

# 1 **Response of soil microbiome composition to selection on** 2 **methane oxidation rate**

3 Andrew H. Morris<sup>1\*</sup> and Brendan J. M. Bohannan<sup>1</sup>

4 <sup>1</sup> Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

5 \* Correspondence: Andrew H. Morris, [amorris3@uoregon.edu](mailto:amorris3@uoregon.edu)

6 Competing Interests: We declare we have no competing interests.

## 7 **Abstract**

8 Microbiomes mediate important ecosystem functions, yet it has proven difficult to  
9 determine the relationship between microbiome composition and the rate of ecosystem  
10 functions. This challenge remains because it is difficult to manipulate microbiome  
11 composition directly, we often cannot know *a priori* which microbiome members  
12 influence the rate of an ecosystem function, and microbiomes can covary strongly with  
13 other drivers of ecosystem function, such as the environment. To address these  
14 challenges, we imposed artificial selection on whole soil ecosystems over multiple  
15 generations to select for microbial communities with a high rate of CH<sub>4</sub> oxidation. This  
16 approach is potentially powerful because it is biologically “agnostic” in that it makes few  
17 assumptions about which taxa are important to function, and repeated passaging with  
18 fresh substrate weakens the covariance between microbes and the environment. As a  
19 response to selection, we observed a 50.7% increase in CH<sub>4</sub> oxidation rate per passage

20 relative to a control that experienced random selection. We estimated that 31.5% of the  
21 variation in CH<sub>4</sub> oxidation rate in these soils can be attributed to microbiome variation  
22 (though this was not significant). We also found that selection did not enrich for known  
23 CH<sub>4</sub> oxidizers; instead, 12 families not known to oxidize CH<sub>4</sub>, including  
24 *Fimbriimonadaceae*, *Cytophagaceae*, and *Diplorickettsiaceae*, were enriched by  
25 selection. This result is in contrast to the typical assumption that the rate of an ecosystem  
26 function is limited by the final step in the associated microbial pathway. Our study  
27 demonstrates that variation in microbiome composition can contribute to variation in the  
28 rate of ecosystem function independent of the environment and that this may not always  
29 be limited by the final step in a pathway. This suggests that manipulating microbiome  
30 composition directly without altering the environment could be a viable strategy for  
31 managing ecosystem functions.

## 32 **Introduction**

33 Microbiomes mediate a variety of important ecosystem functions relevant to human  
34 health, agriculture, and global change. As a result, there is great interest in understanding  
35 how to manipulate the microbiome to achieve desirable outcomes within these domains  
36 (1–3). However, for microbiome manipulations to be successful, variation in the  
37 microbiome must contribute directly to variation in the magnitude of the function of  
38 interest independent of other factors. Many studies have attempted to document such a  
39 relationship (4–9). However, it is difficult to isolate the direct effect of variation in  
40 microbiome composition from other drivers of variation in ecosystem function, such as

41 the indirect effect of the environment on function through microbiome assembly. Here,  
42 we overcome these limitations by using a selection approach to estimate the degree to  
43 which an ecosystem function varies with microbiome composition.

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

61         There have been two general categories of approaches that investigators have used  
62 to estimate the degree to which an ecosystem function varies with microbiome  
63 composition: comparative and manipulative. Comparative studies sample natural

64 variation in an ecosystem function across different habitats and simultaneously measure  
65 variation in community composition. Investigators can then correlate ecosystem function  
66 with aspects of community composition while attempting to control for environmental  
67 variation. These approaches have documented important relationships between  
68 microbiomes and ecosystem functions. For example, a meta-analysis of these studies  
69 observed a small but significant contribution of the microbiome to variation in ecosystem  
70 function after controlling for environmental variation (10). In addition, studies focusing  
71 on the correlation between the rate of an ecosystem function and the abundance of an  
72 associated marker gene (i.e., a gene that codes for a protein assumed to be involved in the  
73 ecosystem function) sometimes observe a significant correlation, though this relationship  
74 is rare and contingent upon both the function and the ecosystem sampled (11). However,  
75 comparative studies come with unique challenges and limitations. One issue is that  
76 microbiome attributes tend to covary with the abiotic conditions within an environment,  
77 and it is difficult to control for these abiotic variables in order to identify the unique  
78 contribution of the microbiome to ecosystem function. In addition, it is difficult to know  
79 *a priori* which environmental variables or community attributes to measure. Finally,  
80 while these approaches can establish a potential magnitude and direction for these  
81 relationships, it is often difficult to identify the taxa or genes that explain the connection  
82 between composition and function.

83         The other broad category of approaches used to address this question are  
84 manipulative approaches. Manipulative experiments try to alter microbial community  
85 composition and observe the effect on function. For example, reciprocal transplant and  
86 common garden experiments have shown that microbiomes originating from different

87 ecosystems inoculated into the same substrate or introduced into a common environment  
88 display distinct functional rates (4–7). In addition, manipulating diversity by filtering  
89 communities by cell size or through dilution has been shown to alter the rate of  
90 ecosystem functions (8,9,12). However, manipulating the microbiome directly is  
91 challenging, and manipulative approaches often confound community composition with  
92 other factors. For example, reciprocal transplant and common garden experiments can  
93 confound community composition with the abiotic conditions introduced with the  
94 inoculum, while manipulating composition through dilution may confound composition  
95 with biomass (13).

96         In this study, we sought to build on the observations of comparative and  
97 manipulative studies by applying a different approach to the question of whether  
98 microbiome variation contributes to variation in the rate of an ecosystem function. We  
99 used artificial ecosystem selection to select for microbiomes that performed a greater rate  
100 of ecosystem function (14–16). We then tested whether variation in the microbiome  
101 contributed to variation in the rate of ecosystem function and identified microbiome  
102 attributes that might explain this relationship. There are several potential advantages to  
103 this approach for documenting the direct contribution of the microbiome to variation in  
104 ecosystem function and for investigating the mechanisms underlying those relationships.  
105 Through repeated passaging of microbiomes in a common environment, we can weaken  
106 the covariance between microbes and the environment by repeatedly diluting the  
107 influence of variation in abiotic conditions. In addition, our approach eliminates the need  
108 to generate microbiome variation through methods that are confounded with biomass or  
109 cell size. Lastly, by comparing our artificially selected community to a control

110 community resulting from random selection, we can both control for changes in the  
111 environment over time and identify genes or taxa that are associated with the ecosystem  
112 function under selection.

113 We applied artificial ecosystem selection to soil microbiomes by selecting on soil  
114 methane (CH<sub>4</sub>) oxidation rate. We chose this function because CH<sub>4</sub> is a globally  
115 important greenhouse gas and CH<sub>4</sub> oxidation by soil bacteria is the primary biological  
116 sink for atmospheric CH<sub>4</sub> (17). In addition, there is evidence that soil CH<sub>4</sub> oxidation rate  
117 may vary with microbiome composition based on comparative studies in a variety of  
118 arctic and tropical ecosystems (18–21) as well as studies that manipulate methanotroph  
119 richness (22). Finally, methanotrophy is one of the most deeply conserved microbial  
120 physiologies and is represented in a narrow range of taxa, which suggests that the  
121 taxonomic composition of the microbiome is more likely to be associated with the rate of  
122 CH<sub>4</sub> oxidation than other broader or more shallowly conserved functions (2,23).

123 In this study, we used artificial ecosystem selection on CH<sub>4</sub> oxidation rate to  
124 address the following questions: Does variation in the relative abundance of microbial  
125 taxa contribute to variation in soil CH<sub>4</sub> oxidation rate independent of the environment in  
126 our system? Which attributes of the microbiome are associated with variation in CH<sub>4</sub>  
127 oxidation rate, and do these attributes match our assumptions about the factors that  
128 regulate CH<sub>4</sub> oxidation rate in nature?

## 129 **Materials and Methods**

### 130 **Experimental design**

131 We performed an artificial ecosystem selection experiment (*sensu* (14)) by passaging  
132 replicate soil microbiomes. The trait we selected on was soil CH<sub>4</sub> oxidation rate. Soil  
133 microcosms were incubated at room temperature in sealed 500 mL glass jars with a  
134 rubber septum for gas sampling. Each jar was sterilized with 70% ethanol and was  
135 composed of 45 g of autoclaved artificial potting mix, 5 g of living soil inoculum, and 3.5  
136 mL of sterile deionized water to bring the soil to 60% of field capacity. The potting mix  
137 consisted of bark fines, peat moss, pumice, sand, composted manure, and biochar (Lane  
138 Potting Mix, Lane Forest Products, Eugene, OR). The initial soil microbiome inoculum  
139 was sampled from the top 10 cm of an upland mineral soil under a deciduous forest  
140 ecosystem near the University of Oregon campus in Eugene, OR, USA. Each jar was  
141 capped and injected with 4.3 mL of 99% CH<sub>4</sub>, which produced a mean headspace  
142 concentration of 763.9 ppm (SD = 183.1). Twice per week, jars were flushed in a  
143 biosafety cabinet (to avoid contamination) and respiked with CH<sub>4</sub> to maintain aerobic  
144 conditions and elevated CH<sub>4</sub> concentrations.

145 For the selection experiment, we created two lines of soil microcosms with 12 jars  
146 each: a control line with random selection and an experimental line with directional  
147 selection for greater soil CH<sub>4</sub> oxidation rate. The selection line underwent positive  
148 selection where the two or three jars with the highest CH<sub>4</sub> oxidation rate were  
149 homogenized to inoculate the next set of jars. The control line underwent random

150 selection where an equal number of jars as the selection line were chosen at random to  
151 inoculate the next set of jars. The number of jars chosen was based on the distribution of  
152 fluxes among the positive jars: three jars were chosen in all generations except for  
153 passage 3 where two jars were selected. The experiment was carried out over five  
154 passages with an average incubation time per generation of four weeks. Methane  
155 oxidation rates were determined at the end of the incubation period and selection was  
156 performed. For each treatment, the selected jars were homogenized and 5 g of the  
157 homogenized soil was used as the living soil inoculum for the next generation. The next  
158 set of jars were created in an identical manner to the first generation with fresh  
159 autoclaved potting mix and the same moisture and CH<sub>4</sub> content.

## 160 Methane oxidation rate

161 Methane oxidation rates were determined after flushing and spiking jars to 1000 ppm  
162 CH<sub>4</sub>. Headspace samples of 1 mL were collected from each jar immediately after spiking  
163 and then at time points 3, 6, 24, and 48 hours for a 5-point curve. Samples were  
164 immediately injected into a SRI model 8610C gas chromatograph equipped with a flame  
165 ionization detector (SRI Instruments, Torrance, CA, USA) to determine the headspace  
166 CH<sub>4</sub> concentration. We applied a first-order exponential decay function to determine the  
167 rate constant ( $k$ , units = d<sup>-1</sup>; i.e.,  $d\text{CH}_4/\text{dt} = k[\text{CH}_4]$ ) of the exponential decrease in CH<sub>4</sub>.  
168 Oxidation rates are presented as the additive inverse of  $k$  (i.e.,  $-k$ ) so that a more positive  
169 value represents a greater oxidation rate. The jars selected to inoculate passage three for



170 the positive selection treatment had the lowest CH<sub>4</sub> oxidation rate of the twelve jars due  
171 to a calculation error in the rate constant.

## 172 Soil DNA extraction and sequencing

173 A subsample of soil from the starting inoculum and from every jar in passages 2 and 5  
174 was collected and stored at – 80°C. Soil DNA was extracted from 0.25 g soil. Negative  
175 controls were extracted from autoclaved potting mix and DNase-free water. Extractions  
176 were performed using the DNeasy PowerSoil kit (QIAGEN, Düsseldorf, Germany) and  
177 quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc., Waltham,  
178 MA, USA). To estimate the diversity and relative abundance of the bacterial and archaeal  
179 taxa in our soil ecosystems, we sequenced the V4 region of the 16S rRNA gene using the  
180 515F - 806R primer combination (24). PCR mixtures were: 10 µl NEBNext Q5 Hot Start  
181 HiFi PCR master mix, 9.2 µl primer mixture (1.09 µM concentration), and 0.8 µl of DNA  
182 template. Reaction conditions were: 98°C for 30 s (initialization); 35 cycles of 98°C for  
183 10 s (denaturation), 61°C for 20 s (annealing), and 72°C for 20 s (extension); and 72°C for  
184 2 m (final extension). Reactions were performed in triplicate and then combined.  
185 Amplicons were purified twice using 0.8x ratio Mag-Bind RxnPure Plus isolation beads  
186 (Omega Bio-Tek, Norcross, GA, USA). Sequencing libraries were prepared using a dual-  
187 indexing approach (25,26). Amplicon concentrations were quantified using Qubit and  
188 multiplexed at equimolar concentration. Sequencing was performed at the University of  
189 Oregon Genomics Core Facility on the Illumina NovaSeq 6000 with paired-end 150 bp  
190 reads (Illumina, Inc., San Diego, CA, USA).

## 191 Bioinformatics

192 Bioinformatics processing was performed in ‘R’ (27). Demultiplexed sequencing reads  
193 were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs)  
194 (28). Taxonomic assignment was performed using the Ribosomal Database Project naive  
195 Bayesian classifier (29). The presence of contaminants was evaluated using both the  
196 prevalence and frequency methods from ‘DECONTAM’ by comparing samples to  
197 extraction controls of water (30). Decontam identified 16 potential contaminants based on  
198 prevalence and frequency. Visual inspection of abundance-concentration plots indicated  
199 that 9 of these were likely contaminants and these ASVs were removed. Amplicon  
200 sequence variants that were assigned chloroplast or mitochondria taxonomy were  
201 removed prior to analysis.

## 202 Statistical Analysis

203 Statistical analyses were performed in ‘R’ (27). To determine whether there was a  
204 significant change in CH<sub>4</sub> oxidation rate as a response to selection, we tested a difference  
205 in slopes between the selection and control lines. Residuals did not meet the assumptions  
206 of constant variance and normal distribution. Therefore, CH<sub>4</sub> oxidation rates were log<sub>10</sub>  
207 transformed prior to analysis. Following transformation, these assumptions were met.  
208 First, we tested if there was a difference of slopes between the selection line and the  
209 control based on the interaction between passage and treatment. To test the interaction,  
210 we fit two nested models with and without the interaction term and compared them using

211 an F-test with the ‘anova’ function. We then present the slopes for each treatment, which  
212 represented the change in CH<sub>4</sub> oxidation rate per passage as a response to selection.

213 We estimated the proportion of variation in CH<sub>4</sub> oxidation rate due to variation in  
214 the microbiome as the regression of divergence between the positive line and the control  
215 on the cumulative selection differential (31). This estimate is analogous to estimates of  
216 “microbiability” from the animal breeding literature, which quantifies the variation in a  
217 host trait that is due to microbiome variation (32). The slope of the regression of  
218 divergence on cumulative selection differential provides an estimate of realized  
219 microbiability ( $h^2 \pm SE$ ). Divergence was calculated as the mean CH<sub>4</sub> oxidation rate of  
220 the positive treatment minus the mean CH<sub>4</sub> oxidation rate of the control in each passage.  
221 The selection differential was calculated as the difference between the mean of the three  
222 selected jars and the mean of all twelve jars in a passage. Cumulative selection  
223 differential was calculated as the sum of the selection differential from all preceding  
224 selection events. We then regressed cumulative divergence on cumulative selection  
225 differential using the ‘lm’ function. We report the slope as percent change by back-  
226 calculating the percent change from the log-transformed data into the original units using  
227 the formula  $(10^\beta - 1) * 100$  where  $\beta$  is the slope.

228 Richness was estimated using the method from (33) with a subsample size of  
229 176,545 calculated via the ‘rarefy’ function in ‘vegan’ (34). We tested a difference in  
230 richness by both passage and treatment with a Kruskal-Wallis test followed by a  
231 pairwise Wilcoxon test. Next, we estimated beta-diversity as the Bray-Curtis dissimilarity  
232 by averaging 100 random subsets with a subsample size of 176,545 using the ‘avgdist’

233 function in ‘vegan’ (34,35). We tested a difference in centroid and dispersion of beta  
234 diversity by passage and treatment using a permutational analysis of variance  
235 (PERMANOVA) with 999 permutations using the ‘adonis2’ function from ‘vegan’ and  
236 tested a difference of group dispersions using ‘betadisper’ and ‘anova’ with 999  
237 permutations (34,36). Lastly, we tested the correlation between CH<sub>4</sub> oxidation rate and  
238 Bray-Curtis dissimilarity in Passage 5 with a distance-based redundancy analysis  
239 (dbRDA) using the ‘dbrda’ function in ‘vegan’ and estimated the p-value using a  
240 permutation F-test with 999 permutations (34,36)

241 To identify taxa that responded to selection on CH<sub>4</sub> oxidation rate, we tested  
242 differential abundance between the two treatments in passage 5. We first grouped ASVs  
243 at the family level. We chose this level of agglomeration because CH<sub>4</sub> oxidation is a  
244 relatively deeply conserved function (23) and is restricted to a handful of bacterial and  
245 archaeal families (37). Therefore, we are most likely to detect an enrichment of  
246 methanotrophs at this taxonomic scale. Any ASVs that lacked a family-level taxonomic  
247 assignment were grouped at a higher taxonomic level. We then subset the samples in  
248 Passage 5 and removed all families with a prevalence of less than 10% in either  
249 treatment. We used three methods for testing differential abundance: ANCOM-II,  
250 ALDEx2, and CORNCOB (38–41). We then identified the consensus taxa that were  
251 significant with all three tests and plotted their relative abundances. For ANCOM-II, we  
252 used the ‘ancom’ function in the ‘ANCOM-BC’ package with a cutoff of  $W = 0.7$   
253 (38,39). For ALDEx2, we used the ‘aldex’ function in the ‘ALDEx2’ package with  
254 Welch’s t-test and we used an effect size of 1 as our significance threshold (40). Finally,  
255 we used CORNCOB with the ‘differentialTest’ function in the ‘corncob’ package with

256 the Wald test and without bootstrapping (41). Lastly, to test differentially abundant  
257 methanotrophs, we subset all ASVs within methanotrophic families and tested their  
258 differential abundance aggregated at the family and genus level using ‘corncob’. For each  
259 test, p-values were adjusted for multiple testing by controlling the false discovery rate  
260 using the Benjamini-Hochberg procedure (42).

## 261 **Results**

### 262 **Response to selection on methane oxidation rate**

263 We observed a response to artificial selection on whole-ecosystem soil CH<sub>4</sub> oxidation rate  
264 (Figure 2; difference of slopes:  $F_{2,113} = 3.85$ ,  $p = 0.02$ ). At the start of the experiment, the  
265 positive selection treatment had a mean CH<sub>4</sub> oxidation rate that was 24% lower than the  
266 control (difference of y-intercepts = -0.34, SE = 0.16,  $t = -2.14$ ,  $p = 0.03$ ). There was no  
267 change in CH<sub>4</sub> oxidation rate in the control over the five passages (slope = -0.01, SE =  
268 0.05,  $t = -0.26$ ,  $p = 0.80$ ). By contrast, the selection treatment had a 50.7% increase in  
269 CH<sub>4</sub> oxidation rate per passage (slope = 0.18, SE = 0.06,  $t = 2.76$ ,  $p = 0.01$ ).

270 To estimate the proportion of variation in CH<sub>4</sub> oxidation rate due to variation in  
271 microbiome composition—i.e., microbiability (as described in the Methods; (32)—we  
272 regressed divergence between the positive selection treatment and the control against the  
273 cumulative selection differential. The microbiability was  $0.31 \pm 0.17$ , though this was not  
274 significant ( $F_{1,2} = 3.44$ ,  $p = 0.20$ ).

## 275 Taxonomic richness

276 Median ASV richness decreased from 3406.6 (778.5) in passage 2 to 1557.8 (157.7) in  
277 passage 5 (Kruskal-Wallis test:  $\chi^2 = 35.4$ ,  $df = 3$ ,  $p < 0.001$ ; pairwise Wilcoxon test:  $p <$   
278  $0.001$ ). However, there was no difference in richness between the selection treatment and  
279 the control in passage 2 or 5 (pairwise Wilcoxon test; Passage 2:  $p = 0.66$ , Passage 5:  $p =$   
280  $0.67$ ). In addition, there was no correlation between richness and  $\text{CH}_4$  oxidation rate  
281 across the two treatments in passage 5 (Spearman's  $\rho = -0.2$ ,  $p = 0.3$ ).

## 282 Community dissimilarity

283 Bray-Curtis dissimilarity of the soil microbiome varied strongly by passage and weakly  
284 by treatment with an interaction between passage and treatment (Figure 3). Passage  
285 explained 55.9% of the variation in Bray-Curtis dissimilarity ( $F_{1,44} = 73.3$ ,  $p = 0.001$ ),  
286 treatment explained 5.9% of the variation ( $F_{1,44} = 7.8$ ,  $p = 0.001$ ), and the interaction  
287 between treatment and passage explained 4.7% of the variation ( $F_{1,44} = 6.2$ ,  $p = 0.003$ ).  
288 There was no difference in dispersion between treatments or passages ( $F_{3,44} = 0.91$ ,  $p =$   
289  $0.45$ ). Finally,  $\text{CH}_4$  oxidation rate was correlated with Bray-Curtis dissimilarity across  
290 both treatments in passage 5 and explained 9.6% of the variation in Bray-Curtis  
291 dissimilarity (dbRDA:  $F_{1,22} = 2.34$ ,  $p = 0.010$ )

## 292 Taxa that responded to selection

293 To identify taxa that responded to selection on soil  $\text{CH}_4$  oxidation rate, we tested the  
294 differential relative abundance of families in the selected jars relative to the control jars

295 within passage 5 using three methods and then plotted the taxa identified by all three  
296 methods. We identified 12 families that were enriched or depleted in the selection  
297 treatment relative to the control (Figure 4).

298 Overall, none of the families enriched in the selection treatment contain known  
299 methanotrophs. Several taxa identified had a higher taxonomic designation that contains  
300 methanotrophs, for example, the Gammaproteobacteria class had a large effect size. The  
301 Gammaproteobacteria include the type I and type X methanotrophs in the families  
302 *Methylococcaceae* and *Methylothermaceae* (43). However, the Gammaproteobacteria is  
303 among the most diverse groups in the Prokaryotes, so this is not strong evidence for a  
304 selection response by methanotrophs (44). In addition, the *Puniceicoccaceae* is a member  
305 of the phylum Verrucomicrobia. The Verrucomicrobia is a diverse group that contain  
306 known methanotrophs as well as ammonia-oxidizing bacteria (45). Other than these two  
307 groups, none of the other taxa enriched in the selection treatment are known to be related  
308 to methanotrophs. Two groups in the Armatimonadales were enriched in the selection  
309 treatment including the family *Fimbriimonadaceae* and an unclassified ASV from the  
310 order Armatimonadales (46). *Cytophagaceae* was also enriched in the selection treatment  
311 and contains a number of mainly aerobic heterotrophs that can digest a variety of  
312 macromolecules (47). The remaining families include the uncultured family 0319-6G20,  
313 *Diplorickettsiaceae*, *Rhodospirillaceae*, and an unclassified Kapabacteriales.

314 We did not identify any methanotrophic families in the overall differential  
315 abundance analysis. However, we wanted to look more closely at the known  
316 methanotrophs in our dataset to be sure that they did not have an effect. To do this, we  
317 subset the ASVs in our dataset that were in families that contained methanotrophs. Only

318 two families were represented: *Methylophilaceae* and *Beijerinckiaceae*. Aggregating  
319 reads at the family level, neither family was differentially abundant between the two  
320 treatments. However, aggregated at the genus level, a group of unclassified genera in the  
321 *Beijerinckiaceae* were depleted in the selection treatment and the genus *Rhodoblastus*, a  
322 member of the *Beijerinckiaceae*, was enriched in the selection treatment. While many  
323 *Beijerinckiaceae* are methanotrophs, several taxa in this family have lost the ability to  
324 oxidize CH<sub>4</sub> and it appears that *Rhodoblastus* species are not able to grow on CH<sub>4</sub>, though  
325 they can grow on methanol (48,49). Based on this analysis, it appears that no  
326 methanotrophs were enriched in the positive selection treatment.

## 327 **Discussion**

328 We used artificial ecosystem selection to estimate the contribution of variation in  
329 microbiome composition to variation in the rate of an ecosystem function, CH<sub>4</sub> oxidation  
330 in soil, independent of environmental variation. Understanding how and to what degree  
331 microbiome variation contributes to variation in ecosystem function is important for  
332 many reasons. For example, successful microbiome manipulations require that the  
333 manipulated microbiome contribute to variation in ecosystem function independent of  
334 other drivers of ecosystem variation (such as variation in environmental conditions). This  
335 is because the drivers of variation in ecosystem functions can interact in complicated  
336 ways (Figure 1); for example, environmental variation can indirectly contribute by  
337 providing conditions that select for microbial groups that in turn alter the rates of  
338 ecosystem functions. Determining the independent contribution of microbiome variation



339 to ecosystem function is crucial because if microbiome composition is driven primarily  
340 by environmental conditions, then introducing a desirable taxon through microbiome  
341 manipulation without altering the environment will likely be unsuccessful at shifting the  
342 targeted ecosystem function. The artificial selection approach is different from the  
343 comparative and manipulative approaches used in past attempts at answering this  
344 question, because it can control for both the direct effect of environment on function as  
345 well as the indirect effect on environment via its impact on microbiome assembly.

346 In our study, we observed an increase in CH<sub>4</sub> oxidation rate in the selection  
347 treatment relative to the control, which demonstrates that there was a response to  
348 selection. Given that we observed a response to selection, we conclude that variation in  
349 the microbiome contributes to variation in the CH<sub>4</sub> oxidation rate independent of the  
350 environment. This suggests that microbiome manipulations could be an effective  
351 approach for altering the rate of CH<sub>4</sub> oxidation in this soil, and that the artificial selection  
352 approach may be useful in determining the potential for microbiome manipulations for  
353 other functions in other ecosystems.

354 Given that variation in the microbiome is associated with variation in the rate of an  
355 ecosystem function in our system, a reasonable follow-up question is “how much  
356 variation in ecosystem function is associated with microbiome variation in this system?”  
357 One way to estimate this is to determine how much the recipient jars resemble the  
358 selected donor jars that were used to inoculate them (31). We can calculate the response  
359 to selection as the difference between two successive passages in their mean CH<sub>4</sub>  
360 oxidation rate. We will denote this as  $R$ . We can also calculate the strength of selection as  
361 the difference in mean CH<sub>4</sub> oxidation rate between the twelve jars in one generation and

362 the three jars chosen for selection in that generation, which we will call the selection  
363 differential and denote as  $S$ . If we plot the cumulative  $R$  against the cumulative  $S$ , the  
364 slope of this relationship will equal the proportion of variation explained by the  
365 microbiome. If the change in mean function from passage one to passage two ( $R$ ) is equal  
366 to the difference in mean function between the twelve jars in passage one and the three  
367 jars selected to inoculate passage two ( $S$ ), then we would conclude that 100% of the  
368 variation is due to variation in the microbiome. Likewise, if recipients do not resemble  
369 the donors in their mean  $\text{CH}_4$  oxidation rate and simply wander randomly, then we would  
370 conclude that all of the variation is due to the environment or technical variation.

371 The relationship between microbiome variation and ecosystem function variation is  
372 analogous to the concept of “heritability” (50) used by quantitative geneticists, or more  
373 precisely the concept of “microbiability” (32) proposed by microbiome scientists who  
374 study host-associated microbiomes. Although rarely used in the study of environmental  
375 microbiomes, this concept could be very useful for understanding and manipulating  
376 microbially-mediated functions in a variety of ecosystems. In our experiment, variation in  
377 microbiome taxonomic composition statistically explained (i.e., was associated with)  
378 31.5% of the variation we observed in the rate of  $\text{CH}_4$  oxidation, though this was not  
379 significant. However, we did observe a significant divergence between the positive  
380 selection and control lines, which suggests that the imposed selection and passaging of  
381 microbiomes was sufficient to generate variation in soil  $\text{CH}_4$  oxidation rate. Future studies  
382 with greater replication could more precisely estimate the microbiability. This suggests  
383 that there is substantial potential for altering this ecosystem function through microbiome  
384 manipulation in this soil. It is very likely that the “environmental microbiability” will be

385 different for other ecosystem functions in this soil and for CH<sub>4</sub> oxidation in other soils.  
386 However, our experiment demonstrates that this relationship is measurable and provides  
387 an example of how this can be accomplished.

388 We next wanted to determine which aspects of the microbiome might explain the  
389 divergence in CH<sub>4</sub> oxidation rate between the two treatments. There are three inter-  
390 related ways that microbiomes could have responded to selection in this experiment: gain  
391 or loss of taxa, changes in the relative abundances of taxa, or changes within the genomes  
392 of the constituent taxa. We surveyed microbiome variation via 16s rRNA ribotyping in  
393 our experiment, which allowed us to deeply sample taxonomic diversity but did not allow  
394 us to directly address whether taxa in this experiment evolved genomic changes as a  
395 result of selection. However, if such genomic changes resulted in increased persistence or  
396 abundance of the population with these changes, this would be detectable. Therefore, we  
397 will focus on the first two possibilities.

398 Richness at the ASV level did not vary between the two treatments and there were  
399 relatively few taxa gained or lost in the selection treatment and none of these were  
400 prevalent across the 12 jars in passage 5. Therefore, the gain or loss of species is unlikely  
401 to explain the increase in CH<sub>4</sub> oxidation rate. However, we found that Bray-Curtis  
402 dissimilarity was greater between the two treatments in Passage 5 than within each  
403 treatment and was correlated with CH<sub>4</sub> oxidation rate, which suggests that changes in the  
404 relative abundance of taxa could explain the response to selection.

405 Even though we observed an increase in CH<sub>4</sub> oxidation rate in the selection  
406 treatment and a difference in composition between the two treatments, we did not observe  
407 an increase in the relative abundance of known methanotrophs. This was surprising given

408 that CH<sub>4</sub> consumption is not a common trait among microbes and that it is often assumed  
409 that the rate of an ecosystem function is limited by the final enzymatic step in the  
410 underlying metabolic pathway (11). In certain ecosystems, CH<sub>4</sub> production and  
411 consumption are correlated with the abundance of methanogens and methanotrophs as  
412 estimated from marker genes (18,19). However, our results suggest that in this system  
413 ecosystem-scale CH<sub>4</sub> oxidation rates can be altered by non-methanotrophs, perhaps  
414 through ecological interactions with methanotrophic species, or by unknown  
415 methanotrophs. This suggests that simple assumptions about how microbes contribute to  
416 rate variation in ecosystem function may not apply universally, and it demonstrates the  
417 importance of using biologically “agnostic” approaches (that make few starting  
418 assumptions) to linking microbial taxa to ecosystem functions (51). Artificial ecosystem  
419 selection is an important example of such an approach.

420       There is increasing interest in using artificial selection for understanding and  
421 manipulating the microbiomes associated with plants and animals (a.k.a., “microbiome  
422 breeding”; (52)). Our study demonstrates that artificial ecosystem selection can also be an  
423 important tool for exploring the relationship between microbiome composition and  
424 ecosystem function in non-host systems. This approach can provide unique information  
425 about the independent contribution of microbiomes to ecosystem functions. Such  
426 information is crucial if we are to successfully manipulate environmental microbiomes to  
427 alter ecosystem functions, whether to improve crop productivity (53) or ameliorate the  
428 impacts of environmental change (54).

## 429 **Acknowledgments**

430 This project was supported by the National Science Foundation Graduate Research  
431 Fellowship Program (grant no. DGE 1255832) and the ARCS Foundation Florence and  
432 Mike Nudelman Scholarship. Figure 1 was created with BioRender.com.

## 433 **Competing Interests**

434 We declare we have no competing interests.

## 435 **Data Availability Statement**

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

## 441 **References**

- 442 1. Conrad R. Soil microorganisms as controllers of atmospheric trace gases (H<sub>2</sub>, CO,  
443 CH<sub>4</sub>, OCS, N<sub>2</sub>O, and NO). *Microbiological Reviews*. 1996 Dec;60(4):609–40.
- 444 2. Schimel JP, Gullledge J. Microbial community structure and global trace gases. *Global  
445 Change Biology*. 1998;4(7):745–58.

- 446 3. Crowther TW, Hoogen J van den, Wan J, Mayes MA, Keiser AD, Mo L, et al. The  
447 global soil community and its influence on biogeochemistry. *Science*. 2019  
448 Aug;365(6455).
- 449 4. Cavigelli MA, Robertson GP. The Functional Significance of Denitrifier Community  
450 Composition in a Terrestrial Ecosystem. *Ecology*. 2000;81(5):1402–14.
- 451 5. Balser TC, Firestone MK. Linking microbial community composition and soil  
452 processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry*.  
453 2005 Apr;73(2):395–415.
- 454 6. Waldrop MP, Firestone MK. Response of Microbial Community Composition and  
455 Function to Soil Climate Change. *Microbial Ecology*. 2006 Nov;52(4):716–24.
- 456 7. Strickland MS, Lauber C, Fierer N, Bradford MA. Testing the functional significance  
457 of microbial community composition. *Ecology*. 2009;90(2):441–51.
- 458 8. Wagg C, Bender SF, Widmer F, Heijden MGA van der. Soil biodiversity and soil  
459 community composition determine ecosystem multifunctionality. *Proceedings of the*  
460 *National Academy of Sciences*. 2014 Apr;111(14):5266–70.
- 461 9. Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA. Fungal-  
462 bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature*  
463 *Communications*. 2019 Oct;10(1):4841.

- 464 10. Graham EB, Knelman JE, Schindlbacher A, Siciliano S, Breulmann M, Yannarell A,  
465 et al. Microbes as Engines of Ecosystem Function: When Does Community Structure  
466 Enhance Predictions of Ecosystem Processes? *Frontiers in Microbiology*. 2016;7.
- 467 11. Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, et al.  
468 Relationships between protein-encoding gene abundance and corresponding process are  
469 commonly assumed yet rarely observed. *The ISME Journal*. 2015 Aug;9(8):1693–9.
- 470 12. Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, et al. An  
471 examination of the biodiversityEcosystem function relationship in arable soil microbial  
472 communities. *Soil Biology and Biochemistry*. 2001 Oct;33(12):1713–22.
- 473 13. Reed HE, Martiny JBH. Testing the functional significance of microbial composition  
474 in natural communities. *FEMS Microbiology Ecology*. 2007;62(2):161–70.
- 475 14. Swenson W, Wilson DS, Elias R. Artificial ecosystem selection. *Proceedings of the*  
476 *National Academy of Sciences*. 2000 Aug;97(16):9110–4.
- 477 15. Blouin M, Karimi B, Mathieu J, Lerch TZ. Levels and limits in artificial selection of  
478 communities. *Ecology Letters*. 2015;18(10):1040–8.
- 479 16. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. Selection on soil  
480 microbiomes reveals reproducible impacts on plant function. *The ISME journal*. 2015  
481 Mar;9(4):980–9.

- 482 17. Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, et al.  
483 Three decades of global methane sources and sinks. *Nature Geoscience*. 2013  
484 Oct;6(10):813–23.
- 485 18. Meyer KM, Morris AH, Webster K, Klein AM, Kroeger ME, Meredith LK, et al.  
486 Belowground changes to community structure alter methane-cycling dynamics in  
487 Amazonia. *Environment International*. 2020 Dec;145:106131.
- 488 19. Meyer KM, Hopple AM, Klein AM, Morris AH, Bridgham SD, Bohannon BJM.  
489 Community structure Ecosystem function relationships in the Congo Basin methane cycle  
490 depend on the physiological scale of function. *Molecular Ecology*. 2020;29(10):1806–19.
- 492 20. Freitag TE, Prosser JI. Correlation of Methane Production and Functional Gene  
493 Transcriptional Activity in a Peat Soil. *Applied and Environmental Microbiology*. 2009  
494 Nov;75(21):6679–87.
- 495 21. Freitag TE, Toet S, Ineson P, Prosser JI. Links between methane flux and  
496 transcriptional activities of methanogens and methane oxidizers in a blanket peat bog.  
497 *FEMS Microbiology Ecology*. 2010 Jul;73(1):157–65.
- 498 22. Schnyder E, Bodelier PLE, Hartmann M, Henneberger R, Niklaus PA. Positive  
499 diversity-functioning relationships in model communities of methanotrophic bacteria.  
500 *Ecology*. 2018;99(3):714–23.



- 501 23. Martiny AC, Treseder K, Pusch G. Phylogenetic conservatism of functional traits in  
502 microorganisms. *The ISME Journal*. 2013 Apr;7(4):830–8.
- 503 24. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ,  
504 et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per  
505 sample. *Proceedings of the National Academy of Sciences*. 2011 Mar;108(Supplement  
506 1):4516–22.
- 507 25. Fadrosch DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved  
508 dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina  
509 MiSeq platform. *Microbiome*. 2014 Dec;2(1):1–7.
- 510 26. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a  
511 Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon  
512 Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental*  
513 *Microbiology*. 2013 Sep;79(17):5112–20.
- 514 27. R Core Team. *R: A language and environment for statistical computing*. Vienna,  
515 Austria: R Foundation for Statistical Computing; 2018.
- 516 28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:  
517 High-resolution sample inference from Illumina amplicon data. *Nature Methods*. 2016  
518 Jul;13(7):581–3.

- 519 29. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian Classifier for Rapid  
520 Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and*  
521 *Environmental Microbiology*. 2007 Aug;73(16):5261–7.
- 522 30. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical  
523 identification and removal of contaminant sequences in marker-gene and metagenomics  
524 data. *Microbiome*. 2018 Dec;6(1):1–14.
- 525 31. Falconer DS, MacKay TFC. *Introduction to quantitative genetics*. Harlow : Prentice  
526 Hall; 1996.
- 527 32. He Y, Tiezzi F, Jiang J, Howard J, Huang Y, Gray K, et al. Exploring methods to  
528 summarize gut microbiota composition for microbiability estimation and phenotypic  
529 prediction in swine. *Journal of Animal Science*. 2022 Sep;100(9):skac231.
- 530 33. Hurlbert SH. The Nonconcept of Species Diversity: A Critique and Alternative  
531 Parameters. *Ecology*. 1971;52(4):577–86.
- 532 34. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. *Vegan:*  
533 *Community ecology package*. 2019.
- 534 35. Bray JR, Curtis JT. An Ordination of the Upland Forest Communities of Southern  
535 Wisconsin. *Ecological Monographs*. 1957;27(4):325–49.
- 536 36. McArdle BH, Anderson MJ. *Fitting Multivariate Models to Community Data: A*  
537 *Comment on Distance-Based Redundancy Analysis*. *Ecology*. 2001;82(1):290–7.

- 538 37. Kang CS, Dunfield PF, Semrau JD. The origin of aerobic methanotrophy within the  
539 Proteobacteria. *FEMS Microbiology Letters*. 2019 May;366(9):fnz096.
- 540 38. Kaul A, Mandal S, Davidov O, Peddada SD. Analysis of Microbiome Data in the  
541 Presence of Excess Zeros. *Frontiers in Microbiology*. 2017 Nov;8:2114.
- 542 39. Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction.  
543 *Nature Communications*. 2020 Jul;11(1):3514.
- 544 40. Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB.  
545 Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq,  
546 16S rRNA gene sequencing and selective growth experiments by compositional data  
547 analysis. *Microbiome*. 2014 May;2(1):15.
- 548 41. Martin BD, Witten D, Willis AD. Modeling microbial abundances and dysbiosis with  
549 beta-binomial regression. *The Annals of Applied Statistics*. 2020 Mar;14(1):94–115.
- 550 42. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and  
551 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B*  
552 (Methodological) [Internet]. 1995 [cited 2023 Jun 13];57(1):289–300. Available from:  
553 <http://www.jstor.org/stable/2346101>
- 554 43. Stein LY, Roy R, Dunfield PF. Aerobic Methanotrophy and Nitrification: Processes  
555 and Connections. In: eLS. John Wiley & Sons, Ltd; 2012.

- 556 44. Garrity GM, Bell JA, Lilburn TG. Class III. Gammaproteobacteria class. Nov., p. 1.  
557 In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey's manual of systematic  
558 bacteriology. Second. New York, NY: Springer; 2005.
- 559 45. Freitag TE, Prosser JI. Community Structure of Ammonia-Oxidizing Bacteria within  
560 Anoxic Marine Sediments. *Applied and Environmental Microbiology*. 2003  
561 Mar;69(3):1359–71.
- 562 46. Im W-T, Hu Z-Y, Kim K-H, Rhee S-K, Meng H, Lee S-T, et al. Description of  
563 *Fimbriimonas ginsengisoli* gen. Nov., sp. Nov. Within the Fimbriimonadia class nov., of  
564 the phylum Armatimonadetes. *Antonie van Leeuwenhoek*. 2012 Aug;102(2):307–17.
- 565 47. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W. The Family Cytophagaceae. In:  
566 Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. *The*  
567 *Prokaryotes: Other Major Lineages of Bacteria and The Archaea*. Berlin, Heidelberg:  
568 Springer; 2014. pp. 577–93.
- 569 48. Tamas I, Smirnova AV, He Z, Dunfield PF. The (d)Evolution of methanotrophy in  
570 the Beijerinckiaceae comparative genomics analysis. *The ISME Journal*. 2014  
571 Feb;8(2):369–82.
- 572 49. Dedysh SN, Haupt ES, Dunfield PF. Emended description of the family  
573 Beijerinckiaceae and transfer of the genera *Chelatococcus* and *Camelimonas* to the  
574 family Chelatococcaceae fam. Nov. *International Journal of Systematic and Evolutionary*  
575 *Microbiology*. 2016;66(8):3177–82.

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

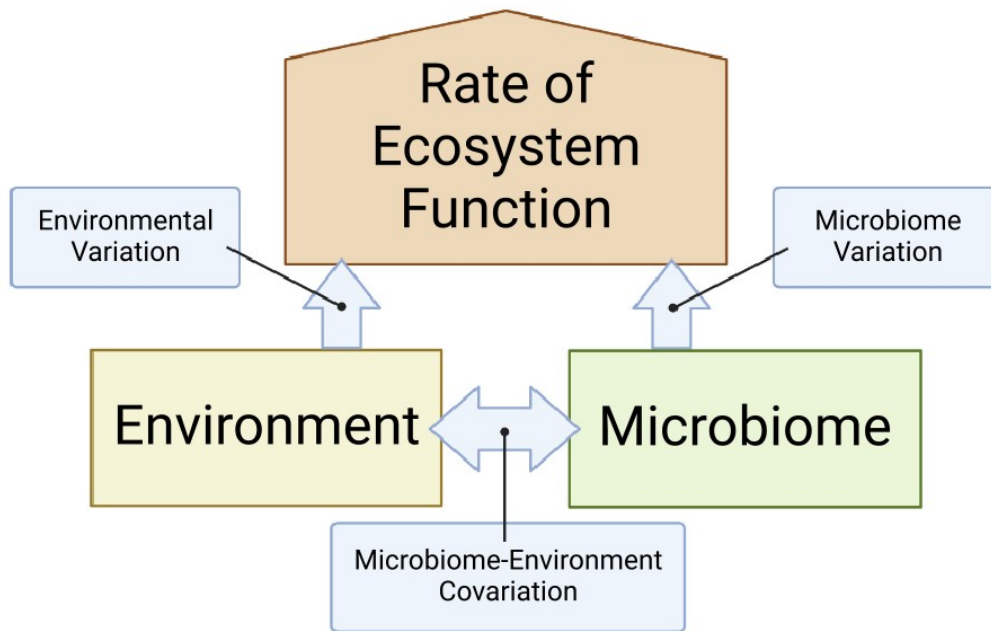
578 51. Morris A, Meyer K, Bohannan B. Linking microbial communities to ecosystem  
579 functions: What we can learn from genotypePhenotype mapping in organisms.  
580 Philosophical Transactions of the Royal Society B: Biological Sciences. 2020  
581 May;375(1798):20190244.

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

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

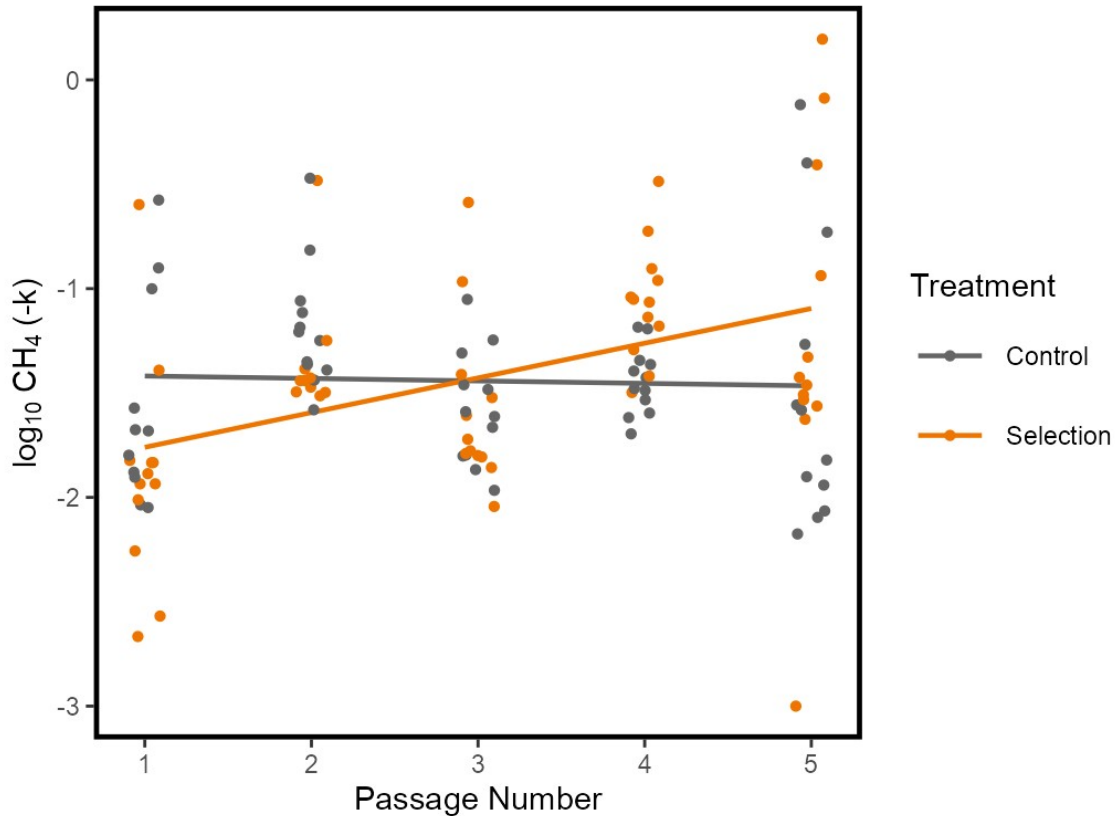
587 54. Jansson JK, Hofmockel KS. Soil microbiomes and climate change. Nature Reviews  
588 Microbiology. 2020 Jan;18(1):35–46.

## 589 **Figures**



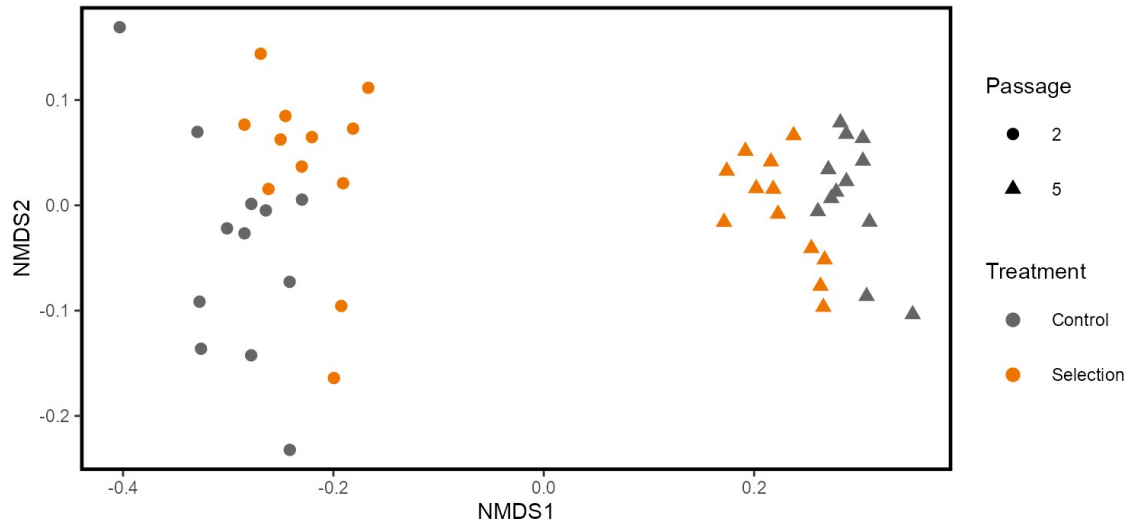
590

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



599

600 Figure 2: Response to selection on soil CH<sub>4</sub> oxidation rate. The y-axis is CH<sub>4</sub> oxidation  
601 rate as the log<sub>10</sub> of the additive inverse of the first-order exponential decay constant *k*  
602 (i.e.,  $-k$ ) with units day<sup>-1</sup> so that a more positive value represents a higher CH<sub>4</sub> oxidation  
603 rate. Orange points and regression line are for the positive selection treatment and gray  
604 points and regression line are for the control. There was a significant difference of slopes  
605 between the positive selection treatment and the control ( $F_{2,113} = 3.85$ ,  $p = 0.02$ ).



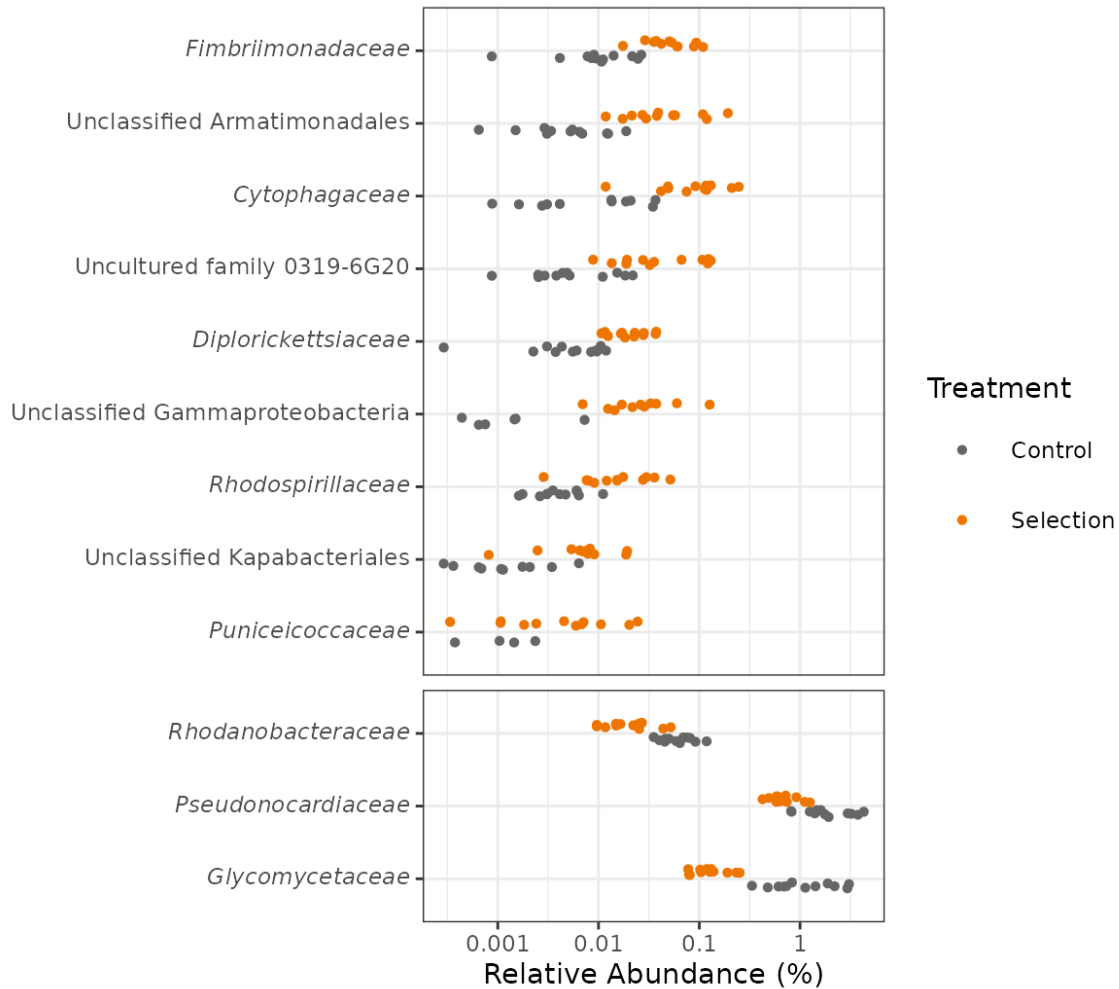
607 Figure 3: Non-metric multidimensional scaling plot of beta diversity for all jars.

608 Dissimilarities are rarefied Bray-Curtis dissimilarity averaged over 100 subsamples.

609 Orange points are the positive selection treatment and gray points are the control. Circles

610 are passage 2 and triangles are passage 5.





611

612 Figure 4: Differentially abundant family-level taxa identified by ANCOM-II, ALDEx2,  
613 and CORNCOB. Values on the x-axis are relative abundances on a log<sub>10</sub> scale. Taxa in  
614 the top panel are enriched in the positive selection treatment relative to the control and  
615 taxa in the bottom panel are depleted in the positive selection treatment relative to the  
616 control. Taxa are sorted by their effect size with taxa at the top having the largest positive  
617 effect size and taxa at the bottom with the largest negative effect size.