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 any ecosystem function. This challenge remains because it is difficult to manipulate microbiome composition directly, we cannot know a priori which members of the microbiome limit the rate of an ecosystem function, and microbiomes covary with 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 13 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.

# 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). 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 (2–4). While there is evidence that variation in the microbiome contributes to variation in ecosystem functions, describing this relationship has been elusive (5,6).

One reason for this difficulty is that it is challenging to directly manipulate microbiome composition and diversity the way we can for macroorganismal communities. Instead, we must use genetics through marker gene or metagenomic sequencing to characterize the microbiome. However, studies that measure the taxonomic or functional diversity of a microbiome through these approaches rarely find a correlation between diversity and function (6). Another approach is to quantify cells or genes using quantitative PCR. Studies such as this still rarely find a correlation between the abundance of a functional group and the rate of the corresponding process (5). As a result, it remains unclear how and when microbiome variation contributes to variation in the rate of ecosystem functions.

Another likely reason that microbiome-ecosystem function relationships have been difficult to document is that the most common approaches for looking for a relationship requires some prior knowledge of the likely causal links between microbiomes and ecosystem functions. For example, many studies have looked for relationships with the abundance or diversity of a “marker gene” for a particular function. Marker genes are microbial genes that code 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. What is needed are approaches to microbiome-ecosystem function mapping that do not require this degree of prior knowledge.

Yet another likely reason that microbiome-ecosystem function relationships have been difficult to document is that microbiomes can be related to ecosystem functions in two related but distinct ways that historically have been difficult to separate. One way is that microbiomes may simply be conduits through which the environment drives function. That is to say that the environmental conditions completely regulate the attributes of the microbiome and a shift in the environment causes a shift in microbiome attributes, such as the abundance of a microbial functional group. This shift results in a change in the rate of an ecosystem function. In this case, microbiome attributes would be statistically associated with variation in ecosystem function, but also strongly covary with environmental conditions. Therefore, there would be no association between microbiomes and ecosystem functions independent of the environmental conditions. Under this scenario, knowing anything about the microbiome would not necessarily provide you with information regarding the causes of the change in ecosystem function or improve one’s ability to predict changes in ecosystem function. Furthermore, attempting to alter ecosystem function by altering the microbiome without changing the underlying environmental conditions would likely be fruitless.

Another possibility is that the microbiome itself alters the rate of ecosystem function independent of the environment. In this case, one cannot predict the rate of ecosystem function without understanding changes in the microbiome. In addition, managing the microbiome through inoculation or selective antibiotics would potentially be an effective approach for enhancing or mitigating certain changes to ecosystem functions. These two scenarios are not mutually exclusive, nor are they likely unique to microbes, but methods to determine the relative importance of these two scenarios have not until recently been employed.

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 (7). 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 (8,9). Microbiomes have been shown to respond to selection at the ecosystem-trait level (8), and some ecosystem-scale microbiome traits have been shown to be transferable through microbiome inoculations (9). 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 (10). 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 (7,11). Such an approach has the potential to expand our ability to model microbial ecosystem-function relationships more accurately and allow us to manage ecosystems for particular outcomes (12,13). Here, we use this approach to estimate how much of the variation in 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 (14,15). In addition, soil microbiome phylogenetic variation is a strong predictor of CH4 oxidation in forests and pastures of the Brazilian Amazon (16). 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 (13,17). 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 without 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 8) 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. All other passages correctly used the top three jars.

## 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 (18). 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 (19,20). 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’ (21). Demultiplexed sequencing reads were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs) (22). Taxonomic assignment was performed using the Ribosomal Database Project naive Bayesian classifier (23). The presence of contaminants was evaluated using both the prevalence and frequency methods from ‘DECONTAM’ (24). 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.

## Statistical Analysis

Statistical analyses were performed in ‘R’ (21). 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 mid-offspring on mid-parent (25). The mid-parent was the mean for all three selected jars and the mid-offspring was the mean for all jars 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 (26) with a subsample size of 177,130 using the ‘rarefy’ function in ‘vegan’ (27). 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 177,130 using the ‘avgdist’ function in ‘vegan’ (27,28). 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 (27,29). 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 (27,29)

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 pulled out 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 (30,31). 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 (32). Finally, we used CORNCOB with the ‘differentialTest’ function in the ‘corncob’ package with the Wald test and without bootstrapping (33). 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: F~27.3895504,log\_ch4 ~ passage \* treat~ = 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).

## Heritability of methane oxidation

We estimated heritability as the regression of mid-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 = 0.24, 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 3427.0 (783.8) in Passage 2 to 1579.0 (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 = 0.3).

## 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 = 0.005). 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 = 0.015)

## 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 13 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* (34). However, the Gammaproteobacteria is among the most diverse groups in the Prokaryotes, so this is not strong evidence for a selection response by methanotrophs (35). 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 (36). 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 (37). *Cytophagaceae* was also enriched in the Positive treatment and contains a number of mainly aerobic heterotrophs that can digest a variety of macromolecules (38). 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 (40). Based on this analysis, it appears that no methanotrophs were enriched in the Positive treatment.

# New Discussion

We observed a response to selection on whole-ecosystem soil methane oxidation rate, which demonstrates that variation in the microbiome can generate variation in the rate of ecosystem function independent of the underlying environmental conditions. 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. To address that 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 addition, we observed a large heritability for methane oxidation rate, which suggests that microbiome variation explains a large portion of the variation in methane oxidation rate relative to environmental variation in this system. To estimate how much of the variation in methane oxidation rate can be explained by the presence, absence, and relative abundances of specific microbial taxa, we calculated the narrow-sense heritability of methane oxidation rate, which was 1.08 for the Positive 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 the 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. A difference in composition between the two treatments could be a direct result of selection for organisms that contribute to CH4 oxidation or a result of ecological drift over the course of the experiment. In the former case, that would suggest that changes in the relative abundance of specific microbial taxa could explain the response to selection on CH4 oxidation rate.

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 contrasts with the usual assumption that the final step in a metabolic pathway is the rate-limiting step (citation?). In certain ecosystems, methane oxidation rate is correlated with the abundance of methanotrophs as estimated from marker genes (Cite. I know that one methanogenesis paper, not sure about methanotrophy). 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 (cite rocca, maybe some of the methane work.) Since we did not observe an increase in the relative abundance of methanotrophs in the treatment that displayed an increase in methane oxidation rate, we may have to consider other microbial functional groups within an ecosystem to understand what drives changes in the rate of ecosystem functions.

One of the goals of this study was to determine how much of the variation in ecosystem function is due to variation in the microbiome. In this experiment, we eliminated as much environmental variation as possible. This was intentional, because we wanted to determine whether it was possible for variation in the microbiome to generate variation in methane oxidation rate independent of the environment. As a result, our heritability estimates are likely to be greater than they would be in natural ecosystems with greater environmental variation. Future work could estimate heritability in experimental systems that manipulate environmental variation or across natural ecosystems to evaluate how much of the variation in ecosystem function can be explained by microbiome variation outside the lab. This experiment also had a relatively small sample size which resulted in heritability estimates with wide error bars. Increasing replication would help us more accurately estimate this parameter and give us a better understanding of the relative importance of environmental variation and microbiome variation in the rate of microbiome-mediated ecosystem functions.

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. Future work could analyze an artificial ecosystem selection experiment using genome-resolved metagenomics to try to detect strain-level changes in the constituent taxa. This would help evaluate whether certain metabolic pathways were enriched in the positive selection treatment or if functional genes changed in frequency 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 and future analyses with greater replication should test the role of multiple taxa simultaneously by, for example, using machine learning methods (41)

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. 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.

# Old Discussion

We observed a response to selection on whole-ecosystem soil CH4 oxidation rate. This type of response has been observed for other functions, such as chloroaniline degradation in water (8), but this is the first instance, to our knowledge, of selection being performed directly on soil biogeochemical cycling. With this experimental design, we attempted to create multiple ecosystems that were very similar with respect to the type and quantity of soil, soil moisture content, and the headspace concentration of CH4. we further controlled for environmental variation across replicates by having twelve replicates per treatment, using controlled laboratory conditions, and by having a random selection treatment as a control. Therefore, the observed response to selection on CH4 oxidation rate is likely due to changes in the microbiome.

To investigate the relationship between microbiome structure and ecosystem function, we wanted to determine how much of the variation in soil CH4 oxidation was attributable to variation in the microbiome. This ecological question is analogous to the problem of estimating heritability in quantitative genetics (7). Variation in an organismal trait is determined by the sum of genetic variation, environmental variation, and the interaction between the two (25). One goal of quantitative genetics is to determine the proportion of phenotypic variation attributable to genetic variation. This is commonly estimated as the narrow-sense heritability defined as the additive genetic variance. This goal can be achieved through artificial selection experiments with selection on the character of interest (25).

The heritability we observed in the Positive treatment was large ( = 1.08) relative to other studies of biological heritability (42). However, given the small sample size and wide confidence intervals (95% CI: 0.40, 1.75) it is likely that a larger study could more precisely estimate the true heritability. We intentionally eliminated much of the environmental variation that would be present in natural ecosystems, but it is unclear how much jar-to-jar variation there is in the composition of the soil substrate. Given these data, though, we conclude that variation within the soil microbiome has a very strong effect on soil CH4 oxidation rates independent of any environmental variation.

The negative heritability observed in the Neutral treatment could indicate a negative genetic correlation between CH4 oxidation rate and traits associated with persistence in a jar (43). The conditions in the jar favor organisms that can grow in potting mix, reproduce within a three week incubation, and survive passaging between jars. These traits might be negatively correlated with a community’s ability to oxidize CH4 indicating a potential trade-off (43). This trade-off would result in a negative heritability of CH4 oxidation rate in the Neutral treatment, which did not experience selection on CH4 oxidation rate.

Since there was a response to selection, we wanted to identify which taxa responded to selection. That is, the taxa that were enriched in the Positive treatment relative to the Neutral treatment. Studies of microbiome structure-function relationships typically quantify marker genes for the final enzyme in a pathway to try to predict the rate of flux through that pathway. However, of the 37 taxa identified by the differential abundance test, none were members of a family known to oxidize CH4. This suggests that, in this system, the taxa that perform the final step in the CH4 oxidation pathway do not limit the rate of flux through the pathway. Traits that we assume are important for regulating function (such as the diversity or relative abundance of methanotrophs estimated from phylogenetic marker genes) may not be important for determining variation in function. Perhaps once a sufficient number of methanotrophs are present, the process itself is limited by other metabolic processes in the ecosystem, such as nitrogen cycling mediated by non-methanotrophic organisms.

We found that 24 taxa were enriched in the Positive treatment, which might indicate that the soil CH4 oxidation rate in these jars is controlled by multiple taxa. This conclusion is analogous to the conclusion in quantitative genetics that most traits at the organismal level are the result of the interaction between many independent genes. Indeed, very few traits or genetic diseases are the result of a single gene or mutation (44,45). This results in the use of polygenic risk scores for predicting phenotype or disease risk in humans (46,47). An analogous conclusion can be drawn for predicting the rate of ecosystem function from the presence or relative abundance of microbial taxa. The rate of an ecosystem function at the whole-ecosystem level is the result of interactions among a variety of disparate taxa with different traits. To better understand microbiome structure-function relationships, our results suggest that we should move away from single marker genes and instead investigate the role of multiple functional groups in determining the function of ecosystems whether it is CH4 flux from soil or host-microbiome health.

Taxonomic richness dropped precipitously between Passage 2 and Passage 5. There are several explanations for this drop in diversity. The initial community was a diverse microbiome sampled from a natural soil. With the initial inoculation and at each subsequent passaging, the ecosystem was subsampled to 10% (5 g of living soil was combined with 45 g of sterilized substrate). This subsampling likely explains a large part of the drop in diversity. In addition, beyond the imposed selection regime based on CH4 oxidation rate, these soil microbiomes were under a variety of selection pressures such as persistence in a jar, survival during the transfer from one jar to the next, and the ability to colonize a new jar in a couple of weeks, to name a few. These selection pressures would have further filtered the microbiome for taxa that could persist in this laboratory environment.

In addition to the drop in richness, there was also a decrease in beta diversity among the jars as a result of passaging. This could be due to the homogenization step between each passage. Once the jars were selected to generate the next set of jars, the soil from those jars was homogenized and this homogenate was used to inoculate the next set of jars. The goal of this step was to “shuffle” membership in the microbiomes among the jars in order to test different combinations and relative abundances of taxa in terms of their effect on CH4 oxidation rate. This likely also made the communities more similar in their membership by increasing the likelihood that each taxon was represented in each jar. Another explanation for this biotic homogenization is the aforementioned selection for persistence in a jar. These selection pressures would have favored the subset of taxa suited to these laboratory conditions regardless of whether they were in the Positive or Neutral selection line. This can be seen by the relative similarity of the Positive and Neutral jars in Passage 5. Despite the Positive treatment undergoing selection on CH4 oxidation rate, both the Positive and Neutral jars were experiencing selection on a variety of other traits that made the overall soil microbiomes appear similar.

One caveat that is important to keep in mind is that 16S rRNA genes are not markers of functional characteristics of microorganisms. They are simply phylogenetic markers useful for determining the relative position of taxa in a phylogenetic tree. In addition, assigning functions to taxa based solely on their taxonomy is a fraught exercise because many microbial functions are not very deeply conserved, are spread across disparate lineages of the tree of life, or are easily transferred between distantly related taxa. However, methanotrophy and methanogenesis are two of the most deeply conserved microbial traits (17). This results in part from the fact that these functions require multiple genes to perform and are therefore not easy to evolve independently or to transfer between lineages through horizontal gene transfer. However, in order to be confident that the response we observed was not simply due to a relative increase in methanotrophs, we would need to apply a functional approach to characterizing the microbiome, for example, by sequencing whole metagenomes.

Future research in this area should use methods that are able to detect functions and metabolic pathways within the community that respond to selection. For example, genome-resolved metagenomics could identify pathways that are enriched in the Positive selection treatment. This would further advance our understanding of the exact traits that are enriched in high-flux ecosystems and therefore could be markers of ecosystem function. In addition, future studies could build on experimental results such as these to sample a natural gradient of ecosystem CH4 fluxes to determine whether the taxa identified in our selection experiment could be useful indicators of CH4 flux in the field. These studies would allow us to build a picture of the multiple traits that drive variation in ecosystem function at the whole-ecosystem level.

We performed an artificial ecosystem selection experiment on whole-ecosystem CH4 oxidation rate to determine whether variation in microbiome composition can contribute to variation in ecosystem function. We observed a significant response to selection on CH4 oxidation rate - the first example of an experiment performing selection on soil biogeochemical cycling. In addition, we observed strong heritability of CH4 flux between passages, suggesting that variation in microbiome composition could be a major source of variation in CH4 flux in ecosystems. Surprisingly, the taxonomic groups that experienced selection on CH4 oxidation rate were not enriched in methanotrophs. This suggests that understanding a single functional group is insufficient for predicting the effect of microbiome composition on the rate of ecosystem function. Instead, we may need to investigate alternative functional groups or multiple interacting taxa to understand the role of microbiomes in ecosystem function. While it has often been assumed that microorganisms play a major role in determining variation in ecosystem function, this has not previously been shown empirically. This experiment demonstrates that variation in microbiome composition can contribute to considerable variation in 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

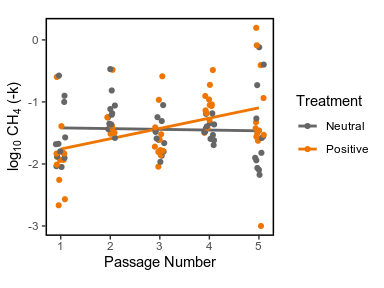


Figure 1: Response to selection on soil CH4 oxidation rate fit by ordinary least squares regression. The y-axis is the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1. The x-axis is the passage number. The orange line and points are the Positive treatment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

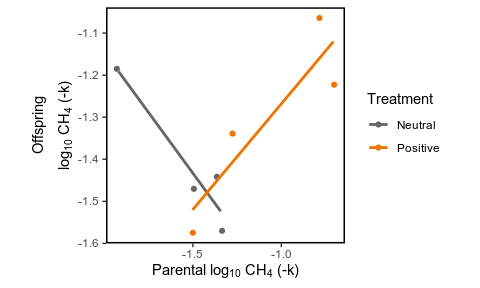


Figure 2: Ordinary least squares regression of mid-offspring CH4 flux on mid-parent CH4 flux ( with units day-1). Mid-parent is the mean of the jars selected to inoculate the next passage. Mid-offspring is the mean of all twelve jars produced in one passage. The orange lines and points are the Positive treatment and the gray lines and points are the Neutral treatment

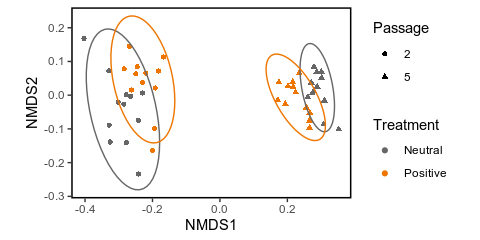


Figure 3: Principal component analysis plot of beta diversity for all jars. Dissimilarities are based on rarefied Bray-Curtis dissimilarity. Colors represent treatment and shapes represent passage number.

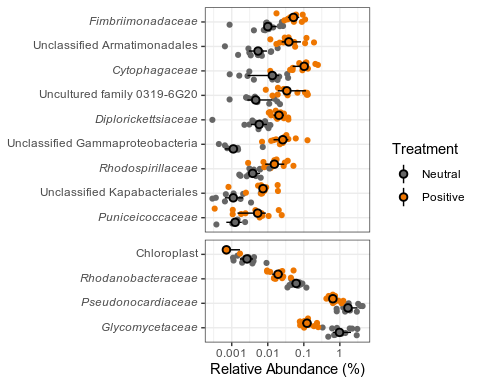


Figure 4: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2, and CORNCOB. Values on the x-axis are relative abundances on a log10 scale. Points are colored by treatment. Large points with error bars are the median relative abundance and interquartile range. Taxa in the top panel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