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 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 microcosms to select for increased soil methane oxidation rate. We observed a strong response to selection with a 51% increase in methane oxidation rate per round over five rounds of selection. This suggests that a large portion 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 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 this relationship can be revealed via artificial ecosystem selection.

# 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 described for a variety of macroorganismal communities. For example, plant species richness correlates with productivity and marine community diversity correlates with functions such as production, consumption, and nutrient release (4–6). While there is some evidence that variation in microbiomes can contribute to variation in ecosystem functions, documenting this relationship has been elusive (1,7,8).

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. Microbial ecologists are thus often restricted to using comparative methods, in which microbiome composition and diversity is surveyed via environmental DNA and then correlated with the rate of an ecosystem function {cite examples}. However, such studies are rarely successful (8). In an attempt to increase the likelihood of detecting such a correlation, microbial ecologists may focus their survey efforts on a particular group of microbes thought to be involved in the ecosystem function of interest. For example, 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 a particular function. But this approach assumes that one can identify the “right” marker gene a priori which, given how little is understood about microbial diversity, seems highly unlikely. And in fact these studies are rarely successful (7).

Another likely reason that microbiome-ecosystem function relationships have been difficult to document is that 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.

The problem of connecting microbiome attributes to the rate of an ecosystem function is analogous to the problem of connecting genomic variation to phenotypic traits in organisms, and solutions to this problem may be found by exploring this analogy (9). For example, one approach to the problem of genotype-phenotype mapping is to perform artificial selection on a trait of interest and identify genes that respond to selection. Such genes are likely to be causally linked to the trait under selection. An analogous approach for exploring microbiome-ecosystem function relationships is gaining acceptance among microbiologists (10–12). Microbiomes have been shown to respond to selection at the ecosystem-trait level (10), and some ecosystem-scale microbiome traits have been shown to be transferable through microbiome inoculations (11). Applying artificial selection to whole microbiomes could be a novel approach for quantifying how much of the variation in the rate of an ecosystem function could be attributed independently to microbiome variation (13). In addition, this approach could be used to identify microbiome attributes, such as genes or taxa, associated with the rate of an ecosystem function, although, to our knowledge this has not previously been accomplished (9,14). Such an approach has the potential to expand our ability to more accurately model microbial ecosystem-function relationships and allow us to manage ecosystems for particular outcomes (2,15).

We use this approach to estimate how much of the variation in an ecosystem function - the flux of CH4 gas between the soil and atmosphere - can be attributed to variation in the microbiome and to identify microbial markers associated with this ecosystem function. Soil CH4 oxidation is a suitable function for this study because there is evidence that it may vary with microbiome attributes. For example, variation in CH4 emissions in arctic permafrost is correlated with the transcriptional activity of certain methanogens (16,17). In addition, soil microbiome phylogenetic variation is a strong predictor of CH4 oxidation in forests and pastures of the Brazilian Amazon and the Congo Basin (18,19). Finally, methanogenesis and methanotrophy are two of the most deeply conserved microbial physiologies and are 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 CH4 flux than other broader or more shallowly conserved functions (2,20). This suggests that microbiome variation might drive variation in CH4 oxidation in soils.

We applied artificial ecosystem selection on soil microbiomes by selecting on the ecosystem-scale CH4 oxidation rate. Using this approach, we were able to estimate how much of the variation in CH4 oxidation rate was associated with variation in the microbiome. To identify which microorganisms could be markers of CH4 oxidation rate, we compared the composition of the artificially selected microbiomes to a control set of microbiomes with random selection. We then evaluated whether these markers met our underlying assumptions about which taxa limit the rate of ecosystem function.

# Materials and Methods

## Experimental design

We performed an artificial ecosystem selection experiment (sensu 10) 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 chosen 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.

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). The jars were then homogenized, 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 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. Fluxes were calculated from a first-order exponential decay function as decay constant with units day-1. 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 for the positive treatment in passage 2 had the lowest CH4 oxidation rate of the twelve jars due to a calculation error in the CH4 oxidation rate. 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 (12).

## 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 (21). 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 (22,23). 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’ (24). Demultiplexed sequencing reads were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs) (25). Taxonomic assignment was performed using the Ribosomal Database Project naive Bayesian classifier (26). The presence of contaminants was evaluated using both the prevalence and frequency methods from ‘DECONTAM’ (27). 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 taxonomy were removed prior to analysis.

## Statistical Analysis

Statistical analyses were performed in ‘R’ (24). 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 the difference of slopes between the positive and neutral treatments by fitting two nested models with and without the interaction between treatment and passage using ‘lm’. We compared these models using an F-test with the ‘anova’ function. We then fit the full model with the interaction term to estimate the slopes for each treatment, which represented the change in CH4 oxidation rate per passage as a response to selection.

To estimate the proportion of variance in CH4 oxidation rate due to variation in the microbiome, we estimated narrow-sense heritability () as the regression of offspring on mid-parent (28). The mid-parent was the mean for all three selected jars and the offspring was the CH4 oxidation rate for each jar produced by those parents. First, we tested if there was an effect of treatment on the heritability estimate. We compared nested models with and without treatment using an F-test test with the ‘anova’ function. We then fit the full model to estimate the heritability of the Positive and Neutral treatments.

Richness was estimated using the method from (29) with a subsample size of 177,127 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 177,127 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 keeping ASVs that lacked a family-level taxonomic assignment by grouping them at a higher 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. 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 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 51% increase in CH4 oxidation rate per passage (slope = 0.18, SE = 0.06, t = 2.76, p = 0.01).

We estimated the proportion of variation in methane oxidation due to variation in the microbiome as the regression of offspring on mid-parent (Figure 2). Offspring CH4 oxidation rates were correlated with parental CH4 oxidation rates in both the Positive treatment (slope = 1.08, SE = 0.35, t = 3.10, p = 0.00) and the Neutral treatment (slope = -0.58, SE = 0.29, t = -2.02, p = 0.05).

## Taxonomic richness

Median ASV richness decreased from 3426.2 (783.9) in Passage 2 to 1577.2 (156.8) 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 = 0.6). 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 = 0.4).

## 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 = 0.013)

## 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 as a response to selection. Each generation of soil ecosystems was created from the same pool of sterile soil substrate and a small inoculum from the previous generation. Therefore, it is unlikely that this response to selection was due to changes in the abiotic conditions of the ecosystem. In addition, the response was only observed in the Positive treatment and not in the Neutral treatment, which shows that this was not a side effect of passaging in jars. Therefore, we conclude that variation in the microbiome can generate variation in ecosystem functions independent of the environment.

In quantitative genetics, heritability quantifies the degree to which variation in a trait is due to variation in genotype (28). This is often estimated as the regression of offspring phenotype on parental phenotype. Thus, a significant correlation between the trait values of parents and offspring indicates that the trait is at least partially genetically determined. To estimate the variation in an ecosystem trait due to variation in the microbiome, we can perform a similar estimation by comparing the trait values of the “offspring” jars in one generation to the trait value of the “parental” jars chosen for selection in the previous generation. This “ecosystem heritability” then quantifies how much of the variation in ecosystem function is due to variation in the microbiome (12,13,44).

We quantified how much of the variation in methane oxidation rate was due to variation in the microbiome by regressing the methane oxidation rates of the 12 offspring jars against the mean methane oxidation rate of the three jars selected in the previous generation We observed a heritability of 1.08 (add SE/CI) for the Positive selection treatment. A heritability of 1 would indicate that a trait is entirely genetically determined and thus offspring would have the exact same value of that trait as their parents. A heritability greater than 1 indicates that offspring exceed the value of their parents. This is unlikely in the context of a genomic trait, but microbiomes are not limited by the constraints of genomes because microbes are not alleles and communities are not chromosomes. Therefore, complementarity within a microbial community could result in offspring communities that exceed the trait values of their “parent” ecosystems. The large heritability we observed indicates that microbiome variation is a large contributor to variation in methane oxidation in this experimental system.

Next, we wanted to address what aspects of the microbiome, in terms of alpha diversity, beta diversity, or individual 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 (7). In certain ecosystems, methane production and consumption are correlated with the abundance of methanogens and methanotrophs as estimated from marker genes (19). 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 (7). Our results suggest that the rate of methane oxidation 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.

As more is understood about the role of the microbiome in regulating ecosystem functions and the specific taxa involved, more detailed experiments could selectively add or remove important taxa or manipulate their relative abundance to start to estimate the quantitative relationship between specific taxa and the rate of an ecosystem function. Both our differential abundance analysis and this reductionist approach to manipulating individual taxa assumes that taxa act individually on ecosystem function. However, it is much more likely that the microbiome affects the rate of ecosystem function through the interactions of multiple taxa. We are unable to evaluate this possibility given our small sample size, but future analyses with greater replication should test the role of multiple taxa simultaneously by, for example, using machine learning methods (49).

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. Surprisingly, we did not observe an increase in the relative of abundance of methanotrophs as a response to selection. This suggests that the rate of methane oxidation could be altered by non-methanotrophs. Future research could estimate heritability under greater environmental variation to understand how much of the variation in methane oxidation rate is due to variation in the microbiome in a natural ecosystem. In addition, future selection experiments could use genome-resolved metagenomics to observe within-strain evolution and test changes in the functional pathways of the microbiome as a response to selection to determine what aspects of the microbiome explain this increase in oxidation rate. 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.

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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

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# Figures

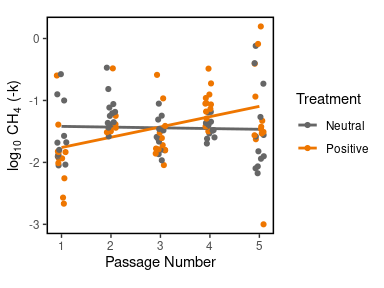


Figure 1: Response to selection on soil CH4 oxidation rate fit by ordinary least squares regression. The y-axis is the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1. The x-axis is the passage number. The orange line and points are the Positive treatment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

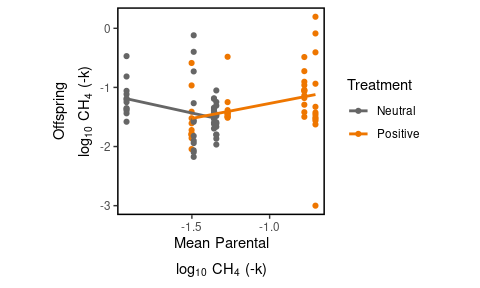


Figure 2: Ordinary least squares regression of offspring CH4 flux on mid-parent CH4 flux ( with units day-1). Mean parental is the mean of the jars selected to inoculate the next passage. Offspring is the oxidation rate of each jar in the next passage. The orange lines and points are the Positive treatment and the gray lines and points are the Neutral treatment

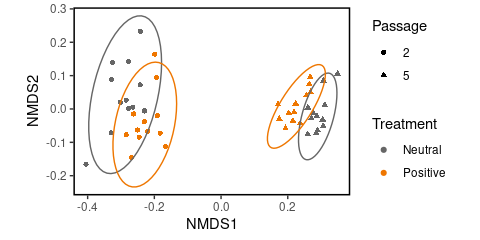


Figure 3: Principal component analysis plot of beta diversity for all jars. Dissimilarities are based on rarefied Bray-Curtis dissimilarity. Colors represent treatment and shapes represent passage number.

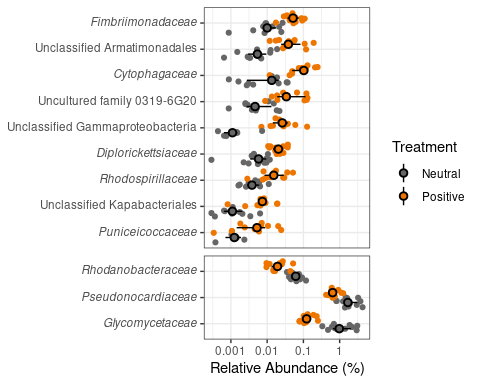


Figure 4: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2, and CORNCOB. Values on the x-axis are relative abundances on a log10 scale. Points are colored by treatment. Large points with error bars are the median relative abundance and interquartile range. Taxa in the top panel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