concluded beef cattle feedlot manure and compost can be effectively utilized in no-till corn production systems when the correct N availability factor is used. Although more field-level experiments are needed to be performed to confirm the benefits of the livestock-based products regarding the crop productivity, our study could help to promote the transition from an agricultural production based on chemical inputs to a more sustainable paradigm in which agricultural wastes can be recycled.

5. Conclusions

The survival rates of the microbes originated from the woodchip compost in soils were relatively higher than those of other livestock waste-based products. The number of bacteria applied to the soils from livestock waste-based products was approximately 100-fold higher for compost than digestate when applied with a commonly practiced rate. The microbial communities in compost were more diverse than in digestate. The family-level community structures were relatively similar between woodchip compost and the soil than the other livestock waste-based products and the soils. These facts (abundance and diversity of livestock waste-based products and family-level similarity between the product and the soil) could explain the stronger impact of woodchip compost on soil microbes. Regarding inorganic-N concentrations, only digestate application to the soil dramatically increased, but this did not affect the microbial diversity and community structure. We suggest to analyze not only the chemical properties but also the microbial properties of livestock waste-based products before the application, as the impact of livestock waste-based products' microbial communities on soil microbial communities might depend on the microbial properties of livestock waste-based products. To the authors' knowledge, this is the first study that focused on the interaction between the microbial communities in livestock waste-based products and the soils after the products were applied, with the growth of potatoes. This study will contribute to the sustainable management of agroecosystems utilizing microbial abilities.

Data Availability

Sequence data are available on the NCBI Sequence Read Archive under accession number PRJNA663956. All of the original data related to the current study are available through contacting the corresponding author (Yoshitaka Uchida; uchiday@chem.agr.hokudai.ac.jp).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

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Supplementary Materials

Figure S1: experimental design. Livestock waste-based products (digestate, wheat straw compost, and woodchip compost) were applied to three treatments (Dig, Whe, and Woo) on week 0. After 2 weeks of the application, chemical fertilizer was applied to all treatments except Con, and potato seed (*Solanum tuberosum*) was planted to all treatments. Bulk soil was sampled on 0 (just before the application), 1, 6, 10, and 14 weeks after the application. Rhizospheric soil and plant materials were sampled on week 14. Figure S2: relative abundance of microbial phyla detected in (A) sampled soils ($n = 4$) and (B) livestock waste-based product sources (digestate, wheat straw compost, and woodchip compost) ($n = 3$). A vertical bar chart was obtained by averaging the relative abundance across the biological replicates for each sample and by cutting phylum whose relative abundance was less than 2% × number of

samples. In (A), data are divided by sampling time: w0, week 0; w1, week 1; w6, week 6; w10, week 10; and w14, week 14. Figure S3: Venn diagrams of the number of shared and unique family numbers between (A) the original soil and the digestate, (B) the original soil and the wheat straw compost, and (C) the original soil and the woodchip compost. The families with the relative abundance of more than 1% were used for this analysis. The bar figures below the Venn diagrams express the relative abundance of the "shared families (colored)" and "unique families or families less than 1% (gray)," within the whole bacterial abundance. Figure S4: Venn diagrams of the number of shared and unique OTU numbers among (A) Che, Dig, and digestate, (B) Che, Whe, and wheat straw compost, and (C) Che, Woo, and woodchip compost on week 1. Same group of the treatments on different timings is shown in D, E, and F (week 6) and in G, H, and I (week 10). The values in the brackets (percentage values) are the relative abundances within the total bacterial abundance in the Dig (A, D, and G), Woo (B, E, and H), and Whe (C, F, and I). The relative abundances of each OTU differ; thus, the OTU number values and the percentage values are not proportional. Figure S5: relative abundance of microbial phyla shared between one of the treatments with livestock waste-based products (Dig, Whe, or Woo) and each original livestock waste-based product source (digestate, wheat straw compost, or woodchip compost) on week 1. The total percentage values for each bar are derived from A: 1.19% (shared between Dig and digestate in Figure S4A), B: 9.04% (shared between Whe and Wheat straw compost in Figure S4B), and C: 5.99% (shared between Woo and woodchip compost in Figure S4C). Vertical bar charts were obtained by averaging the relative abundance across the biological replicates for each sample ($n = 4$). Table S1: application rates of the nutrients (N/P/K) in each treatment from both chemical fertilizer and livestock waste-based products. Table S2: unique families observed in original digestate compared to original soil. Table S3: unique families observed in original wheat straw compost compared to original soil. Table S4: unique families observed in original woodchip compost compared to original soil. Table S5: OTUs significantly changed in Woo compared to Che on week 1. Table S6: OTUs significantly changed in Woo compared to Che on week 6. Table S7: OTUs significantly changed in Woo compared to Che on week 10. Table S8: OTUs significantly changed in Whe compared to Che on week 1. (*Supplementary Materials*)

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