Artificial ecosystem selection reveals relationships between microbiome composition and ecosystem function

Andrew H. Morris1\* and Brendan J. M. Bohannan1

1 Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA  
\* Correspondence: Andrew H. Morris, amorris3@uoregon.edu  
Competing Interests: We declare we have no competing interests.

# 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 members of a microbiome 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 methane oxidation rate. We observed a strong response to selection with a 50.7% increase in methane oxidation rate per passage. In addition, we estimated that 50.2% of the variation in methane oxidation rate in these soils can be attributed to microbiome variation. We also found that selection did not enrich for known methane oxidizers; rather microbes that responded to selection included members of 12 families not known to oxidize methane, such as *Fimbriimonadaceae*, *Cytophagaceae*, and *Diplorickettsiaceae*. 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 functions in ecosystems, and there is great interest in understanding how attributes of microbiomes may influence variation in ecosystem functions (1–3). Biodiversity-ecosystem function relationships have been documented for a variety of macroorganismal communities. For example, plant species richness correlates with productivity and marine community diversity correlates with production, consumption, and nutrient release (4–6). However, we have been unsuccessful in documenting similar relationships for microbial communities. While there is some evidence that microbiomes from different ecosystems display different functional rates (7,8), adding microbiome attributes to ecosystem models adds very little to total variance explained (9). Therefore, we still lack a detailed understanding of what functions are altered by microbiome composition and what attributes of the microbiome explain this relationship.

There are several reasons for this difficulty. One reason is that it is challenging to manipulate microbiome composition and diversity directly. Experimental manipulations of community composition and diversity have been central to documenting the relationships between ecosystem functions and the biodiversity of plants and animals (5), but this is seldom possible with microbiomes. Most microorganisms cannot yet be cultured and most available methods for manipulation of microbiomes (e.g., antibiotics, dilution, etc.) are not sufficiently specific to manipulate individual microbial lineages. In addition, many studies have looked for relationships between an ecosystem function and the abundance or diversity of a microbial gene that codes for an enzyme or enzyme-subunit known to be involved in that function. However, it is difficult to identify the “right” marker gene a priori and for most functions in most ecosystems there is no correlation between the abundance of a marker gene and the rate of the corresponding process (10). Lastly, microbiome-ecosystem function relationships have been difficult to document because microbiomes often covary with other drivers of variation in ecosystem functions, such as environmental conditions or resources. Such covariation can make it impossible to determine whether microbiomes directly contribute to functional variation. Understanding the degree to which microbiomes directly influence functional variation is crucial if scientists are to develop microbiome-focused methods for altering or maintaining ecosystem functions.

One way to overcome these challenges is to manipulate the relationship between microbiome biodiversity and ecosystem function using artificial ecosystem selection (11–14). Microbiomes have been shown to respond to selection at the ecosystem-trait level (11), and some ecosystem-scale microbiome traits have been shown to be transferrable through microbiome inoculations (13). Therefore, applying artificial selection to whole microbiomes could be a novel approach for describing a more detailed microbiome-ecosystem function relationship. This approach works by subsampling a microbiome to inoculate multiple replicate ecosystems that vary in microbiome composition and then measuring the rate of ecosystem function for each replicate. The investigator then selects the replicates with the highest (or lowest) rates and subsamples those ecosystems to passage them to a new set of ecosystems. By holding environmental conditions relatively constant among the replicates and from passage to passage (or by using isogenic hosts, in the case of host-associated microbiomes), investigators can determine whether variation in the microbiome contributes to variation in the rate of ecosystem function independent of the environment.

A valuable feature of this approach is that it can be used to estimate how much of the variation in ecosystem function is due to microbiome variation. By calculating the slope of the regression between the “donor” ecosystems selected in one passage and the “recipient” ecosystems inoculated by those donors in the next passage, we can quantify an estimate of “ecosystem heritability” (12,15). The concept of heritability has been borrowed from quantitative genetics where heritability is defined as *the ratio of additive genetic variance to the total phenotypic variance* and is commonly estimated as *the slope of the linear regression between offspring phenotype and parental phenotype* (16,17). Here we apply this concept to donor and recipient communities in an artificial ecosystem selection experiment to estimate *the variance in ecosystem function due to microbiome variance*. We do not assume that environmental variation is zero, instead we quantify the proportion of variation due to microbiome effects. If the slope of the regression is zero, we would conclude that environmental variation among the replicates and technical error are the primary drivers of variation in ecosystem function. On the other hand, a significant slope between donors and recipients would demonstrate that microbiome variation generates variation in the rate of an ecosystem function. We then use our artificially selected microbiomes to investigate what aspects of microbiome variation explain this response. By “microbiome variation” we mean aspects of microbial diversity commonly measured by microbial ecologists, such as the relative abundance of taxonomic groups inferred from amplicon sequence variants. With this approach, we can not only determine whether an ecosystem function is influenced by microbiome variation, but also estimate how much of the variation and which taxa are associated with higher rates of ecosystem function.

We applied artificial ecosystem selection to soil microbial communities by selecting on soil methane oxidation rate. We chose this function because methane is a globally important greenhouse gas and methane oxidation by soil bacteria is the primary biological sink for atmospheric methane (18). In addition, there is evidence that soil methane oxidation rate may vary with microbiome composition. For example, soil microbiome phylogenetic variation is a strong predictor of methane oxidation in forests and pastures of the Brazilian Amazon as well as soils of the Congo Basin (19,20). Finally, methanotrophy is one of the most deeply conserved microbial physiologies and is represented in a narrow range of taxa and so the taxonomic composition of the microbiome is more likely to be associated with the rate of methane oxidation than other broader or more shallowly conserved functions (2,21). This suggests that microbiome variation might drive variation in methane oxidation in soils. One other study has estimated ecosystem heritability for an environmental microbiome. Blouin et al. (12) selected for water microbiomes with low carbon dioxide respiration rates and estimated a heritability of 52%. However, this appeared to be driven primarily by biomass, not microbiome composition. Here we want to build on this work by selecting for higher rates of a more narrow function that is less likely to be driven by total biomass. That way we can begin to assess whether variation in microbiome composition is important for understanding variation in ecosystem functions.

In this study, we evaluated whether variation in the microbiome contributes to variation in the rate of ecosystem functions. To address this problem, we applied artificial ecosystem selection to methane oxidation rate of the soil microbiome and answered the following questions: Does variation in the relative abundance of microbial taxa contribute to variation in soil methane oxidation rate in the lab? How much of the variation in soil methane oxidation rate can be attributed to the microbiome? What attributes of the microbiome regulate variation in methane oxidation rate? And do these attributes match our assumptions about what regulates methane oxidation rate in nature?

# Materials and Methods

## Experimental design

We performed an artificial ecosystem selection experiment (*sensu* 11) by passaging replicate soil microbiomes. The trait we selected on was CH4 oxidation rate. This experiment had two selection lines with twelve jars each for a total of twenty-four jars per passage. One line was a positive selection line where the two or three jars with the highest CH4 oxidation rate were homogenized to inoculate the next set of positive jars. The other line was a neutral selection line where an equal number of jars as the positive line were chosen at random to inoculate the next set of neutral 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 positive or neutral selection line. Jars were flushed and respiked with methane twice per week to maintain aerobic conditions and elevated CH4 concentrations and were incubated at ambient temperature for approximately three weeks. Methane oxidation rates were determined at the end of the incubation period. For the positive treatment, the three jars with the greatest CH4 oxidation rates were chosen to inoculate the next generation. For the neutral treatment, three jars were randomly selected to inoculate the next generation. 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 methane. 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 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 (14).

## 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 (22). 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 (23,24). 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’ (25). Demultiplexed sequencing reads were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs) (26). Taxonomic assignment was performed using the Ribosomal Database Project naive Bayesian classifier (27). The presence of contaminants was evaluated using both the prevalence and frequency methods from ‘DECONTAM’ (28). 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’ (25). 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 positive and neutral selection 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 positive and neutral treatments 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 ecosystem heritability of methane oxidation rate, which is the proportion of variance in CH4 oxidation rate due to variation in the microbiome, as the regression of mid-recipient on mid-donor (17). The mid-donor was the mean for all three selected jars and the mid-recipient was the mean methane oxidation rate for the twelve jars produced by those three donors. First, we tested if there was a difference of slopes between the positive and neutral treatments. To test this, we fit two nested models with and without the treatment term and compared them using an F-test with the ‘anova’ function. We then present the slopes for each treatment, which represented the ecosystem heritability or the proprotion of total variance in methane oxidation rate due to microbiome variance.

First, we tested if there was an effect of treatment by comparing nested models with and without treatment using an F-test with the ‘anova’ function. We then fit the full model to estimate the slope of the relationship between recipient and donor for the Positive and Neutral treatments.

Richness was estimated using the method from (29) with a subsample size of 176,545 using the ‘rarefy’ function in ‘vegan’ (30). 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’ (30,31). 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 (30,32). 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 (30,32)

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 (33–36). 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 (33,34). 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 (35). Finally, we used CORNCOB with the ‘differentialTest’ function in the ‘corncob’ package with the Wald test and without bootstrapping (36). 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 1; difference of slopes: F2,113 = 1.86, p = 3.85). At the start of the experiment, the Positive treatment had a mean CH4 oxidation rate that was 24% lower than the Neutral treatment (difference of y-intercepts = -0.34, SE = 0.16, t = -2.14, p = 0.03). There was no change in CH4 oxidation rate of the Neutral treatment over the five passages (slope = -0.01, SE = 0.05, t = -0.26, p = 0.80). By contrast, the Positive treatment had a 50.7% increase in CH4 oxidation rate per passage (slope = 0.18, SE = 0.06, t = 2.76, p = 0.01).

We estimated the ecosystem heritability defined as the proportion of variation in methane oxidation due rate to variation in the microbiome as the regression of mid-recipient on mid-donor (Figure 2). Recipient CH4 oxidation rates were correlated with donor CH4 oxidation rates in both the Positive treatment (slope = 0.50, SE = 0.24, t = 4.43, p = 0.01) and the Neutral treatment (slope = -0.58, SE = 0.20, t = -2.89, p = 0.04).

## 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 Positive and the Neutral treatment 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 3). Passage explained 55.8% 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 = 2.35, p = 0.009)

## 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 Positive jars relative to the Neutral 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 Positive treatment relative to the Neutral treatment (Figure 4).

Overall, none of the families enriched in the Positive 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* (37). However, the Gammaproteobacteria is among the most diverse groups in the Prokaryotes, so this is not strong evidence for a selection response by methanotrophs (38). 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 (39). Other than these two groups, none of the other taxa enriched in the Positive treatment are known to be related to methanotrophs. Two groups in the Armatimonadales were enriched in the Positive treatment including the family *Fimbriimonadaceae* and an unclassified ASV from the order Armatimonadales (40). *Cytophagaceae* was also enriched in the Positive treatment and contains a number of mainly aerobic heterotrophs that can digest a variety of macromolecules (41). 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 they did not have an effect. To do this, we subset all of 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 Positive treatment and the genus *Rhodoblastus*, a member of the *Beijerinckiaceae*, was enriched in the Positive treatment. While many *Beijerinckiaceae* are methanotrophs, several taxa in this family have lost the the ability to oxidize CH4 and it appears that *Rhodoblastus* species are not able to grow on CH4, though they can grow on methanol (43). Based on this analysis, it appears that no methanotrophs were enriched in the Positive treatment.

# Discussion

While it is well understood that the microbiome mediates a variety of ecosystem functions, a fundamental question in microbial ecology is whether we need to understand variation in the composition of microbiomes to understand variation in the rate of ecosystem functions (1,2). To address this question, we performed artificial ecosystem selection on soil microbiomes by selecting for ecosystems with high methane oxidation rates. We found that there was a significant increase in methane oxidation rate in the Positive selection treatment relative to the Neutral selection treatment. This demonstrates that passaging the microbiome through artificial ecosystem selection resulted in a shift in the mean methane oxidation rate of the soil microbiome. This suggests that variation in the microbiome can generate variation in ecosystem functions independent of the environment.

We quantified how much of the variation in methane oxidation rate was due to variation in the microbiome by regressing the mean methane oxidation rate of the 12 recipient jars against the mean methane oxidation rate of the three donor jars selected to inoculate the recipients. We observed a heritability of 50.2% for the Positive selection treatment. This indicates that 50.2% of the variation in methane oxidation rate can be attributed to variation in the microbiome. This is comparable to the ecosystem heritability of carbon dioxide emissions from water (12). Notably, we observed a negative heritability in the Neutral treatment (-57.6%). Negative heritability estimates are not uncommon in quantitative genetics (44). This can occur for random selection controls in artificial selection experiments and may indicate that methane oxidation rate is negatively correlated with other traits associated with persistence in jars. The heritability we observed in the Positive treatment indicates that a large portion of the variation in methane oxidation rate is due to variation in the microbiome in our experimental system.

Given that variation in the microbiome is driving the observed variation in methane oxidation rate, we next wanted to address what aspects of the microbiome, in terms of alpha diversity, beta diversity, or the relative abundance of taxa, explain this contribution of the microbiome to variation in methane oxidation rate. There are three ways that the microbiome could respond to selection in this experiment: gain or loss of species, changes in the relative abundances of species, or changes within the genomes of the constituent species. Using 16S rRNA gene sequences, we are unable to address the third possibility of whether taxa in this experiment could have evolved changes in their genomes as a result of selection. 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 Positive 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 methane 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 methane 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 methane oxidation rate in the Positive 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 methane 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 a metabolic pathway (10). In certain ecosystems, methane production and consumption are correlated with the abundance of methanogens and methanotrophs as estimated from marker genes (19,20). However, it does not appear generally true that the rate of an ecosystem function is limited by the abundance of the gene that encodes the final step in that pathway (10). Our results suggest that ecosystem-scale methane oxidation rates can be altered by non-methanotrophs, perhaps through ecological interactions with methanotrophic species.

Another important question is whether selection at the ecosystem scale resulted in within-strain evolution. Using 16S rRNA gene sequencing we are unable to resolve genomic changes within taxa that may have undergone evolution. Therefore, it is possible that the response to selection was driven by changes within the genomes of methanotrophs. But given the slow growth rate of methanotrophs, often requiring weeks to years of serial batch culturing for isolation, this seems like an unlikely explanation (45–47). Future work could analyze an artificial ecosystem selection experiment using genome-resolved metagenomics to try to detect strain-level changes in the constituent taxa. However, reconstructing genomes from in situ soil samples has been difficult to achieve and may require enrichment techniques, particularly for methanotrophs which are a relatively rare component of the soil microbiome (48). If these challenges could be overcome for soil metagenomics, we could begin to evaluate whether ecosystem selection results in strain-level evolution or enriches for certain metabolic pathways within the microbiome.

A fundamental question in microbial ecology is whether variation in the microbiome contributes to variation in ecosystem function. We performed artificial ecosystem selection on soil methane oxidation rate to test the relationship between microbiome variation and soil methane oxidation rate. We observed a response to selection on soil methane oxidation rate and found a high heritability for methane oxidation, which suggests that variation in the microbiome contributes to variation in soil methane oxidation rate independent of the environment. This shows that manipulating the microbiome could be a viable strategy for mitigating or enhancing certain ecosystem functions. Surprisingly, we did not observe an increase in the relative abundance of methanotrophs as a response to selection. This suggests that the rate of methane oxidation could be altered by non-methanotrophs, possibly through ecological interactions with methanotrophs. Future research should investigate other aspects of microbiome variation, such as strain-level evolution or functional gene composition, to begin to describe a more detailed mapping between the microbiome and ecosystem function. This study demonstrates that soil methane oxidation rate can vary greatly with variation in the microbiome and opens up future research opportunities to establish a more detailed understanding of the relationship between the microbiome and ecosystem function.

# Acknowledgments

This project was supported by the National Science Foundation Graduate Research Fellowship Program (grant no. DGE 1255832) and the ARCS Foundation Florence and Mike Nudelman Scholarship.

# 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)](https://doi.org/10.1128/mr.60.4.609-640.1996). Microbiological Reviews. 1996 Dec;60(4):609–40.

2. Schimel JP, Gulledge J. [Microbial community structure and global trace gases](https://doi.org/10.1046/j.1365-2486.1998.00195.x). 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](https://doi.org/10.1126/science.aav0550). Science. 2019 Aug;365(6455).

4. Cardinale BJ, Srivastava DS, Emmett Duffy J, Wright JP, Downing AL, Sankaran M, et al. [Effects of biodiversity on the functioning of trophic groups and ecosystems](https://doi.org/10.1038/nature05202). Nature. 2006 Oct;443(7114):989–92.

5. Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, et al. [A global synthesis reveals biodiversity loss as a major driver of ecosystem change](https://doi.org/10.1038/nature11118). Nature. 2012 Jun;486(7401):105–8.

6. Gamfeldt L, Lefcheck JS, Byrnes JEK, Cardinale BJ, Duffy JE, Griffin JN. [Marine biodiversity and ecosystem functioning: What’s known and what’s next?](https://doi.org/10.1111/oik.01549) Oikos. 2015;124(3):252–65.

7. Balser TC, Firestone MK. [Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest](https://doi.org/10.1007/s10533-004-0372-y). Biogeochemistry. 2005 Apr;73(2):395–415.

8. Strickland MS, Lauber C, Fierer N, Bradford MA. [Testing the functional significance of microbial community composition](https://doi.org/10.1890/08-0296.1). Ecology. 2009;90(2):441–51.

9. 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?](https://doi.org/10.3389/fmicb.2016.00214) Frontiers in Microbiology. 2016;0.

10. 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](https://doi.org/10.1038/ismej.2014.252). The ISME Journal. 2015 Aug;9(8):1693–9.

11. Swenson W, Wilson DS, Elias R. [Artificial ecosystem selection](https://doi.org/10.1073/pnas.150237597). Proceedings of the National Academy of Sciences. 2000 Aug;97(16):9110–4.

12. Blouin M, Karimi B, Mathieu J, Lerch TZ. [Levels and limits in artificial selection of communities](https://doi.org/10.1111/ele.12486). Ecology Letters. 2015;18(10):1040–8.

13. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. [Selection on soil microbiomes reveals reproducible impacts on plant function](https://doi.org/10.1038/ismej.2014.196). The ISME journal. 2015 Mar;9(4):980–9.

14. Sánchez Á, Vila JCC, Chang C-Y, Diaz-Colunga J, Estrela S, Rebolleda-Gomez M. [Directed Evolution of Microbial Communities](https://doi.org/10.1146/annurev-biophys-101220-072829). Annual Review of Biophysics. 2021;50(1):323–41.

15. Goodnight CJ. [Heritability at the ecosystem level](https://doi.org/10.1073/pnas.97.17.9365). Proceedings of the National Academy of Sciences. 2000 Aug;97(17):9365–6.

16. Bell EA. [Heritability in retrospect](https://doi.org/10.1093/oxfordjournals.jhered.a108840). Journal of Heredity. 1977 Sep;68(5):297–300.

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

18. Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, et al. [Three decades of global methane sources and sinks](https://doi.org/10.1038/ngeo1955). Nature Geoscience. 2013 Oct;6(10):813–23.

19. 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](https://doi.org/10.1016/j.envint.2020.106131). Environment International. 2020 Dec;145:106131.

20. 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](https://doi.org/10.1111/mec.15442). Molecular Ecology. 2020;29(10):1806–19.

21. Martiny AC, Treseder K, Pusch G. [Phylogenetic conservatism of functional traits in microorganisms](https://doi.org/10.1038/ismej.2012.160). The ISME Journal. 2013 Apr;7(4):830–8.

22. 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](https://doi.org/10.1073/pnas.1000080107). Proceedings of the National Academy of Sciences. 2011 Mar;108(Supplement 1):4516–22.

23. 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](https://doi.org/10.1186/2049-2618-2-6). Microbiome. 2014 Feb;2(1):6.

24. 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;

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

26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. [DADA2: High-resolution sample inference from Illumina amplicon data](https://doi.org/10.1038/nmeth.3869). Nature Methods. 2016 Jul;13(7):581–3.

27. Wang Q, Garrity GM, Tiedje JM, Cole JR. [Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy](https://doi.org/10.1128/AEM.00062-07). Applied and Environmental Microbiology. 2007 Aug;73(16):5261–7.

28. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. [Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data](https://doi.org/10.1186/s40168-018-0605-2). Microbiome. 2018 Dec;6(1):226.

29. Hurlbert SH. [The Nonconcept of Species Diversity: A Critique and Alternative Parameters](https://doi.org/10.2307/1934145). Ecology. 1971;52(4):577–86.

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

31. Bray JR, Curtis JT. [An Ordination of the Upland Forest Communities of Southern Wisconsin](https://doi.org/10.2307/1942268). Ecological Monographs. 1957;27(4):325–49.

32. McArdle BH, Anderson MJ. [Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis](https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2). Ecology. 2001;82(1):290–7.

33. Kaul A, Mandal S, Davidov O, Peddada SD. [Analysis of Microbiome Data in the Presence of Excess Zeros](https://doi.org/10.3389/fmicb.2017.02114). Frontiers in Microbiology. 2017 Nov;8:2114.

34. Lin H, Peddada SD. [Analysis of compositions of microbiomes with bias correction](https://doi.org/10.1038/s41467-020-17041-7). Nature Communications. 2020 Jul;11(1):3514.

35. 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](https://doi.org/10.1186/2049-2618-2-15). Microbiome. 2014 May;2(1):15.

36. Martin BD, Witten D, Willis AD. [Modeling microbial abundances and dysbiosis with beta-binomial regression](https://doi.org/10.1214/19-AOAS1283). The Annals of Applied Statistics. 2020 Mar;14(1):94–115.

37. Stein LY, Roy R, Dunfield PF. [Aerobic Methanotrophy and Nitrification: Processes and Connections](https://doi.org/10.1002/9780470015902.a0022213). In: eLS. John Wiley & Sons, Ltd; 2012.

38. 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.

39. Freitag TE, Prosser JI. Community Structure of Ammonia-Oxidizing Bacteria within Anoxic Marine Sediments. Applied and Environmental Microbiology. 2003 Mar;

40. 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](https://doi.org/10.1007/s10482-012-9739-6). Antonie van Leeuwenhoek. 2012 Aug;102(2):307–17.

41. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W. [The Family Cytophagaceae](https://doi.org/10.1007/978-3-642-38954-2_382). 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. p. 577–93.

42. Tamas I, Smirnova AV, He Z, Dunfield PF. [The (d)evolution of methanotrophy in the Beijerinckiaceaea comparative genomics analysis](https://doi.org/10.1038/ismej.2013.145). The ISME Journal. 2014 Feb;8(2):369–82.

43. Dedysh SN, Haupt ES, Dunfield PFY2016. [Emended description of the family Beijerinckiaceae and transfer of the genera Chelatococcus and Camelimonas to the family Chelatococcaceae fam. nov.](https://doi.org/10.1099/ijsem.0.001167) International Journal of Systematic and Evolutionary Microbiology. 2016;66(8):3177–82.

44. Steinsaltz D, Dahl A, Wachter KW. [On Negative Heritability and Negative Estimates of Heritability](https://doi.org/10.1534/genetics.120.303161). Genetics. 2020 Jun;215(2):343–57.

45. Whittenbury R, Phillips KC, Wilkinson JFY1970. [Enrichment, Isolation and Some Properties of Methane-utilizing Bacteria](https://doi.org/10.1099/00221287-61-2-205). Microbiology. 1970;61(2):205–18.

46. Hirayama H, Abe M, Miyazaki M, Nunoura T, Furushima Y, Yamamoto H, et al. [Methylomarinovum caldicuralii gen. Nov., Sp. Nov., A moderately thermophilic methanotroph isolated from a shallow submarine hydrothermal system, and proposal of the family Methylothermaceae fam. nov.](https://doi.org/10.1099/ijs.0.058172-0) International Journal of Systematic and Evolutionary Microbiology. 2014;64(Pt\_3):989–99.

47. Kim J, Kim DD, Yoon S. [Rapid isolation of fast-growing methanotrophs from environmental samples using continuous cultivation with gradually increased dilution rates](https://doi.org/10.1007/s00253-018-8978-5). Applied Microbiology and Biotechnology. 2018 Jul;102(13):5707–15.

48. Delmont TO, Eren AM, Maccario L, Prestat E, Esen ÖC, Pelletier E, et al. [Reconstructing rare soil microbial genomes using in situ enrichments and metagenomics](https://doi.org/10.3389/fmicb.2015.00358). Frontiers in Microbiology. 2015 Apr;6.

# Figures

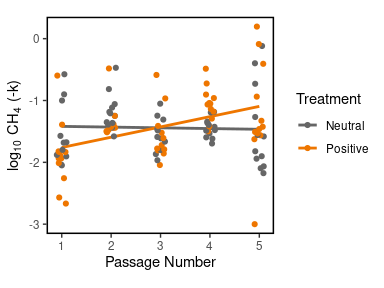


Figure 1: Response to selection on soil CH4 oxidation rate. The y-axis is the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1. The x-axis is the passage number. The orange line and points are the Positive treatment and the gray line and points are the Neutral treatment.

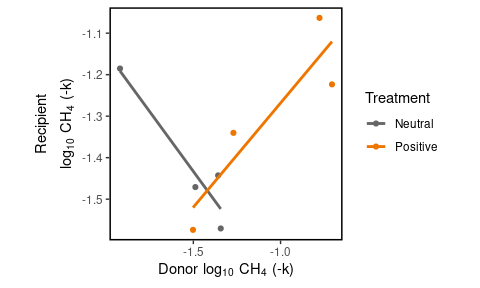


Figure 2: Ecosystem heritability as the regression of mid-recipient methane flux on mid-donor methane flux ( with units day-1). Donor is the mean oxidation rate of the three jars selected to inoculate the next passage. Recipient is the mean oxidation rate of the 12 jars in the next passage. The orange lines and points are the Positive treatment and the gray lines and points are the Neutral treatment. Slope of the regression line estimates the proportion of variation in methane oxidation rate due to variation in the microbiome. For the Positive treatment, the variance explained by the microbiome was 50.2% (slope = 0.502, SE = 0.24) and for the Neutral treatment the variance explained by the microbiome was -57.6% (slope = -0.576, SE = 0.20).

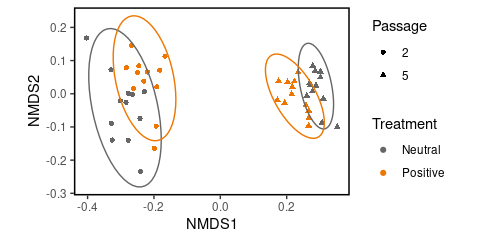


Figure 3: Principal component analysis plot of beta diversity for all jars. Dissimilarities are based on rarefied Bray-Curtis dissimilarity. Colors represent treatment and shapes represent passage number.

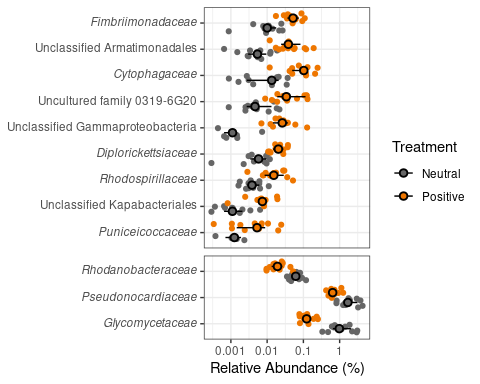


Figure 4: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2, and CORNCOB. Values on the x-axis are relative abundances on a log10 scale. Points are colored by treatment. Large points with error bars are the median relative abundance and interquartile range. Taxa in the top panel are enriched in the Positive treatment and taxa in the bottom panel are depleted in the Positive treatment. 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.