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 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 ecosystem functions. This suggests that understanding variation in microbiome composition might be important for predicting the rates of ecosystem functions (1–3). Many studies have attempted to document such a relationship (4–9). However, it is difficult to isolate the effect of variation in microbiome composition from other drivers of variation in ecosystem function. For example, microbiome manipulations often confound variation in diversity, composition, and abundance. Likewise, comparative studies must control for abiotic variation statistically to identify the direct effect of microbiome variation. This approach relies on measuring the relevant abiotic variables and cannot account for unmeasured environmental variation. Here, we overcome these limitations by using a selection approach to estimate the degree to which an ecosystem function varies with microbiome composition.

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 community structure while trying 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 functional marker gene 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 some unique challenges and limitations. One issue is that microbiome attributes tend to covary with the abiotic conditions within an environment, and whether and how the investigator controls for these variables can affect the conclusions of such a comparative study. 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. Each of the manipulative approaches applied confound community composition with other factors. For example, reciprocal transplant and common garden experiments confound community composition with the abiotic conditions carried on 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 variation in the microbiome 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 microbiome-function relationships and for investigating potential mechanisms underlying those relationships. By passaging microbiomes over multiple rounds using a common environment, we can weaken the covariance between microbes and the environment by repeatedly diluting abiotic effects. In addition, by inoculating our ecosystems with equally sized subsamples, we eliminate 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 significant genes or taxa associated with our selection treatment to establish a potential mechanism that explains this variation.

We applied artificial ecosystem selection to soil microbiomes 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 as well as possibly the main driver of temporal methane dynamics (17). In addition, there is evidence that soil methane 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 methane oxidation than other broader or more shallowly conserved functions (2,23).

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 (14) 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 four 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 (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 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 proportion of variation in methane oxidation rate due to variation in the microbiome as the regression of divergence between the positive and neutral treatment on the cumulative selection differential (32). Divergence was calculated as the mean methane oxidation rate of the positive treatment minus the mean methane oxidation rate of the neutral treatment 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 then the sum of the selection differential from all preceding selection events. We test this as the regression of divergence on cumulative selection differential using the ‘lm’ function.

Richness was estimated using the method from (33) with a subsample size of 176,545 using 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 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 proportion of variation in methane oxidation rate due to variation in the microbiome by regressing the divergence between the positive and neutral treatments against the cumulative selection differential. We observed a slope of 0.40, which would indicate that 40% of the variation is due to the microbiome, but this was not significant (statistics).

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* (41). However, the Gammaproteobacteria is among the most diverse groups in the Prokaryotes, so this is not strong evidence for a selection response by methanotrophs (42). 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 (43). 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 (44). *Cytophagaceae* was also enriched in the Positive treatment and contains a number of mainly aerobic heterotrophs that can digest a variety of macromolecules (45). 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 (46). Based on this analysis, it appears that no methanotrophs were enriched in the Positive treatment.

# Discussion

In this study, we addressed whether variation in the microbiome contributes to variation in the rate of ecosystem function. To test this, we applied artificial ecosystem selection to soil microbiomes by selecting on the whole-ecosystem soil methane oxidation rate. We observed an increase in methane 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 methane oxidation rate independent of the environment.

The ultimate goal of this study (and much of microbial ecology) could be summarized as “Do microbes matter to ecosystem function (or health)?” and there are at least three ways we could address that question. One would be to ask whether microorganisms mediate function. This question is perhaps trivial. In the case of methane emissions from soil, we know that microorganisms produce and consume methane. We could also ask what would happen if we removed the microorganisms. Again, this question is trivial. There are no known abiotic reactions in soil that consume methane (outside of the troposphere, the stratosphere, and the marine boundary layer (17)). Therefore, removing the microbiome would likely eliminate methane production and consumption. Then, the third question we could ask is whether *variation* in the microbiome is associated with *variation* in the rate of ecosystem function. The answer to this question is not so obvious.

If we look at variation in the rate of a microbially-mediated ecosystem function across a set of habitats, that variation could be associated with variation in the abiotic environmental conditions, variation in the composition of microbiomes, or some interaction or covariance between the two (see Figure 5). To address the question of whether variation in the microbiome matters for ecosystem function, we need to identify the component of variation uniquely associated with the microbiome. If we sample natural variation, as in the comparative approach, it can be difficult to distinguish between the direct effect of the microbiome and the effect of the microbiome due to covariance with the environment. Likewise, with the manipulative approach even though we are placing ecosystem propagules into a common environment or substrate, the propagule itself carries with it environmental conditions that cannot be removed. Generating variation in microbiome composition through filtering or other similar means often confounds composition with other factors, such as biomass or cell size. To overcome these limitations and to isolate the direct effect of the microbiome on ecosystem function, we used artificial ecosystem selection.

To perform artificial ecosystem selection, we generate a set of ecosystems in bottles using a common, sterile substrate and inoculate them with a propagule containing a diverse microbiome that is a subsample of an existing microbiome. This generates variation in microbiome composition among the ecosystems. We can then measure the rate of an ecosystem function for each bottle and select the bottles with the highest (or lowest) rate of ecosystem function. The selected ecosystems are then homogenized and used as the propagule to inoculate a new set of ecosystems containing the same sterile substrate as the initial set. By subsampling the microbiome, we can generate variation in the composition of the microbiome that is independent of cell size or biomass. And by passaging the microbiome through the same sterile substrate over multiple rounds of selection, we can progressively minimize the effect of the abiotic conditions from the donor environment on both the rate of function and the composition of the microbiome. In this way, over multiple rounds of selection, we can observe whether varying the microbiome can generate variation in the rate of our ecosystem function. If we observe no change in ecosystem function from passage to passage despite our imposed selection, we would conclude that this ecosystem function in this set of ecosystems is driven primarily by environmental variation or technical error among the replicates. However, if we observe a response to selection, that is, a shift in the mean ecosystem function between successive passages, we would conclude that variation in the microbiome can generate variation in the rate of ecosystem function. And that is, in fact, what we observed.

Given that variation in the microbiome is associated with variation in ecosystem function, a reasonable follow-up question is “How much variation?” This is a challenging question to answer since the value depends on the population of ecosystems that is sampled and the estimate for one set of ecosystems may not apply to another set. For example, we cannot estimate a percent of variation explained in tropical rainforests and then assume that number applies in polar wetlands. In addition, the magnitude of the estimate will depend on how much variation is sampled. A large is not very interesting if is small. Keeping these caveats in mind, one way to estimate this number is to determine how much the recipient jars resemble the selected donor jars that were used to inoculate them. We can calculate the response to selection as the difference between two successive passages in their mean methane oxidation rate. We will denote this as . We can also calculate the strength of selection as the difference in mean methane oxidation rate between the twelve jars in one generation and the 3 jars chosen for selection in that generation, which we will call the selection differential and denote as . If the change in mean function from passage 1 to passage 2 () is equal to the difference in mean function between the 12 jars in passage 1 and and the 3 jars selected to inoculate passage 2 (), 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 methane oxidation rate and simply wander randomly, then we would conclude that all of the variation is due to the environment or technical variation.

The concept that we have just described is analogous to the concept of heritability from quantitative genetics. Heritability for given a population is defined as the proportion of variation in a phenotype associated with variation in genotype (**???**). This concept has been applied above the population level for groups within a species (48), as well as communities and ecosystems (49,50). One study previously estimated the heritability of a community trait after artificial ecosystem selection using parent-offspring regression and observed a relatively large heritability of 50% (15). However, parent-offspring regression assumes a randomly mating population, which, by definition, does not apply to a selection experiment. In the present study, we estimated the variation explained by the microbiome somewhat differently using the breeder’s equation () and regressing the observed response to selection against the imposed selection differential (Figure 1; (32)). We also observed a relatively large response to selection (40%), though this estimate had large error bars and was not significant so we did not have the power to estimate an accurate percent of variation explained for this experiment.

Asking how much of the variation in ecosystem function is due to variation in the microbiome is a silly question. We cannot answer this questions broadly for all communities or all ecosystems, because the answer depends in large part on the magnitude of variation sampled.

What do I want to get across here:

This estimate is analogous to the concept of heritability from quantitative genetics Define heritability Point out that it’s been applied to communities and ecosystems Mention the different ways of estimating heritability Mention the assumptions inherent in some of these estimates (non-assortative mating; particularly numbers of shared alleles based on kinship) Point out that while heritability is a useful framework to borrow from in order to describe the components of variation, there are limitations in its application Propose that we pursue new theory about how to understand what drives variation in microbiome functions and microbiome-encoded traits

The challenge with applying the concept of heritability outside its original scope is that it is unclear if the assumptions underlying these estimates apply to microbiomes. For a genomic trait, we can rely on the assumption that offspring share, on average, 50% of the alleles of each of their parents. It is less clear if offspring share 50% of the microbes of each of their parents or if it is sufficient for 50% of the microbes to be *present* or if they must be in a similar relative abundance. Estimates of heritability, such as parent-offspring regression and the ACE model rely on specific estimates of the similarity of relatives based on their relatedness. It is unclear if these assumptions apply to the microbiomes of host ecosystems. It is even less clear whether they apply to non-host ecosystems that do not reproduce through sexual recombination.

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 (11). In certain ecosystems, methane production and consumption are correlated with the abundance of methanogens and methanotrophs as estimated from marker genes (18,19). 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 (11). 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 (51–53). 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 (54). 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.

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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

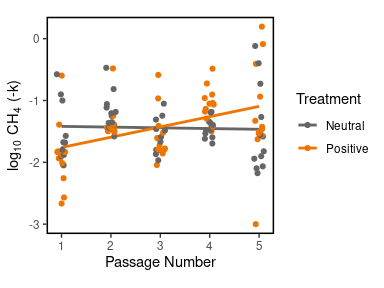


Figure 1: Response to selection on soil CH4 oxidation rate. The y-axis is the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1. The x-axis is the passage number. The orange line and points are the Positive treatment and the gray line and points are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

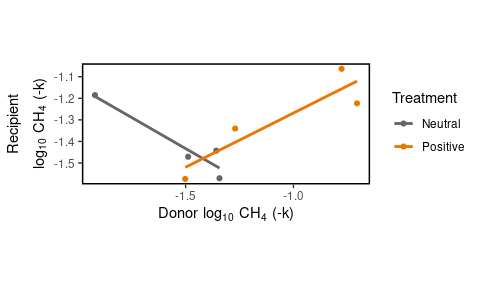


Figure 2: Ecosystem heritability as the regression of mid-recipient methane flux on mid-donor methane flux ( with units day-1). Donor is the mean oxidation rate of the three jars selected to inoculate the next passage. Recipient is the mean oxidation rate of the 12 jars in the next passage. The orange 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).

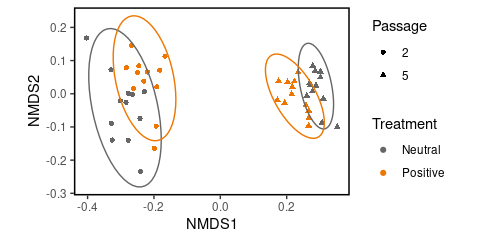


Figure 3: Principal component analysis plot of beta diversity for all jars. Dissimilarities are based on rarefied Bray-Curtis dissimilarity. Colors represent treatment and shapes represent passage number.

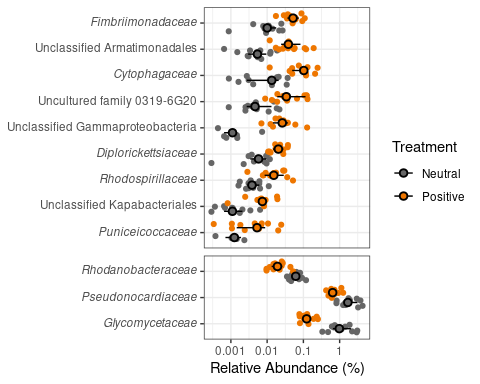


Figure 4: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2, and CORNCOB. Values on the x-axis are relative abundances on a log10 scale. Points are colored by treatment. Large points with error bars are the median relative abundance and interquartile range. Taxa in the top panel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

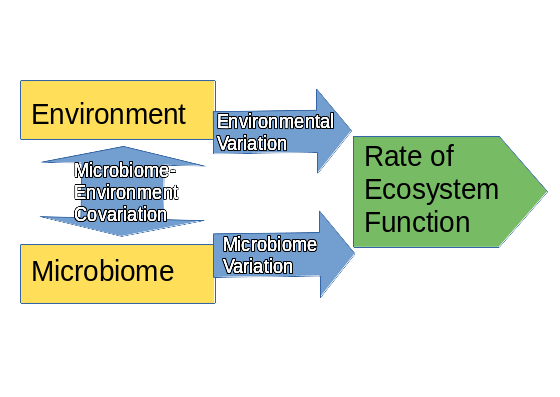


Figure 5. Variation in the rate of an ecosystem function is potentially the result of at least three components: variation in the abiotic environmental conditions, variation in microbiome composition, and the covariance between microbiomes and the environment. It is difficult with traditional study designs 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 interaction between microbiome and the environment, though these relationshps may also exist.