Long term drought and warming alter soil bacterial and fungal communities in an upland heathland

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Short title: Climate change alters microbial communities

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Contribution statement:

FMS carried out the soil sampling for the DNA metabarcoding, all statistical analysis and drafted the manuscript. TG carried out the DNA metabarcoding laboratory analyses and bioinformatic pipelines under the direction of RG. NW carried out the soil sampling and fungal microscopy work under the direction of AS. FMS, SR, DLJ, BAE, and DAR conceived this project and assisted in designing the sampling scheme. SR and BAE maintain the Clocaenog experimental field site. FMS, SR, DLJ, DAR and SC contributed to writing the manuscript.

Abstract

The response of soil microbial communities to a changing climate will impact global biogeochemical cycles, potentially leading to positive and negative feedbacks. However, our understanding of how soil microbial communities respond to climate change and the implications of these changes for future soil function is limited. Here we assess the response of soil bacterial and fungal communities to long-term experimental climate change in a heathland organo-mineral soil. We analysed microbial communities using Illumina sequencing of the 16S rRNA gene and ITS2 region at two depths, from plots undergoing 4- and 18-years of *in-situ* summer drought or warming. We also assessed the colonisation of *Calluna vulgaris* roots by ericoid and dark septate endophytic (DSE) fungi using microscopy after 16 years of climate treatment. We found significant changes in both the bacterial and fungal communities in response to drought and warming, likely mediated by changes in soil pH and electrical conductivity. Changes in the microbial communities were more pronounced after a longer period of climate manipulation. Additionally, the subsoil communities of the long-term warmed plots became similar to the topsoil. Ericoid mycorrhizal colonisation decreased with depth while DSEs increased, however these trends with depth were removed by warming. We largely ascribe the observed changes in microbial communities to shifts in plant cover and subsequent feedback on soil physicochemical properties, especially pH. Our results demonstrate the importance of considering changes in soil microbial responses to climate change across different soil depths and after extended periods of time.

Keywords

Climate change; warming; drought; bacteria; fungi; heathland; mycorrhiza

Highlights

Bacterial and fungal communities change after 18 years of drought and warming

Changes in microbial composition are more pronounced after 18 vs 4 years of treatment

Warming treatments led to loss of depth stratification of communities and soil pH

Introduction

Climate change has and will continue to fundamentally alter global ecosystem functioning. Understanding how ecosystems will respond to future change is essential for societal adaptation as well as mitigation. Soils are a source of major uncertainty in future earth predictions, as we still do not know in sufficient detail how soil biogeochemical cycling, hydrology or biology will change in response to climate change and how these changes will alter climatic feedbacks (Bradford and others 2016). The potential for soils to accentuate or mitigate future climate change is in large part due to the vast quantities of carbon that is currently stored in the soil system (~2400 Pg, three times as much as is in the atmosphere) (Batjes 1996; Stocker and others 2013; Bossio and others 2020). In order to predict how soil systems will change in the future, we have to take into account how climate alters both the soil physicochemical environment as well as changes in the soil biota (e.g. Robinson and others 2019).

Soil microbial communities have responded to climate change manipulations across various experimental systems (Cavicchioli and others 2019; Jansson and Hofmockel 2020). The response of microbial communities to the different climate change related stressors is dependent on the type of climatic stress, the ecosystem type and the identity of the microbial communities. There have been recent suggestions that microbial responses to drought are phylogenetically conserved (Amend and others 2016); though this has not been evidenced by another analysis of multiple independent global studies (Oliverio and others 2016). In general, warming has a stronger impact on fungi than bacteria (García-Palacios and others 2015), and stronger impacts on microbial abundances in colder regions (Chen and others 2015). Meta-analyses of the impact of altering precipitation on microbial abundance and composition found that the impact on biomass depended on the climate, with high precipitation areas being more responsive to drought and low precipitation areas being more responsive to

increased precipitation (Ren and others 2017, 2018; Zhou and others 2018). Drought has been found to result in increased fungal dominance in a temperate heath (Haugwitz and others 2014), and in grassland ecosystems fungi showed higher resilience to drought than bacteria (de Vries and others 2018).

The response of soil microbial communities to climate change has implications for various essential ecosystem functions, including the provision of nutrients to plants through mycorrhizal associations. The response of mycorrhizal associations to experimental drought or warming varies according to the specific type of mycorrhiza (Olsrud and others 2009; Binet and others 2017). There is some evidence that changes in mycorrhizal associations in response to climate change may be more related to changes in plant composition than to changes in mycorrhizal interactions (Rudgers and others 2014). Studies across a variety of climates and ecosystem types have found that altering precipitation can impact the extracellular enzyme activities within soils (Ren and others 2017), resulting in impacts on soil and root respiration and associated soil carbon loss (Crowther and others 2016; Ren and others 2018). The impact of drought upon soil respiration has been found to be dependent on the local climate, with high precipitation areas being more responsive (Reinsch and others 2017). Drought has been found to affect the microbial community impact on litter decomposition across various studies (Allison and others 2013; Martiny and others 2017; Santonja and others 2017; Tóth and others 2017). The legacy of global change persists within the microbial community for several years and impacts their ability to carry out key functions in response to new or altered environments (Martiny and others 2017).

The increasing awareness of the impact of legacy effects upon the ability of an ecosystem to respond to future change makes the use of long-term ecological experiments increasingly important. Long-term climate change has been found to impact plant communities (Fridley and others 2011; Andresen and others 2016), soil respiration (Crowther and others 2016;

100 Domínguez and others 2017b), hydrological behaviour (Robinson and others 2016), soil
101 mesofauna (Petersen 2011; Holmstrup and others 2013), soil microbial communities (Rousk
102 and others 2013; Sayer and others 2017) and whole ecosystem feedbacks (O. Abbasi and others
103 2020). Importantly, many of these impacts emerge only after years of experimental treatment
104 (e.g. Andresen and others, 2016), indicating how essential long-term experiments are for
105 evaluating future climate change. Within this study we will look at the climate manipulation
106 experiment in the Clocaenog Forest, NE Wales, UK which has imposed summer drought and
107 warming treatments over an organo-mineral heathland soil since 1999. There has been a rise
108 in soil respiration in the treatments compared to the controls (Reinsch and others 2017),
109 which has not been matched by changes in the plant community which has shown only a
110 slight shift to greater moss cover in the warming plots (Kröel-Dulay and others 2015). Previous
111 work on this site has found that drought could be impacting the summer fungal community
112 only (Toberman and others 2008), and other studies show limited impact of the warming or
113 drought treatments upon microbial biomass, extracellular enzyme activity, microbial
114 community as measured by phospholipid fatty acid analysis and microbial growth rates
115 (Rousk and others 2013; Domínguez and others 2017a). Within this study our aims were: to
116 characterise the bacterial and fungal communities after four versus eighteen years of
117 persistent summer drought and warming; to see if the microbial communities are altered by
118 the legacy of repeated drought and warming; to assess whether the microbial response can be
119 clearly demarcated into a bacterial versus a fungal response through identifying co-occurrence
120 patterns; to compare changes in the bulk community composition to changes in mycorrhizal
121 associations; and to see if these changes were associated with changes in the soil
122 physicochemical environment. Our hypotheses were:

123 1. The bacterial and fungal communities both show significantly different composition
124 within the plots exposed to long-term summer drought and warming compared to
125 control.

2. The impact of climate treatments on microbial community would be greater after eighteen versus four years of treatment, and would also be greater in the topsoil compared to the subsoil.
3. Fungal communities would show stronger responses to the treatments than bacterial communities, with limited interactions across the Kingdoms identified through network analysis.
4. The root fungal associations are affected by treatment in a manner consistent with that of the response of fungal community composition in the bulk soil.
5. The above hypothesised changes in microbial community composition with treatment and soil depth would be partially mediated by changes in soil pH and water content.

Methods

Site description and sampling

Long term climate manipulations were carried out in North Wales (53°03'N 3°28'W) on a peaty podzol. The mean annual precipitation is 1263 mm, and mean annual temperature 7.4°C. The vegetation is dominated by *Calluna vulgaris*, and the soil consists of a carbon-rich topsoil layer (~8 cm deep, ~87% organic matter) and an underlying layer of gley subsoil (~4 cm deep, ~37% organic matter). Climate manipulations started in 1999, and consisted of a summer drought treatment, a warming treatment as well as un-manipulated control plots. There are three replicate plots per treatment. The treatments are imposed using an automated retractable roof system, with drought plots having the roofs cover the plots during summer rainfall events (~54% of summer rainfall is excluded) and the warming plots having the roofs cover the plots overnight to keep in the heat (0.2°C increase in mean annual temperature). A full description of the experimental set-up is provided in (Beier and others 2004).

150 Soil samples were collected in February 2003 and 2017 using a stainless steel auger. Samples
151 were collected from both the organic topsoil and gleyed mineral subsoil for both years, and in
152 2017 the 2 cm interface between the two soil layers was analysed separately. The samples for
153 each plot were bulked together for topsoil and subsoil in 2003; while three soil cores per plot
154 from 2017 were analysed with the three depths separately. In total, there were 15 samples from
155 2003 and 78 from 2017, as some samples were not able to be included. Soil pH and EC were
156 measured on frozen 2017 soil samples using a Corning 220 pH meter (VWR combination
157 electrode for pH and EC 662-1805; Jenway 4510). Soil pH was measured from a 1:2.5 (w/v) soil-
158 to-0.01 M $CaCl_2$ suspension after equilibration for 0.5 h.

159 Molecular analyses of soil microbial communities

160 *DNA extraction and sequencing*

161 DNA was extracted from 0.2 g frozen field moist soil using a Powersoil® DNA Isolation Kit
162 (Mo Bio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions.
163 Amplicons were generated using a 2-step amplification approach, using Illumina Nextera
164 tagged primers. Bacterial 16S V4 primers 515f GTGYCAGCMGCCGCGGTAA and
165 806r GGACTACNVGGGTWTCTAAT (Walters and others 2016), and Fungal ITS
166 primers GTGARTCATCGAATCTTTG and TCCTCCGCTTATTGATATGC (Ihrmark and others
167 2012) were each modified at 5' end with the addition of Illumina pre-adapter and Nextera
168 sequencing primer sequences. Amplicons were generated using a high-fidelity DNA
169 polymerase (Q5 Taq, New England Biolabs). After an initial denaturation at 95°C for 2
170 minutes, PCR conditions were: denaturation at 95°C for 15 seconds; annealing at temperatures
171 55°C and 52°C for 16S and ITS reactions respectively; annealing times were 30 seconds with
172 extension at 72°C for 30 seconds; repeated for 25 cycles. A final extension of 10 minutes at
173 72°C was included.

174 PCR products were cleaned using a ZR-96 DNA Clean-up Kit (Zymo Research Inc., Irvone, CA)
175 following manufacturer's instructions. MiSeq adapters and 8nt dual-indexing barcode
176 sequences were added during a second step of PCR amplification. After an initial denaturation
177 95°C for 2 minutes, PCR conditions were: denaturation at 95°C for 15 seconds; annealing at
178 temperatures 55°C; annealing times were 30 seconds with extension at 72°C for 30 seconds;
179 repeated for 8 cycles with a final extension of 10 minutes at 72°C.

180 Amplicon sizes were determined using an Agilent 2200 TapeStation system. Libraries were
181 normalized using SequalPrep Normalization Plate Kit (Thermo Fisher Scientific), quantified
182 using Qubit dsDNA HS kit (Thermo Fisher Scientific) and pooled together at equal
183 concentrations. The pooled library was diluted to achieve 400 pM in a 40 µl volume after
184 denaturation and neutralisation. Denaturation was achieved with 4 µl 2 M NaOH for 5
185 minutes followed by neutralisation with 4 µl 2 M HCl. The library was then diluted to its load
186 concentration of 14 pM with HT1 Buffer and 5% denatured PhiX control library. A final
187 denaturation was performed by heating to 96°C for 2 minutes followed by cooling in crushed
188 ice. Sequencing was performed on an Illumina MiSeq using V3 600 cycle reagents. The DNA
189 sequences are available on the European Nucleotide Archive under primary accession
190 reference PRJEB33721.

191 *Molecular Bioinformatics*

192 Illumina demultiplexed sequences for 16S and ITS were processed separately in R using
193 DADA2 (Callahan and others 2016) to quality filter, merge, denoise and assign taxonomies as
194 follows:

195 Amplicons reads were trimmed to 270 and 220 bases, forward and reverse respectively.
196 Filtering settings were maximum number of Ns (maxN) = 0, maximum number of expected
197 errors (maxEE) = (3,5), and amplicon primer sequences removed using trimLeft=c(20,20).
198 Sequences were dereplicated and the DADA2 core sequence variant inference algorithm

199 applied. Forward and reverse reads were then merged using mergePairs function to produce
200 amplicon sequence variants (ASVs). Sequence tables were constructed from the resultant
201 ASVs and chimeric sequences were removed using removeBimeraDenovo default settings.
202 ASVs were subject to taxonomic assignment using assignTaxonomy at default settings;
203 training databases were GreenGenes v13.8 (DeSantis and others 2006; McDonald and others
204 2012) and Unite v7.2 (Kõljalg and others 2005) for 16S and ITS respectively. Fungal taxa were
205 assigned to trophic modes using FunGUILD (Nguyen and others 2016).

206 Root fungal colonisation

207 Soil cores for root assays were extracted in April 2015. Each plot had a single 8 cm diameter
208 core taken to 8 cm depth. The cores were cut into 1 cm subsections, soaked in tap water and
209 *Calluna vulgaris* roots removed by hand and washed to remove soil particles. Root length,
210 diameter and number of tips were measured using WinRHIZO version 3.2 on a flatbed
211 scanner. Proportional colonisation of ericoid mycorrhizae (ErM) and dark septate endophytes
212 (DSE) was estimated using the magnified intersection technique (McGonigle and others 1990).
213 Roots were bleached in 10% KOH for 20 h and then stained with a 5% vinegar-ink solution
214 (Vierheilig and others 1998; Arndal and others 2013). Roots were cut to 1-2 cm in length and 2
215 mm passes made along each root length. At the end of each pass all cells were examined for
216 ErM colonisation or the presence of DSE hyphae. ErM colonisation was categorised as 0%, 0-
217 1%, 1-10%, 10-50%, 50-90% and 90-100% colonisation based upon the classification system of
218 Trouvelet and others (1986). DSE proportional colonisation was calculated as the number of
219 colonised intervals divided by the total number of intervals. Root biomass, length and fungal
220 colonisation data is available online at the NERC Environmental Data Centre (White and
221 others 2019).

222 ## Statistics

223 All statistics were performed in R version 3.6.0 (R Core Team 2020). Bacterial taxa were
224 rarefied to 25000 reads 100 times using the vegan R package (Oksanen and others 2020) and
225 the rounded average used for calculation of richness and diversity indices. The same
226 procedure was used for fungal taxa with rarefaction to 10000 reads. The cut-offs for
227 rarefaction were identified based on evaluation of the read depths of the samples and removal
228 of the samples with considerably lower read depths than the rest of the data (Salter and others
229 2014). In order to account for the structure of the data we modelled the effect of climate
230 treatment, depth and year of collection upon diversity with a Bayesian hierarchical model
231 using the brms package (Bürkner 2017). These models had plot identity nested in year as a
232 grouping factor and normal priors on the population-level effects with mean 0 and standard
233 deviation 5 for fungi and 10 for bacteria. Priors on the group-level effect were student T
234 distributed with shape parameter 3, mean 0 and the above standard deviation. Model
235 diagnostics – i.e. R-hat, effective sample size and graphical posterior predictive checks – were
236 assessed for all models and found to be acceptable. This same procedure was used for the
237 analysis of changes in soil pH and EC with depth and treatment in the 2017 samples, with
238 priors having a standard deviation of 1 for soil pH and 10 for soil EC. Two-dimensional NMDS
239 ordination on the Bray-Curtis distances between taxa based on the rarefied data was used to
240 characterise community composition. The response of community composition to treatment
241 was analysed using PERMANOVA, the homogeneity of dispersion assumption was shown to
242 hold at $p > 0.1$ and plot identity was included as a blocking factor. All figures were plotted
243 using ggplot2 (Wickham 2009)

244 Co-occurrence networks were constructed using the SpiecEasi package in R (Kurtz and others
245 2015). Bacterial and fungal taxa that appeared in over 50% of the 2017 samples were used
246 simultaneously to construct networks that contained intra- and inter-kingdom co-occurrence
247 relationships for each of the control, warming and drought treatments (Tipton and others

248 2018). The network was then plotted and its characteristics determined using the igraph
249 package (Csardi and Nepusz 2006). Node degree and betweenness were used to identify the
250 key taxa within the network.

251 Indicator taxa for the warming and drought treatments were identified through analysing the
252 differential relative abundance of taxa by treatment using the DESeq2 method (Love and
253 others 2014) and the apeglm shrinkage estimator (Zhu and others 2019). Year, climate
254 treatment and soil depth were included as additive terms within the DESeq2 model. The
255 presence of any interaction terms was tested using the likelihood-ratio test, and the absence of
256 any taxa responding to interaction term compared to the reduced model taken as
257 confirmation that no interaction occurred.

258 The impact of depth and treatment on DSE colonisation was modelled using a Bayesian
259 regression model with a zero-inflated beta distribution using the brms package, and with plot
260 identity as a random effect (Bürkner 2017). The impact of depth and treatment upon ErM
261 colonisation was modelled using the brms package as well, as a cumulative ordinal regression
262 model assuming the latent variable to be normally distributed (Bürkner and Vuorre 2019).
263 Plot identity was modelled as a group-level effect, and an interaction between depth and
264 treatment was assumed. The emmeans package was used to obtain contrasts between the
265 treatments given soil depth (Lenth 2021).

266 The relative impact of soil pH, EC, soil moisture and temperature on the soil microbial
267 community composition was established using multivariate hierarchical Bayesian regression
268 models within brms. Soil moisture and temperature were taken from in-situ sensors on the
269 day of sampling. Soil temperature is measured at 5 cm depth with a T107 sensor from
270 Campbell scientific. Soil moisture is measured as volumetric water content with CS616 sensors
271 from Campbell scientific. The numeric predictors (pH, temperature, moisture) were first
272 transformed by centring the mean at zero and dividing by twice the standard deviation so they

were on a similar scale to any binary predictor. All models had plot identity included as a grouping factor. Models were compared using leave-one-out cross validation to estimate pointwise out-of-sample prediction accuracy (Vehtari and others 2017). For the models predicting NMDS scores the models were fit with both NMDS scores as response variables simultaneously so that the residual correlation between the two could be estimated. Data from 2017 only was used within these models due to the absence of pH and EC values from 2003.

Results

Microbial diversity

There were 8818 unique sequences from a total of 4,514,220 reads returned by the 16S primer, of which 8673 were matched to bacteria and 138 to archaea. There were 2539 unique ITS sequences from a total of 5,711,663 reads, of which 2377 matched to fungi. The bacterial data was rarefied to 25000 reads, and the fungal to 10000 reads. There were 28 samples of the 93 that failed to amplify enough fungal DNA for inclusion in the analysis, of which 12 samples had 0 reads and 14 samples had less than 200 reads. Of the failed samples, 15 were from warming plots, compared to 5 and 8 from control and drought plots respectively (Supplementary Table 1). The median read depth of successfully amplified samples was 41110 for bacteria (Q1: 35623, Q3:50001), and 87494 for fungi (Q1: 43626, Q3: 112151).

While both bacterial and fungal richness showed trends with soil depth, sampling year and climate treatment our models showed no clear effects of treatment, depth or year once plot identity was incorporated (Figure 1). Fungal richness tended to be lower in the subsoil samples regardless of year and treatment (50% quantile of subsoil predictions lower than all middle zone and topsoil predictions), however there were no particular trends with treatment or year and all the estimates for every depth/year/treatment combination had their 95% quantile overlap. Bacterial richness within all depth/year/treatment combinations was similar, with the

298 50% quantiles overlapping in all cases. Interestingly, the group-level plot identity factor was
299 estimated to be much greater in the bacterial richness model compared to the fungal richness
300 model (45 compared to 4.6). The response of bacterial and fungal Shannon and inverse
301 Simpson diversity indices to depth, year and treatment followed the same patterns as richness
302 (supplementary figures 1 and 2). Overall, there was no clear response of microbial diversity to
303 climate treatments, soil depth or duration of treatment.

304 Microbial community composition

305 The microbial community composition was impacted by depth and treatment in both
306 sampling periods, with a clear separation by depth and some separation by treatment (Figure
307 2). The warming subsoil shows greater similarity to the topsoil than to the subsoil of the
308 control and drought plots. There was a significant effect of both soil depth and climate
309 treatment upon both bacterial and fungal composition in 2017, but no significant interaction
310 (Bacterial PERMANOVA: $R^2 = 0.287$, $p = 0.001$ (Depth), $p = 0.002$ (Treatment), $p = 0.08$
311 (Interaction); Fungal PERMANOVA: $R^2 = 0.259$, $p = 0.001$ (Depth), $p = 0.001$ (Treatment), $p =$
312 0.39 (Interaction)). The 2003 samples were largely clustered within the 2017 samples, with
313 less visible impact of depth and treatment. However, at the phylum level there was limited
314 change by depth, treatment or year for both bacteria and fungi (Supplementary figures 3 and
315 4). The majority of fungi were not able to be assigned to any trophic mode, likely related to
316 63% of our fungal taxa not being assigned to a genus, and there was limited evidence of
317 change in trophic modes to treatment (Supplementary figure 5).

318 *Network analysis*

319 The nature of the co-occurrence patterns across the microbial data is dependent on whether
320 the bacteria and fungi are considered together or separately. There were 161 bacterial taxa and
321 54 fungal taxa that appeared in over 50% of the samples, and were thus included in the
322 networks (Figure 3, supplementary figure 6). The network construction was run for bacteria

323 and fungi together, bacteria only and fungi only. The majority of the abundant microbial taxa
324 included within our network showed a distinct depth preference, but no effect of treatment
325 (supplementary figure 7). There were many links within the joint network that would not have
326 been found if bacteria and fungi were only considered separately (123 out of a total 428 links,
327 with another 283 within bacteria and 22 within fungi). Inter-kingdom links include four links
328 from a fungal ASV identified as Mortierella humilis to bacterial taxa and two from a fungal
329 ASV identified as Hyaloscypha fuckelii to bacterial taxa, both fungi being probable
330 saprotrophs and none of the bacterial taxa being identified to species level. The bacteria only
331 network had 312 edges and the fungi only network had 10 edges. The interactions between the
332 different microbial communities in our sites act across the kingdom boundaries and ignoring
333 across-kingdom interactions changes specific links and overall network stability
334 (Supplementary figure 6).

335 *Indicator taxa*

336 Analysis of the different relevant abundance of bacterial and fungal taxa in drought and
337 warming compared to control treatments revealed a small number of taxa that responded
338 strongly to the treatments, with no taxa responding differently to the treatment in the
339 different depths or years (table 1, supplementary figure 8). There were more bacterial taxa that
340 declined under the climate change treatments than increased, and over half of the taxa that
341 declined did so under both drought and warming. However, in the fungal communities while
342 the warming treatments caused a decline in more taxa than increased, there were far more
343 fungal taxa that increased under the drought treatment than decreased.

344 Root fungal colonisation

345 The climate treatments impacted overall root biomass and the change in mycorrhizal
346 colonisation of roots with depth as well as the overall microbial community composition.
347 There was a decrease in root biomass and number of root tips with depth (supplementary

348 figure 9). Drought and warming had only a limited effect upon the number of root tips and
349 fine root biomass once depth was accounted for (elpd of all models within standard errors of
350 each other), but did decrease the overall root biomass by ~50% and ~33% respectively (elpd
351 difference 3.7 ± 2.2). The rate of ericoid mycorrhizal colonisation also tended to decrease with
352 depth, with a decline in the number of 50-90% colonised sections and increase in the number
353 of 1-10% colonised sections in both control and drought plots (95% quantile of depth effect -
354 0.18 to 0.10 in control and -0.29 to 0.01 in drought). However, proportionally more roots were
355 colonised by dark septate endophytes at the lower depths. These changes in colonisation with
356 depth were not apparent in the warming plots (95% quantile included 0 for depth effect in
357 warming plots in both models), and overall warming had lower ErM colonisation rates than
358 control and drought (95% HPD -1.39 to -0.44 on probit scale, Figure 4), and higher levels of
359 DSE colonisation at intermediate depths (95% HPD 0.18 to 0.68 on log-odd scale,
360 supplementary figure 10). Overall, compared to the control plots the warming plots had lower
361 rates of fungal colonisation, while drought plots had higher rates of ErM colonisation in the
362 topsoil.

363 ## Impact of soil chemical properties on microbial communities

364 The change in pH and EC with depth was altered by the experimental treatment (Figure 5).
365 Under control conditions pH increases with depth (subsoil was 0.12 units higher than topsoil,
366 95% interval: 0.03 to 0.22), but within the drought and warming plots there was no change in
367 pH with depth (95% interval was -0.10 to 0.10 for drought and -0.16 to 0.03 for warming). EC
368 was lowest in the subsoil in the control and drought plots (control subsoil was 21.2 units
369 below topsoil, 95% interval: 8.6 to 33.0, drought was 31.6, 95% interval 14.1 to 48.1), however
370 in the warming plots there was less change in EC with depth (95% interval 1.4 to 34.9).
371 However, these differences in pH and EC with depth and treatment were limited; with the
372 addition of treatment to the model performing equivalently to the model with depth only

(elpd difference of adding treatment for pH +1.0 ± 3.0, for EC -0.7 ± 1.1). The subsoil of the warming plots had similar conditions to the topsoil throughout the site.

The models suggest that the impact of treatment upon fungal richness was fully mediated by the changes in soil physicochemical environment, while bacterial richness was less impacted by the change in soil properties. No models did particularly well at predicting the bacterial richness, but the best model for investigating bacterial richness had solely climate treatment and soil depth as predictors (Bayes R^2 = 0.227 ± 0.071), with three other equivalent models by elpd (taking standard errors into account) adding subsets of pH, EC, moisture and temperature to the predictors. Bacterial richness was lowest in the intermediate depth and highest in the drought plots. Fungal richness was impacted by the physical properties of the site, with the best model including pH, EC, soil depth, temperature and moisture as predictors (Bayes R^2 = 0.651). The model with only climate treatment and soil depth as predictors was notably worse than all the other models (elpd difference of 3.4 compared to <1 for all other models). Fungal richness decreased with depth and increased with EC and pH. There was also evidence for decreasing richness with moisture and increasing with temperature.

The microbial community composition was still impacted by the treatment after accounting for changes in soil temperature, moisture, pH and EC. The best model had treatment, pH and EC as predictors of bacterial and fungal composition (Figure 6). The relative impact of pH on bacterial composition was higher than that of EC, while the reverse was true for fungal composition. The treatments have resulted in changes in the soil physicochemical structure which have impacted the microbial community composition, but the impact of treatment is only partially mediated by the measured changes in soil chemistry.

396 # Discussion

397 ## Microbial community response to warming and drought

398 Our results show that fungal taxa are more responsive to drought and warming than bacterial
399 taxa, with warming altering soil biological and chemical properties throughout the soil profile.
400 We found that these microbial community responses were dependent upon the duration of
401 treatment, in agreement with our hypothesis. While we found limited evidence of treatment
402 effects after 4 years, previous work on this site after a similar period of time found that
403 drought treatment effects upon fungal communities were limited to the summer treatment
404 period, and our sampling occurred outside this period in the winter season (Toberman and
405 others 2008). Our results indicate that it takes longer than 4 years to develop legacy effects of
406 climate change treatments, and based on comparison to previous studies of microbial growth
407 and biomass at our site this legacy effect may not have been apparent after 13 years of
408 treatment (Rousk and others 2013). Our observed impacts of climate change are at least
409 partially moderated by changes in the soil chemical environment which are likely driven by
410 alterations in soil hydrology and the plant community at the site in response to treatment
411 (Domínguez and others 2015; Robinson and others 2016). The importance of the soil
412 physicochemical properties in mediating climate change impacts upon the microbial
413 community is in agreement with results from other climate change experiments (Deltedesco
414 and others 2020). We have observed changes in the fungal community using both DNA
415 sequencing of the soil and microscopic examination of the mycorrhizal colonisation of
416 *C. vulgaris* roots. This indicates that changes in the bulk soil microbial community reflect
417 changes in the functional capability of the soil microbiome to interact with plants which could
418 alter biogeochemical cycling (Read and Perez-Moreno 2003; Tedersoo and Bahram 2019).

419 Previous studies on the microbial response to long term climate change have largely focused
420 on measuring the microbial biomass response, which is highly dependent on the soil type and

421 climate conditions (Ren and others 2018; Zhou and others 2018). Drought and warming have
422 been found to impact microbial composition more strongly and more persistently than
423 microbial richness in both short and long-term experiments (Sayer and others 2017; Tóth and
424 others 2017; de Vries and others 2018; Yu and others 2018; Birnbaum and others 2019). This is
425 consistent with our finding that both bacterial and fungal richness show limited response to
426 climate change treatments across soil depths and regardless of duration of treatment. Changes
427 in composition in response to long-term drought have been shown in some cases to be driven
428 by changes in the rarer taxa in a calcareous grassland (Sayer and others 2017). This supports
429 our finding that the dominant taxa show limited response to treatment in contrast to their
430 strong depth preference, as demonstrated by the qualitative description of the taxa in the
431 network analysis by colouring according to either depth or treatment preference.

432 Soil depth

433 A variety of observational studies have shown a distinct difference in microbial composition
434 with depth, consistent with our results (Griffiths and others 2003; Serkebaeva and others
435 2013; Delgado-Baquerizo and others 2017; Seuradge and others 2017). The subsoil is generally
436 less biologically active and shows less seasonal variation in its physical conditions and
437 microbial communities (Griffiths and others 2003). Therefore it is no surprise that many
438 studies have found that the topsoil is more strongly linked to the aboveground land use and
439 plant productivity (Delgado-Baquerizo and others 2017; Seuradge and others 2017). However,
440 our results suggest that the impact of climate change can penetrate deeper into the soil,
441 impacting soil physicochemical properties, plant-soil interactions and microbial processes.
442 This change in the stratification with depth of physicochemical properties and microbial
443 communities could be linked to the distinct change in hydrological behaviour and roots over
444 the course of the experiment (Robinson and others 2016). We found that the overall root
445 biomass decreased under drought and warming by around a half and a third respectively in
446 the top soil layers while fine root biomass did not, which agrees with previous results at this

site that showed increased proportion of fine root biomass under drought (Robinson and others 2016). This shift in root allocation away from thicker roots to finer roots could potentially be related to a reduction in the structural strength of the soil or changes in the hydrology such that thicker roots are less important for soil penetration and water uptake while finer roots are maintained for nutrient absorption (Hodge and others 2009). Our soils are admittedly shallow and the changes with depth we have observed are over relatively short distances (Sowerby and others 2008). However, the results still hold relevance for a geographically extensive soil type and are likely to occur in many deeper soil types.

The change in stratification with depth of the soil physical, chemical and biological properties in response to warming may be related to aboveground changes in plant communities, particularly the increased cover of moss in the warming plots (Domínguez and others 2015). Moss acts as a layer of insulation on the soil surface, buffering changes in soil temperature and moisture (Turetsky and others 2012). Mosses have been found to reduce evapotranspiration, increase surface infiltration and influence the partitioning of heat fluxes (Beringer and others 2001; Blok and others 2011). The thermal and hydrological influences of moss cover can lead to differences in belowground soil microbial communities, changing their biomass and activity (Gornall and others 2007; Benavent-González and others 2018), with relevance to global biogeochemical cycling (Porada and others 2014). The insulative properties of moss could be reducing the magnitude of daily and seasonal heat and moisture fluctuations at the soil surface, therefore reducing subtle temperature and hydrological differences between the topsoil and subsoil (Turetsky and others 2012). However, our results indicate that the observed changes are occurring in the subsoil, rather than in the topsoil, which could mean that the changes in water infiltration and heat fluxes are leading to increasing penetration of water and translocation of chemicals within the soil profile.

Evaluating the concept of a bacterial vs a fungal response

The community composition response to drought and warming we have found shows some differences between bacterial and fungal taxa. However the presence of co-occurrence links between specific bacteria and fungi suggest that caution should be used in interpreting these results as a bacterial response vs a fungal response. While the overall abundance and dominance of bacteria and fungi may change in response to climate change this obscures changes in fine-scale dynamics which could be creating specific drought and warming communities that are composed of a combination of both bacteria and fungi. We do find that more fungal taxa respond positively to drought and negatively to warming which indicates that the drought microbial community could consist of relatively more fungi. This is consistent with previous results showing higher tolerance of fungi to drought (Haugwitz and others 2014). Neglecting the inter-Kingdom links could lead to erroneous assumptions about microbial community stability, as suggested by work in the human microbiome (Tipton and others 2018). It is possible that the correlations between bacteria and fungi are driven solely by changes in environmental conditions and do not represent a true microbial interaction, as these co-occurrence networks are prone to this kind of error (Carr and others 2019). However this result does offer an intriguing avenue for future work in establishing whether specific inter-Kingdom interactions such as those we have identified between fungal saprotrophs and bacterial taxa exist and can influence microbial community structure and activity in soils.

Fungal root colonisation

Our results from examination of the fungal root colonisation are in agreement with previous results that have indicated that ErM and DSE colonisation respond differently to experimental warming (Olsrud and others 2009; Binet and others 2017). However, the treatment responses we found differed from previous results, with Olsrud and others (2009) finding no effect of warming on ErM colonisation after 6 years of treatment in a subarctic birch forest. Other

496 results from a Danish heathland with similar experimental set-up to our site on a sandy soil
497 found that ErM colonisation was lower in the warming treatment at 5-10 cm depth compared
498 to drought and control, and that DSE was lower at depth in the drought treatments (Arndal
499 and others 2013). Overall, we found lower ErM colonisation in response to warming, however
500 we found higher ErM colonisation in response to drought which is different from Arndal and
501 others results. These contrasting results could reflect the difference in duration of treatment,
502 differences in soil type and drainage behaviour, or potentially the differences in the plant
503 response to treatment. The implications of shifts in mycorrhizal type for plant community
504 resilience and biogeochemical cycling in response to warming are unclear due to the lack of
505 knowledge on the relative role of DSE versus ErM colonisation upon plant nutrient uptake and
506 stress resilience (Newsham and others 2009). However, there are some suggestions that DSE
507 colonisation may enable the uptake of organic nitrogen compounds and improve resilience to
508 certain stressors (Newsham 2011; Hill and others 2019).

509 Implications for ecosystem function

510 Our analysis has revealed that microbial community shifts in response to climate change
511 occur over decades, not just years, which could have continued, long-term effects upon
512 ecosystem functions. While we have identified clear shifts in some functions – i.e. mycorrhizal
513 colonisation – it is unclear what impact other changes in microbial community composition
514 could have upon ecosystem function and stress resilience. One potential ecosystem function
515 of clear importance to both global carbon cycling and local ecosystem health is soil
516 respiration, and microbial communities are known to be particularly important in
517 determining soil respiration (Davidson and Janssens 2006). Our site has previously been
518 shown to have experienced increases in respiration in response to drought and warming,
519 which could be related to the microbial community composition and fungal root colonisation
520 changes described here (Reinsch and others 2017). It is important to consider that changes in
521 ecosystem function need not be driven by changes in overall microbial biomass or dominant

522 taxa, as rare taxa can be disproportionately important to biogeochemical cycles (Jousset and
523 others 2017). Therefore, the small number of specific taxa we have identified as showing
524 differences between the climate treatments could be directly influencing ecosystem functions,
525 as well as potentially driving shifts in microbial community network connectivity with
526 associated impacts on ecosystem functioning (Wagg and others 2019). The changes we have
527 observed are persisting over the winter season when treatments are not in effect, indicating
528 the possibility of legacy effects of climate change on ecosystem functions mediated by changes
529 in microbial communities.

530 Conclusions

531 We have found that bacterial and fungal community composition and diversity is altered by
532 long-term drought and warming treatments. The impact of simulated climate change is
533 greater after eighteen years of treatment compared to four years. The shift in bulk soil
534 community composition is also reflected by a shift in fungal root colonisation of the dominant
535 *Calluna vulgaris* plant species. These changes are at least partially driven by changes in the
536 distribution of roots throughout the soil profile and changes in the soil chemical environment.
537 In general, fungal taxa appear to be more responsive to the climate change treatments than
538 bacterial taxa but the presence of cross-domain co-occurrence relationships and sensitive taxa
539 in both domains cautions against interpreting our results as a fungal vs a bacterial response.
540 These insights into soil microbial community response to drought and warming can inform
541 our understanding of what drives differences in soil functional response to climate change.

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References

Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, Martiny JBH. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94:714–25.

Amend AS, Martiny AC, Allison SD, Berlemont R, Goulden ML, Lu Y, Treseder KK, Weihe C, Martiny JBH. 2016. Microbial response to simulated global change is phylogenetically conserved and linked with functional potential. ISME J 10:109–18.

Andresen LC, Müller C, de Dato G, Dukes JS, Emmett BA, Estiarte M, Jentsch A, Kröel-Dulay G, Lüscher A, Niu S, Peñuelas J, Reich PB, Reinsch S, Ogaya R, Schmidt IK, Schneider MK, Sternberg M, Tietema A, Zhu K, Bilton MC. 2016. Shifting impacts of climate change: Long-term patterns of plant response to elevated CO2, drought, and warming across ecosystems. Adv Ecol Res 55:437–73.

Arndal MF, Merrild MP, Michelsen A, Schmidt IK, Mikkelsen TN, Beier C. 2013. Net root growth and nutrient acquisition in response to predicted climate change in two contrasting heathland species. Plant Soil 369:615–29.

Batjes NH. 1996. Total carbon and nitrogen in the soils of the world. Eur J Soil Sci 47:151–63.

Beier C, Emmett B, Gundersen P, Tietema A, Peñuelas J, Estiarte M, Gordon C, Gorissen A, Llorens L, Roda F, Williams D. 2004. Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming.

Ecosystems 7:583–97.

Benavent-González A, Delgado-Baquerizo M, Fernández-Brun L, Singh BK, Maestre FT, Sancho LG. 2018. Identity of plant, lichen and moss species connects with microbial abundance and soil functioning in maritime Antarctica. Plant Soil 429:35–52.

Beringer J, Lynch AH, Chapin FSI, Mack M, Bonan GB. 2001. The representation of arctic soils in the land surface model: the importance of mosses. J Clim 14:3324–35.

Binet P, Rouifed S, Jassey VEJ, Toussaint M-L, Chiapusio G. 2017. Experimental climate warming alters the relationship between fungal root symbiosis and Sphagnum litter phenolics in two peatland microhabitats. Soil Biol Biochem 105:153–61.

Birnbaum C, Hopkins AJM, Fontaine JB, Enright NJ. 2019. Soil fungal responses to experimental warming and drying in a Mediterranean shrubland. Sci Total Environ 683:524–36.

Blok D, Heijmans MMPD, Schaepman-Strub G, van Ruijven J, Parmentier FJW, Maximov TC, Berendse F. 2011. The cooling capacity of mosses: controls on water and energy fluxes in a Siberian tundra site. Ecosystems 14:1055–65.

Bossio DA, Cook-Patton SC, Ellis PW, Fargione J, Sanderman J, Smith P, Wood S, Zomer RJ, von Unger M, Emmer IM, Griscom BW. 2020. The role of soil carbon in natural climate solutions. Nat Sustain 3:391–8.

Bradford MA, Wieder WR, Bonan GB, Fierer N, Raymond PA, Crowther TW. 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nat Clim Chang 6:751–8.

Bürkner P-C. 2017. brms: An R package for Bayesian multilevel models using Stan. J Stat Softw 80:1–28.

Bürkner P-C, Vuorre M. 2019. Ordinal regression models in psychology: a tutorial. Adv Methods Pract Psychol Sci 2:77–101.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–3.

Carr A, Diener C, Baliga NS, Gibbons SM. 2019. Use and abuse of correlation analyses in microbial ecology. ISME J 13:2647–55.

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins

604 DA, Jansson JK, Karl DM, Koskella B, Mark Welch DB, Martiny JBH, Moran MA, Orphan
605 VJ, Reay DS, Remais J V., Rich VI, Singh BK, Stein LY, Stewart FJ, Sullivan MB, van Oppen
606 MJH, Weaver SC, Webb EA, Webster NS. 2019. Scientists' warning to humanity:
607 microorganisms and climate change. Nat Rev Microbiol 17:569–86.

608 Chen J, Luo Y, Xia J, Jiang L, Zhou X, Lu M, Liang J, Shi Z, Shelton S, Cao J. 2015. Stronger
609 warming effects on microbial abundances in colder regions. Sci Rep 5:18032.

610 Crowther T, Todd-Brown K, Rowe C, Wieder W, Carey J, Machmuller M, Snoek L, Fang S,
611 Zhou G, Allison S, Blair J, Bridgham S, Burton A, Carrillo Y, Reich P, Clark J, Classen A,
612 Dijkstra F, Elberling B, Emmett B, Estiarte M, Frey S, Guo J, Harte J, Jiang L, Johnson B,
613 Kröel-Dulay G, Larsen K, Laudon H, Lavallee J, Luo Y, Lupascu M, Ma L, Marhan S,
614 Michelsen A, Mohan J, Niu S, Pendall E, Penuelas J, Pfeifer-Meister L, Poll C, Reinsch S,
615 Reynolds L, Schmidth I, Sistla S, Sokol N, Templer P, Treseder K, Welker J, Bradford M.
616 2016. Quantifying global soil C losses in response to warming. Nature 540:104–8.

617 Csardi G, Nepusz T. 2006. The igraph software package for complex network research.
618 Interjournal Complex Sy:1695.

619 Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and
620 feedbacks to climate change. Nature 440:165–73.

621 Delgado-Baquerizo M, Powell JR, Hamonts K, Reith F, Mele P, Brown M V., Dennis PG, Ferrari
622 BC, Fitzgerald A, Young A, Singh BK, Bissett A. 2017. Circular linkages between soil
623 biodiversity, fertility and plant productivity are limited to topsoil at the continental scale.
624 New Phytol 215:1186–96.

625 Deltedesco E, Keiblinger KM, Piepho H-P, Antonielli L, Pötsch EM, Zechmeister-Boltenstern
626 S, Gorfer M. 2020. Soil microbial community structure and function mainly respond to
627 indirect effects in a multifactorial climate manipulation experiment. Soil Biol Biochem
628 142:107704.

629 DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P,
630 Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and
631 workbench compatible with ARB. Appl Environ Microbiol 72:5069–72.

632 Domínguez MT, Holthof E, Smith AR, Koller E, Emmett BA. 2017a. Contrasting response of
633 summer soil respiration and enzyme activities to long-term warming and drought in a
634 wet shrubland (NE Wales, UK). Appl Soil Ecol 110:151–5.

Domínguez MT, Smith AR, Reinsch S, Emmett BA. 2017b. Inter-annual variability of soil respiration in wet shrublands: do plants modulate its sensitivity to climate? Ecosystems 20:796–812.

Domínguez MT, Sowerby A, Smith AR, Robinson DA, Van Baarsel S, Mills RTE, Marshall MR, Koller E, Lebron I, Hall J, Emmett BA. 2015. Sustained impact of drought on wet shrublands mediated by soil physical changes. Biogeochemistry 122:151–63.

Fridley JD, Grime JP, Askew AP, Moser B, Stevens CJ. 2011. Soil heterogeneity buffers community response to climate change in species-rich grassland. Glob Chang Biol 17:2002–11.

García-Palacios P, Vandegehuchte ML, Shaw EA, Dam M, Post KH, Ramirez KS, Sylvain ZA, de Tomasel CM, Wall DH. 2015. Are there links between responses of soil microbes and ecosystem functioning to elevated CO2, N deposition and warming? A global perspective. Glob Chang Biol 21:1590–600.

Gornall JL, Jónsdóttir IS, Woodin SJ, Van der Wal R. 2007. Arctic mosses govern below-ground environment and ecosystem processes. Oecologia 153:931–41.

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. 2003. Influence of depth and sampling time on bacterial community structure in an upland grassland soil. FEMS Microbiol Ecol 46:35–43.

Haugwitz MS, Bergmark L, Priemé A, Christensen S, Beier C, Michelsen A. 2014. Soil microorganisms respond to five years of climate change manipulations and elevated atmospheric CO2 in a temperate heath ecosystem. Plant Soil 374:211–22.

Hill PW, Broughton R, Bougoure J, Havelange W, Newsham KK, Grant H, Murphy D V., Clode P, Ramayah S, Marsden KA, Quilliam RS, Roberts P, Brown C, Read DJ, Deluca TH, Bardgett RD, Hopkins DW, Jones DL. 2019. Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. Knops J, editor. Ecol Lett 22:2111–9.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M. 2009. Plant root growth, architecture and function. Plant Soil 321:153–87.

Holmstrup M, Sørensen JG, Schmidt IK, Nielsen PL, Mason S, Tietema A, Smith AR, Bataillon T, Beier C, Ehlers BK. 2013. Soil microarthropods are only weakly impacted after 13 years of repeated drought treatment in wet and dry heathland soils. Soil Biol Biochem 66:110–8.

666 Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid
667 J, Brandström-Durling M, Clemmensen KE, Lindahl BD. 2012. New primers to amplify
668 the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural
669 communities. FEMS Microbiol Ecol 82:666–77.

670 Jansson JK, Hofmockel KS. 2020. Soil microbiomes and climate change. Nat Rev Microbiol
671 18:35–46.

672 Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, Küsel K, Rillig MC, Rivett
673 DW, Salles JF, van der Heijden MGA, Youssef NH, Zhang X, Wei Z, Hol WHG. 2017.
674 Where less may be more: how the rare biosphere pulls ecosystems strings. ISME J 11:853–
675 62.

676 Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S,
677 Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T.
678 2005. UNITE: a database providing web-based methods for the molecular identification
679 of ectomycorrhizal fungi. New Phytol 166:1063–8.

680 Kröel-Dulay G, Ransijn J, Schmidt IK, Beier C, De Angelis P, de Dato G, Dukes JS, Emmett B,
681 Estiarte M, Garadnai J, Kongstad J, Kovács-Láng E, Larsen KS, Liberati D, Ogaya R, Riis-
682 Nielsen T, Smith AR, Sowerby A, Tietema A, Penuelas J. 2015. Increased sensitivity to
683 climate change in disturbed ecosystems. Nat Commun 6:6682.

684 Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. 2015. Sparse and
685 compositionally robust inference of microbial ecological networks. PLoS Comput Biol
686 11:1–25.

687 Lenth R. 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package
688 version 1.6.2-1. https://CRAN.R-project.org/package=emmeans

689 Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for
690 RNA-seq data with DESeq2. Genome Biol 15:550.

691 Martiny JB, Martiny AC, Weihe C, Lu Y, Berlemont R, Brodie EL, Goulden ML, Treseder KK,
692 Allison SD. 2017. Microbial legacies alter decomposition in response to simulated global
693 change. ISME J 11:490–9.

694 McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL,
695 Knight R, Hugenholtz P. 2012. An improved Greengenes taxonomy with explicit ranks for
696 ecological and evolutionary analyses of bacteria and archaea. ISME J 6:610–8.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115:495–501.

Newsham KK. 2011. A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–93.

Newsham KK, Upson R, Read DJ. 2009. Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2:10–20.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–8.

O. Abbasi A, Salazar A, Oh Y, Reinsch S, Del Rosario Uribe M, Li J, Rashid I, S. Dukes J. 2020. Reviews and syntheses: Soil responses to manipulated precipitation changes - An assessment of meta-analyses. Biogeosciences 17:3859–73.

Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Oliverio AM, Bradford MA, Fierer N. 2016. Identifying the microbial taxa which consistently respond to soil warming across time and space. Glob Chang Biol:1–13.

Olsrud M, Carlsson BÅ, Svensson BM, Michelsen A, Melillo JM. 2009. Responses of fungal root colonization, plant cover and leaf nutrients to long-term exposure to elevated atmospheric CO2 and warming in a subarctic birch forest understory. Glob Chang Biol 16:1820–9.

Petersen H. 2011. Collembolan communities in shrublands along climatic gradients in Europe and the effects of experimental warming and drought on population density, biomass and diversity. Soil Org 83:463–88.

Porada P, Weber B, Elbert W, Pöschl U, Kleidon A. 2014. Estimating impacts of lichens and bryophytes on global biogeochemical cycles. Global Biogeochem Cycles 28:71–85.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey

727 towards relevance? New Phytol 157:475–92.

728 Reinsch S, Koller E, Sowerby A, de Dato G, Estiarte M, Guidolotti G, Kovács-Láng E, Kröel-
729 Dulay G, Lellei-Kovács E, Larsen KS, Liberati D, Peñuelas J, Ransijn J, Robinson DA,
730 Schmidt IK, Smith AR, Tietema A, Dukes JS, Beier C, Emmett BA. 2017. Shrubland
731 primary production and soil respiration diverge along European climate gradient. Sci Rep
732 7:43952.

733 Ren C, Chen J, Lu X, Doughty R, Zhao F, Zhong Z, Han X, Yang G, Feng Y, Ren G. 2018.
734 Responses of soil total microbial biomass and community compositions to rainfall
735 reductions. Soil Biol Biochem 116:4–10.

736 Ren C, Zhao F, Shi Z, Chen J, Han X, Yang G, Feng Y, Ren G. 2017. Differential responses of soil
737 microbial biomass and carbon-degrading enzyme activities to altered precipitation. Soil
738 Biol Biochem 115:1–10.

739 Robinson DA, Hopmans JW, Filipovic V, van der Ploeg M, Lebron I, Jones SB, Reinsch S, Jarvis
740 N, Tuller M. 2019. Global environmental changes impact soil hydraulic functions through
741 biophysical feedbacks. Glob Chang Biol 25:1895–904.

742 Robinson DA, Jones SB, Lebron I, Reinsch S, Domínguez MT, Smith AR, Jones DL, Marshall
743 MR, Emmett BA. 2016. Experimental evidence for drought induced alternative stable
744 states of soil moisture. Sci Rep 6:20018.

745 Rousk J, Smith AR, Jones DL. 2013. Investigating the long-term legacy of drought and warming
746 on the soil microbial community across five European shrubland ecosystems. Glob Chang
747 Biol 19:3872–84.

748 Rudgers JA, Kivlin SN, Whitney KD, Price M V., Waser NM, Harte J. 2014. Responses of high-
749 altitude graminoids and soil fungi to 20 years of experimental warming. Ecology 95:1918–
750 28.

751 Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman
752 NJ, Walker AW. 2014. Reagent and laboratory contamination can critically impact
753 sequence-based microbiome analyses. BMC Biol 12:1–12.

754 Santonja M, Fernandez C, Proffit M, Gers C, Gauquelin T, Reiter IM, Cramer W, Baldy V. 2017.
755 Plant litter mixture partly mitigates the negative effects of extended drought on soil biota
756 and litter decomposition in a Mediterranean oak forest. J Ecol 105:801–15.

757 Sayer EJ, Oliver AE, Fridley JD, Askew AP, Mills RTE, Grime JP. 2017. Links between soil

758 microbial communities and plant traits in a species-rich grassland under long-term
759 climate change. Ecol Evol 7:855–62.

760 Serkebaeva YM, Kim Y, Liesack W, Dedysh SN. 2013. Pyrosequencing-based assessment of the
761 bacteria diversity in surface and subsurface peat layers of a northern wetland, with focus
762 on poorly studied phyla and candidate divisions. Neufeld J, editor. PLoS One 8:e63994.

763 Seuradge BJ, Oelbermann M, Neufeld JD. 2017. Depth-dependent influence of different land-
764 use systems on bacterial biogeography. Baldrian P, editor. FEMS Microbiol Ecol
765 93:fiw239.

766 Sowerby A, Emmett BA, Tietema A, Beier C. 2008. Contrasting effects of repeated summer
767 drought on soil carbon efflux in hydric and mesic heathland soils. Glob Chang Biol
768 14:2388–404.

769 Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V,
770 Midgley PM, editors. 2013. Climate Change 2013: The Physical Science Basis.
771 Contribution of Working Group I to the Fifth Assessment Report of the
772 Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New
773 York, NY, USA: Cambridge University Press

774 Tedersoo L, Bahram M. 2019. Mycorrhizal types differ in ecophysiology and alter plant
775 nutrition and soil processes. Biol Rev 94:1857–80.

776 Tipton L, Müller CL, Kurtz ZD, Huang L, Kleerup E, Morris A, Bonneau R, Ghedin E. 2018.
777 Fungi stabilize connectivity in the lung and skin microbial ecosystems. Microbiome 6:12.

778 Toberman H, Freeman C, Evans C, Fenner N, Artz RRE. 2008. Summer drought decreases soil
779 fungal diversity and associated phenol oxidase activity in upland Calluna heathland soil.
780 FEMS Microbiol Ecol 66:426–36.

781 Tóth Z, Táncsics A, Kriszt B, Kröel-Dulay G, Ónodi G, Hornung E. 2017. Extreme effects of
782 drought on composition of the soil bacterial community and decomposition of plant
783 tissue. Eur J Soil Sci:504–13.

784 Trouvelet A, Kough JL, Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un
785 système radiculaire. Recherche de méthodes d'estimation ayant une signification
786 fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S, editors. Physiological and
787 Genetical Aspects of Mycorrhizae. INRA Press, Paris. pp 217–21.

788 Turetsky MR, Bond-Lamberty B, Euskirchen E, Talbot J, Frolking S, McGuire AD, Tuittila E-S.

789 2012. The resilience and functional role of moss in boreal and arctic ecosystems. New
790 Phytol 196:49–67.

791 Vehtari A, Gelman A, Gabry J. 2017. Practical Bayesian model evaluation using leave-one-out
792 cross-validation and WAIC. Stat Comput 27:1413–32.

793 Vierheilig H, Coughlan AP, Wyss U, Piche Y. 1998. Ink and vinegar, a simple staining
794 technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–7.

795 de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A,
796 Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason KE, Oliver A, Ostle N, Prosser
797 JI, Thion C, Thomson B, Bardgett RD. 2018. Soil bacterial networks are less stable under
798 drought than fungal networks. Nat Commun 9:3033.

799 Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA. 2019. Fungal-bacterial
800 diversity and microbiome complexity predict ecosystem functioning. Nat Commun
801 10:4841.

802 Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA,
803 Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R. 2016. Improved bacterial 16S
804 rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for
805 microbial community surveys. mSystems 1:e00009-15.

806 White N, Seaton FM, Reinsch S, Smith AR, Brooks MR, Emmett BA. 2019. Calluna vulgaris
807 root length and fungal colonisation data from the Climoor long-term climate change
808 experiment in Clocaenog forest, UK (2015).

809 Wickham H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

810 Yu H, Ma Q, Liu X, Xu Z, Zhou G, Shi Y. 2018. Short- and long-term warming alters soil
811 microbial community and relates to soil traits. Appl Soil Ecol 131:22–8.

812 Zhou Z, Wang C, Luo Y. 2018. Response of soil microbial communities to altered precipitation:
813 a global synthesis. Glob Ecol Biogeogr 27:1121–36.

814 Zhu A, Ibrahim JG, Love MI. 2019. Heavy-tailed prior distributions for sequence count data:
815 removing the noise and preserving large differences. Bioinformatics 35:2084–92.

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818 # Tables

819 Table 1: Number of taxa that responded to warming or drought

		Warming	Drought	Both
Bacteria (n=4936)	Increase	104 (2.2%)	213 (4.4%)	48
	Decrease	320 (6.7%)	295 (6.1%)	189
Fungi (n=1704)	Increase	99 (5.8%)	167 (9.8%)	42
	Decrease	169 (9.9%)	99 (5.8%)	46

820

821 The number of taxa (percentage of all taxa) that responded to warming, drought or both

822 treatments compared to control at a $p < 0.1$ significance level.

823

824 # Figures legends

825 Figure 1: Change in bacterial richness per 25000 rarefied reads (a) and fungal richness per
826 10000 rarefied reads (b) with soil depth (topsoil, subsoil), year (2003, 2017) and treatment
827 (Control, Drought, and Warming). The Bayesian hierarchical model with plot identity as a
828 group-level effect found that the 50% quantiles for every treatment/year/depth combination
829 overlapped for bacteria (a), and although the subsoil fungal diversity tended to be lower than
830 the topsoil diversity the 95% quantiles for every treatment/year/depth combination
831 overlapped for fungi (b).

832 Figure 2: NMDS of the bacterial (top) and fungal communities (bottom) for 2003 (left) and
833 2017 (right). Stress was 0.142 for the fungal and 0.127 for the bacterial NMDS. Note that
834 changes by treatment are more evident for the 2017 samples than the 2003. Centroids of each
835 treatment and soil depth combination are represented by larger circles, with lines from this
836 centroid to every sample in this grouping, represented by the smaller circles. For the 2017 data
837 only there were significant effects of depth and treatment: Bacteria: $R^2 = 0.219$, $p = 0.001$
838 (Depth), $p = 0.002$ (Treatment), $p = 0.12$ (Interaction); Fungi: $R^2 = 0.186$, $p = 0.001$ (Depth), p
839 $= 0.002$ (Treatment), $p = 0.38$ (Interaction).

840 Figure 3: Microbial co-occurrence networks for bacteria and fungi together (A), bacteria only
841 (B) and fungi only (C). Black links are positive, grey negative connections. The nodes are
842 clustered together according to the Fruchterman Reingold algorithm (for graphical simplicity
843 the fungal layout is based on the unweighted edges). Nodes are coloured by their preference
844 for different depths: green are topsoil specialists, red subsoil specialists and blue transition
845 zone specialists. Data from 2017 only.

846 Figure 4: Changes in proportion of each ErM colonisation category with depth. Note that there
847 is overall lower colonisation in the warming treatment compared to control at intermediate
848 depths (-0.94 on probit scale, 95% HPD -1.39 to -0.44) and higher in the drought treatment

(0.157, 95% HPD -0.33 to 0.64). There is also limited change with depth within the warming treatment, a slight decrease in the high proportional colonisation categories in the control treatment and a larger change within the drought plots.

Figure 5: The change in pH and EC with soil depth (T = Topsoil, M = intermediate, S = Subsoil) and climate treatments in 2017. pH was higher in the subsoil compared to topsoil in control plots (0.12, 95% CI 0.03-0.22), but drought showed no difference (0.00, 95% CI -0.10-0.10) and pH in warming plots tended to decrease with depth (-0.07, 95% CI -0.16-0.03). EC increased with depth in all three treatments (95% CI: control -8 to -33; drought -14 to -48; warming -1 to -35).

Figure 6: The parameter estimates for the impact of soil depth, EC, pH and climate treatment on bacterial (top row) and fungal (bottom row) NMDS scores. The circles are the mean estimate, the thick bars the standard error and the thin bars the 90% CI. Data presented is from 2017 only. The impact of depth is represented as the impact of the subsoil (DepthsS) and topsoil (DepthT) relative to the transition zone, and the impact of treatment represented relative to control.