Microbiome Aggregated Traits and Assembly are More Sensitive to Soil Management than Diversity

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Abstract. How soil is managed, particularly for agriculture, exerts stresses upon soil microbiomes resulting in altered community structures and functional states. Understanding how soil microbiomes respond to combined stresses is important for predicting system performance under different land use scenarios, aids in identification of the most environmentally benign managements and provides insight into how system function can be recovered in degraded soils. We use a long-established field experiment to study the effects of combined chronic disturbance of the magnitude of organic carbon inputs with acute effects of physical disturbance by tillage. We show that because of the variety of ways it can be assessed, biodiversity – here based on microbial small subunit ribosomal RNA genes – does not provide a consistent view of community change. In contrast, aggregated traits associated with soil microbiomes indicate general loss of function, measured as a reduction of average genome lengths, associated with chronic reduction of organic inputs in arable or bare fallow soils and altered growth strategies associated with ribosomal RNA operon copy number in prokaryotes, as well as a switch to pathogenicity in fungal communities. In addition, pulse disturbance by soil tillage is

associated with an increased influence of stochastic processes upon prokaryote community assembly, but fungicide used in arable soils results in niche assembly of fungal communities compared to untilled grassland. Overall, bacteria, archaea and fungi do not share a common response to land management change and estimates of biodiversity do not capture important facets of community adaptation to stresses adequately.

Importance.

Changes in soil microbiome diversity and function brought about by land management are predicted to influence a range of environmental services provided by soil, including provision of food and clean water. However, opportunities to compare the long-term effects of combinations of stresses imposed by different management approaches are limited. We exploit a globally unique fifty-year field experiment, demonstrating that soil management practises alter microbiome diversity, community traits and assembly. Grassland soil microbiomes are dominated by fewer - but phylogenetically more diverse - prokaryote phylotypes which sustain larger genomes than microbiomes in arable or bare fallow soil maintained free of plants. Dominant fungi in grassland soils are less phylogenetically diverse than those in arable or fallow soils. Soil tillage increases stochastic processes in microbiome assembly: this, combined with reduced plant biomass, presents opportunities for organisms with a capacity for pathogenesis to become established in stressed soils.

Introduction.

One consequence of the biodiversity of microorganisms in soils (1,2) is that historically, responses of below-ground communities to environmental or land use change were thought to be largely inconsequential to ecosystem processes (3). This stemmed from an assumption that although functional diversity in soils can be high, it is typically exceeded by the number of extant soil microbial species. It is generally assumed from this richness of species that soil biological systems have high levels of functional redundancy. However, soil microbial community composition and function have been shown to be sensitive to land use and climactic change including CO_2 increases, inorganic fertilization, temperature changes and carbon amendments (4). Recovery of community function to pre-disturbance states is typically limited, particularly by long-term (chronic) disturbances (5). Understanding the effects of land management upon soil microbial diversity is important because soil microbes are responsible for the provision of a significant number

of environmental services (6,7). While the previous two decades have seen an increase in our understanding of the effects of individual physical or chemical disturbance upon microbial populations in soil, there is still limited information relating to the more realistic combined effects of physical and chemical or chronic and acute disturbances (5). Arguably, the greatest disturbances to soil and associated microbial communities result from agricultural practices. Agricultural management is associated with losses of soil organic carbon (8); harvesting limits the input of plant material, typically to just roots and stubble in arable systems, and tillage accelerates microbial decomposition of soil organic matter. Associated mechanical activity also induces soil compaction. Comparison of soils from permanently untilled grassland and arable field experiments (9) indicate that grassland soils show greater physical stability (to compression and wet/dry cycles) and biological functional stability (to temperature and metal toxicity). The loss of stability in arable soils is largely related to management effects on soil organic carbon (9).

Identifying any effects of disturbance arising from agricultural practice upon the phylogenetic assemblage and diversity of soil microbial communities is not trivial. Carbon turnover in soil typically occurs over decennial temporal scales (10). Studies of the effects of persistent soil management must account for such long temporal scales if they are to assess maximal changes in communities (5). This limits the practicality of laboratory-based experiments, but controlled field manipulations lasting many decades provide opportunities to investigate community responses to the combination of disturbances brought about by altered land management (11). One example of such field-scale manipulation is the Rothamsted Highfield Ley-Arable experiment, set on soil that has been under permanent grass since at least 1838. The experiment compares original grassland with continuous arable management (established in 1948) as well as bare fallowed soil, kept free of vegetation and other organic inputs (established in 1959) in the same soil and exposed to identical climatic conditions. Over this period, bare fallowed soils have become depleted in labile organic carbon and enriched in persistent organic carbon (12), and total organic carbon has been reduced to a greater extent than in arable soil. There has also been observable progressive shifts, from grassland to arable and bare fallow, in the distribution of organic carbon between different pools in the three soil managements, particularly a relative decline in discrete organic particles independent of stable soil aggregates, and a corresponding increase in the proportion of organic particles

encapsulated in stable aggregates (13). Confirmation of this apparent shift in soil structure has been provided by high-resolution X-ray computed tomography (14).

This long-established field experiment presents a unique opportunity to study the combined effects of press disturbance on the magnitude of organic carbon inputs (estimated at approximately 78 Mg ha^{-1} $annum^{-1}$ from perennial grass and forbs to grassland soils, 46 Mg ha^{-1} $annum^{-1}$ derived from annual wheat straw to arable soils, and none in bare fallow soils (10)) with pulse effects of physical disturbance by tillage (once a year in arable soils, three to four times a year in bare fallowed soils and never in grassland soils) upon microbial communities: contemporaneous grassland effectively represents the pre-disturbance state of arable and bare fallow soils, but also accounting for time as a covariate. We generated shotgun metagenome datasets from DNA extracted directly from soils subject to the three land managements. Metagenome reads with homology to prokaryotic or fungal SSU rRNA genes were not clustered but analysed individually using an evolutionary placement algorithm. This approach not only increases the accuracy of taxonomic identification but also considers the complete range of biodiversity represented in sequenced organisms. We used DNA extracted from soils subject to the contrasting regimes to test three hypotheses relating to the structure and phylogenetic diversity of soil prokaryotic and fungal communities: first, that reduced opportunity space (including reduced bioavailability of nutrients) resulting from arable and bare fallow managements will be reflected in reduced diversity of microbial communities compared to communities associated with grassland; secondly, the reduced opportunity space, particularly as is relates to the diversity of organic matter inputs, will also be reflected in reduced average genome lengths observed in prokaryotes associated with arable and bare fallow soils and environment-associated shifts in 16S rRNA gene copy number; and thirdly, that physical disturbance associated with arable and bare fallow managements will result in greater heterogeneity of community assemblages (*i.e.* β-diversity) between individual plots due to the influence of stochastic processes upon community assembly.

Results

Community-Aggregated traits – There was a significant difference in average genome lengths associated with metagenomes from each land management (ANOVA, $F_{2,6} = 36.7$, $p = 0.0004$, $\omega^2 = 0.888$). AGL was 596.3 kb and 1.204 Mb larger in grassland soil than arable or bare fallow soils, respectively (Fig. 1A). Significant differences between land

managements were also observed for 16S rRNA gene copy number (ANOVA, $F_{2,6} = 10.9$, $p = 0.0100$, $\omega^2 = 0.688$). ACN was significantly greater in bare fallow soil than either arable or grassland soils (Fig. 1B).

SSU rRNA gene phylogenetic placement – Bacteria in soils associated with the three land managements were dominated by Acidobacteria including *Luteitalea pratensis* (Vicinamibacteraceae, Acidobacteria subdivision 6), *Candidatus* Solibacter usitatus (Solibacteraceae, Acidobacteria subdivision 3), *Chloracidobacterium thermophilum* (Chloracidobacterium, Acidobacteria subdivision 4), the Gemmatimonadete *Gemmatirosa kalamazoonesis* and the Verrucomicrobium *Ca*. Xiphinematobacter sp. (Supplementary Fig. 1). A second, less numerous cluster of phylogenetic placements was associated with organisms of the Terrabacteria group, including *Fimbriimonas ginsengisoli* (Armatimonadetes) and *Thermobaculum terrenum* (unclassified Terrabacteria group) among others. The most abundant Proteobacteria were *Rhodoplanes* sp. Z2-YC6860 (Rhizobiales) and *Sphingomonas ginsengisoli* (Sphingomonadales), both α-Proteobacteria and the unclassified β-Proteobacterium GR16-43. Archaea were dominated by *Ca*. Korarchaeum cryptofilum and the closely related *Ca*. Prometheoarchaeum syntrophicum which outnumbered other placements (Supplementary Fig. 2). Other abundant organisms included *Ca*. Mancarchaeum acidiphilum, the Thermoprotei Crenarchaeotes *Caldivirga maquilingensis*, *Pyrobaculum arsenaticum* and *Sulfolobus acidocaldarius* and the Euryarchaeotes *Methanobrevibacter ruminantium* (Methanobacteriales), *Methanopyrus kandleri* (Methanopyrales) and *Methanococcus vannielii* (Methanococcales). There were fewer dominant taxa for fungi than for bacteria or archaea (Supplementary Fig. 3). The most abundant fungus in all soils was *Conidiobolus obscurus*, a member of the Zoopagomycota. Other abundant fungi included *Brunneoclavispora bambusae* (Dothideomycetes), *Gongronella orasabula* (Mucoromycetes), *Cornuvesica acuminate* (Sordariomycetes) and *Yarrowia osloensis* (Saccharomycetes).

Abundance-sensitive measures of SSU rRNA sequence diversity – Estimates of sample coverage (*C*) for each gene were not significantly different across the land managements (Supplementary Fig. 4) indicating that direct sample comparison was reasonable. The three marker genes present in the soils were not censused equally. For the bacterial 16S rRNA gene, *C* ranged from 97.0 to 98.5%. This was less than estimates for the archaeal

16S rRNA gene (C = 99.8 – 99.9%), but greater than estimates for the fungal 18S rRNA gene (C = 94.4 – 97.1%).

To test the hypothesis that the reduced niche space of arable and bare fallow soils is reflected in reduced microbial diversity compared to grassland, we examined abundance-sensitive sequence diversity for each marker gene. Individual- (Fig. 2) and sample coverage-based (Supplementary Fig. 5) estimates of sequence richness (${}^0D$) indicated considerable overlap in the estimate 95% confidence intervals and no consistent effect of treatment. This was particularly evident for prokaryotic 16S rRNA genes. There were no significant effects of land management upon ${}^0D$ for any gene (largest $\omega^2 = 0.383$, fungal 18S rRNA gene, ANOVA $F_{2,6} = 3.8$, $p = 0.086$). Differences between land managements were more evident for ${}^1D$ and ${}^2D$ which were kingdom dependent. There was a significant land management effect upon ${}^1D$ associated with the bacterial 16S rRNA gene (ANOVA $F_{2,6} = 9.1$, $p = 0.015$, $\omega^2 = 0.642$). Grassland was associated with significantly lower ${}^1D$ than soils from the other managements (smallest difference, grassland *vs*. arable $Q = 5.1$, $p = 0.025$). There was a more pronounced management effect on ${}^2D$ (ANOVA $F_{2,6} = 48.1$, $p < 0.001$, $\omega^2 = 0.913$), grassland again being associated with significantly lower diversity than the other soils (smallest difference, grassland *vs*. arable $Q = 10.7$, $p < 0.001$) which were equally diverse. Diversity of the archaeal 16S rRNA gene was also influenced significantly by management (ANOVA ${}^1D$ - $F_{2,6} = 8.3$, $p = 0.019$, $\omega^2 = 0.619$; ${}^2D$ - $F_{2,6} = 8.2$, $p = 0.019$, $\omega^2 = 0.615$). For both measures, arable soils were significantly more diverse than bare fallow soils (smallest difference ${}^2D$, $Q = 5.7$, $p = 0.016$) but there was no significant difference between grassland and arable soil diversities. For the fungal 18S rRNA gene, a significant influence of land management was again apparent (ANOVA ${}^1D$ - $F_{2,6} = 7.0$, $p = 0.027$, $\omega^2 = 0.573$; ${}^2D$ - $F_{2,6} = 7.1$, $p = 0.026$, $\omega^2 = 0.575$). For ${}^1D$, grassland was significantly more diverse than either arable or bare fallow soils (smallest difference, grassland *vs*. bare fallow $Q = 4.4$, $p = 0.049$), however in the case of ${}^2D$, only the difference between grassland and arable soils was significant ($Q = 5.0$, $p = 0.028$). The trends indicated that grassland soils were associated with significantly lower diversity of common (${}^1D$) and dominant (${}^2D$) bacterial sequences. This was reversed for fungi, where grassland was associated with the highest ${}^1D$ and ${}^2D$ sequence diversities. There was also considerable variation between grassland replicates. For these genes, diversity in arable and bare fallow soils was similar. Archaeal sequence abundance distributions were markedly different from those observed for bacteria and fungi in the sense that the greatest

sequence diversities were observed in soils managed as arable. Analysis of abundance-sensitive sequence diversity provides insight into sequence abundance distributions associated with soils from the different treatments. No phylogenetic information is considered, even though it is inherent in the sequences upon which the analysis is based.

Phylogeny-sensitive measures of SSU rRNA sequence diversity – As an additional test, we calculated sequence phylogenetic diversity using a one-parameter family of α-diversity measures - $BWPD_\theta$ - based upon phylogenetic placement of metagenome reads on each reference marker gene phylogram. Profiles show the phylogenetic diversity of increasingly more abundant organisms, akin to ${}^qD$ described above: $BWPD_0$ takes no account of sequence abundance, while $BWPD_1$ considers the most abundant sequences. Resulting profiles are shown in Supplementary Fig. 6. They demonstrate a common, highly uneven phylogenetic diversity–abundance distribution but with observable differences between land uses.

These differences are illustrated best by considering the extremes of PD profiles: $BWPD_0$ (Faith's phylogenetic diversity, representing the sum of lengths of phylogram branches spanning all community members), and its abundance-weighted extension ($BWPD_1$) shown in Fig. 3. As with the response of ${}^0D$ above, there was no significant effect of land management upon $BWPD_0$ associated with any biomarker gene, although a clear consistent trend of arable soils being associated with the lowest PD was evident. This observed lack of a treatment effect upon $BWPD_0$ may reflect a remarkable resistance of soil microbiome PD to environmental change. However, alternatively it may reflect a relative lack of statistical power of comparing three replicates *per* land management. Irrespective of this, ω^2 estimates suggested that archaeal $BWPD_1$ was the least sensitive to the different treatments, consistent with observations derived from ${}^qD$ measures of sequence diversity. There was a significant effect of management upon archaeal 16S rRNA gene $BWPD_1$, and ω^2 estimates suggested that archaea were in this case the most sensitive to the imposed managements when both phylogeny and abundance were considered. $BWPD_1$ was significantly lower in arable soil ($6.05 \pm 6.47\text{x}10^{-3}$, mean ± standard error) than in grassland ($6.16 \pm 3.98\text{x}10^{-3}$, $Q = 9.5$, $p = 0.0013$) or bare fallow ($6.15 \pm 15.2\text{x}10^{-3}$, $Q = 9.0$, $p = 0.0018$) soils. There was no significant difference between grassland or bare fallow soil archaeal $BWPD_1$. There was also a significant effect of treatment upon $BWPD_1$ associated with the fungal 18S rRNA gene. In this case, grassland soil was associated with lower

230 $BWPD_1$ (3.61 ± 0.213) than either arable (4.54 ± 0.197) or bare fallow (4.68 ± 0.324) soils.
231 There was, however, no statistically significant difference between bare fallow and
232 grassland soils (Q = 4.2, p = 0.055), showing the extremes of fungal $BWPD_1$.
233
234 *Comparison of SSU rRNA gene sequence assemblages* – Our third hypothesis related to
235 processes controlling community assembly in disturbed soils, predicting that physical
236 disturbance (tillage) associated with arable and bare fallow management would result in
237 greater assemblage heterogeneity than is observed for undisturbed grassland soils. To test
238 this, we generated Kantorovich-Rubinstein (KR) distance metrics, based upon the
239 distribution of homologous reads associated with each land management on reference
240 phylograms. We calculated the multivariate KR deviation of each replicate community
241 from each land management centroid in Euclidean space (phylogenetic dispersion). The
242 rationale was that where a combination of disturbances resulted in strong environmental
243 filtering, phylogenetic dispersion would be lower than that for grassland soil. Where
244 community assembly in disturbed soil was subject to a strong influence of stochastic
245 processes, phylogenetic dispersion would be greater than in grassland soil. The observed
246 relationships between the communities in each soil are shown in Fig. 4. In bare fallow
247 soils there is greater phylogenetic dispersion than is observed in grassland soils, although
248 there is overlap of 95% confidence intervals around the means. This provides evidence of
249 an increased influence of stochastic processes in bacterial community assembly in bare
250 fallow soils than grassland soils. Bacterial community phylogenetic dispersion in arable
251 soils is indistinguishable from grassland soil communities. The trend of increased
252 community phylogenetic dispersion in disturbed soils is more evident for archaea, where
253 phylogenetic dispersion is greater within arable and bare fallow soil communities. In this
254 instance, the 95% confidence intervals suggest significantly greater dispersion between
255 communities in bare fallow than grassland soils. The response of fungal soil communities
256 to disturbance is not consistent with an increased influence of stochasticity observed for
257 prokaryotes. There was significantly less phylogenetic dispersion between fungal
258 communities in arable compared to communities in grassland soils. This suggests
259 increased environmental filtering during community assembly. Environmental filtering
260 was not observed for fungal communities in bare fallow soils which were associated with
261 similar phylogenetic dispersion as grassland soil communities.

A significant effect of land management upon sequence assemblages of bacterial 16S rRNA (PERMANOVA, *pseudo-*$F_{2,6}$ = 16.3, p_{perm} = 0.0034), archaeal 16S rRNA (PERMANOVA, *pseudo-*$F_{2,6}$ = 8.0, p_{perm} = 0.0036) and fungal 18S rRNA (PERMANOVA, *pseudo-*$F_{2,6}$ = 3.0, p_{perm} = 0.0105) genes was detected. *Post hoc* pair-wise comparisons indicated that prokaryote assemblages were significantly different between all land managements; in both cases the smallest *pseudo-t* was associated with the arable *vs.* bare fallow comparison (bacteria, *pseudo-t* = 3.0, p_{MC} = 0.0084; archaea *pseudo-t* = 0.1, p_{MC} = 0.0301). Land management differences were more limited for the fungal 18S rRNA gene. In this case, only the comparison of assemblages in arable and grassland soils indicated a significant difference (*pseudo-t* = 2.2, p_{MC} = 0.0291). Associated CAP analyses are shown in Supplementary Fig. 7.

To identify taxa responsible for the observed distinctiveness between land managements, we used edge-PCA to identify phylogram branches across which there was a high level of between-sample heterogeneity. Ordination of bacterial and archaeal 16S rRNA gene assemblages separated the land managements clearly in two dimensions (Fig. 5 and 6). On edge-PCA axis 1, bacteria such as *Ca.* Xiphinematobacter, *Rhodoplanes* sp. and the δ-Proteobacterium *Sorangium cellulosum* and the Crenarchaeotes *Sulfolobus* sp. and *Metallosphaera* sp. were more associated with grassland soils. The Actinobacteria *Mycolicibacterium* sp. and bacterium IMCC26256, the Chloroflexia *Roseiflexus* sp., the α-Proteobacteria *Azospirillum* sp. and *Sphingomonas* sp., the β-Proteobacteria *Massilia* sp. and *Methyloversatilis* sp., the δ-Proteobacterium *Polyangium brachysporum* and the Gemmatimonadetes *Gemmatirosa kalamazoonensis* the Crenarchaeote *Sulfurisphaera tokodaii*, the Euryarchaeotes *Pyrococcus* sp., *Methanothrix soehngenii* and *Methanocaldococcus* sp. and the Thaumarchaeote *Ca*. Nitrosotenuis were all associated more with bare fallow soil. On the second axis, *Roseiflexus* sp., *Rhodoplanes* sp., *Sphingomonas* sp., the Planctomycete *Gemmata obscuriglobus* and the Actinobacterium *Streptomyces* sp., *Methanocaldococcus* sp. and other Methanomada group Euryarchaeotes including *Methanococcus paludis*, *Methanobrevibacter* spp. and *Methanobacterium* sp., the Halobacteria Euryarchaeaotes *Natronococcus occultus* and *Natronomonas* sp., and the Nitrososphaerales Thaumarchaeotes *Ca*. Nitrosocosmicus and *Nitrososphaera viennensis* were all more associated with arable soil.

Edge-PCA ordination of fungal 18S rRNA gene assemblages revealed a distinctly different treatment distribution than observed for 16S rRNA genes (Fig. 7). Treatment

differences were distributed only across the first axis, separating grassland assemblages from arable and bare fallow assemblages. Taxa most associated with grassland were the Agaricomycetes (Basidiomycota) *Amanita pruitii* and *Clitopilus brunnescens* and the Eurotiomycetes (Ascomycota) *Aspergillus cremeus, Cladophialophora* sp. and *Auxarthron* sp. Arable and bare fallow soils were most associated with the Saccharomycete (Ascomycota) *Yarrowia lipolytica*, the Agaricomycete *Cantharellus cascadensis*, the Kickxellomycete (Zoopagomycota) *Coemansia biformis*, the Sordariomycetes (Ascomycota) *Ophiocordyceps tiputini*, *Cornuvesica crypta*, *Sporidesmium olivaceoconidium, Peroneutypa mackenziei* and *Irenopsis crotonicola*, and the Dothideomycete (Ascomycota) *Acidomyces acidophilum*. Ecological guilds associated with these taxa (Table I) suggest grassland soil was associated more with ectomycorrhizal and saprotrophic fungi, whereas taxa more associated with arable and bare fallow soils were microfungal in growth habit, and had the capacity to pathotrophy, associating with animals, plants and lichens.

Discussion

The Highfield Ley-Arable experiment soils studied here have experienced consistent management for sufficiently long periods of time for the complete extent of microbial community response to become apparent. While grassland soils effectively represent the original soil community traits, structures and phylogeny, soils managed as arable or bare fallow continue to experience combinations of press (different levels of plant inputs) and pulse (different levels of tillage, addition of wheat seed-associated fungicide-insecticide) disturbance. Despite these long-term combinations of disturbance, the prokaryotic and fungal communities in all soils are dominated by a limited number of abundant organisms, several of which share partner-dependent lifestyles. For example, *Ca*. Xiphinematobacter sp., one of the more abundant bacteria in all metagenomes (Supplementary Fig. 1, consistently one of the twenty most abundant bacterial species), is an obligate mutualist endosymbiont of a group of migratory plant root-ectoparasitic nematodes, *Xiphinema americanum sensu lato* (16). It has been identified in forty-nine of the sixty-one nominal species comprising the *X. americanum s. l.* complex (17). The organism was more abundant in grassland and arable than bare fallow soils (Fig. 5) and this is consistent with 16S rRNA amplicon sequencing of these microbiomes which identified a Verrucomicrobium as being associated with significantly different abundance between the three soils (18). Of the dominant archaeal species, two are dependent upon associations with other organisms.

328 *Ca*. Prometheoarchaeum syntrophicum MK-D1 is a slow growing organism that degrades
329 amino acids syntrophically with other archaea - *Halodesulfovibrio* and *Methanogenium* in the
330 original co-cultures (19). A second organism, *Ca*. Mancarchaeum acidiphilum MIA14,
331 lacks any genes of the central carbohydrate metabolic pathways, but degrades proteins and
332 amino acids as part of obligate mutualistic partnerships with Thermoplasmatales archaea
333 (20). The most abundant fungus in all soils was the entomopathogen *Conidiobolus obscurus,*
334 which produce conidia that infect aphids (21,22). Another abundant microfungus,
335 *Cornuvesica acuminata*, requires metabolites (possibly siderophores) from other fungi for
336 growth (23).
337 Compared to grasslands composed of mixed forb and grass plant species, arable
338 and bare fallow soils provide severely limited breadths of niche space for microbes: limited
339 diversity of plant species and reduced ranges of organic inputs. Our first hypothesis
340 predicted that reduced opportunity space in arable and bare fallow soils would be
341 associated with changes to prokaryotic community-aggregated traits: average genome
342 length and 16S rRNA gene copy number. The effect of land management upon these
343 CATs was marked. Prokaryotic microbiomes of arable and bare fallow soils were
344 associated with significantly shorter AGL than grassland microbiomes (Fig. 1). Assuming
345 an average prokaryote gene length of 0.924 kb (24), the 596.3 kb and 1.204 Mb reductions
346 of arable and bare fallow AGL represent losses of approximately 645 and 1,300 genes *per*
347 genome compared to prokaryotes in grassland soil. This suggests strong genome
348 streamlining (25) driven by a pervasive bias towards greater numbers of nucleotide
349 deletions than insertions in the absence of strong selective pressures to maintain genes (26).
350 In the absence of the wide variety of organic inputs in grassland soils, a great number of
351 genes are lost and the less diverse the inputs the greater number of lost genes. However,
352 the 16S rRNA gene copy number suggests that microbiome responses to inputs is altered
353 between soils. Gene copy number was significantly greater in bare fallow soil than either
354 arable or grassland soils suggesting a shift in ecological strategy. Bacteria with greater
355 numbers of rRNA operons show more rapid responses to substrate inputs (27). Together,
356 these CATs suggest that microbiomes in arable and bare fallow soils have lost a significant
357 number of genes (and associated functions) but maintain a greater number of rRNA
358 operons enabling a more rapid response to organic inputs when they occur. Comparing
359 ω^2 between CATs indicates that AGL is more sensitive to stressors than 16S rRNA gene
360 copy number.

Our second hypothesis predicted that reduced niche space would be reflected in lower diversity of prokaryotic and fungal communities typifying each disturbed soil. We generated abundance- and phylogeny-sensitive diversity measures that suggest a nuanced response of biodiversity to land management. Abundance-insensitive measures (${}^0D$ and $BWPD_0$) indicated limited differences in phylotype richness (Fig. 2) or phylogenetic diversity (Fig. 3). There was a consistent effect of land management upon $BWPD_0$ – "feature diversity" (28) – where arable soil was associated with the lowest, and grassland with the highest $BWPD_0$ for each SSU rRNA gene. It is remarkable that $BWPD_0$ associated with arable soil was consistently lower than even that associated with bare fallow soil. This lack of any statistically significant effect of land management upon phylotype richness or $BWPD_0$ could be a result of the low statistical power of the experiment however, richness and PD cannot be estimated in a robust fashion (29) and our results may reflect this. ${}^1D$, ${}^2D$ and $BWPD_1$ are all estimated with greater certainty and these parameters indicate significant land management effects upon diversity. Grassland soils are associated with significantly lower numbers of common and dominant sequence phylotypes suggesting a more uneven community profile. However, $BWPD_1$ (Fig. 3) suggests that this reduced number of dominant phylotypes were associated with greater PD than the dominant phylotypes in arable or bare fallow soils. For the fungal 18S rRNA gene this distribution was reversed: grassland soils were associated with a greater number of common and dominant phylotypes (Fig. 2), but dominant phylotypes were significantly less phylogenetically diverse than dominant phylotypes in disturbed soils (Fig. 3). The greatest number of archaeal 16S rRNA gene phylotypes were observed in arable soils (Fig. 2). These were associated with significantly lower $BWPD_1$ than either grassland or bare fallow soils (Fig. 3). Prokaryotic communities appeared to have a common phylogeny-sensitive response to land management. This assessment provides several salient observations: disturbance in soil systems does not result in consistently reduced measures of diversity; abundance- and phylogeny-sensitive measures of diversity are necessary to generate a complete view of soil microbiome responses to disturbance; and, community responses are kingdom specific.

In addition to these observations regarding diversity, comparison of soils subject to different management suggests that shifts in community structure typically do not involve dominant phylotypes. Few phylotypes associated with large edge-PCA eigenvalues in Figs. 5 – 7 were dominant as indicated in Supplementary Fig. 1. Exceptions to this

observation were the nematode endosymbiont *Ca*. Xiphinematobacter sp. which was more numerous in grassland than bare fallow soils, and *Gemmatirosa kalamazoonesis*, a representative of a group of extremely abundant soil bacteria (Gemmatimonadetes) well-adapted to arid conditions (30) which was more numerous in bare fallow soil than grassland, consistent with previous 16S rRNA amplicon sequencing of these soils (18). A second organism most numerous in bare fallow soils was *Methyoloversatilis* sp. which grows on single-carbon compounds (31) suggesting that organisms adapted to arid conditions or capable of utilizing simple carbon substrates were typical of bacteria in bare fallowed soils. Arable soils were associated with significantly higher ^{1}D and ^{2}D, associated with greater numbers of Methanomada and Halobacteria Euryarchaeotes as well as of ammonia oxidising *Nitrososphaera viennensis* and *Ca*. Nitrosocosmicus sp. These latter organisms suggest that the response of archaea to arable management may reflect regular nitrogen fertilization of these soils. The response of fungi to land management was distinct from that of prokaryotes since the difference in communities were expressed on only one edge-PCA dimension separating grassland from the disturbed soils (Fig. 7). Ectomycorrhizal *Amanita pruitii* and saprotrophic *Clitopilus brunnescens* were less numerous in disturbed soils than grassland. Most fungal species identified as more numerous in arable and bare fallow soils had microfungal or yeast-like growth forms (Table I), possibly because of the effect of physical disturbance arising from tillage upon ectomycorrhizal fungi (32,33). Fungal species which became more numerous in disturbed soils were predominantly pathotrophs of insects (*Ophiocordyceps tiputini*), plants (*Acidomyces acidophilum, Cornuvesica crypta, Irenopsis crotonicola* and *Peroneutypa mackenziei*) and lichens (*Sporidesmium olivaceoconidium*). The differences in phylotype assemblages observed between the land managements reflect the predicted selection pressures within the soils and organismal traits.

Of equal interest to the effects of land management upon microbial diversity is the issue of how disturbance influences microbiome assembly, testing our third hypothesis. Our data support the proposition that physical pulse disturbance by tillage in arable and bare fallow soils results in increased prokaryotic phylogenetic dispersion than in non-tilled grassland soils (Fig. 4). This is indicative of an increased role for species neutral assembly where community structures result from stochastic colonization and extinction processes and are influenced less by species traits (34,35). This stochasticity is likely to arise as tillage disrupts community assembly once *per* year in arable soils but three or four times in bare fallow soils. Phylogenetic dispersion increases with the frequency of tillage (Fig. 4).

Assembly is re-established following tillage, but colonization is influenced by localized abundance of potential colonizers and the assemblage of organisms remaining which can exert an influence upon potential immigrating species – termed priority effects (36). Despite this increased stochasticity, prokaryote phylotype assemblages in arable and bare fallow soils are distinct, from grassland and each other (Figs. 5 and 6, Supplementary Fig. 6), suggesting several possible phylotype assemblages, dependent upon priority effects and the degree of disturbance, even under the same environmental conditions and species pool. However, given the consistent disturbance it is unlikely that the phylotype assemblages represent stable endpoints, but more likely reflect alternative transient states (37). Phylotype assemblages are dependent upon disturbance periodicity. Although we have not tested it, observation of a greater role for stochasticity in phylotype assembly in disturbed soils suggests that they may be more susceptible to immigration of pathogens, a potential problem in arable soils. Soil structure and phylotype assemblages may contribute to the significantly reduced yields observed when wheat is grown in the bare fallow soil studied here (18). For fungal assemblages there was no evidence of increased dispersion in response to tillage. Instead, phylogenetic dispersion was reduced significantly in arable soil compared to grassland (Fig. 4). This suggests strong environmental filtering of phylotypes (niche assembly). This filtering of fungal phylotypes cannot be due to tillage, since phylogenetic dispersion of fungal assemblages in bare fallow soils was equivalent to grassland. Instead, the fungicide prothioconazole (2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-3-thione) added as a wheat seed coat is likely to exert a significant selection pressure on fungi in arable soils resulting in the observed increase in fungal niche assembly.

In summary, after a minimum of fifty-two years of continuous management, soils experiencing combinations of chemical and physical press and pulse disturbances harboured distinctly different microbial communities than undisturbed grassland soil. The effects of each imposed management upon SSU rRNA gene phylotype diversity were kingdom dependent. The observations were also dependent upon whether diversity metrics considered SSU rRNA gene phylogenies. As an example, grassland bacterial phylotype distribution was highly uneven and the soils were associated with the fewest number of dominant phylotypes which were however more phylogenetically diverse than the greater number of dominant phylotypes in disturbed arable and bare fallow soils. At the other extreme, grassland had the greatest number of dominant fungal phylotypes, but

these were associated with reduced phylogenetic diversity compared to arable and bare fallowed soils. We also observed a distinct influence of different disturbance types upon the assembly of communities in disturbed soils. Physical disturbance by tillage increased the influence of stochastic process upon assembly leading to apparently stable transient states of the prokaryotic communities. Fungal community assembly was not influenced by physical disturbance but showed a strong influence of niche assembly probably sue to fungicide incorporation in arable soils.

Material and Methods

Soils – We analysed soil from plots of the Rothamsted Highfield Ley-Arable field experiment (00:21:48 °W, 51:48:18 °N). The soil is a silty clay loam (25% clay: 62% silt: 13% sand) (Chromic Luvisol according to FAO criteria). We sampled plots which had been managed consistently as bare fallow for fifty-two years, arable for sixty-two years (continuous winter wheat, *Triticum aestivum* L., at the time of sampling cv. "Hereward" seed treated with Redigo Deter, a combination fungicide-insecticide, Bayer Crop Science) or mixed grass swards since at least 1838. Grassland and arable plots were established as 300 m² plots, randomly distributed between four in-field blocks. Bare fallow plots were added later in 1959.

DNA Extraction and Metagenome Sequencing - Soil was collected from triplicate plots for each treatment to a depth of 10-cm. using a 3-cm. diameter corer. The top 2-cm. of soil containing root mats and other plant detritus was discarded. Ten cores per plot were pooled and thoroughly mixed whilst sieving through a 2-mm. mesh; samples were then frozen at -80 °C. All implements were cleaned with 70% ethanol (vol./vol.) between sampling/sieving soil from each plot. Soil community DNA was extracted from a minimum of 2 g soil using the MoBio PowerSoil® DNA isolation kit (Mo Bio Laboratories, Inc. Carlsbad, CA) with three replicates for each soil treatment. When necessary, extracts from individual replicates were pooled to provide sufficient material for sequencing. 10 µg of high-quality DNA was provided for sequencing for each of the nine plots. Shotgun metagenomic sequencing of DNA was provided by Illumina® (Great Abington, UK) using a HiSeq™ 2000 sequencing platform, generating 150-base, paired-end reads. The generated sequences were limited to a minimum quality score of 25 and a minimum read length of 70-bases using Trimmomatic (38). After filtering to remove substandard sequences, the average metagenome size for each soil was 4.96×10^8 reads for grassland, 2.86×10^8 for arable and 2.88×10^8 for bare fallow soils.

Estimation of community-aggregated traits - We selected two community-aggregated traits (CAT) to test our hypothesis regarding the opportunity space provided by the treatments studied. Firstly, we generated information regarding the average genome length (AGL) of prokaryotes in each soil metagenome using the ags.sh binary (39). The process proceeds in several steps. First, the abundance of a set of thirty-five single-copy genes are enumerated and coverage estimated as the total number of annotated bases divided by each gene length. These largely translation-associated marker genes occur only very occasionally as duplicates within genomes, are considered both essential for cellular life and very ancient, evolve at a slow rate and code for basal cellular processes, exhibiting little variation across phyla (40). The number of genomes present in each metagenome is then calculated as the average coverage of the thirty-five single copy genes. AGL is derived from the ratio of the number of bases to the number of genomes. Secondly, we calculated the average copy number of the 16S rRNA gene using the acn.sh binary (39) which estimates the 16S rRNA gene coverage as the ratio of bases annotated as belonging to the 16S rRNA gene using SortMeRNA version 2.0 (41) and the 16S rRNA gene length (1,542 bases from *Escherichia coli*) and this value is then divided by the number of genomes in the metagenome described above to estimate the average copy number.

SSU rRNA gene phylogenetic placement - Each of the metagenomes generated in this study were analysed to assess the phylogenetic diversity of bacterial, archaeal and fungal SSU rRNA genes. Nucleotide-based profile hidden Markov models (pHMM) were generated from multi-sequence alignments (MSAs) of reference sequences of each gene using HMMBUILD, part of the HMMER suite version 3.1 (42). All MSAs were generated using the 1PAM/κ = 2 scoring matrix and the *E-INS-i* iterative refinement algorithm in MAFFT version 7.3 (43). For 16S rRNA genes, pHMMs were generated from alignment of a set of 7,245 bacterial and 266 archaeal curated reference sequences associated with PAPRICA version 0.5.2 (44), built November 2019. For the fungal 18S rRNA gene, a pHMM was generated from 2,447 reference sequences downloaded from the National Center for Biotechnology Information's curated Fungal 18S Ribosomal RNA RefSeq Targeted Loci Project, built February 2020. Metagenome reads with homology to each pHMM were identified using HMMSEARCH and a $1x10^{-5}$ Expect-value (*E*) cut-off. Each homologous read was assigned to branches of maximum likelihood (ML) phylograms generated from the respective reference gene sets using RAxML version 8.2.4 (45). Phylogenetic placement of exact sequence variants was implemented using EPA-NG

version 0.3.6 (46) and visualized using iTOL version 5.5 (47). Gene sequence placements can be translated into robust relative abundance and phylogenetic relatedness estimates of organisms using the taxonomic labelling of phylogram branches.

Statistical Analyses – To test our hypotheses, we generated several gene assemblage-related metrics, including gene sequence richness and phylogenetic diversity, abundance-sensitive measures of sequence and phylogenetic diversity using a one-parameter family of diversity measures, balance-weighted phylogenetic diversity ($BWPD_\theta$, (48)) and phylogeny-based distance metrics for assemblage comparison between treatments. Sample size- and coverage-based interpolation and extrapolation of ${}^qD$ of each SSU rRNA gene was performed using iNEXT version 2.0.20 (49) in R version 3.6.1, treating each read as a point mass concentrated on the highest-weight placement. Extrapolation of ${}^qD$ was extended to the greater of the maximum number of sequences across all samples or twice the number of sequences in the smallest sample; 77,805 bacterial 16S rRNA sequences, 62,304 archaeal 16S rRNA sequences and 15,153 fungal 18S rRNA sequences. Estimates of associated 95% confidence intervals were based on 399 bootstrap samples (50).

Estimates of gene sequence similarity-sensitive phylogenetic diversity (PD) based upon placement of homologous metagenomic reads were assessed by computing a measure incorporating abundance, using the FPD binary in GUPPY version 1.1 (part of the PPLACER code (51), accounting for reference ML tree pendant branch length. The effects of different land managements upon $BWPD_0$ and $BWPD_1$ were analysed using one-factor analysis of variance (ANOVA) after testing for homogeneity of variances using Levene's test and normality using the Shapiro-Wilk test. We calculated omega squared (ω^2) as an estimate of the extent to which variance in the response variable was accounted for by the treatment (effect size). The experimental design was limited by having only three replicate plots *per* land management and as a result low statistical power (increasing the likelihood of Type II error). Where significant treatment effects were identified, *post-hoc* pair-wise comparisons were performed using the Tukey-Kramer Studentized Q statistic, following the Copenhaver-Holland procedure of sequentially rejective multiple comparisons (52) to control family-wise Type I error. All parametric tests were performed using PAST version 4.02 (53). An α of 0.05 was considered significant.

To assess prokaryotic 16S rRNA and fungal 18S rRNA gene-based β-diversity between land managements, Kantorovich-Rubinstein (KR) metrics of phylogenetic distance were calculated from phylogenetic placements of metagenome reads using the

559 KRD binary associated with GAPPA version 0.4.0 (54), treating each query as a point
560 mass concentrated on the highest-weight placement. The KR distance metric, which is
561 allied to the weighted-UniFrac measure (55), compares gene assemblage distributions on
562 a phylogram in units of nucleotide substitutions *per* site, a biologically meaningful
563 approach to comparing communities. Comparison of β-diversity dispersion of KR
564 phylogenetic distance metrics within and between land management was performed using
565 a multivariate analogue of Levene's test for homogeneity of multivariate variances, the
566 PERMDISP test (56). Differences in gene assemblages based upon KR distance metrics
567 were tested using permutational multivariate analysis of variance (PERMANOVA, (57)).
568 In addition, the distinctiveness of bacterial, archaeal and fungal phylogenetic assemblages
569 associated with each land management was tested in multivariate space using canonical
570 analysis of principle coordinates (CAP, (58)), maximising the success of a leave-one-out
571 allocation to land management to determine the appropriate number of axes to include in
572 the test. CAP-based hypothesis testing was based upon the sum of canonical eigenvalues.
573 For all multivariate tests, probability estimation was based upon 99,999 permutations
574 (denoted as p_{perm}). Where PERMANOVA indicated a significant treatment effect, pair-
575 wise comparisons were performed. However, since the number of observations was
576 insufficient to allow a reasonable number of permutations, Monte Carlo probabilities
577 (denoted p_{MC}) were calculated based upon an asymptotic permutation distribution.
578 Multivariate tests were performed using PRIMER PERMANOVA+ version 7.0.13
579 (PRIMER-e, Auckland, New Zealand).
580 Unconstrained ordination based upon principal component analysis of the difference in
581 placement masses across reference phylograms - termed edge-PCA (59) - was used for
582 graphical representation of phylogeny-based differences between treatments in a two-
583 dimensional plane using the EDGEPCA binary in GAPPA, treating each query as a point
584 mass concentrated on the highest-weight placement. An advantage of edge-PCA is that
585 branches associated with placements contributing to eigenvalues on each axis, and thus
586 organisms contributing to the observed differences, can be identified. For fungal taxa
587 identified by edge-PCA to be characteristic of the difference land managements, we used
588 the FUNGuild version 1.1 annotation tool (60) to associate taxa with ecological guilds.

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Figure 1. Aggregated Traits of microbiomes associated with soil of the Highfield Ley-Arable field experiment. The average genome length (A) and 16S rRNA gene copy number (B) determined from shotgun metagenomes generated from grassland (green), arable (yellow) and bare fallow (brown) soils. In each case the mean value and standard error of the mean is shown. Comparisons associated with significant trait differences are indicated by dashed lines and the associated Tukey-Kramer Studentized *Q* and probability (*p*) are given.

Figure 2. Sample size-based interpolation (solid line) and extrapolation (dashed line) of SSU rRNA gene phylotype diversity of order q, ${}^{q}D$: $q = 0$ (species richness, left panel), $q = 1$ (Shannon diversity, middle panel) and $q = 2$ (Simpson diversity, right panel). Data points represent the observed ${}^{q}D$ and number of phylotypes for each data set. Shaded areas represent the 95% confidence intervals of the diversity estimates. Diversity is presented as the effective number of species. Data for bacterial and archaeal 16S rRNA gene and the fungal 18S rRNA gene are shown for grassland (green) arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment. The observed range in sample coverage (*C*) for each gene is given. Individual sample coverages are shown in Supplementary Figure 1.

Figure 3. Comparison of phylogenetic diversity of SSU rRNA gene phylotype assemblages associated with grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment based upon a one-parameter family of diversity measures, $BWPD_\theta$, that interpolates between classical phylogenetic diversity (PD, $\theta = 0$, left panel) and an abundance-weighted extension of PD ($\theta = 1$, right panel). The mean and standard error of the mean $BWPD_\theta$ are shown together with results of a one-factor analysis of variance and observed effect size (ω^2) are shown for each gene. $BWPD_\theta$ profiles are shown in Supplementary Fig. 6.

Figure 4. Phylogenetic dispersion associated with SSU rRNA phylotype assemblages in grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment. Phylogenetic dispersion was estimated based upon the multivariate deviation of each replicate community from the centroid of each land management group in Euclidean space, based upon Kantorovich-Rubinstein phylogenetic distances between each phylotype assemblage. The mean ± 95% confidence intervals are shown for each soil.

Figure 5. Ordination of bacterial 16S rRNA gene phylotype assemblages shown in Fig. 1a, exploiting the underlying phylogenetic nucleotide sequence structure (edge-PCA). Phylotype assemblages associated with grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment are separated across both edge-PCA axes. Edges associated with large eigenvectors are shown in each axis-associated colour-coded phylogram and corresponding to the axis colour scales. Phylotypes associating more with grassland, arable or bare fallow soils are identified.

Figure 6. Ordination of archaeal 16S rRNA gene phylotype assemblages shown in Fig. 1b, exploiting the underlying phylogenetic nucleotide sequence structure (edge-PCA). Phylotype assemblages associated with grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment are separated across both edge-PCA axes. Edges associated with large eigenvectors are shown in each axis-associated colour-coded phylogram and corresponding to the axis colour scales. Phylotypes associating more with grassland, arable or bare fallow soils are identified.

Figure 7. Ordination of fungal 18S rRNA gene phylotype assemblages shown in Fig. 1c, exploiting the underlying phylogenetic nucleotide sequence structure (edge-PCA). Phylotype assemblages associated with grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment are separated across both edge-PCA axes. Edges associated with large eigenvectors are shown in each axis-associated colour-coded phylogram and corresponding to the axis colour scales. Phylotypes associating more with grassland, arable or bare fallow soils are identified.

Table I. Predictions of trophic mode, growth form and ecological guild for the fungal species identified with shifts in community assemblages between grassland, arable and bare fallow soils shown in Fig. 8. Predictions are taken from FUNGuild version 1.0.

Supplementary Figure 1. Phylogenetic comparison of bacterial 16S rRNA phylotype assemblages in grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment. The most abundant organisms are identified on branch tips of each maximum likelihood SSU rRNA gene phylogram. Placement symbol size is scaled to reflect relative abundance across the nine samples. Replicates for each land management are represented by different placement shapes – circle, square or triangle.

Supplementary Figure 2. Phylogenetic comparison of archaeal 16S rRNA phylotype assemblages in grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment. The most abundant organisms are identified on branch tips of each maximum likelihood SSU rRNA gene phylogram. Placement symbol size is scaled to reflect relative abundance across the nine samples. Replicates for each land management are represented by different placement shapes – circle, square or triangle.

Supplementary Figure 3. Phylogenetic comparison of fungal 18S rRNA phylotype assemblages in grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment. The most abundant organisms are identified on

branch tips of each maximum likelihood SSU rRNA gene phylogram. Placement symbol size is scaled to reflect relative abundance across the nine samples. Replicates for each land management are represented by different placement shapes – circle, square or triangle

Supplementary Figure 4. Sample size-based interpolation (solid line) and extrapolation (dashed line) of SSU rRNA gene phylotype coverage. Data points represent the observed coverage and number of phylotypes for each data set. Shaded areas represent the 95% confidence intervals of the coverage estimates. Data for bacterial and archaeal 16S rRNA genes and the fungal 18S rRNA gene are shown for grassland (green) arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment.

Supplementary Figure 5. Sample coverage-based interpolation (solid line) and extrapolation (dashed line) of SSU rRNA gene phylotype diversity of order q, ${}^{q}D$: $q = 0$ (species richness, left panel), $q = 1$ (Shannon diversity, middle panel) and $q = 2$ (Simpson diversity, right panel). Data points represent the observed ${}^{q}D$ and coverage for each data set. Shaded areas represent the 95% confidence intervals of the diversity estimates. Diversity is presented as the effective number of species. Data for bacterial and archaeal 16S rRNA genes and the fungal 18S rRNA gene are shown for grassland (green) arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment.

Supplementary Figure 6. Comparison of phylogenetic diversity profiles of SSU rRNA gene phylotype assemblages associated with grassland (green), arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment based upon a one-parameter family of diversity measures, $BWPD_{\theta}$, that interpolates between classical phylogenetic diversity (PD, $\theta = 0$) and an abundance-weighted extension of PD ($\theta = 1$).

Supplementary Figure 7. Discriminant analysis employing canonical analysis of principal coordinates (CAP) of SSU rRNA phylotype assemblages based upon Kantorovich-Rubinstein phylogenetic distance metrics. Phylotype assemblages associated with grassland (green) arable (yellow) and bare fallow (brown) soils of the Highfield Ley-Arable field experiment are shown. For each ordination, two CAP axes were defined, based upon maximising a leave-one-out allocation success to *a priori* land management groups. The results of permutation tests of the significance of the canonical relationships using the trace statistic (sum of canonical eigenvalues) are shown.

TABLE I

Genus	Association	Trophic Mode	Growth Form	Guild
Acidomyces	arable/fallow	Pathotroph; Saprotroph; Symbiotroph	Microfungus	Endophyte; Plant Pathogen; Unknown Saprotroph; Wood Saprotroph
Amanita	grassland	Symbiotroph	Agaricoid	Ectomycorrhizal
Aspergillus	grassland	Pathotroph; Saprotroph; Symbiotroph	Microfungus	Animal Pathogen; Endophyte; Plant Saprotroph; Soil Saprotroph; Undefined Saprotroph; Wood Saprotroph
Auxarthron	grassland	Saprotroph	not known	Undefined Saprotroph
Cantharellus	arable/fallow	Symbiotroph	Cantherelloid	Ectomycorrhizal
Cladophialophora	grassland	Saprotroph	Facultative Yeast	Undefined Saprotroph
Clitopilus	grassland	Saprotroph	Agaricoid	Undefined Saprotroph
Coemansia	arable/fallow	Saprotroph	not known	Undefined Saprotroph
Cornuvesica	arable/fallow	Pathotroph; Saptroroph	Microfungus	Plant Pathogen; Wood Saprotroph
Irenopsis	arable/fallow	Pathotroph	not known	Plant Pathogen
Ophiocordyceps	arable/fallow	Pathotroph; Symbiotroph	Microfungus	Animal Pathogen; Endophyte
Peroneutypa	arable/fallow	Pathotroph	not known	Plant Pathogen
Sporidesmium	arable/fallow	Pathotroph	Microfungus	Lichen Parasite
Yarrowia	arable/fallow	Saprotroph	Yeast	Undefined Saprotroph

Fig. 1

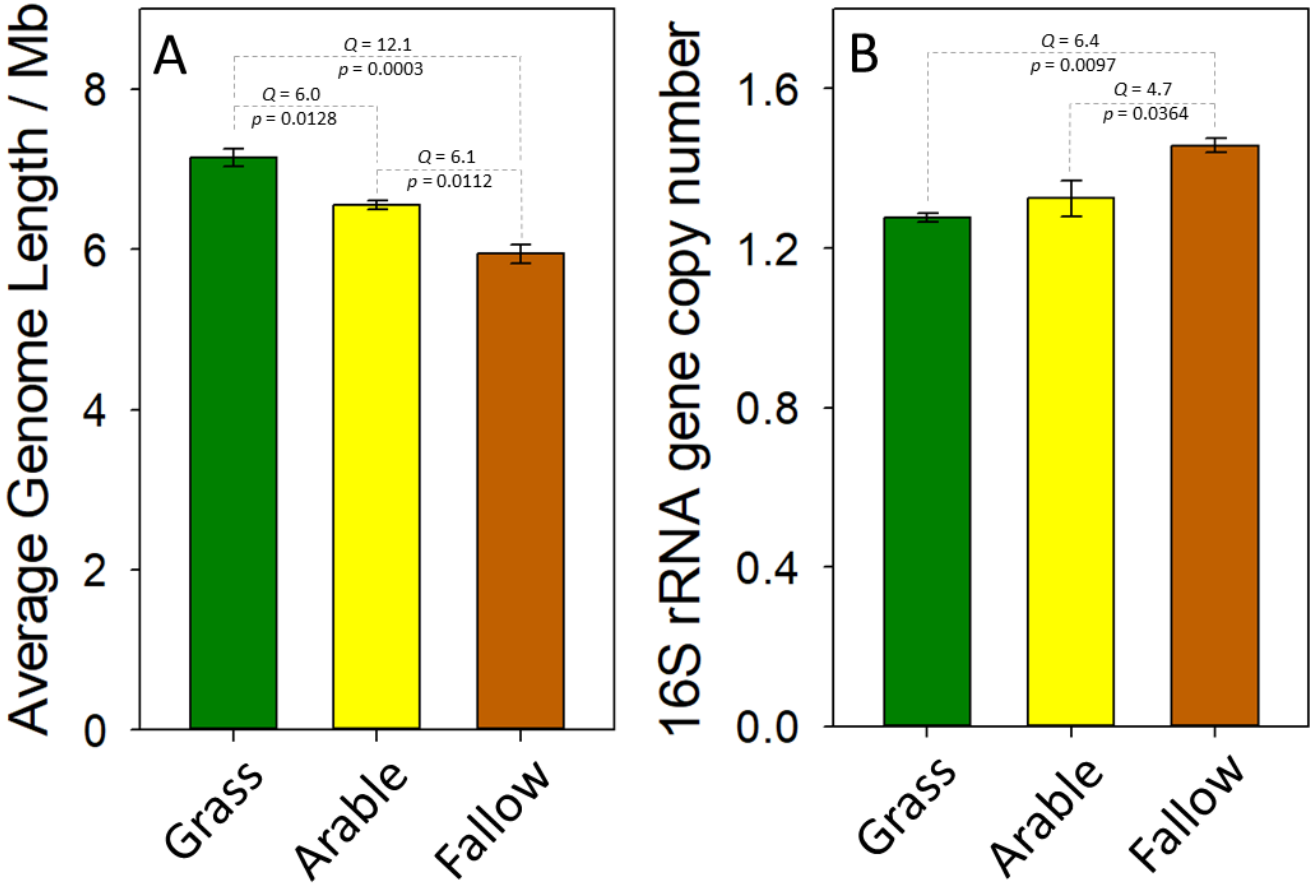

A
Average Genome Length / Mb
Q = 12.1
p = 0.0003
Q = 6.0
p = 0.0128
Q = 6.1
p = 0.0112
0
2
4
6
8
Grass
Arable
Fallow
B
16S rRNA gene copy number
Q = 6.4
p = 0.0097
Q = 4.7
p = 0.0364
0.0
0.4
0.8
1.2
1.6
Grass
Arable
Fallow

Fig. 2

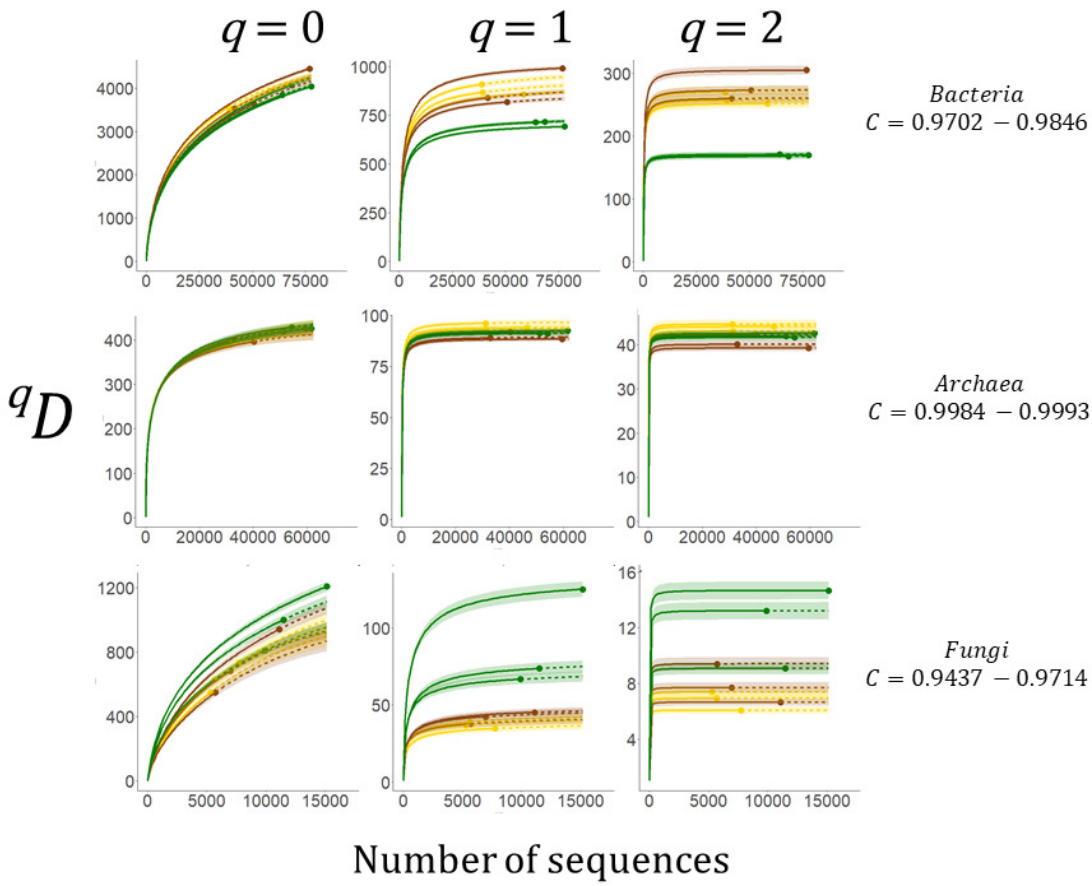

$q = 0$
$q = 1$
$q = 2$
^{q}D
Bacteria
$C = 0.9702 - 0.9846$
Archaea
$C = 0.9984 - 0.9993$
Fungi
$C = 0.9437 - 0.9714$
Number of sequences

Fig. 3

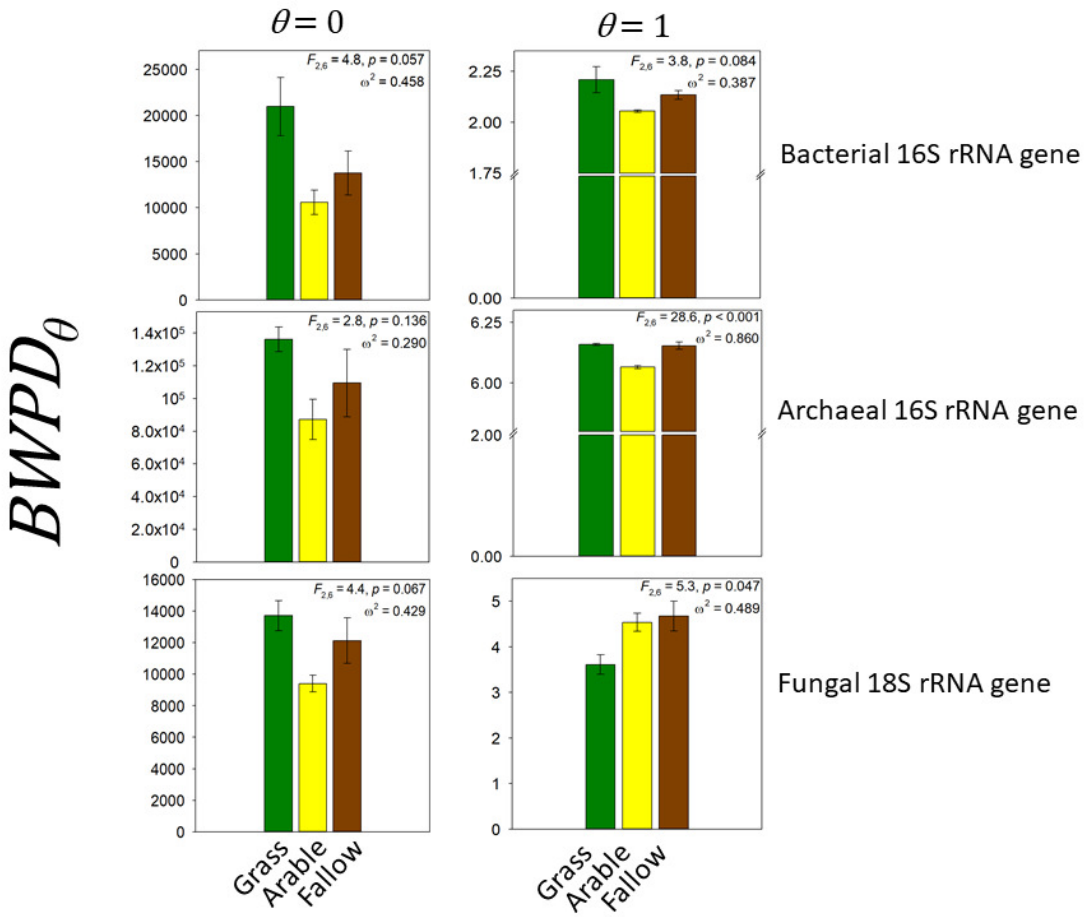

$BWPD_\theta$
$\theta = 0$
$\theta = 1$
$F_{2,6} = 4.8, p = 0.057$
$\omega^2 = 0.458$
$F_{2,6} = 3.8, p = 0.084$
$\omega^2 = 0.387$
Bacterial 16S rRNA gene
$F_{2,6} = 2.8, p = 0.136$
$\omega^2 = 0.290$
$F_{2,6} = 28.6, p < 0.001$
$\omega^2 = 0.860$
Archaeal 16S rRNA gene
$F_{2,6} = 4.4, p = 0.067$
$\omega^2 = 0.429$
$F_{2,6} = 5.3, p = 0.047$
$\omega^2 = 0.489$
Fungal 18S rRNA gene
Grass
Arable
Fallow
Grass
Arable
Fallow

Fig. 4

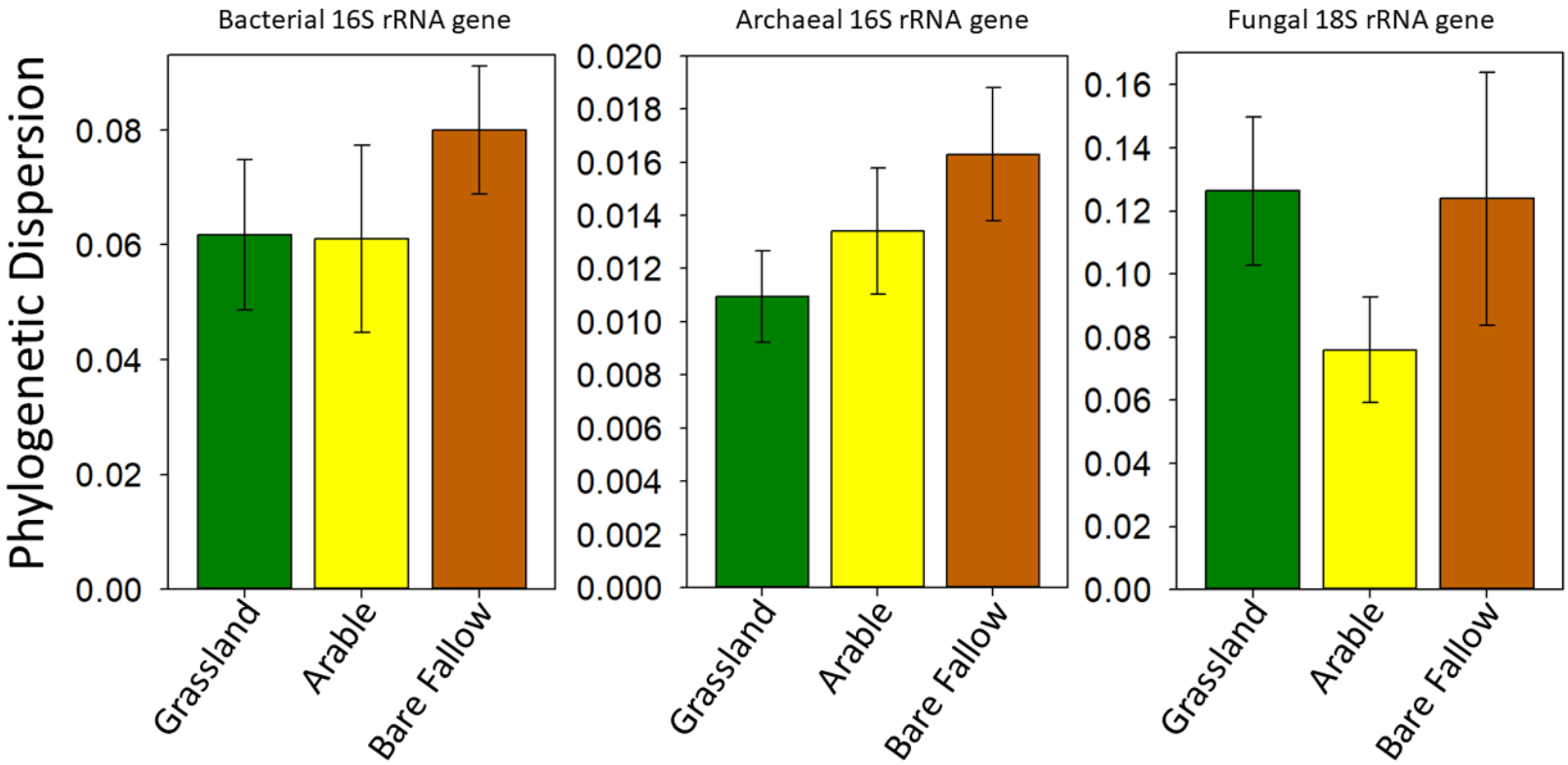

Bacterial 16S rRNA gene
Archaeal 16S rRNA gene
Fungal 18S rRNA gene
Phylogenetic Dispersion
0.00
0.02
0.04
0.06
0.08
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
0.018
0.020
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
Grassland
Arable
Bare Fallow

Fig. 5

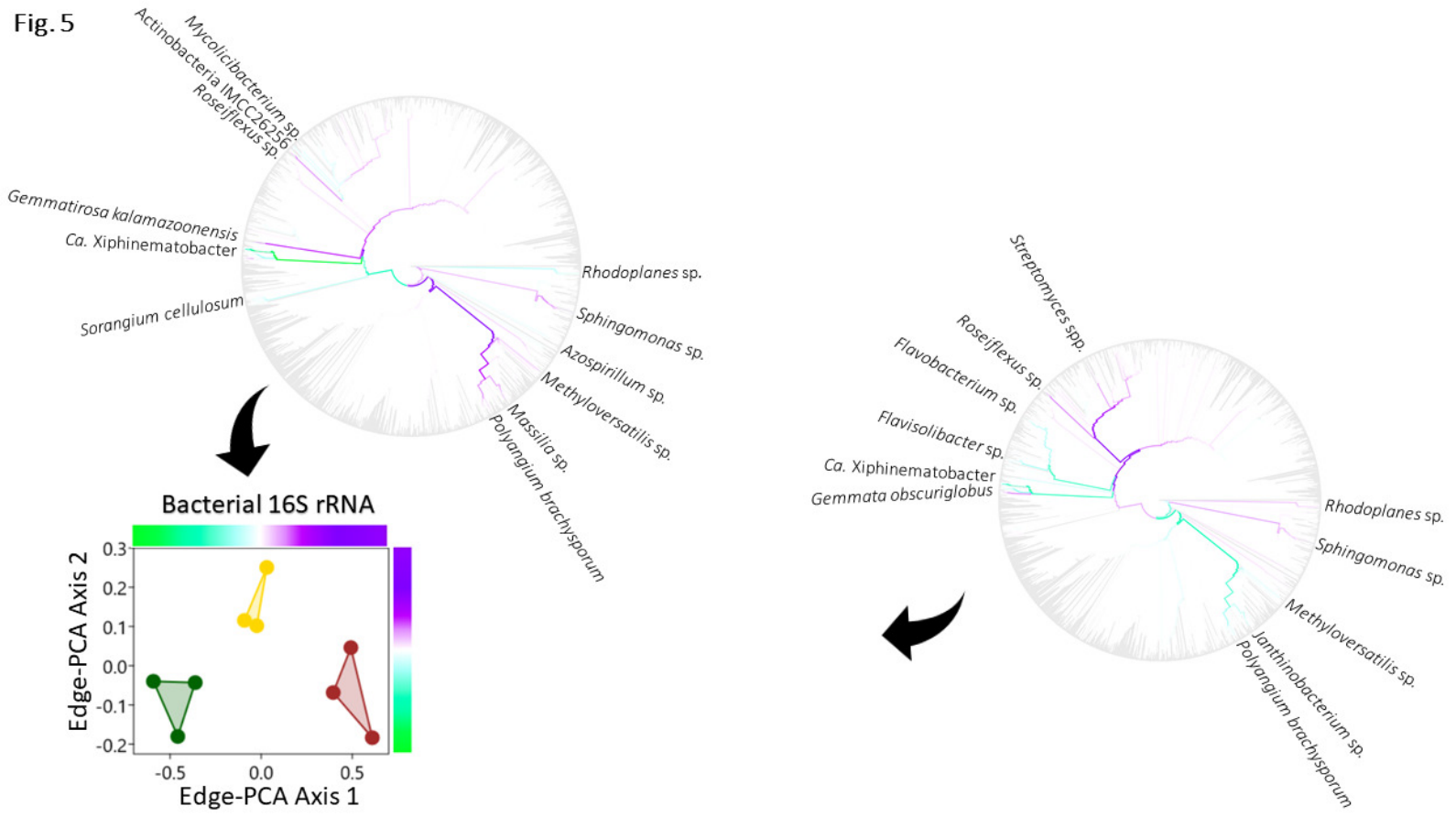

Mycolicibacterium sp.
Actinobacteria IMCC26256
Roseiflexus sp.
Gemmatirosa kalamazoonensis
Ca. Xiphinematobacter
Sorangium cellulosum
Rhodoplanes sp.
Sphingomonas sp.
Azospirillum sp.
Methyloversatilis sp.
Massilia sp.
Polyangium brachysporum
Bacterial 16S rRNA
Edge-PCA Axis 2
Edge-PCA Axis 1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.5
0.0
0.5
Streptomyces spp.
Roseiflexus sp.
Flavobacterium sp.
Flavisolibacter sp.
Ca. Xiphinematobacter
Gemmata obscuriglobus
Rhodoplanes sp.
Sphingomonas sp.
Methyloversatilis sp.
Janthinobacterium sp.
Polyangium brachysporum

Fig. 6

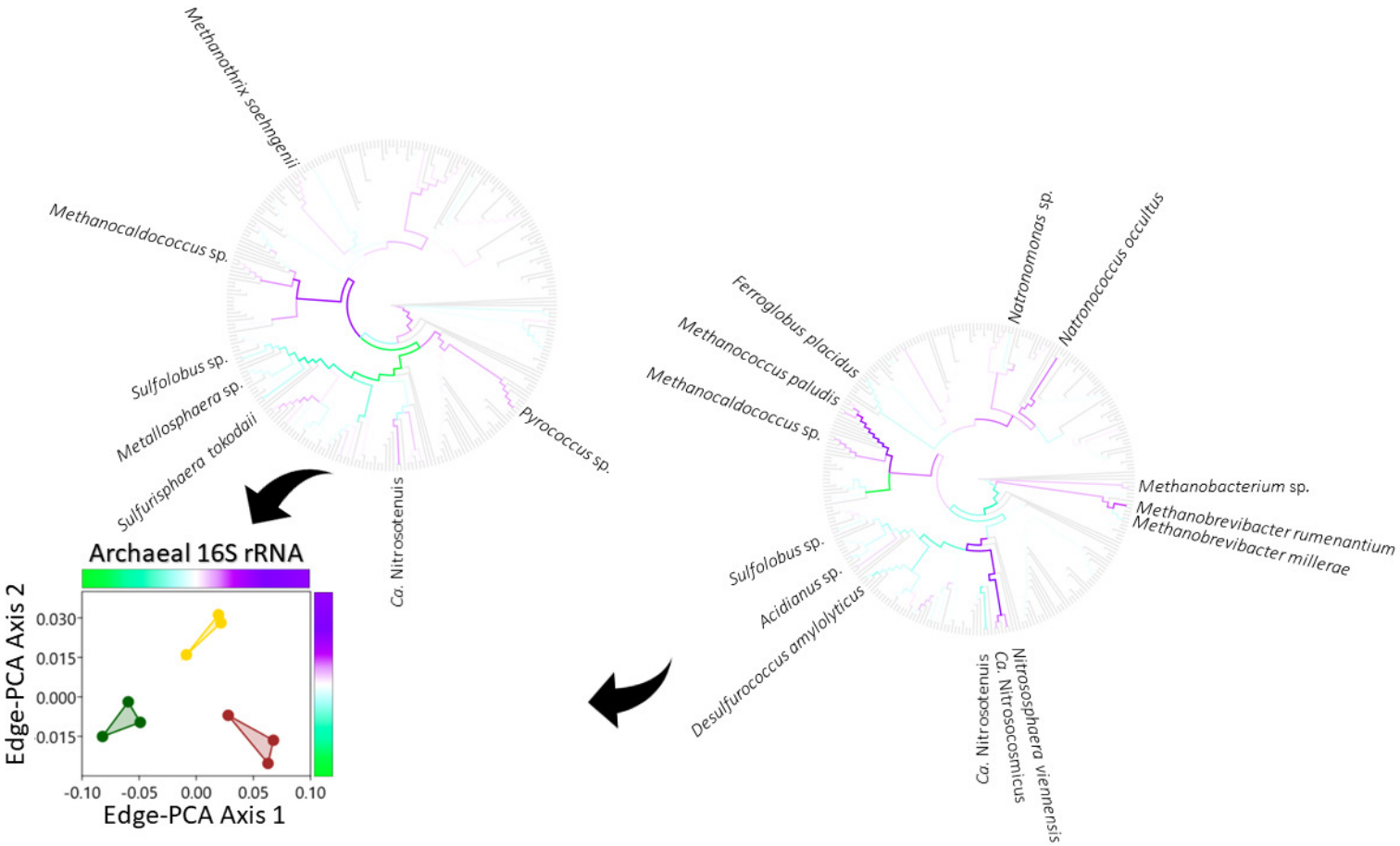
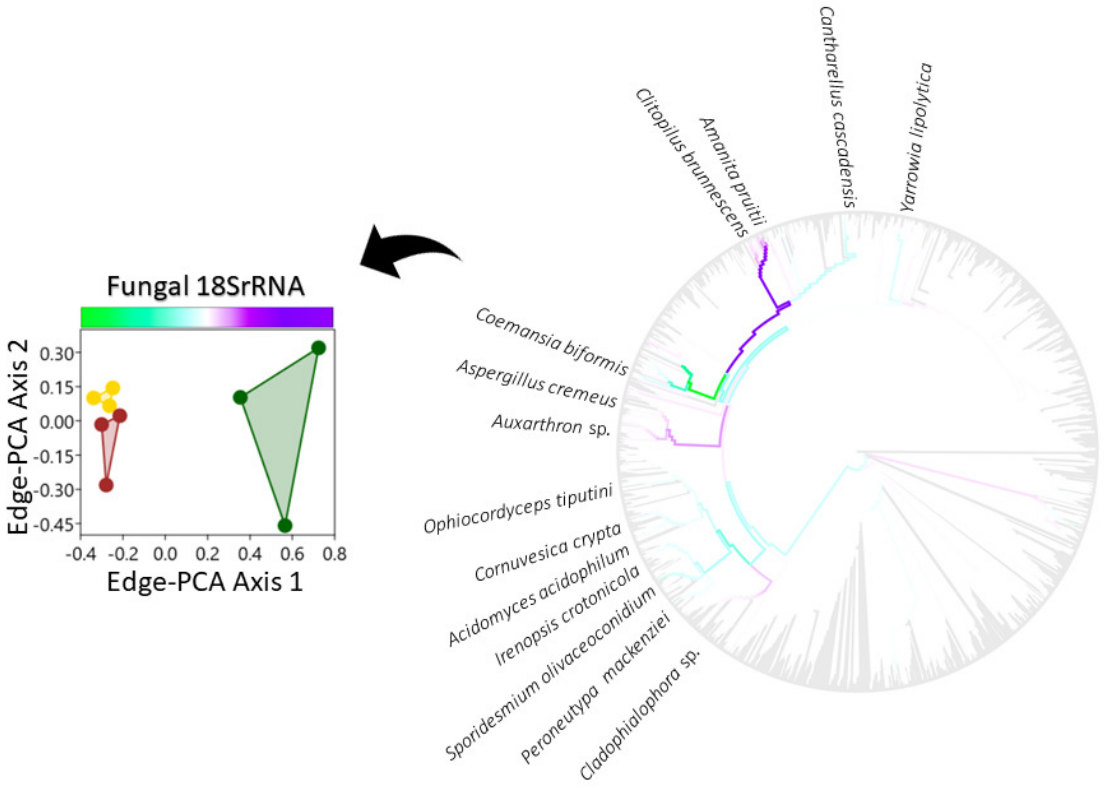

Methanothrix soehngenii
Methanocaldococcus sp.
Sulfolobus sp.
Metallosphaera sp.
Sulfurisphaera tokodaii
Ca. Nitrosotenuis
Pyrococcus sp.
Archaeal 16S rRNA
Edge-PCA Axis 2
0.030
0.015
0.000
0.015
-0.10
-0.05
0.00
0.05
0.10
Edge-PCA Axis 1
Ferroglobus placidus
Methanococcus paludis
Methanocaldococcus sp.
Sulfolobus sp.
Acidianus sp.
Desulfurococcus amylolyticus
Natronomonas sp.
Natronococcus occultus
Methanobacterium sp.
Methanobrevibacter rumenantium
Methanobrevibacter millerae
Ca. Nitrosotenuis
Ca. Nitrosocosmicus
Nitrososphaera viennensis

Fig. 7


Fungal 18SrRNA
Edge-PCA Axis 2
0.30
0.15
0.00
-0.15
-0.30
-0.45
-0.4 -0.2 0.0 0.2 0.4 0.6 0.8
Edge-PCA Axis 1
Clitopilus brunnescens
Amanita pruitii
Cantharellus cascadensis
Yarrowia lipolytica
Coemansia biformis
Aspergillus cremeus
Auxarthron sp.
Ophiocordyceps tiputini
Cornuvesica crypta
Acidomyces acidophilum
Irenopsis crotonicola
Sporidesmium olivaceoconidium
Peroneutypa mackenziei
Cladophialophora sp.