Artificial ecosystem selection reveals relationships between microbiome composition and ecosystem function

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# Abstract

Microbiomes mediate important ecosystem functions, yet it has proven difficult to determine the relationship between microbiome composition and the rate of ecosystem functions. This challenge remains because it is difficult to manipulate microbiome composition directly, we often cannot know *a priori* which microbiome members influence the rate of an ecosystem function, and microbiomes can covary strongly with other drivers of ecosystem function, such as the environment. We propose that artificial ecosystem selection can overcome these challenges. Artificial ecosystem selection involves creating replicate ecosystems, selecting replicates with a desired ecosystem-level trait, and using the microbiomes from the selected ecosystems to inoculate a new set of ecosystems. We performed artificial ecosystem selection on replicate soil microbiomes to select for increased soil CH4 oxidation rate. We observed a strong response to selection with a 50.7% increase in CH4 oxidation rate per passage. We estimated that 31.5% of the variation in CH4 oxidation rate in these soils can be attributed to microbiome variation. We also found that selection did not enrich for known CH4 oxidizers; instead, 12 families not known to oxidize CH4, including *Fimbriimonadaceae*, *Cytophagaceae*, and *Diplorickettsiaceae*, were enriched by selection. This result is in contrast to the typical assumption that the rate of an ecosystem function is limited by the final step in the associated microbial pathway. Our study demonstrates that variation in microbiome composition can contribute to variation in the rate of ecosystem function and that manipulating microbiome composition could be a viable strategy for managing ecosystem functions.

# Introduction

Microbiomes mediate a variety of important ecosystem functions relevant to human health, agriculture, and global change. As a result, there is great interest in understanding how to manipulate the microbiome to achieve desirable outcomes within these domains (1–3). However, for microbiome manipulations to be successful, variation in the microbiome must contribute directly to variation in the magnitude of the function of interest independent of other factors. Many studies have attempted to document such a relationship (4–9). However, it is difficult to isolate the direct effect of variation in microbiome composition from other drivers of variation in ecosystem function, such as the indirect effect of the environment on function through microbiome assembly. Here, we overcome these limitations by using a selection approach to estimate the degree to which an ecosystem function varies with microbiome composition.

Altering ecosystem functions via microbiome manipulations requires that the microbiome contributes to variation in ecosystem function independent of other drivers of ecosystem variation, such as variation in environmental conditions. This is because the drivers of variation in ecosystem functions can interact in complicated ways (Figure 1). Variation in the microbiome can contribute directly to variation in ecosystem function, for example, if a microbial population is replaced by one with a greater enzyme efficiency. In addition, environmental conditions can contribute indirectly to ecosystem function via covariance with the microbiome, for example, by providing conditions that select for microbial groups that in turn alter the rates of ecosystem functions. In this scenario, identifying the change in microbial community composition without adequately controlling for the environmental conditions would incorrectly attribute the change in ecosystem function to the microbiome when it is ultimately an indirect effect of the environment. Determining the independent contribution of microbiome variation to ecosystem function is crucial because if microbiome composition is driven primarily by environmental conditions, then introducing a desirable taxon through microbiome manipulation without altering the environment will likely be unsuccessful at shifting the targeted ecosystem function.

There have been two general categories of approaches that investigators have used to estimate the degree to which an ecosystem function varies with microbiome composition: comparative and manipulative. Comparative studies sample natural variation in an ecosystem function across different habitats and simultaneously measure variation in community composition. Investigators can then correlate ecosystem function with aspects of community composition while attempting to control for environmental variation. These approaches have documented important relationships between microbiomes and ecosystem functions. For example, a meta-analysis of these studies observed a small but significant contribution of the microbiome to variation in ecosystem function after controlling for environmental variation (10). In addition, studies focusing on the correlation between the rate of an ecosystem function and the abundance of an associated marker gene (i.e., a gene that codes for a protein assumed to be involved in the ecosystem function) sometimes observe a significant correlation, though this relationship is rare and contingent upon both the function and the ecosystem sampled (11). However, comparative studies come with unique challenges and limitations. One issue is that microbiome attributes tend to covary with the abiotic conditions within an environment, and it is difficult to control for these abiotic variables in order to identify the unique contribution of the microbiome to ecosystem function. In addition, it is difficult to know *a priori* which environmental variables or community attributes to measure. Finally, while these approaches can establish a potential magnitude and direction for these relationships, it is often difficult to identify the taxa or genes that explain the connection between composition and function.

The other broad category of approaches used to address this question are manipulative approaches. Manipulative experiments try to alter microbial community composition and observe the effect on function. For example, reciprocal transplant and common garden experiments have shown that microbiomes originating from different ecosystems inoculated into the same substrate or introduced into a common environment display distinct functional rates (4–7). In addition, manipulating diversity by filtering communities by cell size or through dilution has been shown to alter the rate of ecosystem functions (8,9,12). However, manipulating the microbiome directly is challenging, and manipulative approaches often confound community composition with other factors. For example, reciprocal transplant and common garden experiments can confound community composition with the abiotic conditions introduced with the inoculum, while manipulating composition through dilution may confound composition with biomass (13).

In this study, we sought to build on the observations of comparative and manipulative studies by applying a different approach to the question of whether microbiome variation contributes to variation in the rate of an ecosystem function. We used artificial ecosystem selection to select for microbiomes that performed a greater rate of ecosystem function (14–16). We then tested whether variation in the microbiome contributed to variation in the rate of ecosystem function and identified microbiome attributes that might explain this relationship. There are several potential advantages to this approach for documenting the direct contribution of the microbiome to variation in ecosystem function and for investigating the mechanisms underlying those relationships. By repeated passaging of microbiomes in a common environment, we can weaken the covariance between microbes and the environment by repeatedly diluting the influence of variation in abiotic conditions. In addition, our approach eliminates the need to generate microbiome variation through methods that are confounded with biomass or cell size. Lastly, by comparing our artificially selected community to a control community resulting from random selection, we can both control for changes in the environment over time and identify genes or taxa that are associated with the ecosystem function under selection.

We applied artificial ecosystem selection to soil microbiomes by selecting on soil methane (CH4) oxidation rate. We chose this function because CH4 is a globally important greenhouse gas and CH4 oxidation by soil bacteria is the primary biological sink for atmospheric CH4 (17). In addition, there is evidence that soil CH4 oxidation rate may vary with microbiome composition based on comparative studies in a variety of arctic and tropical ecosystems (18–21) as well as studies that manipulate methanotroph richness (22). Finally, methanotrophy is one of the most deeply conserved microbial physiologies and is represented in a narrow range of taxa, which suggests that the taxonomic composition of the microbiome is more likely to be associated with the rate of CH4 oxidation than other broader or more shallowly conserved functions (2,23).

In this study, we used artificial ecosystem selection on CH4 oxidation rate to address the following questions: Does variation in the relative abundance of microbial taxa contribute to variation in soil CH4 oxidation rate independent of the environment in our system? Which attributes of the microbiome are associated with variation in CH4 oxidation rate, and do these attributes match our assumptions about the factors that regulate CH4 oxidation rate in nature?

# Materials and Methods

## Experimental design

We performed an artificial ecosystem selection experiment (*sensu* (14)) by passaging replicate soil microbiomes. The trait we selected on was CH4 oxidation rate. This experiment had a selection line and a control line with twelve jars each for a total of twenty-four jars per passage. The selection line underwent positive selection where the two or three jars with the highest CH4 oxidation rate were homogenized to inoculate the next set of jars. The control line underwent random selection where an equal number of jars as the selection line were chosen at random to inoculate the next set of jars. The number of jars chosen was based on the distribution of fluxes among the positive jars, i.e., we chose the top three jars unless only two jars had considerably greater CH4 oxidation rates based on visual inspection of histograms. The experiment was carried out over five passages until a significant divergence in functional rates was observed between the two selection treatments based on a difference of slopes.

The initial soil microbiome was sampled from the top 10 cm of an upland mineral soil under a deciduous forest ecosystem near the University of Oregon campus in Eugene, OR, USA. Incubations were performed in 500 mL mason jars with rubber septa installed in the lids. Each jar was sterilized with 70% ethanol to which was added 45 g of autoclaved potting mix, 5 g of living soil, and 3.5 mL of sterile deionized water to bring them to 60% of field capacity. The potting mix was Lane Potting Mix from Lane Forest Products (Eugene, OR, USA). Each jar was then capped and injected with 4.3 mL of 99% CH4 to bring the headspace concentration to approximately 1000 ppm CH4. To create the two treatment groups, twenty-four jars were created in an identical manner and then randomly assigned to either the selection or control line. Jars were flushed and respiked with CH4 twice per week to maintain aerobic conditions and elevated CH4 concentrations and were incubated at ambient temperature for approximately four weeks. Methane oxidation rates were determined at the end of the incubation period and selection was performed. For each treatment, the selected jars were homogenized and 5 g of the homogenized soil was used to inoculate the next set of jars, which represents a 10% subsampling. The next set of jars were created in an identical manner to the first generation with fresh autoclaved potting mix and the same moisture and CH4 content.

## Methane oxidation rate

Methane oxidation rates were determined after flushing and spiking jars to approximately 1000 ppm CH4. Headspace samples of 1 mL were collected from each jar immediately after spiking and then at time points 3, 6, 24, and 48 hours for a 5-point curve. Samples were immediately injected into a SRI model 8610C gas chromatograph equipped with a flame ionization detector (SRI Instruments, Torrance, CA, USA) to determine the headspace CH4 concentration. We applied a first-order exponential decay function to determine the rate constant (k, units = d−1; i.e., dCH4/dt = k[CH4]) of the exponential decrease in CH4 Oxidation rates are presented as the additive inverse of (i.e., ) so that a more positive value represents a greater oxidation rate. The jars selected in passage two for the positive selection treatment had the lowest CH4 oxidation rate of the twelve jars due to a calculation error in the rate constant. Despite this error, we still observed a strong response to selection demonstrating the power of this technique. In addition, this likely reintroduced diversity lost during the selection process, potentially increasing the variation available to be selected upon, as is recommended for microbiome selection experiments (24).

## Soil DNA extraction and sequencing

A subsample of soil from the starting inoculum and from every jar in passages 2 and 5 was collected and stored at C. Soil DNA was extracted from 0.25 g soil. Negative controls were extracted from autoclaved potting mix and DNase-free water. Extractions were performed using the DNeasy PowerSoil kit (QIAGEN, Düsseldorf, Germany) and quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). To estimate the diversity and relative abundance of the bacterial and archaeal taxa in our soil ecosystems, we sequenced the V4 region of the 16S rRNA gene using the 515F - 806R primer combination (25). PCR mixtures were: 10 l NEBNext Q5 Hot Start HiFi PCR master mix, 9.2 l primer mixture (1.09 M concentration), and 0.8 l of DNA template. Reaction conditions were: C for 30 s (initialization); 35 cycles of C for 10 s (denaturation), C for 20 s (annealing), and C for 20 s (extension); and C for 2 m (final extension). Reactions were performed in triplicate and then combined. Amplicons were purified twice using 0.8x ratio Mag-Bind RxnPure Plus isolation beads (Omega Bio-Tek, Norcross, GA, USA). Sequencing libraries were prepared using a dual-indexing approach (26,27). Amplicon concentrations were quantified using Qubit and multiplexed at equimolar concentration. Sequencing was performed at the University of Oregon Genomics Core Facility on the Illumina NovaSeq 6000 with paired-end 150 bp reads (Illumina, Inc., San Diego, CA, USA).

## Bioinformatics

Bioinformatics processing was performed in ‘R’ (28). Demultiplexed sequencing reads were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs) (29). Taxonomic assignment was performed using the Ribosomal Database Project naive Bayesian classifier (30). The presence of contaminants was evaluated using both the prevalence and frequency methods from ‘DECONTAM’ (31). Decontam identified 16 potential contaminants based on prevalence and frequency. Visual inspection of abundance-concentration plots indicated that 9 of these were likely contaminants and these ASVs were removed. Amplicon sequence variants that were assigned chloroplast or mitochondria taxonomy were removed prior to analysis.

## Statistical Analysis

Statistical analyses were performed in ‘R’ (28). To determine whether there was a significant change in CH4 oxidation rate as a response to selection, we tested a difference in slopes between the selection and control lines. Residuals did not meet the assumptions of constant variance and normal distribution. Therefore, CH4 oxidation rates were log10 transformed prior to analysis. First, we tested if there was a difference of slopes between the selection line and the control based on the interaction between passage and treatment. To test the interaction, we fit two nested models with and without the interaction term and compared them using an F-test with the ‘anova’ function. We then present the slopes for each treatment, which represented the change in CH4 oxidation rate per passage as a response to selection.

We estimated the proportion of variation in CH4 oxidation rate due to variation in the microbiome as the regression of divergence between the positive line and the control on the cumulative selection differential (32). Divergence was calculated as the mean CH4 oxidation rate of the positive treatment minus the mean CH4 oxidation rate of the control in each passage. The selection differential was calculated as the difference between the mean of the three selected jars and the mean of all twelve jars in a passage. Cumulative selection differential was calculated as the sum of the selection differential from all preceding selection events. We then regressed cumulative divergence on cumulative selection differential using the ‘lm’ function.

Richness was estimated using the method from (33) with a subsample size of 176,545 calculated via the ‘rarefy’ function in ‘vegan’ (34). We tested a difference in richness by both passage and treatment with a Kruskal-Wallace test followed by a pairwise Wilcoxon test. Next, we estimated beta-diversity as the Bray-Curtis dissimilarity by averaging 100 random subsets with a subsample size of 176,545 using the ‘avgdist’ function in ‘vegan’ (34,35). We tested a difference in centroid and dispersion of beta diversity by passage and treatment using a permutational analysis of variance (PERMANOVA) with 999 permutations using the ‘adonis2’ function from ‘vegan’ and tested a difference of group dispersions using ‘betadisper’ and ‘anova’ with 999 permutations (34,36). Lastly, we tested the correlation between CH4 oxidation rate and Bray-Curtis dissimilarity in Passage 5 with a distance-based redundancy analysis (dbRDA) using the ‘dbrda’ function in ‘vegan’ and estimated the p-value using a permutation F-test with 999 permutations (34,36)

To identify taxa that responded to selection on CH4 oxidation rate, we tested differential abundance between the two treatments in passage 5. We first grouped ASVs at the family level. Any ASVs that lacked a family-level taxonomic assignment were grouped at a higher taxonomic level. We then subset the samples in Passage 5 and removed all families with a prevalence of less than 10% in either treatment. We used three methods for testing differential abundance: ANCOM-II, ALDEx2, and CORNCOB (37–40). We then identified the consensus taxa that were significant with all three tests and plotted their relative abundances. For ANCOM-II, we used the ‘ancom’ function in the ‘ANCOM-BC’ package with a cutoff of W = 0.7 (37,38). For ALDEx2, we used the ‘aldex’ function in the ‘ALDEx2’ package with Welch’s t-test and we used an effect size of 1 as our significance threshold (39). Finally, we used CORNCOB with the ‘differentialTest’ function in the ‘corncob’ package with the Wald test and without bootstrapping (40). Lastly, to test differentially abundant methanotrophs, we subset all ASVs within methanotrophic families and tested their differential abundance aggregated at the family and genus level using ‘corncob’.

# Results

## Response to selection on methane oxidation rate

We observed a response to artificial selection on whole-ecosystem soil CH4 oxidation rate (Figure 2; difference of slopes: F2,113 = 3.85, p = 0.02). At the start of the experiment, the positive selection treatment had a mean CH4 oxidation rate that was 24% lower than the control (difference of y-intercepts = -0.34, SE = 0.16, t = -2.14, p = 0.03). There was no change in CH4 oxidation rate in the control over the five passages (slope = -0.01, SE = 0.05, t = -0.26, p = 0.80). By contrast, the selection treatment had a 50.7% increase in CH4 oxidation rate per passage (slope = 0.18, SE = 0.06, t = 2.76, p = 0.01).

To estimate the proportion of variation in CH4 oxidation rate due to variation in microbiome composition, we regressed divergence between the positive selection treatment and the control against the cumulative selection differential (Figure 3). This estimate is analogous to estimates of “microbiability” from the animal breeding literature, which quantifies the variation in a host trait that is due to microbiome variation (41). The slope of the regression of divergence on cumulative selection differential provides an estimate of realized microbiability (h2 ± SE), which in our experiment was 0.31 ± 0.17, though this was not significant (F1,2 = 3.44, p = 0.20).

## Taxonomic richness

Median ASV richness decreased from 3406.6 (778.5) in passage 2 to 1557.8 (157.7) in passage 5 (Kruskal-Wallace test: = 35.4, df = 3, p < 0.001; pairwise Wilcoxon test: p < 0.001). However, there was no difference in richness between the selection treatment and the control in passage 2 or 5 (pairwise Wilcoxon test: p = 0.7). In addition, there was no correlation between richness and CH4 oxidation rate across the two treatments in passage 5 (Spearman’s rho = -0.2, p = 0.3).

## Community dissimilarity

Bray-Curtis dissimilarity of the soil microbiome varied strongly by passage and weakly by treatment with an interaction between passage and treatment (Figure 4). Passage explained 55.9% of the variation in Bray-Curtis dissimilarity (F1,44 = 73.3, p = 0.001), treatment explained 5.9% of the variation (F1,44 = 7.8, p = 0.001), and the interaction between treatment and passage explained 4.7% of the variation (F1,44 = 6.2, p = 0.003). There was no difference in dispersion between treatments or passages (F3,44 = 0.91, p = 0.45). Finally, CH4 oxidation rate was correlated with Bray-Curtis dissimilarity across both treatments in passage 5 and explained 9.6% of the variation in Bray-Curtis dissimilarity (dbRDA: F1,22 = 2.34, p = 0.010)

## Taxa that responded to selection

To identify taxa that responded to selection on soil CH4 oxidation rate, we tested the differential relative abundance of families in the selected jars relative to the control jars within passage 5 using three methods and then plotted the taxa identified by all three methods. We identified 12 families that were enriched or depleted in the selection treatment relative to the control (Figure 5).

Overall, none of the families enriched in the selection treatment contain known methanotrophs. Several taxa identified had a higher taxonomic designation that contains methanotrophs, for example, the Gammaproteobacteria class had a large effect size. The Gammaproteobacteria include the type I and type X methanotrophs in the families *Methylococcaceae* and *Methylothermaceae* (42). However, the Gammaproteobacteria is among the most diverse groups in the Prokaryotes, so this is not strong evidence for a selection response by methanotrophs (43). In addition, the *Puniceicoccaceae* is a member of the phylum Verrucomicrobia. The Verrucomicrobia is a diverse group that contain known methanotrophs as well as ammonia-oxidizing bacteria (44). Other than these two groups, none of the other taxa enriched in the selection treatment are known to be related to methanotrophs. Two groups in the Armatimonadales were enriched in the selection treatment including the family *Fimbriimonadaceae* and an unclassified ASV from the order Armatimonadales (45). *Cytophagaceae* was also enriched in the selection treatment and contains a number of mainly aerobic heterotrophs that can digest a variety of macromolecules (46). The remaining families include the uncultured family 0319-6G20, *Diplorickettsiaceae*, *Rhodospirillaceae*, and an unclassified Kapabacteriales.

We did not identify any methanotrophic families in the overall differential abundance analysis. However, we wanted to look more closely at the known methanotrophs in our dataset to be sure that they did not have an effect. To do this, we subset the ASVs in our dataset that were in families that contained methanotrophs. Only two families were represented: *Methylacidiphilaceae* and *Beijerinckiaceae*. Aggregating reads at the family level, neither family was differentially abundant between the two treatments. However, aggregated at the genus level, a group of unclassified genera in the *Beijerinckiaceae* were depleted in the selection treatment and the genus *Rhodoblastus*, a member of the *Beijerinckiaceae*, was enriched in the selection treatment. While many *Beijerinckiaceae* are methanotrophs, several taxa in this family have lost the ability to oxidize CH4 and it appears that *Rhodoblastus* species are not able to grow on CH4, though they can grow on methanol (47,48). Based on this analysis, it appears that no methanotrophs were enriched in the positive selection treatment.

# Discussion

We used artificial ecosystem selection to estimate the contribution of variation in microbiome composition to variation in the rate of an ecosystem function, CH4 oxidation in soil, independent of environmental variation. Understanding how and to what degree microbiome variation contributes to variation in ecosystem function is important for many reasons. For example, successful microbiome manipulations require that the manipulated microbiome contribute to variation in ecosystem function independent of other drivers of ecosystem variation (such as variation in environmental conditions). This is because the drivers of variation in ecosystem functions can interact in complicated ways (Figure 1); for example, environmental variation can indirectly contribute by providing conditions that select for microbial groups that in turn alter the rates of ecosystem functions. Determining the independent contribution of microbiome variation to ecosystem function is crucial because if microbiome composition is driven primarily by environmental conditions, then introducing a desirable taxon through microbiome manipulation without altering the environment will likely be unsuccessful at shifting the targeted ecosystem function. The artificial selection approach is different from the comparative and manipulative approaches used in past attempts at answering this question, because it can control for both the direct effect of environment on function as well as the indirect effect on environment via its impact on microbiome assembly.

In our study, we observed an increase in CH4 oxidation rate in the selection treatment relative to the control, which demonstrates that there was a response to selection. Given that we observed a response to selection, we conclude that variation in the microbiome contributes to variation in the CH4 oxidation rate independent of the environment. This suggests that microbiome manipulations could be an effective approach for altering the rate of CH4 oxidation in this soil, and that the artificial selection approach may be useful in determining the potential for microbiome manipulations for other functions in other ecosystems.

Given that variation in the microbiome is associated with variation in the rate of an ecosystem function in our system, a reasonable follow-up question is “how much variation in ecosystem function is associated with microbiome variation in this system?” One way to estimate this is to determine how much the recipient jars resemble the selected donor jars that were used to inoculate them (32). We can calculate the response to selection as the difference between two successive passages in their mean CH4 oxidation rate. We will denote this as . We can also calculate the strength of selection as the difference in mean CH4 oxidation rate between the twelve jars in one generation and the three jars chosen for selection in that generation, which we will call the selection differential and denote as . If we plot the cumulative against the cumulative , the slope of this relationship will equal the proportion of variation explained by the microbiome. If the change in mean function from passage one to passage two () is equal to the difference in mean function between the twelve jars in passage one and the three jars selected to inoculate passage two (), then we would conclude that 100% of the variation is due to variation in the microbiome. Likewise, if recipients do not resemble the donors in their mean CH4 oxidation rate and simply wander randomly, then we would conclude that all of the variation is due to the environment or technical variation.

This relationship between microbiome variation and ecosystem function variation is analogous to the concept of “heritability” (49) used by quantitative geneticists, or more precisely the concept of “microbiability” (41) proposed by microbiome scientists who study host-associated microbiomes. Although rarely used in the study of environmental microbiomes, this concept could be very useful for understanding and manipulating microbially-mediated functions in a variety of ecosystems. In our experiment, variation in microbiome taxonomic composition statistically explained (i.e., was associated with) 31.5% of the variation we observed in the rate of CH4 oxidation. This suggests that there is substantial potential for altering this ecosystem function through microbiome manipulation in this soil. It is very likely that the “environmental microbiability” will be different for other ecosystem functions in this soil and for CH4 oxidation in other soils. However, our experiment demonstrates that this relationship is measurable and provides an example of how this can be accomplished.

Given that variation in the microbiome is driving substantial variation in CH4 oxidation rate in our system, we next wanted to determine which aspects of the microbiome best explain this contribution of microbiome variation to CH4 oxidation rate. There are three inter-related ways that microbiomes could have responded to selection in this experiment: gain or loss of taxa, changes in the relative abundances of taxa, or changes within the genomes of the constituent taxa. We surveyed microbiome variation via 16s rRNA ribotyping in our experiment, which allowed us to deeply sample taxonomic diversity but did not allow us to directly address whether taxa in this experiment evolved genomic changes as a result of selection. However, if such genomic changes resulted in increased persistence or abundance of the population with these changes, this would be detectable. Therefore, we will focus on the first two possibilities.

Richness at the ASV level did not vary between the two treatments and there were relatively few taxa gained or lost in the selection treatment and none of these were prevalent across the 12 jars in passage 5. Therefore, the gain or loss of species is unlikely to explain the increase in CH4 oxidation rate. However, we found that Bray-Curtis dissimilarity was greater between the two treatments in Passage 5 than within each treatment and was correlated with CH4 oxidation rate, which suggests that changes in the relative abundance of taxa could explain the response to selection.

Even though we observed an increase in CH4 oxidation rate in the selection treatment and a difference in composition between the two treatments, we did not observe an increase in the relative abundance of methanotrophs. This was surprising given that CH4 consumption is not a common trait among microbes and that it is often assumed that the rate of an ecosystem function is limited by the final enzymatic step in the underlying metabolic pathway (11). In certain ecosystems, CH4 production and consumption are correlated with the abundance of methanogens and methanotrophs as estimated from marker genes (18,19). However, our results suggest that ecosystem-scale CH4 oxidation rates can be altered by non-methanotrophs, perhaps through ecological interactions with methanotrophic species. This suggests that simple assumptions about how microbes contribute to rate variation in ecosystem function may not apply universally, and it demonstrates the importance of using biologically “agnostic” approaches (that make few starting assumptions) to linking microbial taxa to ecosystem functions (50). Artificial ecosystem selection is an important example of such an approach.

There is increasing interest in using artificial selection for understanding and manipulating the microbiomes associated with plants and animals (a.k.a., “microbiome breeding”; (51)). Our study demonstrates that artificial ecosystem selection can also be an important tool for exploring the relationship between microbiome composition and ecosystem function in non-host systems. This approach can provide unique information about the independent contribution of microbiomes to ecosystem functions. Such information is crucial if we are to successfully manipulate environmental microbiomes to alter ecosystem functions, whether to improve crop productivity (52) or ameliorate the impacts of environmental change (53).

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# Competing Interests

We declare we have no competing interests.

# Data Availability Statement

The 16S rRNA sequencing data generated during the current study are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA832314, <https://www.ncbi.nlm.nih.gov/sra/PRJNA832314>. The metadata generated during the current study as well as the scripts to recreate the analysis are available on Github, <https://github.com/amorris28/artificial_ecosystem_selection>.

# Supplement

# References

1. Conrad R. Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). Microbiological Reviews. 1996 Dec;60(4):609–40.

2. Schimel JP, Gulledge J. Microbial community structure and global trace gases. Global Change Biology. 1998;4(7):745–58.

3. Crowther TW, Hoogen J van den, Wan J, Mayes MA, Keiser AD, Mo L, et al. The global soil community and its influence on biogeochemistry. Science. 2019 Aug;365(6455).

4. Cavigelli MA, Robertson GP. The Functional Significance of Denitrifier Community Composition in a Terrestrial Ecosystem. Ecology. 2000;81(5):1402–14.

5. Balser TC, Firestone MK. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry. 2005 Apr;73(2):395–415.

6. Waldrop MP, Firestone MK. Response of Microbial Community Composition and Function to Soil Climate Change. Microbial Ecology. 2006 Nov;52(4):716–24.

7. Strickland MS, Lauber C, Fierer N, Bradford MA. Testing the functional significance of microbial community composition. Ecology. 2009;90(2):441–51.

8. Wagg C, Bender SF, Widmer F, Heijden MGA van der. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences. 2014 Apr;111(14):5266–70.

9. Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications. 2019 Oct;10(1):4841.

10. Graham EB, Knelman JE, Schindlbacher A, Siciliano S, Breulmann M, Yannarell A, et al. Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? Frontiers in Microbiology. 2016;7.

11. Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, et al. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. The ISME Journal. 2015 Aug;9(8):1693–9.

12. Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, et al. An examination of the biodiversityEcosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry. 2001 Oct;33(12):1713–22.

13. Reed HE, Martiny JBH. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology. 2007;62(2):161–70.

14. Swenson W, Wilson DS, Elias R. Artificial ecosystem selection. Proceedings of the National Academy of Sciences. 2000 Aug;97(16):9110–4.

15. Blouin M, Karimi B, Mathieu J, Lerch TZ. Levels and limits in artificial selection of communities. Ecology Letters. 2015;18(10):1040–8.

16. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME journal. 2015 Mar;9(4):980–9.

17. Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, et al. Three decades of global methane sources and sinks. Nature Geoscience. 2013 Oct;6(10):813–23.

18. Meyer KM, Morris AH, Webster K, Klein AM, Kroeger ME, Meredith LK, et al. Belowground changes to community structure alter methane-cycling dynamics in Amazonia. Environment International. 2020 Dec;145:106131.

19. Meyer KM, Hopple AM, Klein AM, Morris AH, Bridgham SD, Bohannan BJM. Community structure Ecosystem function relationships in the Congo Basin methane cycle depend on the physiological scale of function. Molecular Ecology. 2020;29(10):1806–19.

20. Freitag TE, Prosser JI. Correlation of Methane Production and Functional Gene Transcriptional Activity in a Peat Soil. Applied and Environmental Microbiology. 2009 Nov;75(21):6679–87.

21. Freitag TE, Toet S, Ineson P, Prosser JI. Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. FEMS Microbiology Ecology. 2010 Jul;73(1):157–65.

22. Schnyder E, Bodelier PLE, Hartmann M, Henneberger R, Niklaus PA. Positive diversity-functioning relationships in model communities of methanotrophic bacteria. Ecology. 2018;99(3):714–23.

23. Martiny AC, Treseder K, Pusch G. Phylogenetic conservatism of functional traits in microorganisms. The ISME Journal. 2013 Apr;7(4):830–8.

24. Sánchez Á, Vila JCC, Chang C-Y, Diaz-Colunga J, Estrela S, Rebolleda-Gomez M. Directed Evolution of Microbial Communities. Annual Review of Biophysics. 2021;50(1):323–41.

25. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences. 2011 Mar;108(Supplement 1):4516–22.

26. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014 Dec;2(1):1–7.

27. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology. 2013 Sep;79(17):5112–20.

28. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018.

29. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016 Jul;13(7):581–3.

30. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology. 2007 Aug;73(16):5261–7.

31. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018 Dec;6(1):1–14.

32. Falconer DS, MacKay TFC. Introduction to quantitative genetics. Harlow : Prentice Hall; 1996.

33. Hurlbert SH. The Nonconcept of Species Diversity: A Critique and Alternative Parameters. Ecology. 1971;52(4):577–86.

34. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: Community ecology package. 2019.

35. Bray JR, Curtis JT. An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecological Monographs. 1957;27(4):325–49.

36. McArdle BH, Anderson MJ. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. Ecology. 2001;82(1):290–7.

37. Kaul A, Mandal S, Davidov O, Peddada SD. Analysis of Microbiome Data in the Presence of Excess Zeros. Frontiers in Microbiology. 2017 Nov;8:2114.

38. Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction. Nature Communications. 2020 Jul;11(1):3514.

39. Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome. 2014 May;2(1):15.

40. Martin BD, Witten D, Willis AD. Modeling microbial abundances and dysbiosis with beta-binomial regression. The Annals of Applied Statistics. 2020 Mar;14(1):94–115.

41. Difford G, Lassen J, Lovendahl P. Genes and microbes, the next step in dairy cattle breeding. In: EAAP-67th annual meeting 2016. Wageningen Academic Publishers; 2016. pp. 285–5.

42. Stein LY, Roy R, Dunfield PF. Aerobic Methanotrophy and Nitrification: Processes and Connections. In: eLS. John Wiley & Sons, Ltd; 2012.

43. Garrity GM, Bell JA, Lilburn TG. Class III. Gammaproteobacteria class. Nov., p. 1. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey’s manual of systematic bacteriology. Second. New York, NY: Springer; 2005.

44. Freitag TE, Prosser JI. Community Structure of Ammonia-Oxidizing Bacteria within Anoxic Marine Sediments. Applied and Environmental Microbiology. 2003 Mar;69(3):1359–71.

45. Im W-T, Hu Z-Y, Kim K-H, Rhee S-K, Meng H, Lee S-T, et al. Description of Fimbriimonas ginsengisoli gen. Nov., sp. Nov. Within the Fimbriimonadia class nov., of the phylum Armatimonadetes. Antonie van Leeuwenhoek. 2012 Aug;102(2):307–17.

46. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W. The Family Cytophagaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes: Other Major Lineages of Bacteria and The Archaea. Berlin, Heidelberg: Springer; 2014. pp. 577–93.

47. Tamas I, Smirnova AV, He Z, Dunfield PF. The (d)Evolution of methanotrophy in the Beijerinckiaceaea comparative genomics analysis. The ISME Journal. 2014 Feb;8(2):369–82.

48. Dedysh SN, Haupt ES, Dunfield PF2. Emended description of the family Beijerinckiaceae and transfer of the genera Chelatococcus and Camelimonas to the family Chelatococcaceae fam. Nov. International Journal of Systematic and Evolutionary Microbiology. 2016;66(8):3177–82.

49. Lynch M, Walsh B. Genetics and Analysis of Quantitative Traits. Oxford University Press; 1998.

50. Morris A, Meyer K, Bohannan B. Linking microbial communities to ecosystem functions: What we can learn from genotypePhenotype mapping in organisms. Philosophical Transactions of the Royal Society B: Biological Sciences. 2020 May;375(1798):20190244.

51. Mueller UG, Linksvayer TA. Microbiome breeding: Conceptual and practical issues. Trends in Microbiology. 2022 Oct;30(10):997–1011.

52. Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. Manipulating the soil microbiome to increase soil health and plant fertility. Biology and Fertility of Soils. 2012 Jul;48(5):489–99.

53. Jansson JK, Hofmockel KS. Soil microbiomes and climate change. Nature Reviews Microbiology. 2020 Jan;18(1):35–46.

# Figure Captions

Figure 1: Variation in the rate of an ecosystem function is the result of at least three components: variation in the abiotic environmental conditions, variation in microbiome composition, and the covariance between microbiomes and the environment. The arrows represent causal relationships between the components. It is important to isolate the direct effect of the microbiome from the effect of the environment via covariance with the microbiome. Here, we attempt to isolate the effect of the microbiome through artificial selection on microbiome composition. For simplicity, we omitted the reverse arrows as well as the interactions, though these relationships may also exist.

Figure 2: Response to selection on soil CH4 oxidation rate. The y-axis is CH4 oxidation rate as the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1 so that a more positive value represents a higher CH4 oxidation rate. Closed points are the positive selection treatment and open points are the control. The solid line is the regression for the positive selection treatment and the dotted line is the regression for the control. There was a significant difference of slopes between the positive selection treatment and the control (F2,113 = 3.85, p = 0.02).

Figure 3: Divergence between the positive selection line and the control line over the cumulative selection differential. Values are CH4 oxidation rate as the additive inverse of the first-order exponential decay constant k (day-1). Regression of divergence on cumulative selection differential provides an estimate of realized heritability (h2 ± SE) and the slope of the regression was 0.31 ± 0.17, though this was not significant (F1,2 = 3.44, p = 0.20).

Figure 4: Non-metric multidimensional scaling plot of beta diversity for all jars. Dissimilarities are rarefied Bray-Curtis dissimilarity averaged over 100 subsamples. Closed points are the positive selection treatment and open points are the control. Triangles are passage 2 and circles are passage 5.

Figure 5: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2, and CORNCOB. Values on the x-axis are relative abundances on a log10 scale. Taxa in the top panel are enriched in the positive selection treatment relative to the control and taxa in the bottom panel are depleted in the positive selection treatment relative to the control. Taxa are sorted by their effect size with taxa at the top having the largest positive effect size and taxa at the bottom with the largest negative effect size.