



Article

Fermented Coffee Grounds Diminish Livestock Odors: A Microbiome Study

Min-Sueng Kim ¹, Sang-Ho Kim ², Minsoo Jeong ³, Min-Kyu Park ³, YoungJae Jo ³, GI-Ung Kang ³, Da-Ryung Jung ⁴, Chang-Il Lee ² and Jae-Ho Shin ^{1,3,4,*}

¹ Department of Integrative Biology, Kyungpook National University, Daegu 41566, Korea; chahun4270@knu.ac.kr

² GyeongSangbukdo Government Public Institute of Health & Environment, Yeongcheon 38874, Korea; 0024ja@korea.kr (S.-H.K.); lci@korea.kr (C.-I.L.)

³ Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea; minsoo0326@knu.ac.kr (M.J.); pmk601313@knu.ac.kr (M.-K.P.); dudwo7573@gmail.com (Y.J.); kanggiung5748@gmail.com (G.-U.K.)

⁴ Department of Biomedical Convergence Science & Technology, Kyungpook National University, Daegu 41566, Korea; amugae1210@knu.ac.kr

* Correspondence: jhshin@knu.ac.kr; Tel.: +82-53-950-5716; Fax: +82-53-953-7233

Abstract: Livestock odors are unavoidable problems in modern industrial society. We foresaw a role for fermented organic wastes in controlling odorous gases. In this study, we applied fermented coffee grounds to the floor area of a dairy cow barn and assessed alterations in odor compounds and a microbial shift over a period of three weeks. The treatment dramatically reduced ten odor compounds (more than 50%), highlighting the utility of fermented coffee grounds as an excellent product to reduce odors derived from cow manure. By the end of the treatment, the microbial consortium showed increases in rare families whose prevalence and abundance before the treatment had been low. Network analysis manifested 23 bacterial families dominant in fermented coffee grounds, negatively connected with odorous compounds, indicating potential odor-reducing bacterial families. This study provides an insight into using bacteria at the community level as a treatment to solve an environmental issue; simultaneously, it suggests proper usage of organic wastes by recycling them as fermenters for beneficial bacteria.

Keywords: agro-wastes; microbial fermentation; odor control; microbiome



Citation: Kim, M.-S.; Kim, S.-H.; Jeong, M.; Park, M.-K.; Jo, Y.; Kang, G.-U.; Jung, D.-R.; Lee, C.-I.; Shin, J.-H. Fermented Coffee Grounds Diminish Livestock Odors: A Microbiome Study. *Agronomy* **2021**, *11*, 1914. <https://doi.org/10.3390/agronomy11101914>

Academic Editors: San-Lang Wang, Anh Dzung Nguyen, Van Bon Nguyen and Chien Thang Doan

Received: 19 August 2021

Accepted: 18 September 2021

Published: 24 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Animal manure producing malodorous gases provokes serious issues in rural society, such as legal disputes or the burden of farmers to fit into legal norms [1,2]. To solve these difficulties, numerous approaches have been introduced, such as physical and chemical methods [3,4].

Furthermore, the biological approaches, especially utilizing microorganisms' activity to degrade odor compounds, are notable in that it is the eco-friendly way to contribute to sustainable agriculture. For example, microorganism-embedded instruments have been widely introduced, such as biofilters, biotrickling, and bioscrubbers [5–7]. Furthermore, several studies have shown the potentiality of agricultural wastes as feedstock mixed into the manure for bio-combusting, thereby solving both the odor issue and organic waste disposal [8–10].

The production of coffee, one of the most popular beverages globally, generates vast quantities of by-products, namely “spent coffee grounds (SCG)” and a burden for the industry concerning its disposal. Several studies have examined the properties of SCG. In particular, McNutt et al. reported the plentiful nutritional residues in SCG, represented as sugars, lignin, and proteins [11]. Garcia et al. mentioned that the particles of SCG have a high surface-to-volume [12] ratio. All things considered, we arrived at the idea of using

SCG as the material to nourish and enrich the microorganisms that might diminish odorous compounds.

2. Materials and Methods

2.1. Study Design and Field Testing

The field test location was 933-25, Jangsu-ro, Hwasan-myeon, Yeongcheon-si, Gyeongsangbuk-do, Republic of Korea. We obtained spent coffee grounds at cafes, restaurants, and coffee factories in Daegu province. The obtained spent coffee grounds had undergone typical serial processing for roasting, grinding, and coffee extraction. Subsequently, we covered spent coffee grounds with a plastic cover to minimize the inflow of contaminants. We then fermented covered spent coffee grounds for two weeks to yield FCGs (fermented coffee grounds). The floor of the experimental barn was then treated with one ton of FCG. The FCG-treated area measured 100 m² and was divided into six replicate plots so that any microbial shift would be well represented regardless of spatial variation. The overview of experiment steps is illustrated in Figure 1. The field test started on 5 April 2021 and lasted for three weeks. Meteorological data for the province where the field test was performed were acquired from the Korea Meteorological Administration [13]. The average temperature was 12.66 ± 2.89 °C, and the average precipitation was 0.46 ± 2.15 mm during the test period (see Table S1).

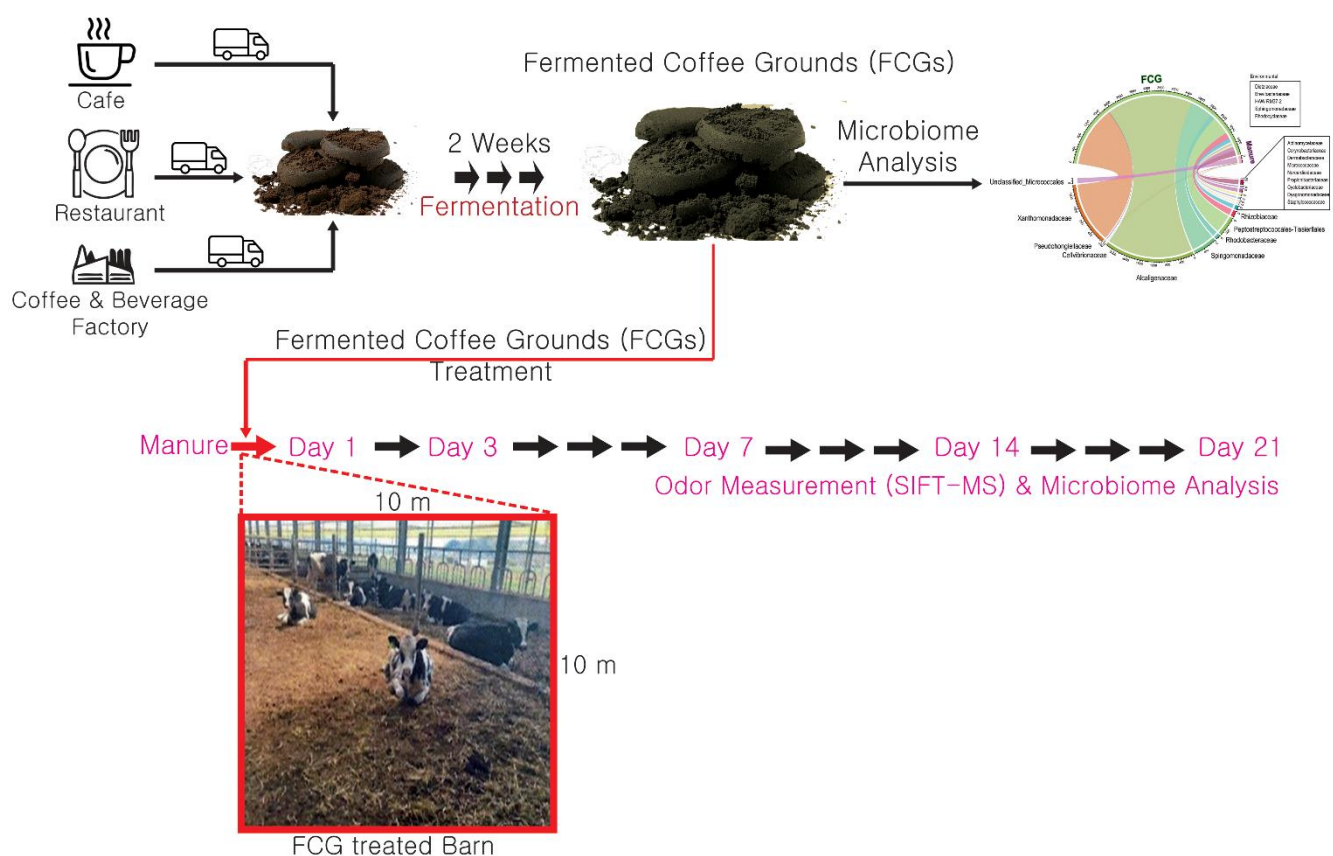


Figure 1. The experimental scheme. The steps of the experiment are illustrated.

2.2. Odor Measurement and Sampling

The odorous gases were measured in the barn utilizing an SYFT Voice 200 Ultra Advanced SIFT Mass Spectrometer device (Thomson Environmental Systems, Kirrawee NSW 2232, Australia). We gathered ten liters of air from a point 50 cm above the barn floor at six locations at each of seven-time points: one day before FCG treatment, on the day of the treatment, and then on day 1, day 3, week 1, week 2, and week 3 after treatment. At the same times and locations, FCG and cow manure samples were also obtained to monitor the

microbial composition as the experiment proceeded. As soon as samples were acquired, they were stored frozen at below -80°C .

2.3. DNA Extraction

After the field test and sampling were completed, the whole samples were transferred to the Department of Applied Biosciences, Kyungpook National University. DNA quality and quantity were checked by electrophoresis and NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Total DNA was extracted with QIAamp PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocols.

2.4. Library Preparation and High Throughput Sequencing

The V4-V5 hypervariable regions of 16S rRNA coding genes on the total DNA extracted were amplified, and barcode sequences were attached through polymerase chain reaction (PCR) to identify the origin of each DNA sequence. PCR cycle specifications and reagents were as previously published [14]. After checking the sizes of products through electrophoresis, the concentration of each library was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). All libraries were then pooled into the exact quantities of DNA to obtain a uniform amount of data after sequencing. The pooled library was purified with QIAquick Gel Extraction Kit (QIAGEN, Germany), the sequences were nonspecifically amplified, and the dimers were removed. After purification, sequencing was performed in the Illumina MiSeq system (Illumina, San Diego, CA, USA).

2.5. Bioinformatical Analysis

From the high throughput sequencing, a total of 2,392,152 reads, with an average of 56,956 reads per sample, were acquired. The data was imported into the Quantitative Insights Into Microbial Ecology 2 version 2020.11 software [15], and trimming and chimeric sequence removal was carried out using the QIIME DADA2 plugin [16]. The parameter for trimming length was set to discard sequences shorter than 299 bp, which was the average length of total outputs. Due to the satisfactory quality of sequencing, the Q score was not considered in the trimming. Except for the trimming length, software default parameters were assigned. Subsequently, taxonomic information was assigned on the reads using the q2-feature-classifier plugin [17]. Then, reads classified as 'Unassigned', 'Archaea', and 'Eukaryota' were discarded to meet the purpose of the study focusing on the bacterial kingdom. As a result, a total of 1,176,865 quality-controlled and bacteria-only reads were retrieved. We confirmed that the output satisfactorily represented the original bacterial composition in samples using a rarefaction curve. Finally, we rarified all read counts into 10,577, the minor count among samples [Figure S1].

2.6. Statistical Analysis

Various tools and software were employed to analyze the processed data. Bray-Curtis dissimilarity was calculated in the Vegan package in R [18]. Alpha diversity and prevalence of bacterial families of interest were acquired using a microbiome package [19], and the co-occurrence network was analyzed and visualized using CoNet software in cytoscape_v3.8.2 [20]. The threshold of representing edges was 0.7 in Spearman correlation and $p < 0.05$ in Fisher's Z statistic. To validate statistical robustness, Wilcoxon signed-rank test was applied for pairwise comparison, and for global validation, Kruskal-Wallis one-way analysis of variance was calculated. To represent the results clearly, we visualized our data with several visualization tools. Results for the odorous compound analysis were visualized utilizing ggplot2 [21], the Vegan package for principal coordinates analysis plots, and Microsoft Excel (Microsoft, Redmond, WA, USA) for barplots, indicating shifts in bacterial family composition. To represent and compare the sources of potential odor reducers, we adopted the circlize R package [22], and to generate a heatmap showing the shift of bacterial prevalence and abundance, we applied the gplots package [23].

3. Results

3.1. Overview of Odor Reduction

Among 23 odor compounds listed by the Minister of Environment, Republic of Korea [24], 10 showed a reduction of more than 50% after FCG treatment (Figure 2). In detail, ammonia and hydrogen sulfide, widely recognized livestock-derived odor compounds [25], showed 86.93% and 93.81% reduction rates, respectively. Volatile fatty acids (considered an anaerobic metabolism by-product [26]), propionic acid, and butyric acid showed reductions of 100%. Concentrations of aldehydes and aromatic compounds were dramatically reduced by the treatment.

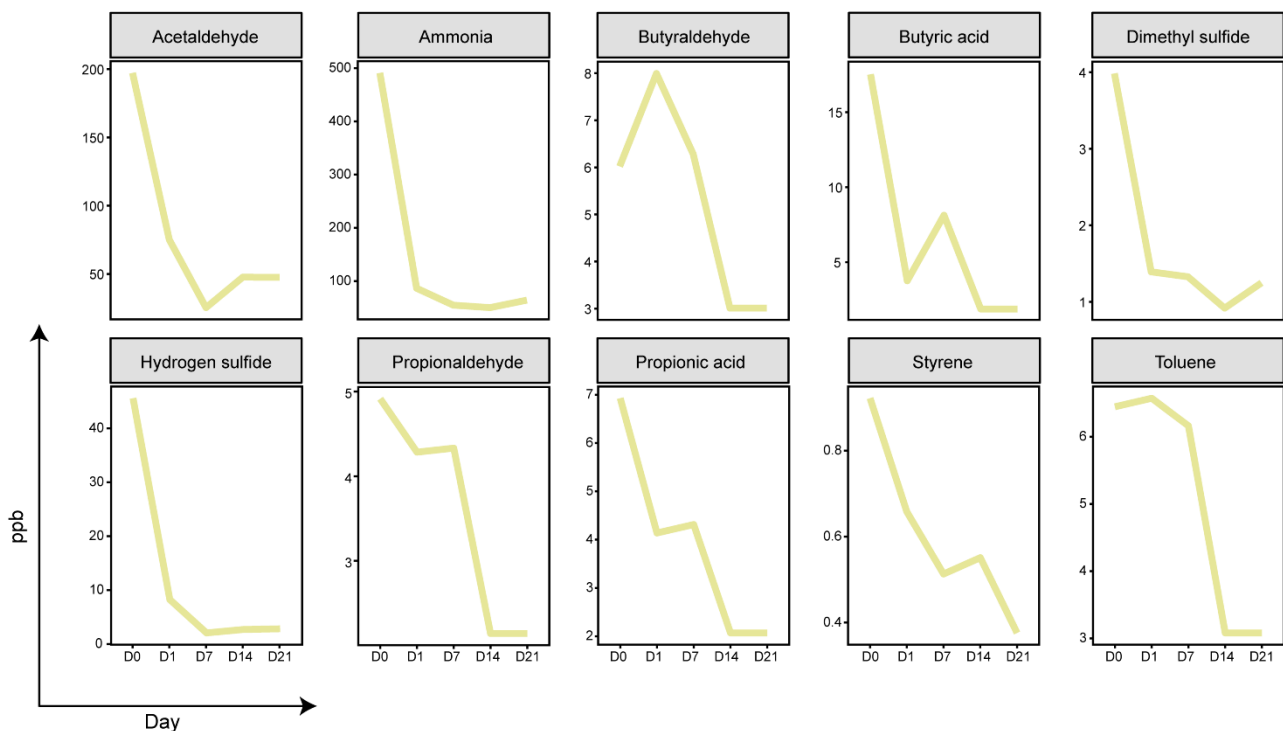


Figure 2. Reduction of odorous chemicals after fermented coffee ground (FCG) treatment. The concentrations of odorous chemicals are measured in parts per billion (ppb), and the representing points are before FCG treatment (“manure”) and days 1, 7, 14, and 21 after treatment.

3.2. Microbial Shift after the Treatment

We monitored the shift in microbial compositions and distances using beta diversity analysis in principal coordinates analysis (Bray–Curtis dissimilarity). One day after the treatment, we observed a change in microbiota toward the FCG, implying that the treatment introduced FCG microbes into the manure. As time passed, the microbial community of the cow manure showed a gradual heterogeneity with that in the FCG. Finally, the treated cow manure microbiota showed a slight regression trend toward the original, untreated cow manure (Figure 3A). Interestingly, the degree of change in the microbial shift decreased significantly until the endpoint (Figure 3B). The microbial composition of the barn excreta initially showed a similarity with the FCG due to the introduction of FCG-deriving bacteria. However, the microbial consortium acquired its new structures as time passed. Moreover, the novel environment formed by the treatment resulted in the selective growth of bacteria. These shifts suggest that an altered environment, on account of FCG mixing, induced the growth of odor-diminishing bacteria through the provision of a favorable environment.

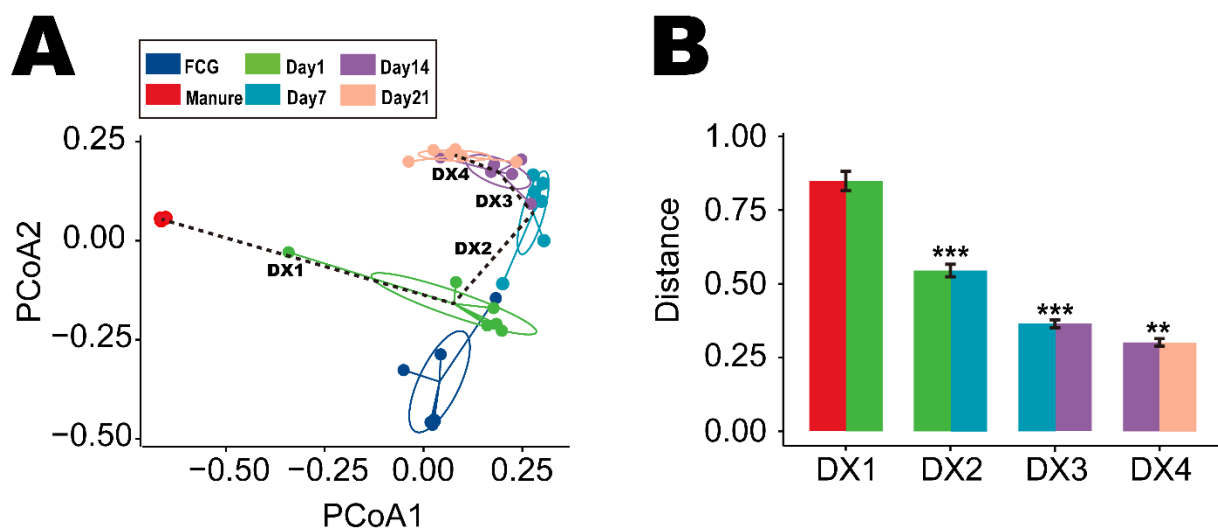


Figure 3. Bray–Curtis distances among groups. (A) Projection displayed by principal coordinates analysis (PCoA) based on Bray–Curtis distances represents the shift of microbial composition over time. The dotted line links the time points. (B) Comparison of distances of cow manure up to day 1 (DX1), day 1 to day 7 (DX2), day 7 to day 14 (DX3), and day 14 to day 21 (DX4). To track the significance of these distances, the Wilcoxon signed-rank test was applied. ** $p < 0.01$ and *** $p < 0.001$.

3.3. Alteration of Alpha Diversity and Core Bacteria

We analyzed alpha diversity using three indexes: richness, inverse Simpson, and Shannon. For all indexes, the microbial diversity of the excreta dropped significantly ($p < 0.01$) directly after mixing with FCG. This result implies a dramatically altered environment arose via bactericidal effects on the manure. However, after mixing FCG, the diversity gradually recovered. Therefore, there were no significant differences with untreated samples with the inverse Simpson and Shannon indexes at the endpoint. However, in the Shannon index, endpoint sample diversity was still lower than that of the untreated sample, and a gradually increasing trend was also observed (Figure 4A). We performed prevalence analysis to track the alterations of core bacterial families in FCG and manure. We assigned the threshold of core bacteria as 75% occurrence among the sample groups with the cut-off value as a relative abundance of 1%. At the start point, FCG showed fewer core families, which means that the composition of FCG mainly consisted of rare bacterial families with high variation among sample replicates. Members of the FCG core bacteria and manure core bacteria are enumerated in Table S2.

Conversely, cow manure showed a high prevalence of core bacteria. After mixing, the cow manure-dominant core bacteria dramatically decreased, and FCG core bacteria increased. Subsequently, FCG core bacteria prevalence dropped until the endpoint. Interestingly, the rare families with low prevalence and abundance in FCG and cow manure samples showed increasing trends seven days after the start of treatment, maintaining abundance until the endpoint (Figure 4B). This suggests that the alteration of the environment via the treatment reshaped the ecology of the bacterial consortium. Following the shifts in alpha diversity mentioned above, the original bacteria community in the dairy cow feces suffered dramatic declines after treatment. In the recovery process, the existing bacterial families rarely seemed to thrive in the altered environment arising from FCG treatment.

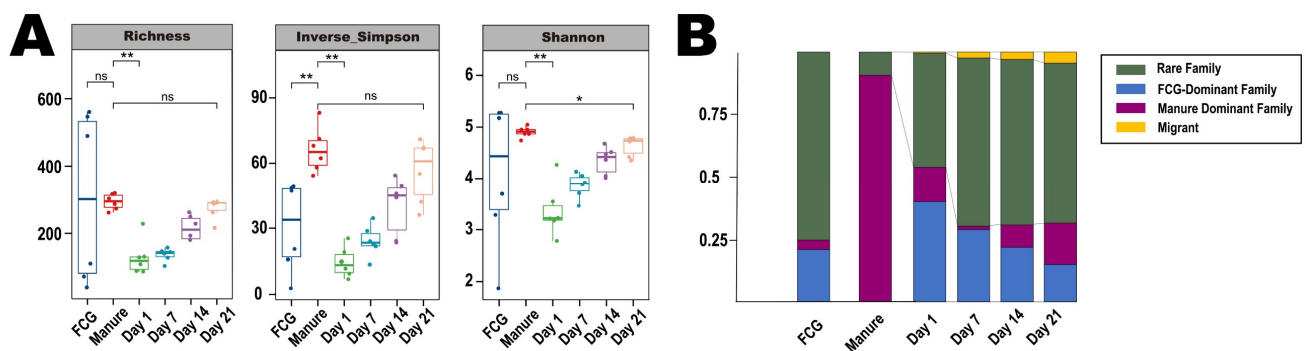


Figure 4. The alteration of alpha diversity and bacterial family composition. (A) Richness, inverse Simpson, and Shannon diversities were calculated and compared using boxplots. The components of comparisons are fermented coffee grounds (FCG), manure, and days 1, 7, 14, and 21 after treatment. The Wilcoxon signed-rank test was applied to monitor the difference in alpha diversity of FCG and manure as well as the decrease in diversity after one day of the treatment and to manifest the recovery of diversity at the endpoint of the experiment (21 days after treatment). ns (nonsignificant) $p > 0.05$, * $p < 0.05$, ** $p < 0.01$. (B) Whole bacterial families were categorized by prevalence in FCG and cow manure samples as FCG-dominant, manure-dominant, rare, and migrant. The shifts of families associated with each group are represented as relative abundance.

3.4. Selection of Potential Odor Reducers

While we monitored the bacterial consortium at the community level to track the alteration of bacterial ecology, we also deepened the resolution to single bacterial families to select potential odor reducers (POR) using network analysis. We specified 23 PORs and examined the contributions of each. Toluene was the odor chemical most affected by bacteria, linked with eight PORs with opposing edges. Conversely, there was no negative relationship between ammonia and bacteria, implying no effects of bacteria in reducing it. Additionally, we tracked the helper bacterial families linked with PORs in positive edges.

Interestingly, the network cluster, which showed vigorous interactions between PORs and odor chemicals, included more helper families (10 families) compared with the other cluster with poor interactions (2 families) (Figure 5A). This result suggests that symbiotic support between bacterial families contributed to the active PORs' inhibition of odorous compounds. To measure the source of such PORs, we examined the sum abundance of PORs in FCG and cow manure sample replicates. Total counts of PORs were 6998 in FCG and cow manure sample recounts. PORs derived from FCG occupied 6779 counts (96.87%) and 14 families out of 23 PORs, but the dominant PORs in cow manure samples only occupied 162 counts (2.31%) and three families. The remaining six PORs had no presence in either FCG or cow manure samples, and it was assumed that their source was from the external environment (Figure 5B).

FCG-derived PORs overwhelmed those from cow manure both in number and abundance. The strengthened resolution of analysis focusing on individual families provides substantial evidence that treatment with FCG propagated the biological factors contributing to odor removal.

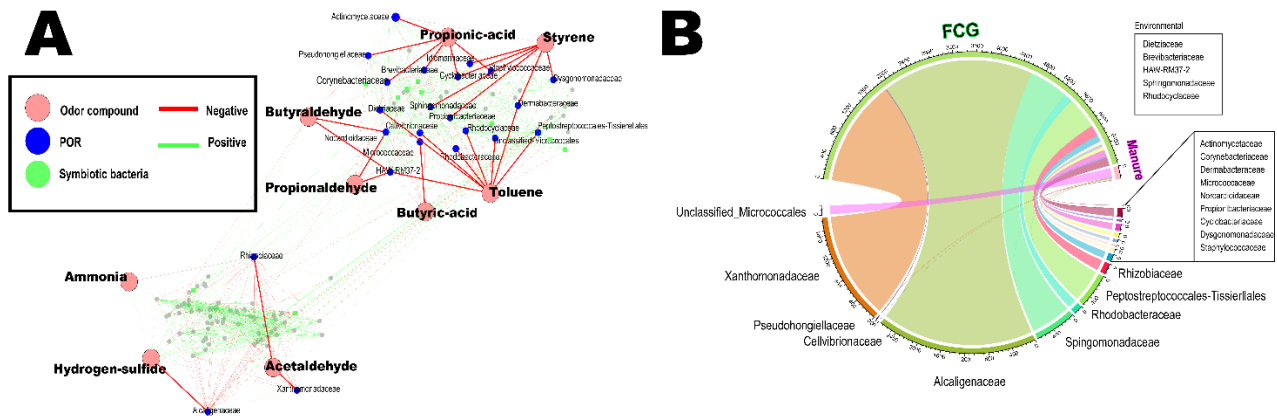


Figure 5. Network and potential odor reducer (POR) distribution. (A) The nodes of the network plot are differentiated by shape and color into the bacterial families, ecological roles, and contribution to odor reduction. Red and green edges imply negative and positive correlations, respectively. Significantly, the negative correlation between PORs and odor compounds is highlighted in solid red lines. (B) The plot indicates the sum abundance (normalized by rarefaction) of PORs in two initial sample groups: fermented coffee grounds and manure.

3.5. Alteration of PORs after the Treatment

The prevalence analysis examined the microbial community shift after FCG treatment. It revealed that FCG initially exhibited rare and few core families, after which there were increasing trends of abundances of rare families following treatment. Interestingly, the PORs followed similar trends. In FCG and cow manure samples, the mean prevalence of PORs was 7.25%, which was under the threshold of judging the core family (75%), and the mean relative abundance of PORs was 0.47%. However, some PORs showed increases in abundance and prevalence after being mixed with odorous wastes. At the endpoint of the experiment, the mean prevalence of PORs was 41%, and the relative abundance was 1.21%. In particular, among 23 PORs, eight families (*Corynebacteriaceae*, *Dysgonomonadaceae*, *Rhodobacteraceae*, *Alcaligenaceae*, *Cellvibrionaceae*, *Xanthomonadaceae*, *Micrococcaceae*, and *Rhizobiaceae*) were classified as core families at the endpoint. This result indicates that the treatment successfully induced engraftment of PORs (Figure 6).

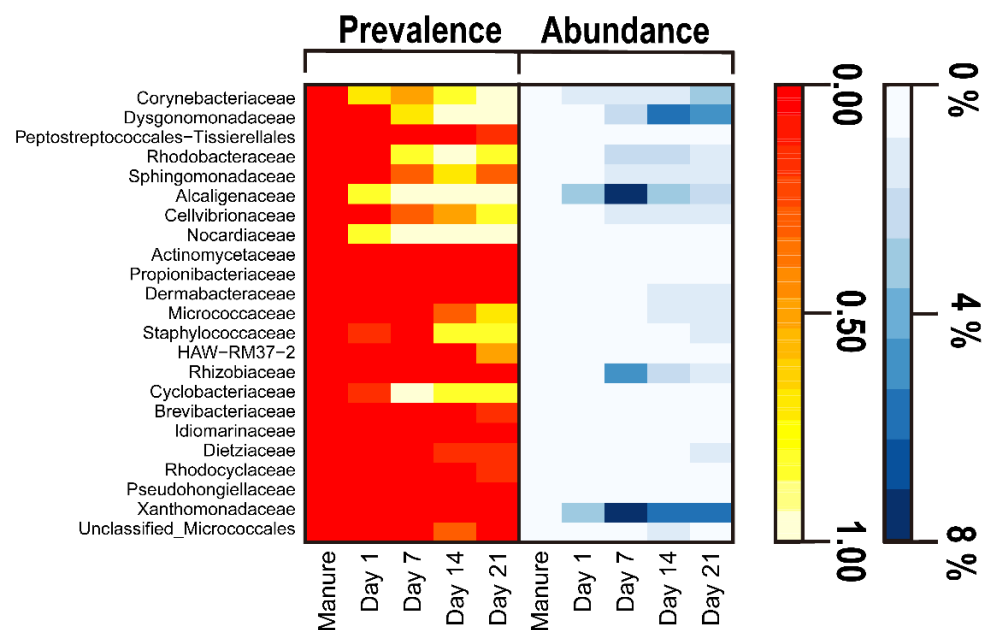


Figure 6. Heatmap showing changes in prevalence and abundance of potential odor reducers.

4. Discussion

The bioinformatical approach enabled us to track the change in the microbiome with gradual odor decline. Illustrated with network analysis, the correlation of bacterial families with individual odorous chemicals was investigated. Ultimately, further assessing the odor removal performance in a single strain of bacteria, we could correlate the microbiome dynamic with the odor compounds. Thus, we could reach a conclusion empathizing the potentiality of community-leveled bacteria treatment in the livestock odor issues.

Multiple approaches to diminish odorous compounds utilizing bacteria have been reported [27,28]. Despite the effectiveness, the determinant of the satisfiable result of treatment might depend on the environmental factors that can be unfavorable to the bacterial strain in the activity. However, our study inserted “FCG microbiome” into the smelly manure by introducing the sum of functional genes and ecology [29] that are naturally generated from coffee ground fermentation without isolation of bacterial strains. It is manifested by our network analysis that detected the helper bacterial strains linked to PORs with a positive correlation. This indicates that the microbial ecology generated by FCG treatment strengthened the activity and engraftment of PORs. This empathizes the potentiality of microbiome treatment in the environmental area.

Our analysis focusing on the alteration of bacterial proportions after the FCG treatment strengthens the potentiality of FCG treatment. In the study performed by Dennehy et al., the co-digestion of food waste and pig manure resulted in the decline of coliforms [30], indicating the reorganization of microbial consortium derived from the introduction of organic materials and microorganisms. Similarly, we monitored the decrease of bacterial family dominance in manure samples after the FCG treatment. This commonality between our study and the previous report implies that by introducing a bacterial community, the original microbiome might be converted, ultimately shattering the properties upheld before the treatment of FCG.

Furthermore, deepening the sight into the rare bacterial families, our study showed that the trends in the abundance and prevalence of PORs, which rarely existed at the FCG, dramatically increased after being introduced into the manure. According to Shade et al., the temporal blooming of rare taxa in the environment has an essential role in the ecosystem [31]. Moreover, Kellermayer et al. suggested that after fecal microbiota transplantation, the operation (transplanting the healthy donor’s fecal microbiota to the colitis patients) and the expansion of rare taxa might have contributed to the relief of the inflammatory response of patients [32]. These studies allude that the growth of rare taxa derived from environmental alteration can play a critical functional role in the environment. Meanwhile, the parallel results of this study manifest our theory that the growth of rare families in FCG induced odor removal.

Duan et al. have performed a study designed to reduce the volatile fatty acid (VFA) emissions which are considered part of livestock malodor in chicken manure. In this study, bamboo biochar was introduced into the manure, and VFA concentration was assessed. The conclusion was that the biochar addition increased bacterial taxa that negatively correlated with VFA concentration [33]. This suggests that the shift of environment, derived from physicochemical properties of biochar, came up with the proliferation of bacteria that metabolize VFA, thus lessening the odor intensities. Putting our study aside from this previous study, it is highly reasonable that the physicochemical properties of FCG drove the environment of manure to benefit the growth of PORs. Further, it inspires us to deepen our knowledge of the physicochemical features of FCG to manifest it.

Summing up these previous studies, it is plausible that FCG might be the material that can efficiently process manure odors. However, various limitations exist in this study. For example, the mechanisms by which PORs reduce odors are still elusive. In addition, the robustness of statistics might be insufficient due to the absence of aerial replication correspondent to the biological replicates. To solve these problems, research focusing on gene components of PORs that can manifest the functional pathways contributing to odor reduction is needed. At the same time, *in vitro* experiments with separated treatment

replicates in controlled conditions would permit the acquisition of the replication of odor data. Finally, this short-term study is still insufficient to reveal the effect and efficiency of FCG treatment in durable applications. Therefore, long-term monitoring with repetitive treatment is required.

Nevertheless, the strength of this study has illustrated a potentially valuable way of utilizing FCG. The success of the FCG treatment is an excellent example of recycling the wastes derived from the food industry. Ultimately, this study will draw attention to the usage of agricultural and industrial organic wastes to be an effective material as an environmentally-friendly fermenter.

5. Conclusions

The FCG treatment used in our study produced a notable decrease in odor intensities in cow manure. Alluding to the robust enrollment of community-based bacterial engraftment for odor inhibition, FCG is a potentially useful material in livestock areas as an odor controller. Reflecting current trends in microbiome study, the introduction of a beneficial microbial community, and not just the bacteria themselves, but also functioning gene components and ecological states can potentially stabilize the subsequent community. Our pilot study aimed to solve an environmental issue through microbiome treatment while providing an alternative use for waste coffee grounds as a reactor generating beneficial bacteria, offering a route for the useful disposal of industrial and agricultural wastes as environmentally-friendly material for bioremediation.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11101914/s1>, Figure S1: Rarefaction curve. Table S1: Temperature and precipitation at each sampling date. Table S2: The list of FCG core family and manure core family.

Author Contributions: Conceptualization, M.-S.K. and J.-H.S.; methodology, M.-S.K., M.J., S.-H.K., and Y.J.; software, M.-S.K., M.J. and D.-R.J.; data curation, M.-S.K., M.-K.P. and S.-H.K.; writing—original draft preparation, M.-S.K. and G.-U.K.; writing—review and editing, C.-I.L. and J.-H.S.; visualization, M.-S.K. and D.-R.J.; funding acquisition, J.-H.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food (Grant No. 918010-4), Ministry of Agriculture, Food and Rural Affairs, South Korea. Students who participated in this work, M.J., M.P., Y.J. and D.J., were supported by a project to train professional personnel in biological materials by the Ministry of Environment, South Korea.

Data Availability Statement: The raw data presented in this study are available in the NCBI Sequence Read Archive (Accession: PRJNA753203).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hooiveld, M.; Dijk, C. Odour annoyance in the neighbourhood of livestock farming perceived health and health care seeking behaviour. *AAEM* **2015**, *22*, 55–61. [[CrossRef](#)] [[PubMed](#)]
2. Mielcarek, P.; Rzeźnik, W. Odor emission factors from livestock production. *Pol. J. Environ. Stud.* **2015**, *24*, 27–35. [[CrossRef](#)]
3. Schiffman, S.S. Livestock odors implications for human health and well-being. *J. Anim. Sci.* **1998**, *76*, 1343–1355. [[CrossRef](#)] [[PubMed](#)]
4. Naseem, S.; King, A.J. Ammonia production in poultry houses can affect health of humans, birds, and the environment—techniques for its reduction during poultry production. *ESPR* **2018**, *25*, 15269–15293. [[CrossRef](#)] [[PubMed](#)]
5. Ren, B.; Zhao, Y. Current Status and Outlook of Odor Removal Technologies in Wastewater Treatment Plant. *Waste Biomass Valorization* **2019**, *10*, 1443–1458. [[CrossRef](#)]
6. Yasuda, T.; Waki, M. Characterization of the denitrifying bacterial community in a full-scale rockwool biofilter for compost waste-gas treatment. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 6779–6792. [[CrossRef](#)] [[PubMed](#)]
7. Haosagul, S.; Prommeenate, P. Sulfide-oxidizing bacteria community in full-scale bioscrubber treating H₂S in biogas from swine anaerobic digester. *Renew. Energy* **2020**, *150*, 973–980. [[CrossRef](#)]
8. Bardi, M.J.; Rad, H.A. Simultaneous synergistic effects of addition of agro-based adsorbent on anaerobic co-digestion of food waste and sewage sludge. *J. Mater. Cycles Waste Manag.* **2020**, *22*, 65–79. [[CrossRef](#)]

9. Beniche, I.; Hungria, J. Effects of C/N ratio on anaerobic co-digestion of cabbage, cauliflower, and restaurant food waste. *Biomass Conv. Bioref.* **2021**, *11*, 2133–2145. [CrossRef]
10. Elsayed, M.; Diab, A. Methane production from anaerobic co-digestion of sludge with fruit and vegetable wastes: Effect of mixing ratio and inoculum type. *Biomass Conv.* **2021**, *11*, 989–998. [CrossRef]
11. McNutt, J.; He, Q. Spent coffee grounds: A review on current utilization. *J. Ind. Eng. Chem.* **2019**, *71*, 78–88. [CrossRef]
12. Garcia, C.V.; Kim, Y.T. Spent Coffee Grounds and Coffee Silverskin as Potential Materials for Packaging: A Review. *J. Polym. Environ.* **2021**, *29*, 2372–2384. [CrossRef]
13. Korea Meteorological Administration. Available online: <https://www.kma.go.kr/eng/index.jsp> (accessed on 18 August 2021).
14. Kang, G.U.; Ibal, J.C. Alteration of the soil microbiota in ginseng rusty roots: Application of machine learning algorithm to explore potential biomarkers for diagnostic and predictive analytics. *J. Agric. Food Chem.* **2021**, *69*, 8298–8306. [CrossRef] [PubMed]
15. Bolyen, E.; Rideout, J.R. Reproducible, interactive, scalable and extensible microbiome data science using QIIME2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
16. Callahan, B.J.; McMurdie, P.J. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
17. Bokulich, N.A.; Kaehler, B.D. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [CrossRef]
18. Dixon, P. VEGAN, a package of R functions for community ecology. *Appl. Veg. Sci.* **2003**, *14*, 927–930. [CrossRef]
19. Bioconductor (2012–2019). Available online: <https://bioconductor.org/packages/release/bioc/html/microbiome.html> (accessed on 1 August 2021).
20. Raes, J.; Faust, K. CoNet app: Inference of biological association networks using Cytoscape. *Food Res.* **2019**, *5*, 1519.
21. Wickham, H. ggplot2. *Comput. Stat. Data Anal.* **2011**, *3*, 180–185. [CrossRef]
22. Gu, Z.; Gu, L. Circlize implements and enhances circular visualization in R. *Bioinformatics* **2014**, *19*, 2811–2812. [CrossRef]
23. rdr.io. Available online: <https://rdr.io/cran/gplots/> (accessed on 28 November 2020).
24. Ministry of Environment. Available online: <https://www.keco.or.kr/en/main/index.do> (accessed on 1 August 2021).
25. Park, J.; Kang, T. Evaluation of short-term exposure levels on ammonia and hydrogen sulfide during manure-handling process at livestock farms. *Saf. Health Work* **2020**, *11*, 109–117. [CrossRef] [PubMed]
26. Kuruti, K.; Nakkasunchi, S. Rapid generation of volatile fatty acids (VFA) through anaerobic acidification of livestock organic waste at low hydraulic residence time (HRT). *Bioresour. Technol.* **2017**, *2389*, 188–193. [CrossRef]
27. Liu, W.C.; Ye, M. Application of complex probiotics in swine nutrition—a review. *Ann. Anim. Sci.* **2017**, *18*, 335–350. [CrossRef]
28. Mahardhika, B.P.; Mutia, R. Efforts to reduce ammonia gas in broiler chicken litter with the use of probiotics. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *399*, 012012. [CrossRef]
29. Rybakova, D.; Berg, G. Microbiome definition re-visited: Old concepts and new challenges. *Microbiome* **2020**, *8*, 103.
30. Dennehy, C.; Lawlor, P.G. Anaerobic co-digestion of pig manure and food waste; effects on digestate biosafety, dewaterability, and microbial community dynamics. *Water Manag.* **2018**, *71*, 532–541. [CrossRef]
31. Shade, A.; Jones, S.E. Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. *mBio* **2014**, *4*, e01371-14. [CrossRef]
32. Kellermayer, R.; Nagy-Szakal, D. Serial Fecal Microbiota Transplantation Alters Mucosal Gene Expression in Pediatric Ulcerative Colitis. *Am. J. Gastroenterol.* **2015**, *110*, 604–606. [CrossRef]
33. Li, F.; Yu, H. The quality of compost was improved by low concentrations of fulvic acid owing to its optimization of the exceptional microbial structure. *Bioresour. Technol.* **2021**, *342*, 125843. [CrossRef]