Selection, succession and stabilization of soil microbial consortia

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ABSTRACT

Soil microorganisms play fundamental roles in cycling of soil carbon, nitrogen and other nutrients, yet we have a poor understanding of how soil microbiomes are shaped by their nutritional and physical environment. Here we investigated the successional dynamics of a soil microbiome during 21-weeks of enrichment on chitin and its monomer, *N-acetylglucosamine*. We examined succession of the soil communities in a physically heterogeneous soil matrix as well as a highly mixed liquid medium. The guiding hypothesis was that the initial species richness would influence the tendency for the selected consortia to stabilize and maintain relatively constant community structure over time. We also hypothesized that long term, substrate-driven growth would result in consortia with reduced species richness as compared to the parent microbiome and that this process would be deterministic with relatively little variation between replicates. We found that the initial species richness does influence the long-term community stability in both liquid media and soil and that lower initial richness results in a more rapid convergence to stability. Despite use of the same soil inoculum and access to the same major substrate, the resulting community composition differed greatly in soil compared to liquid medium. Hence, distinct selective pressures in soils relative to homogenous liquid media exist and can control community succession dynamics. This difference is likely related to the fact that soil microbiomes are more likely to thrive, with fewer compositional changes, in a soil matrix compared to liquid environments.

IMPORTANCE

The soil microbiome carries out important ecosystem functions, but interactions between soil microbial communities have been difficult to study due to the high microbial diversity and

49 complexity of the soil habitat. Here we successfully obtained stable consortia with reduced
50 complexity that contained species found in the original source soil. These consortia and the
51 methods used to obtain them can be a valuable resource for exploration of specific mechanisms
52 underlying soil microbial community ecology. The results of this study also provide new
53 experimental context to better inform how soil microbial communities are shaped by new
54 environments and how a combination of initial taxonomic structure and physical environment
55 influences stability.
56

INTRODUCTION

Soil microbiomes are among the most diverse microbial communities on the planet (1, 2) and the majority of soil microbes have not yet been cultivated or studied under laboratory conditions. This, and other confounding properties, such as extreme spatial heterogeneity, make it difficult to study how soil microorganisms interact within natural communities (3). Despite this, a deeper understanding of the ecological properties that control the structure and function of soil microbiomes is needed as they underpin almost every terrestrial food web (4), regulate many elements of Earth's biogeochemical cycles (5) and are fundamental for growth of healthy crops and bioenergy feedstocks (6).

Well established studies estimate that annual CO_2 emissions from soil microbial respiration are ten times greater than the CO_2 produced by fossil fuel utilization (5). Therefore, small changes in the soil carbon cycle – a process linked to microbiome functioning – can have large impacts on atmospheric CO_2 concentrations. The cycling of complex biopolymers that are both produced and stored in soils largely influences the flux of CO_2 to the atmosphere. Of these, chitin, an insoluble β-1, 4-linked polymer of *N-acetylglucosamine* (NAG) (7, 8), is a major substrate for soil microbial activity (9) and represents a linkage between the carbon and nitrogen cycles in soils (10-12). Chitin is omnipresent in soil and is an important biopolymer synthesized by fungi (13) and many insects. However, little is known about how chitin and NAG can select for soil-specific bacterial and fungal taxa and influence the structure of microbial communities that are involved in their decomposition.

Successional dynamics of soil microbiomes are related to changes in substrate availability and are crucial to predicting ecosystem development (14-20). During primary succession, early colonizing taxa shape available niche space by regulating pH and nutrient

80 availability (16, 17, 21). However, the feedbacks and processes driving successional patterns
81 constitute fundamental knowledge gaps in understanding trajectories of ecosystem development
82 (16, 19). Microbial succession patterns can be influenced by available resources, including
83 nutrient pools (19, 22), physiochemistry (23), and vegetation (24). Additionally, it is well known
84 that soil moisture is a key determinant of microbial metabolism (25-27). Less is known about
85 how the physical environment, with respect to soil or liquid-like conditions, affect microbial
86 community succession and stability. The relative stability of microbial communities through
87 early succession and thereafter is key to understanding and predicting microbial responses to
88 perturbation (28-31). While the immense complexity of soil microbiomes has hindered many
89 efforts to describe the succession dynamics to ecosystem functioning, organic matter chemistry
90 has been identified as a key driver of primary succession (32).

91 In this study, we aimed to investigate processes underlying soil microbial community
92 succession by monitoring microbial community development in a sterile soil matrix enriched
93 with NAG. Comparisons were made over the course of 15-weeks of succession to a liquid
94 medium culture derived from the same inoculum. In this way, environmental successional
95 trajectories of the soil microbiome were directly compared to community development using
96 traditional, liquid-based culturing methods that omit the heterogenous chemical and spatial
97 landscapes associated with the soil matrix.

98 We hypothesized that initial species richness would influence the succession of the
99 consortia and their ability to stabilize with a relatively constant taxonomic structure over time.
100 Specifically, we anticipated that consortia with lower species richness during the initial phases of
101 succession would display higher tendencies to converge towards smaller changes in community
102 structure between successive time points. We also hypothesized that long term selection by NAG

would result in soil microbial consortia with reduced complexity as compared to the parent soil microbiome and that this process would be deterministic with relatively little variation between replicates during enrichment.

To test these hypotheses, we investigated the influence of initial richness and physical environment on the progression of chitin/NAG enriched soil microbial consortia. We designed soil microbiome enrichment experiments with the expectation that dilution and long-term selection on chitin/NAG would dramatically reduce the initial community richness when compared to the native soil. One of our aims was to use this procedure to obtain simplified, naturally adapted consortia that can serve as a valuable experimental resource that can be shared for recapitulating some soil microbiome behaviors. We also expected and found that the consortia that did emerge from this long-term succession experiment showed distinct differences based on the physical environment (soil verses liquid). This study has improved our understanding about the succession and stability of microbial communities in soil. Generally, these results show that the final stability of and the extent of species richness were directed by the length of succession, the initial richness, and the culturing environment.

RESULTS

Enrichment of a native soil microbiome on chitin. Native soil was supplemented in triplicate with 3 concentrations of chitin (10, 50 and 100 ppm) for six-weeks to select for naturally co-existing soil populations capable of using chitin as a carbon and/or nitrogen substrate. Respiration was monitored during the enrichment as a proxy for soil microbial activity during chitin decomposition. The highest respiration was observed for the highest chitin concentration

and therefore the 100 ppm treatments were used to inoculate longer term enrichments supplemented with NAG.

The dominant bacterial phyla in the native soil communities were *Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi* and *Bacteroidetes*; there were few archaea identified in high relative abundance (Fig. 1) (Supplemental Fig. S1A). The dominant fungi were *Ascomycota* (Supplemental Fig. S1B). The native soil bacterial richness had a mean of 818.5 ± 75.6 16S OTUs (Supplemental Fig. S1C). The native soil bacterial community also exhibited high evenness, with a Simpson's Evenness score of 0.3; the most abundant OTU accounted for less than 4% of all observations. In comparison to the 16S results, the fungal richness and evenness in the native soil were much lower (Supplemental Fig. S1D). The mean ITS OTU count was 128 ± 39, with a Simpson's Evenness score of only 0.069, and the top two OTUs together comprised 41% of the observed fungal community.

Following the initial 6-week chitin enrichment, the bacterial community structure shifted to a higher relative abundance of *Firmicutes* and *Acidobacteria* and less *Actinobacteria,* although *Proteobacteria* continued to maintain the highest relative abundance (Supplemental Fig. 1A). Additionally, there were shifts in the fungal communities, with a higher relative abundance of *Mortierellomycota* and decreased *Ascomycota* compared to the native soil (Supplemental Fig. 1B). Bacterial richness remained statistically unchanged ($p = 0.7624$, native soil = 823.5 ± 78.5; $n = 2$, chitin enriched soil = 801.7 ± 26.35; $n = 3$). However, bacterial evenness decreased by 55%, indicating that chitin supplementation selected for a subset of populations. The fungal species richness and evenness remained essentially unchanged by the chitin supplementation indicating that the native fungal taxa were less responsive to chitin compared to the bacteria.

148 **The structure and taxa of soil and liquid-based consortia.** After chitin enrichment, subsequent
149 extended enrichment was carried out over 15-weeks using 100 ppm NAG as the major carbon
150 and nitrogen source. The enrichments were performed in two parallel tracks using the same
151 source inoculum (soil enriched for 6-weeks with 100 ppm chitin); in both gamma-irradiated
152 (sterile) soil and liquid M9 medium. The total time for the experiment using chitin enrichment
153 followed by NAG enrichment was 21-weeks. This experimental design was used to optimize
154 opportunities for selection of reduced complexity, naturally co-existing soil consortia and to
155 determine the influence of the physical matrix on the enrichment process. While the physical
156 differences between soil and liquid are paramount, it is important to note other differences
157 including carbon/nitrogen sources or pH that may also have an effect on the succession of
158 resulting consortia.

159 The NAG enrichments were initiated by serial 10-fold dilution of the chitin enriched soil
160 (dilutions ranged from 10^{-1} to 10^{-4}) into the irradiated sterile soil and into liquid M9, both
161 containing 100 ppm of NAG. The relative abundances of both 16S and ITS OTUs differed
162 between serial dilutions and treatment conditions over the course of the experiment (Fig. 1).
163 *Proteobacteria* remained the dominant bacterial phylum during the succession period in both the
164 liquid and soil treatments (Fig. 1A). However, the NAG enriched liquid environment showed a
165 greater degree of change compared to proportions of the most abundant taxa in the native source
166 soil. In the NAG enriched liquid medium, members of the *Proteobacteria* and *Ascomycota* phyla
167 dominated the bacterial and fungal communities, respectively. In contrast, there was a higher
168 diversity of phyla represented in the NAG enriched soil environment over time. In these samples
169 we observed increases in typical soil bacteria that are generally difficult to cultivate; namely
170 *Planctomycetes* and *Verrucomicrobia*. *Planctomycetes* was negligible in all matching liquid

171 incubations and *Verrucomicrobia* was only present to a comparable degree in the least diluted
172 liquid sample (10^{-1}). Simultaneously, we observed depletion of *Acidobacteria* and *Actinobacteria*
173 in the NAG enriched soil. We also detected a greater number of fungal phyla in communities
174 grown on the NAG enriched soil compared to its liquid counterpart, with relatively high
175 proportions of *Mortierellomycota*, *Basidiomycota* and unidentified fungi at the end of the
176 incubation period (Fig. 1B).

177 As the 15-week NAG enrichments were being regularly sampled for gDNA and
178 respiration, we employed sterile controls to monitor contamination (Supplementary Fig. S2).
179 This enabled us to detect cross contamination between samples as growth in our soil and liquid
180 media controls. This was inferred from non-zero respiration measurements and the recovery of
181 gDNA from liquid media (gDNA was always present in sterile soil). The cross contamination
182 was first observed at week-5 (Supplemental Figure S2). The most common OTU identified from
183 the controls was of the genus *Pseudomonas*. This OTU was present in the native soil and chitin
184 enrichments, indicating that it was intrinsic to the experiential system and native to the parent
185 microbiome (Supplemental Figure S3). Although the sterile controls lacked any viable growth at
186 the onset of the incubations (as determined by plate counting), the *Pseudomonas* OTU
187 introduced during the incubations was able to grow and dominate the liquid sterile controls as
188 well as the more dilute liquid samples (10^{-3} and 10^{-4}). However, although present, the
189 *Pseudomonas* OTU did not establish itself to high relative levels within the higher richness liquid
190 samples or any of the soil samples, likely due to the complexity and stability of the existing
191 microbial communities already present in these sites.

192 We anticipated that long-term selection by NAG in a sterile soil or liquid M9
193 environment would result in soil microbial consortia with reduced complexity as compared to

194 both the native soil microbiome and the chitin enriched soil microbiome. Over all, this was found
195 to be true although the initial species richness of the inoculum also played a major role. We
196 manually reduced the complexity of the inoculum by controlling the initial species-richness
197 through dilutions. A comparison of the species-richness measured on the first sampling date
198 (week-0) across dilutions in NAG enriched liquid media showed that the dilutions were
199 successful in reducing the richness of the initial inoculum (Fig. 1C and Supplemental Table S1).
200 It is very likely that a corresponding initial drop in richness was also happening with the soil
201 dilutions, although this could not be confirmed by amplicon analysis due to DNA amplification
202 from soil microbes that were likely killed during the gamma irradiation process (33, 34). In the
203 liquid incubations, the observed 16S and ITS OTU counts from the 10^{-3} and 10^{-4} dilutions
204 gradually decreased over time; however, the 10^{-1} and 10^{-2} dilutions revealed sharp decreases in
205 species richness on the first week, followed by a rebounding trend through week-15. This drop
206 and rebounding effect after week-3 was also observed across all of the dilutions associated with
207 the NAG enriched soil. Fungal richness measurements followed similar patterns as those seen for
208 the bacterial richness. By the end of 15-weeks the NAG enriched soil microbiome richness was
209 reduced by approximately 35-70% (depending on dilution) compared to the original native soil
210 (Fig. 1 C, D) and the NAG enriched liquid microbiome richness was reduced by approximately
211 37-88%. This represents a considerable decrease in species complexity from the initial native and
212 chitin-enriched soil microbiomes and a demonstration that a combination of dilution and long-
213 term selection on specific carbon sources can lead to consortia with reduced species complexity.
214
215 **Physical environment and initial species richness influences stability.** The stability of the
216 enriched consortia was measured by comparing beta diversity over time. Specifically, we used

measures of weighted UniFrac distance (35) between samples that occurred sequentially as a measure of phylogenetic volatility (Fig. 2), where consortia with lower volatility are defined as those showing a more similar community structure from one time point to the next (36). This represents a way to measure how much the community is changing from week-to-week, which is related to the taxonomic compositional stability over time (Supplemental Figure S4). By using this metric, it was clear that while enrichment on both NAG containing soil and liquid media led to stable consortia, those enriched within the liquid environment became relatively stable more quickly as compared to those in the enriched soil (Fig. 2). Consortial stability also depended on the complexity of the initial inoculum (Supplemental Table S2) (Fig. 2), a factor that was controlled by dilution of the chitin enriched input soil. Samples inoculated with the 10^{-4} dilution (lowest initial richness) showed the greatest tendency to stabilize.

The consortia became more stable starting at week-5 with the maximum stability reached by the end of the experiment on week-15. However, differences were observed based on succession in liquid versus soil environments. The NAG enriched soil microbial communities showed an initial drop in volatility (weeks 1 – 2) followed by a rise in volatility through weeks 3 – 5 (Fig 2). After 5-weeks of enrichment in soil with NAG, the composition of the soil microbiome did not change significantly (Fig. 2) and volatility continued to drop as the experiment progressed. In contrast, NAG enriched liquid microbiomes initially exhibited an extreme drop in volatility over the first two weeks and thereafter showed either a consistent volatility measurement near 0.10 (dilutions 10^{-3} and 10^{-4}) or a continual gradual drop in volatility over the remaining 13-weeks down to a minimum of 0.15 (dilutions 10^{-1} and 10^{-2}). Bacterial volatility showed a consistent increase around near week-11, which also corresponded with the observed decreases in the relative abundance of OTUs assigned as *Verruucomicrobia* and

240 *Bacteroidetes* in the soil consortia (Fig. 1). More diverse microbial communities were enriched
241 and stabilized in soil as compared to the liquid incubations. This demonstrates that the physical
242 environment was a significant factor for the stability and compositional convergence of
243 microbial consortia. These results show that the final stability of the consortia and the extent of
244 species richness were directed by the length of succession, initial richness and culturing
245 environment.

246

247 **Biological and physical variables underpinning observed beta diversity.** Respiration and
248 volatility of the enriched communities were compared to phylogenetic composition over time via
249 ordination by canonical analysis of principal coordinates using weighted Unifrac distance
250 between rarefied samples (Fig. 3). As described earlier, changes (volatility) in community
251 composition between time points were measured as the weighted UniFrac distance between
252 subsequent time points (Fig. 2). In all environments, the volatility vector points were in the
253 direction of early stage incubation samples (Fig. 3), where large changes in the community
254 structure occurred between time points (Supplemental Figure S4). In liquid media incubations,
255 the contribution of respiration for the dissimilarity between samples become more prominent in
256 the later stages of incubation time courses. The dominant phyla from both kingdoms were
257 assessed with respect to incubation time and treatment condition. In the soil, *Proteobacteria* and
258 *Firmicutes* co-varied with volatility (Fig. 3A), as they were most abundant in the volatile initial
259 samples and slowly decreased over time. However, in the liquid media *Firmicutes* and
260 *Bacteroidetes* were closely aligned with volatility (Fig. 3B). Also, *Proteobacteria* became
261 dominant over time in the liquid media incubations; in particular those that were originally
262 inoculated with higher dilutions of the chitin-enrichment that had a lower initial species richness.

263 For the fungi in the soil culture, volatility co-varied primarily with *Mortierellomycota* and
264 *Chytridiomcota*, motile saprotrophs with chitin-containing cells walls that are found in wet soils
265 (Fig. 3C) (37).
266
267
268 **DISCUSSION**
269 Selective enrichment of soil microbes with specific carbon substrates resulted in the formation
270 of distinct microbial consortia that displayed reduced complexity. Those consortia that developed
271 in NAG enriched soil were also representative of the native soil microbiome used as the
272 inoculum. A primary finding from this study was that the initial species richness influenced
273 successional patterns that were enriched with a specific carbon/nitrogen source in both NAG
274 enriched liquid media and soil incubations. Because the experiment was well replicated (8
275 biological replicates per treatment) we also confirmed our hypothesis that substrate-driven soil
276 community succession is deterministic in that all of the replicates for a given soil dilution
277 resulted in similar endpoint communities (Supplemental Figure S4 and Table S3). This result
278 was observed in both liquid media and soil substrates, although the taxonomic structure of
279 endpoint consortia was controlled by hydro-physical and other matrix-associated differences
280 between soil and liquid media. The end-point microbial community structure was well explained
281 by the initial dilution condition and this influence was more pronounced on the liquid than the
282 soil treatment condition (Supplemental Table S2). At the end of the enrichment period, the soil
283 NAG enrichments showed higher species richness compared to equivalent liquid treatments,
284 despite having identical inoculations, and were also more representative of microbiomes from
285 the original native soil with respect to community composition. The persistence of members of

the original soil microbiome was consistent across dilutions for the NAG enriched soil. The 10^{-1} dilution had the highest species similarity to the native soil and retained a diverse community, while, at the other extreme, the 10^{-4} dilution represented a much simpler, less rich community.

This approach for developing consortia with reduced complexity is of interest as a method for obtaining simplified model microbiomes, with naturally interacting members that are representative of the native soil system. This similarity to the native soil seen with the NAG enriched soil is likely a result of the experiment being performed with soil microbes in their natural soil substrate, compared to a relatively foreign substrate (liquid). Another recent study also examined shorter term succession of soil microbiomes in liquid (but not soil) and found that soil microbiomes enriched on liquid media are very different from the original source soil microbiome (38). That study was carried out using a variety of carbon sources resulting in microbiomes with reduced complexity, similar to what we show here. Together, these studies confirm that reduced complexity consortia that have community membership representative of soil microbiomes are much more likely to be obtained using a soil-based enrichment compared to a liquid-based enrichment. In addition, our results clearly show differences in the successional dynamics and end-point structures of each consortium with respect to their initial species richness based on the dilution of the chitin enriched soil inoculum.

We found that the richness of the initial soil inoculum strongly impacted the alpha diversity of the resulting microbial consortia over time (Fig. 1). These results support our hypothesis that the initial species richness would influence each consortium's tendency to converge towards smaller changes in community structure between successive time points. Results supporting this hypothesis were observed for the higher dilutions (10^{-3} and 10^{-4}) for all treatments and measurements (Fig. 2). Each consortium's tendency to converge towards smaller

changes in community structure between successive time points was assessed by comparing weighted UniFrac distances between time points and was notably strongest for communities developed in the liquid media and measured by 16S rRNA sequencing as compared to ITS. The generalizability of this stability convergence effect is partially supported through similar findings presented by Shade et al (2014), who showed how rare taxa significantly influence microbial diversity (39). Dilutions are more likely to remove rare taxa and therefore our results provide some additional quantification of the effect presented by Shade et al. (2014). However, in our current study, we cannot fully decouple the effects of reduced initial richness from reduced counts of viable cells that were almost certainly created from the dilution procedure. Hence, an alternative interpretation could be formulated as decreased viable cell numbers in early stages of succession lead to decreased species richness and higher tendencies to converge towards smaller changes in community structure between successive time points.

Both bacterial and fungal populations were selected during the chitin/NAG incubation process. This suggests that the representative populations were able to either metabolize, or through some other means take advantage of the added substrates. Specifically, we found that members of the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes*, *Proteobacteria*, *Verrucomicrobia* were represented in the NAG incubations (Fig. 1a). In addition, the richness of *Verrucomicrobia*, *Bacteroidetes* and *Planctomycetes* increased in soil incubated with NAG compared to native soil (Fig. 1a). Representatives of these phyla were also detected on a previous study of soil enriched with chitin (12). With respect to the fungi, we found that the *Mortierellomycota* phylum increased in relative abundance in the NAG enriched consortia (Fig. 1b). *Mortierellomycota* are members of the Mucoromyceta, based on recent fungal taxonomy (40). They are sporangiferous, generally

saprotrophic, including being able to grow on other fungi, and are found in soil (41). The dominance of these specific bacteria and fungi suggests that their enrichment came due to their ability to use either chitin/NAG or its metabolic byproduct as a substrate.

The occurrence of enriched, stable consortia with dozens to hundreds of members, found here and in a similar study by Goldfarb, et. al. (42), as opposed to selection of a monoculture, suggests that the compositions of the reduced microbial communities are governed by cross-feeding interactions among microbes. In our longer-term soil incubations with NAG the microbiome converged into a less complex microbial community compared to that found in the native soil. This is consistent with the results of the Goldfarb study, which also enriched a simplified microbial community, derived from soil, on single carbon sources. However, unlike the previous study, which used only liquid, we enriched on both liquid and soil and found that enrichments on soil led to a reduced complexity community that is far more representative of the native soil microbiome compared to enrichment on liquid. There are several reasons why structured environments may better facilitate and stabilize social interactions, including the limited dispersal of interacting species, and the physical retention of resources within the soil matrix. The close physical proximity of members of soil consortia in discrete niches would thus facilitate social activities between member populations (e.g. exchange of public goods, quorum sensing and competition). When microbial communities have a single major carbon source only a subset of the community will have the metabolic capability to utilize it as a substrate. For complex substrates, such as chitin, other species will be reliant on primary species to degrade the polymer to simpler compounds, thus selecting for a community that interacts by metabolic cross-feeding, interactions that positively affect both the primary degrading species and the secondary degrading species (43, 44). Positive metabolic interactions between microorganisms residing

355 within communities have been studied in other systems as well; particularly in biofilms where
356 species and cells are in very close proximity and must cooperate for growth (45).

357 Because we monitored the soil enrichments over a relatively long time period, we could
358 determine the time required for the soil microbiomes to reach stable community memberships.
359 Stability was achieved surprisingly rapidly (3 – 5 weeks) and the resulting consortia remained
360 stable over several months. Importantly, the development of stable, reduced complexity,
361 naturally interacting consortia from native soil can provide representative model soil
362 communities for future studies to study the mechanisms underlying species interactions. This
363 valuable resource should enable deciphering of the molecular signaling mechanisms and
364 metabolic interactions used by soil community members to decompose complex carbon
365 substrates in soil. In addition, the information can be used to enhance *in silico* models of soil
366 microbial community interactions that can be used to predict how key taxa and traits can be
367 perturbed by environmental change.

368

369 **Conclusions.** Here we demonstrated that the succession of microbial communities derived from
370 chitin/NAG enriched soil microbiome is strongly influenced by the initial soil microbiome
371 richness and the hydro-physical environment. The initial species richness, which is a proxy for
372 the complexity of a microbiome, at least partially controlled the tendency for a soil-sourced
373 consortium to stabilize and maintain a relatively constant community structure over time.
374 Additionally, the long-term soil enrichments resulted in a reduced complexity representation of
375 the initial soil microbiome diversity and richness. The results of this study inform how soil
376 microbial communities are shaped during succession and how a combination of the initial

taxonomic structure and physical environment influences the tendency for a community to stabilize over time.

MATERIALS AND METHODS

Field sampling and chitin enrichment. Soil was collected in October 2017 from a field site operated by Washington State University, located in Prosser, Washington State USA (46°15'04"N and 119°43' 43"W) site. The soil represents a Warden silt loam that is characterized as a coarse-silty, mixed, superactive and mesic Xeric Haplocambid. The soil represents a marginal soil with low organic matter content (3.7%) and pH = 8. All soil samples were collected in three field replicates. At each site, bulk sampling was accomplished with a shovel within 0 to 20 cm depth from the ground and it was stored in plastic bags at 4^{o}C. To exclude bigger soil aggregates and rocks, samples were sieved with 4 mm mesh size. For each of the three field blocks, three homogeneous replicates of 150 g soil were weighed out in to a 250 ml sterile screwcap bottles. To enrich chitin degrading members of the microbial community, samples were incubated for six weeks in soil augmented with chitin **(***Poly-(1→4)-β-N-acetyl-D-glucosamine*, Sigma-Aldrich, St. Louis, MO, USA**)** at different concentrations (0, 10, 50,100 µg chitin/g soil dry weight). Chitin was mixed and evenly distributed within the soil, and sterile water was added to reflect a 24% field water capacity. Samples were kept in the dark at 20^{o}C. Additionally, 1 g of sample was harvested weekly from each bottle and stored in -80^{o}C for 16S and ITS amplicon analysis.

Gamma irradiated soil. Prosser soil was sterilized with gamma irradiation at 85 kGy additional in two successive applications of or 25 kGy, followed by 60 kGy. Initially, 3000 curie Co-60

source was used in the collimated open beam irradiator. For the second irradiation, 1300 curie Co-60 source was used in the Gamma Bunker, which is a 1.5 $ft.^3$ closed chamber irradiator (46, 47). Sterility of soil was confirmed by plating of several serial dilutions on LB agar plates followed by incubation at 30 ^oC and the lack of growth.

Sterile soil incubations and liquid controls. M9 minimal media and sterile liquid soil extract were prepared as described by Sambrook and Russell (2001) (48) and method of soil analysis-microbiological and biochemical properties (49), respectively. *N-acetyl glucosamine* (NAG) (Sigma-Aldrich, St. Louis, MO, USA) was added into the M9 media to 100 µg/ml. Ten milliliter liquid cultures were setup in 25 ml sterile glass tubes in four successive 10-fold serial dilutions. First, 1 g of actively respiring chitinolytic enriched soil (100 µg chitin/g soil dry weight) was inoculated into the first glass tubes with 9 mL of the M9 media (representing the 10^{-1} dilution) and vortexed for 30 seconds. This solution then was used for the subsequent serial dilutions. Uninoculated controls were also generated and incubated with the dilution samples. Each serial dilution and respective controls were performed in 8 biological replicates. Incubation was performed in the dark at 20 ^oC, shaking at 130 RPM. CO_2 respiration was measured aseptically three times a week. Headspace was aseptically flushed with air after each sample to prevent anaerobic conditions. Additionally, 1 ml of sample was harvested weekly for the first three weeks, followed by biweekly sampling and stored in -80 ^oC for 16S and ITS amplicon analysis. After each sampling period, substrate and moisture levels were refreshed by adding 1 ml of M9 medium.

The soil enrichments were set up using 5.5 g gamma- irradiated soil in 15ml sterile tubes in parallel with their liquid counterparts. The “sterile soil” treatments were prepared by 1 ml soil

423 extract liquid enriched with 100 ppm NAG to each tube containing sterile soil. The soil samples
424 were briefly mixed with a sterile spatula and pre-incubated in the dark at 20^{o}C for two days.
425 After pre-incubation, 0.5 ml inoculum was taken from the liquid serial dilution described above
426 and added to the counterpart sterile soil tubes. The soil enrichments were sealed with filter screw
427 caps (CellTreat, non-pyrogenic and sterilized by gamma irradiation, China) to allow continuous
428 air flow. Each 0.3 g sample was harvested weekly for the first three weeks, followed by biweekly
429 sampling and stored at -80 ^{o}C for downstream molecular measurements.
430
431 **Amplicon sequencing.** Total DNA was extracted using the MoBio PowerSoil DNA isolation kit
432 (Qiagen, Carlsbad, CA) in accordance with the Earth Microbiome Project (EMP) protocols (50).
433 Sequencing was performed on an Illumina MiSeq instrument (Illumina, San Diego, CA).
434 Triplicate, separate 16S and ITS rRNA gene amplification reactions were performed on DNA
435 from each extraction. The 16S primers targeted the V4 hypervariable region of the 16S SSU
436 rRNA gene using the V4 forward primer (515F) and V4 reverse primer (806R) with 0–3 random
437 bases and the Illumina sequencing primer binding site (51). The ITS primers targeted the ITS1
438 region using the ITS1f and ITS2 primers (52).
439
440 **Amplicon analysis.** The Hundo (2017) amplicon processing protocol was used to process 16S
441 and ITS amplicons (53). In brief, sequences were trimmed and filtered of adapters and
442 contaminants using BBDuk2 of the BBTools (´Tools´) package. VSEARCH (54) was used to
443 merge, filter to an expected error rate of 1, dereplicate, and remove singletons before
444 preclustering reads for *de novo* and reference-based chimera checking. Reads were clustered into
445 OTUs at 97% similarity and an OTU table in the BIOM format (55) was constructed by mapping

446 filtered reads back to these clusters. BLAST+ (56) is used to align OTU sequences to the
447 database curated by CREST (57) (SILVA v128 for 16S, UNITE v7 for ITS) and taxonomy was
448 assigned based on the CREST LCA method. Multiple sequence alignment was performed with
449 Clustal Omega (58) and a phylogenetic tree was constructed using FastTree2 (59).
450
451 **Diversity analysis.** Downstream analysis was completed in R (60), using the phyloseq (61) and
452 vegan packages (62). To preserve the maximum consistency within each replicate, samples were
453 rarified to an even depth of 2,000 reads per sample. The observed counts of unique OTUs
454 (species-richness) and Simpson's Evenness were used to characterize alpha diversity (63). In
455 order to assess microbial stability/volatility over time, we implemented the volatility analysis as
456 previously described (36), in which the amount the community change between successive time
457 points was measured with weighted UniFrac distances.
458
459 **Data repository and reproducible analyses.** Genetic sequencing data is available on the Open
460 Science Framework (osf.io) for both 16S and 1TS amplicons as part of this project:
461 https://osf.io/6d5kz/, along with the Rmarkdown processing scripts used to process the data and
462 build annotated feature abundance tables and phylogenic tree using Hundo.
463
464 **ACKNOWLEDGMENT**
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469

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644

645

646 **FIGURE LEGENDS**

647 **Figure 1**: The successional dynamics of microbial consortia. Differences in microbial
648 community structure and alpha diversity are plotted with respect to native soil communities
649 (labelled as 'n'), the 6-week chitin enriched communities (labelled as 'c'), uninoculated control
650 (labelled as 'control') and different serial dilutions of NAG enrichment in liquid and soil
651 treatments for 0-15 weeks. The results are partitioned by initial dilution and incubating
652 conditions (NAG-enrichment in liquid on the top and soil in the bottom). The most abundant
653 bacterial (A) and fungal (B) phyla are shown over the 15-week incubation. The alpha diversity of
654 bacteria (C) and fungi (D) are estimated using species richness and Simpson's evenness.

655

656 **Figure 2**: The influence of physical environment on taxonomic volatility – i.e., the tendency for
657 the community to stabilize with respect to taxa being gained/lost over time. Each graph shows
658 the weighted UniFrac distance for bacteria (A) and fungi (B) calculated between subsequent
659 incubation times and plotted by weeks of incubation, dilution factor and treatment conditions.

660

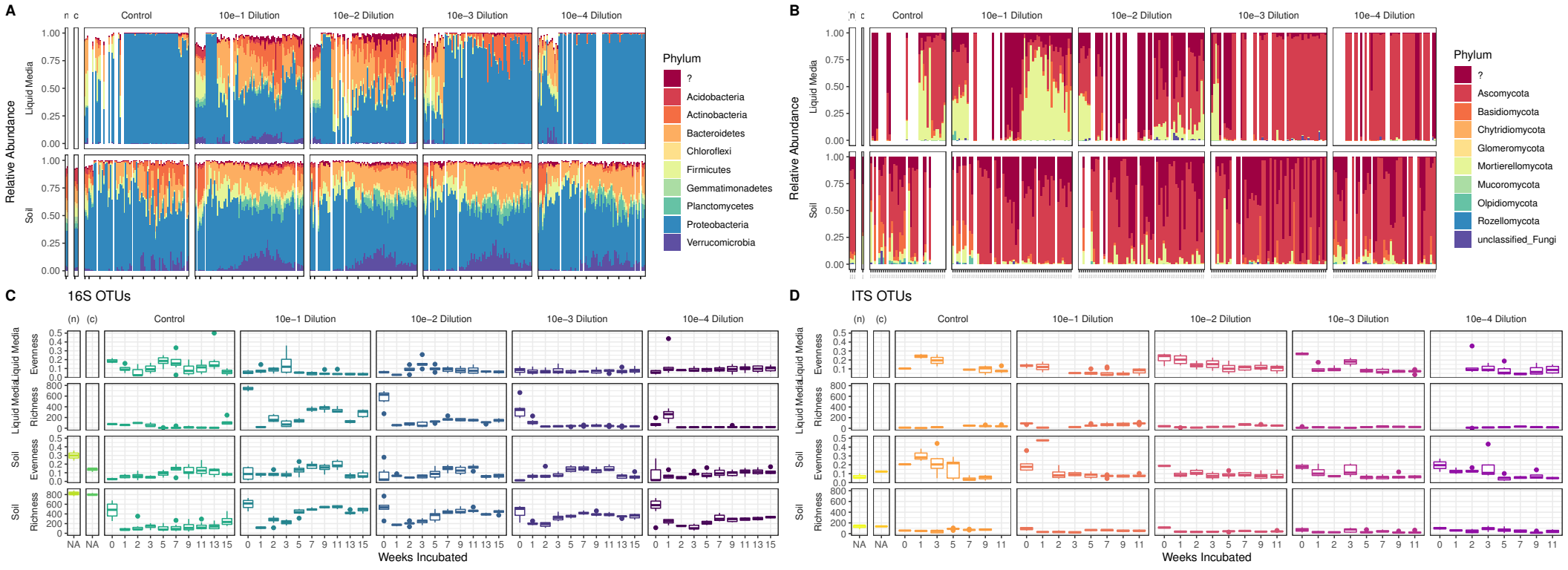
661 **Figure 3**: The biological and physical variables correlated with beta diversity. Ordinations
662 present canonical analysis of principal coordinates (CAP) of weighted UniFrac distances
663 between samples. The percent of variation captured by the vectors is shown on each axis. Each
664 vector has a magnitude (length) and direction of a variable's contributions to the principle
665 components. Vectors represent: respiration CO2/h (orange), volatility (red), and the most
666 abundant phyla (purple).

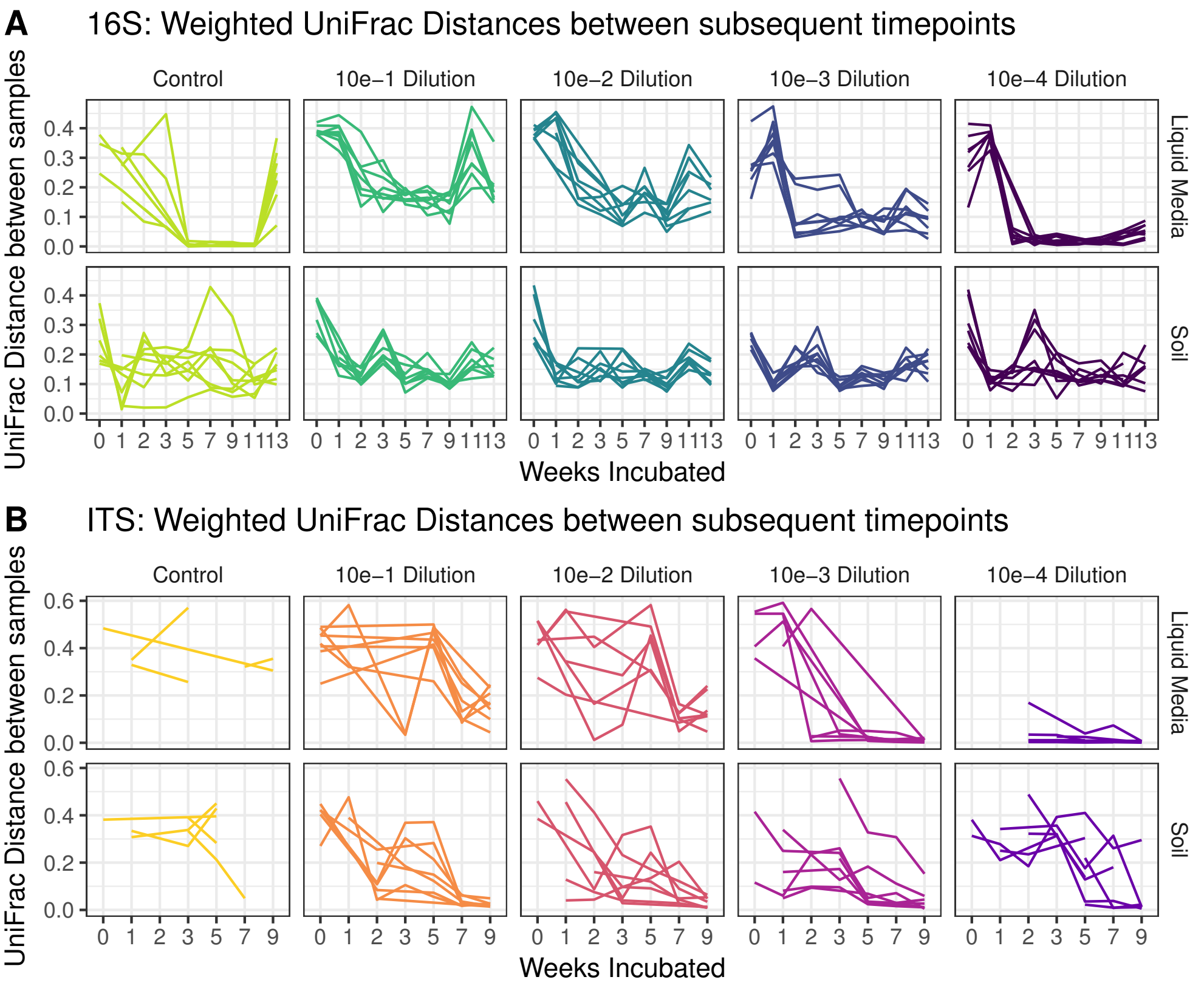
667

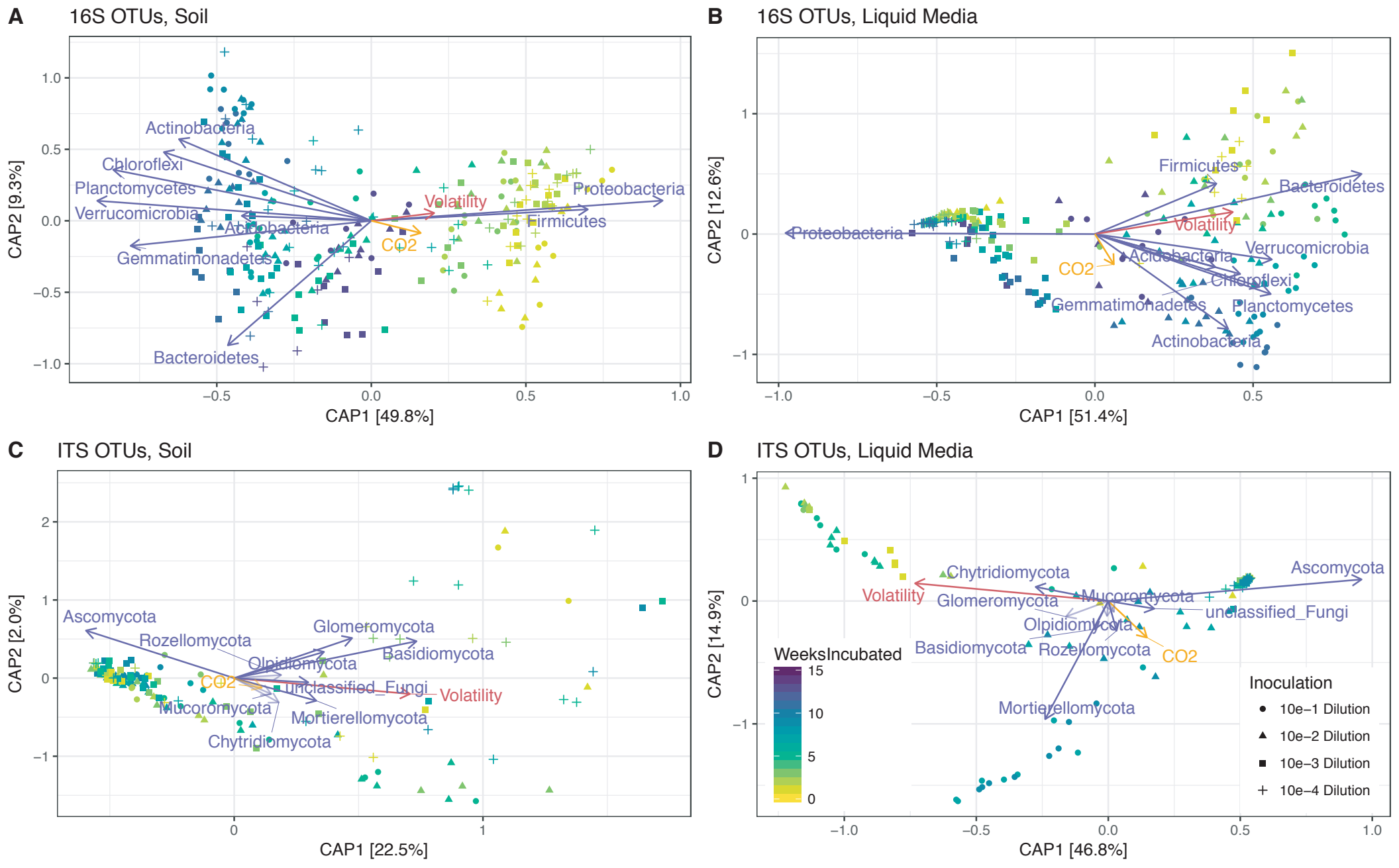
668

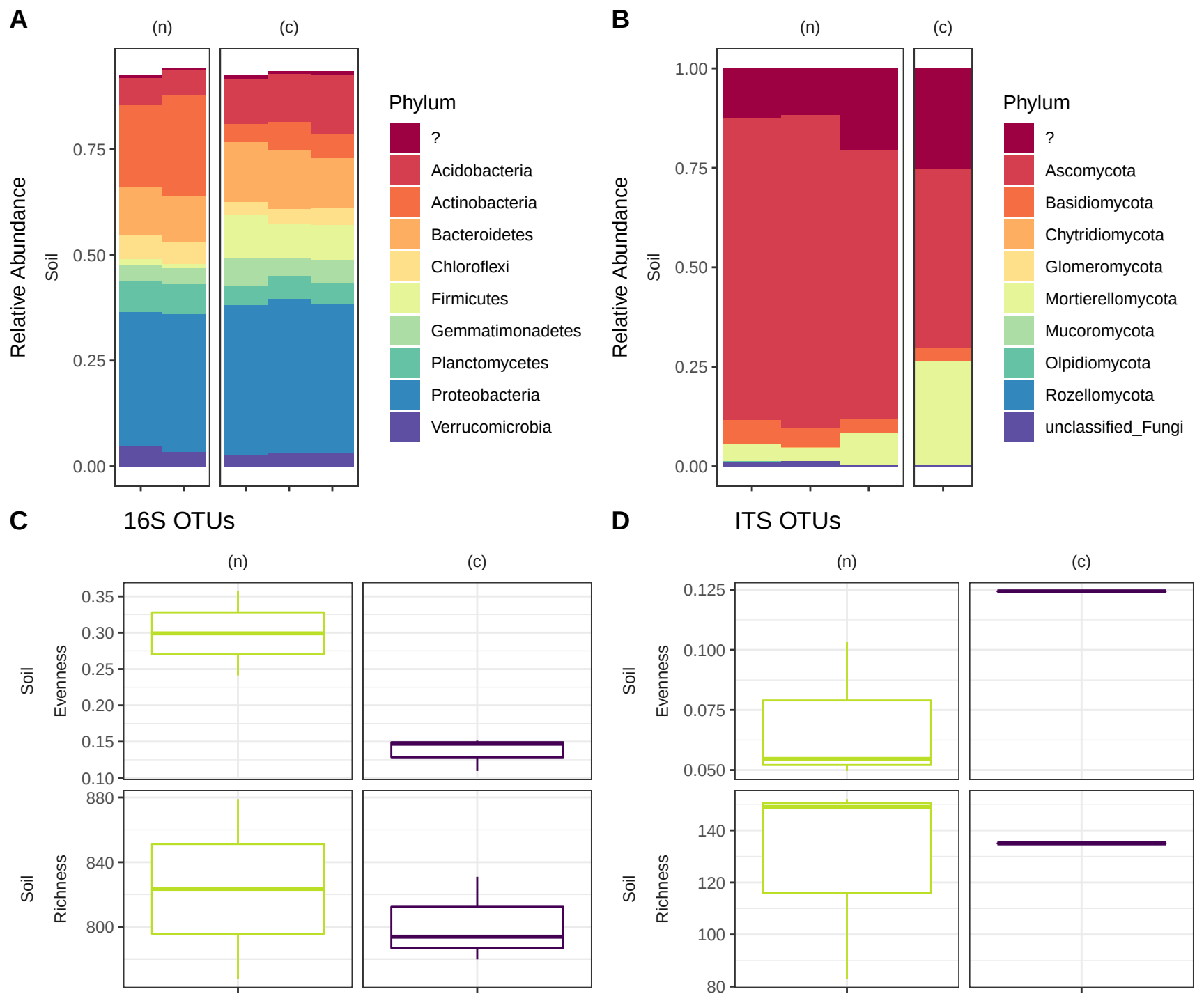
669 **Supplemental Figure S1**: The most abundant bacterial (A) and fungal (B) phyla are plotted for
670 the native soil (labelled as 'n') and the 6-week chitin enriched soil (labelled as 'c'). The alpha
671 diversity of bacteria (C) and fungi (D) are estimated using species richness and Simpson's
672 evenness.
673
674 **Supplemental Figure S2**: Rate of respiration varies over time based on dilution and physical
675 environment. The respiration rate was plotted for both liquid (top) and soil (bottom) NAG
676 enrichment cultures from weeks 2 through 7 to avoid the light spike in CO_2 released immediately
677 after inoculation. Respiration was monitored three times a week with a PP Systems-EGM-4
678 Environmental Gas Monitor (Amesbury, MA). The gas analyzer was setup in static mode to
679 measure CO_2 and monitor respiration in each sample at periodic intervals. To measure CO_2, a 10
680 mL gas aliquot was taken from the incubation container using a sterile plastic syringe which has
681 a control valve.
682
683 **Supplemental Figure S3**: Bacterial OTUs observed from 16S rRNA gene amplicon products in
684 uninoculated controls. The most abundant OTUs shown in the controls were also found in the
685 other samples.
686
687 **Supplemental Figure S4:** Patterns of variation within sample groups and within replicates.
688 A) Beta dispersion (within sample group variation) varies among time points as shown by the
689 pairwise permutation test for homogeneity of multivariate dispersion at each time. Pairwise
690 comparisons of the observed p-values are tabulated for both the 16S and ITS for liquid and soil

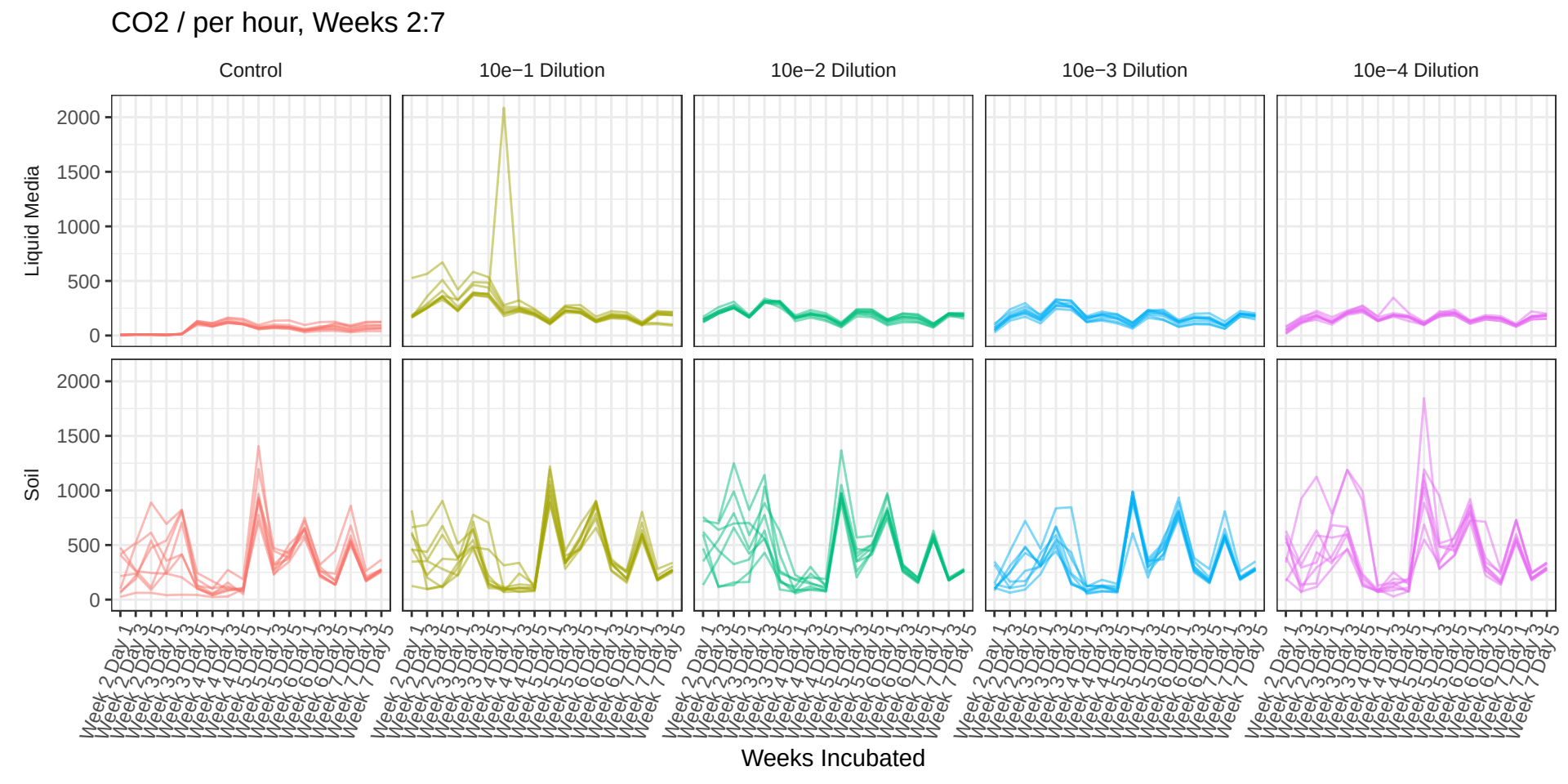
691 treatment conditions. B) A linear regression fit to the pairwise distance between replicates also
692 shows changes in dispersion over time.
693
694 **Supplemental Table S1**: Final richness significantly varies between dilutions. Tukey's HSD test
695 was performed to compare the richness between dilutions, and the p-values are shown here.
696
697 **Supplemental Table S2:** An initial time point species-richness in NAG enriched liquid media
698 across all dilutions and control.
699
700 **Supplemental Table S3**: Community composition of final timepoints can be explained by initial
701 dilution. On each set of endpoints, the adonis test was used to measure how much variation in
702 weighted UniFrac distances between samples could be explained by the initial treatment (R^2) and
703 the probability that this level of similarity between endpoints would be seen by chance alone (p-
704 value).


A
Control
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution
Relative Abundance
Liquid Media
Soil
Phylum
?
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia
B
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Mortierellomycota
Mucoromycota
Olpidiomycota
Rozellomycota
unclassified_Fungi
C
16S OTUs
Evenness
Richness
Weeks Incubated
D
ITS OTUs


A
16S: Weighted UniFrac Distances between subsequent timepoints
Control
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution
Liquid Media
Soil
UniFrac Distance between samples
0.0
0.1
0.2
0.3
0.4
0 1 2 3 5 7 9 11 13
Weeks Incubated
B
ITS: Weighted UniFrac Distances between subsequent timepoints
Control
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution
Liquid Media
Soil
UniFrac Distance between samples
0.0
0.2
0.4
0.6
0 1 2 3 5 7 9
Weeks Incubated


A
16S OTUs, Soil
CAP2 [9.3%]
CAP1 [49.8%]
Actinobacteria
Chloroflexi
Planctomycetes
Verrucomicrobia
Acidobacteria
Gemmatimonadetes
Bacteroidetes
Proteobacteria
Firmicutes
Volatility
CO2
B
16S OTUs, Liquid Media
CAP2 [12.6%]
CAP1 [51.4%]
Firmicutes
Bacteroidetes
Proteobacteria
Volatility
Verrucomicrobia
CO2
Acidobacteria
Chloroflexi
Gemmatimonadetes
Planctomycetes
Actinobacteria
C
ITS OTUs, Soil
CAP2 [2.0%]
CAP1 [22.5%]
Ascomycota
Rozellomycota
Glomeromycota
Olpidiomycota
Basidiomycota
CO2
unclassified_Fungi
Volatility
Mucoromycota
Mortierellomycota
Chytridiomycota
D
ITS OTUs, Liquid Media
CAP2 [14.9%]
CAP1 [46.8%]
Chytridiomycota
Volatility
Glomeromycota
Mucoromycota
Ascomycota
unclassified_Fungi
Olpidiomycota
Basidiomycota
Rozellomycota
CO2
Mortierellomycota
WeeksIncubated
15
10
5
0
Inoculation
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution


A
(n)
(c)
Relative Abundance
Soil
0.00
0.25
0.50
0.75
Phylum
?
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia
B
(n)
(c)
Relative Abundance
Soil
0.00
0.25
0.50
0.75
1.00
Phylum
?
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Mortierellomycota
Mucoromycota
Olpidiomycota
Rozellomycota
unclassified_Fungi
C
16S OTUs
(n)
(c)
Soil
Evenness
0.10
0.15
0.20
0.25
0.30
0.35
Soil
Richness
800
840
880
D
ITS OTUs
(n)
(c)
Soil
Evenness
0.050
0.075
0.100
0.125
Soil
Richness
80
100
120
140


CO2 / per hour, Weeks 2:7
Control
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution
Liquid Media
Soil
2000
1500
1000
500
0
Weeks Incubated

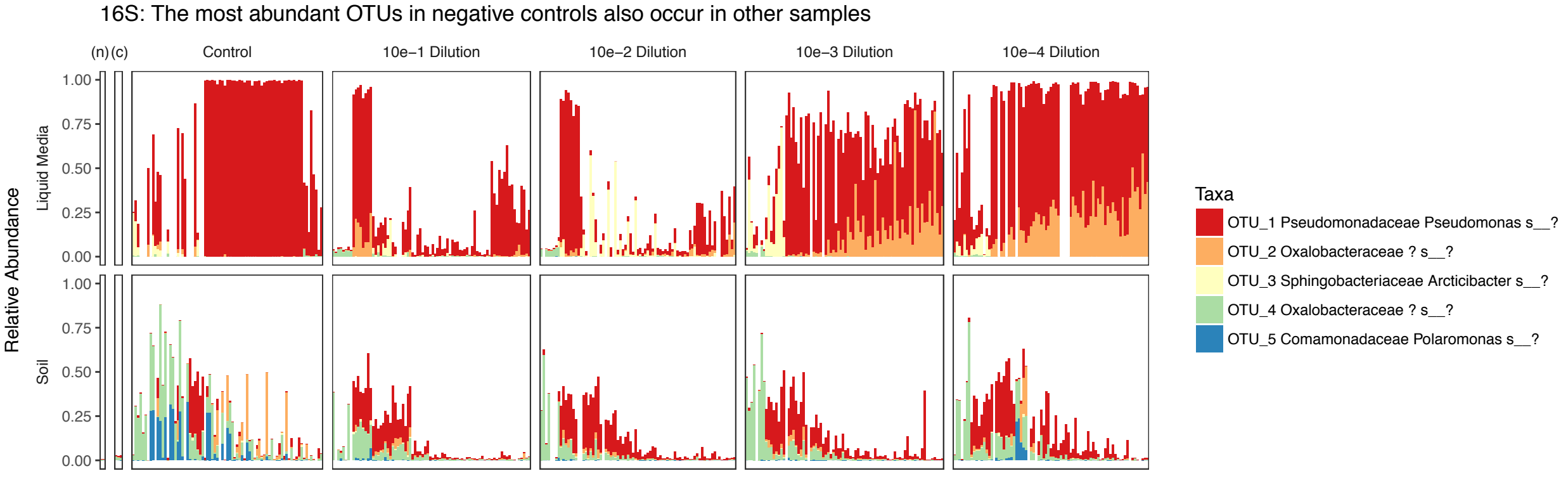

16S: The most abundant OTUs in negative controls also occur in other samples
(n)
(c)
Control
10e−1 Dilution
10e−2 Dilution
10e−3 Dilution
10e−4 Dilution
Relative Abundance
Liquid Media
Soil
1.00
0.75
0.50
0.25
0.00
Taxa
OTU_1 Pseudomonadaceae Pseudomonas s__?
OTU_2 Oxalobacteraceae ? s__?
OTU_3 Sphingobacteriaceae Arcticibacter s__?
OTU_4 Oxalobacteraceae ? s__?
OTU_5 Comamonadaceae Polaromonas s__?

Table S1. Final species richness significantly varies between dilutions. A Tukey's HSD test was performed to compare richness between dilutions, and the p-values are shown here.

Type	Treatment	10e-2 Dilution - 10e-1 Dilution	10e-3 Dilution - 10e-1 Dilution	10e-3 Dilution - 10e-2 Dilution	10e-4 Dilution - 10e-1 Dilution	10e-4 Dilution - 10e-2 Dilution	10e-4 Dilution - 10e-3 Dilution
16S	Soil	0.002598	0	0	0	0	0.1138814
16S	Liquid	0	0	0	0	0	0.0000024
ITS	Soil	0.0007323	0	0.0000002	0.0009251	0.9998387	0.0000002
ITS	Liquid	0	0	0	0	0	0.0014198

Table S2. Community composition of final timepoints is related to the initial condition with respect to dilution. On each set of endpoints, the adonis test was used to measure how much variation in weighted UniFrac distances between samples could be explained by the initial treatment (R2) and the probability that this level of similarity between endpoints would be seen by chance alone (p value).

Type	Treatment	R2	p.value
16S	Inoculated Soil	0.3109907	0.001
16S	Inoculated Liquid	0.7656066	0.001
ITS	Inoculated Soil	0.2955797	0.008
ITS	Inoculated Liquid	0.6843260	0.001

Table S3. Outputs from the tests ofr homogeneity of multivariate dispersions performed with respect to each treatment.

```
> dist.t1 # 16S liquid

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
           Df  Sum Sq   Mean Sq      F N.Perm Pr(>F)
Groups      7 0.10181 0.0145443 1.8706    999  0.076 .
Residuals 230 1.78834 0.0077754
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Pairwise comparisons:
(Observed p-value below diagonal, permuted p-value above diagonal)
           1         2         3         5         7         9        11    13
1            0.8570000 0.9010000 0.5530000 0.8400000 0.8880000 0.8960000 0.030
2  0.8408961           0.7740000 0.6240000 0.6050000 0.6170000 0.6670000 0.008
3  0.9265345 0.7727334           0.5420000 0.9460000 0.9870000 0.9900000 0.056
5  0.5615476 0.6364887 0.5304231           0.3310000 0.3530000 0.4290000 0.003
7  0.8517255 0.6004488 0.9517810 0.3350217           0.9540000 0.9660000 0.010
9  0.8858396 0.6537449 0.9847483 0.3672397 0.9510812           1.0000000 0.013
11 0.8932968 0.6887733 0.9862912 0.4095452 0.9556976 0.9992465           0.017
13 0.0266473 0.0043442 0.0578710 0.0016131 0.0122193 0.0104359 0.0176215
```

> dist.t2 # ITS liquid

```
Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
           Df Sum Sq  Mean Sq      F N.Perm Pr(>F)
Groups      5 0.3227 0.064534 1.4987    999  0.211
Residuals 111 4.7796 0.043060

Pairwise comparisons:
(Observed p-value below diagonal, permuted p-value above diagonal)
          1         2         3         5         7     9
1           0.5380000 0.6650000 0.8540000 0.0590000 0.046
2 0.5282076           0.8820000 0.3270000 0.6480000 0.559
3 0.6468981 0.8809688           0.4620000 0.4420000 0.379
5 0.8500822 0.3317161 0.4825647           0.0200000 0.011
7 0.0566343 0.6407747 0.4688704 0.0165584           0.794
9 0.0434862 0.5295390 0.3830110 0.0091688 0.7722660
```

> dist.t3 # 16S soil

```
Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
           Df  Sum Sq   Mean Sq      F N.Perm Pr(>F)
Groups      7 0.05119 0.0073129 4.7308    999  0.001 ***
Residuals 236 0.36481 0.0015458
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Pairwise comparisons:
(Observed p-value below diagonal, permuted p-value above diagonal)
            1          2          3          5          7          9         11    13
1             8.7700e-01 5.0000e-02 1.0000e-03 6.0000e-02 1.0000e-02 1.0000e-03 0.007
2  8.7035e-01            6.3000e-02 1.0000e-03 8.9000e-02 1.9000e-02 1.0000e-03 0.024
3  3.8715e-02 7.4086e-02            4.0000e-02 7.1400e-01 8.4500e-01 1.9100e-01 0.564
5  5.3112e-06 4.0466e-05 3.7731e-02            6.0000e-03 3.2000e-02 3.2300e-01 0.161
7  5.2127e-02 1.0219e-01 7.0321e-01 6.1211e-03            4.6700e-01 4.6000e-02 0.291
9  6.8351e-03 1.9589e-02 8.2379e-01 3.1001e-02 4.8615e-01            2.0600e-01 0.655
11 7.7709e-05 4.6176e-04 1.8310e-01 3.2948e-01 5.1248e-02 1.9689e-01            0.521
13 9.5279e-03 2.1375e-02 5.6273e-01 1.6736e-01 3.1211e-01 6.5370e-01 5.4121e-01
```

> dist.t4 # ITS soil

```
Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
Groups      5 0.25738 0.051476 2.9098    999  0.015 *
Residuals 139 2.45898 0.017691
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Pairwise comparisons:
(Observed p-value below diagonal, permuted p-value above diagonal)

	1	2	3	5	7	9
1		0.01700000	0.23900000	0.85600000	0.05900000	0.125
2	0.01559845		0.02700000	0.00100000	0.53400000	0.634
3	0.26680358	0.02495234		0.14400000	0.15800000	0.267
5	0.84991942	0.00083406	0.15323977		0.01100000	0.029
7	0.05433724	0.54623469	0.16086438	0.01024285		0.981
9	0.10751451	0.60413746	0.26867577	0.03429609	0.98502734	