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Long-term warming and vegetation change have no impact on microbial resistance to drought, but destabilise microbial communities and microbially-mediated functions

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ABSTRACT

Climate change presents multiple stresses to ecosystems that operate over different timescales, such as long-term warming and short-term drought. It is well established that soil microbial communities are highly responsive to individual stresses, but how they respond combined warming and drought, and how factors such as vegetation change moderate responses, remains uncertain. Here we tested whether long-term passive warming modifies the resistance (amplitude of response) and resilience (degree and duration of recovery) of soil microbial communities to short-term drought. We also tested whether warming effects on microbial resilience to drought are moderated by vegetation composition, and specifically the presence of ericaceous dwarf shrubs, the dominant vegetation type of peatland. This was tested using soil from a nine-year warming and vegetation manipulation experiment established on blanket peatland in northern England. We completed a subsequent laboratory study designed to quantify resistance and resilience of microbial communities and microbial-mediated functions to drought. Neither long-term warming nor shrub removal impacted the resistance of microbial communities to drought. However, resilience of bacterial diversity to drought was decreased by warming (fold change 0.38) and shrub removal (fold change 0.27). Notably the interaction between warming and shrub removal resulted in higher resilience of bacterial diversity than individual treatments (fold change 0.58; warming x shrub removal: $p = 0.008$). Further, warming and shrub removal individually increased the diversity of fungal communities, and reduced resilience of fungal diversity to drought (fold change of warmed against unwarmed 0.11, shrub removal against control 0.39, combination against control 0.59; warming x shrub removal $p = 0.006$). Warming also strongly decreased resilience, but not resistance, of nitrogen-based functions to drought, although shrub removal dampened this effect. Our findings demonstrate potential for long-term warming and vegetation change to modify microbial responses to extreme drought events, with implications for peatland carbon and nitrogen cycling under future climate scenarios.

1. Introduction

Soil microbial communities are increasingly challenged by perturbations associated with anthropogenic environmental change, including abrupt and intense ‘pulse’ perturbations caused by climate extremes (e.g., drought), which are becoming more frequent and intense with climate change (Chiang et al., 2021). However, important uncertainties exist regarding the factors that alter the ability of soil microbial communities and the biogeochemical cycles they drive to resist and recover from drought (Bardgett and Caruso, 2020). It remains unknown, for example, how other climate change factors that are known to cause shifts in the structure and functioning of microbial communities, such as long-term warming (Melillo et al., 2017; Walker et al., 2018; Soong et al., 2021; Chen et al., 2024), alter the ability of soil microbial

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communities to resist and recover from drought. Moreover, uncertainties exist over how microbial communities' responses to drought are moderated by factors such as vegetation structure, which is also responsive to climate and land use change. Indeed, past studies have shown vegetation structure to be an important moderator of the effects or warming on soil microbial communities and their functioning (Ward et al., 2013). But how warming modifies microbial resistance (i.e., the degree to which microbial communities change in response to a perturbation) and resilience (i.e., the rate at which they recover) to drought remains unknown. Consequently, there have been growing calls for studies that consider impacts of multifactor perturbations on soil microbial communities to better understand the range of responses and implications for ecosystem functions such as greenhouse gas emissions and carbon storage (Simon et al., 2020; Bardgett, 2025; Ferguson and Lindo, 2025).

Peatlands in northern latitudes consist of organic soils that are protected from decomposition through cold, waterlogged conditions, and high inputs of phenolic compounds from vegetation that constrain microbial activity (Ward et al., 2015). However, this balance is changing due to rising temperatures and changes in vegetation composition resulting from both land use and climate change (Fry et al., 2023). These combined stresses could have unpredictable and long-lasting impacts on the microbial community and ecosystem functions. Warming, for example, is known to cause shifts in both microbial physiology and community composition due to higher excitation energy for microbial cells, and increased substrate availability due to more effective enzyme binding (Seneca et al., 2021). This may result in higher activity, which can be observed as increased synthesis of extracellular enzymes, and higher rates of nutrient cycling and greenhouse gas emissions (Meng et al., 2020). Studies have also shown, however, that long term warming does not always result in acclimation, meaning that these rates will remain high as long as warming continues (Walker et al., 2018). Further, changes in microbial community composition may result from the release of dormancy, growth from spores and increases in taxa that had previously been undetectable using sequencing (Sorenson and Shade, 2020).

While poorly understood, microbial community responses to warming also have potential to alter the resistance and resilience of soil microbial communities to perturbations, such as drought. Studies across a wide range of ecosystems, including peatlands, demonstrate that warming can cause significant changes in the structure and diversity of soil microbial communities (Ward et al., 2015; Le Geay et al., 2024; Dang et al., 2025), attributes that are expected to influence the stability of microbial communities in the face of perturbations, such as drought (Bardgett and Caruso, 2020; Wagg et al., 2021). For example, increases in the abundance of bacteria relative to fungi (de Vries et al., 2018) and reductions in the diversity of microbial communities (Wagg et al., 2021) have been shown to destabilise microbial communities in response to drought. Moreover, long term changes in microbial community structure and diversity resulting from warming have potential to destabilise community networks (de Vries et al., 2018) and alter the asynchrony of microbial species responses to drought due to differences in their traits related to drought tolerance and recovery (de Vries et al., 2018; Hernandez et al., 2021; Camenzind et al., 2023), with implications for the stability of microbial communities and their functioning (Loreau and De Mazacourt, 2013; Bardgett and Caruso, 2020). Such cumulative effects of climate warming on soil microbial communities over time are hence likely to impact their resistance and resilience to drought events, and in peatlands, potentially have important implications for the storage of carbon through increased decomposer activity (Preece et al., 2019; Philippot et al., 2021; Cordero et al., 2023; Oram et al., 2025).

Changes in vegetation composition, and associated root and litter inputs, are also likely to modify microbial response to warming and drought. Very little is known, however, about how vegetation may moderate interactive effects of warming and drought on microbial community structure and function. Shrub dominated communities such as those found in peatlands are associated with slower rates of nutrient cycling, higher carbon stocks, and slower rates of litter decomposition than grass or moss dominated communities, likely due to the high polyphenol content in their exudates and litter (Ward et al., 2013; Fry et al., 2023). In particular, *Calluna vulgaris*, a dominant shrub of northern peatlands, exudes large quantities of phenolic compounds from its roots (Ward et al., 2013). Phenolics, such as tannins, are large carbon-based molecules produced by plants, which inhibit hydrolase and phenol oxidase activity and could thereby reduce decomposition rates of microbial communities and keep available nutrients low (Dunn et al., 2018). Generally, hydrolases increase, albeit weakly, while oxidases decrease under warming and also in response to drought (Xiao et al., 2018). Shrubs in peatlands also promote slower growing microbes, and protect carbon from decomposition when droughted (Wang et al., 2015). There is potential, therefore, for shifts in vegetation resulting from climate and/or land use change, including reductions in shrub cover resulting from increased grazing pressure and prescribed burning as commonly occurs in northern peatlands (Bardgett et al., 1995), to result in increases in greenhouse gas emissions and losses of stored carbon.

Removal of ericaceous shrubs, such as *C. vulgaris*, will result in the concomitant loss of root-associated ericoid mycorrhizal fungi and increased enzymatic activity and nutrient release (Dunn et al., 2018), thereby vastly altering the soil environment. Previous findings from our field experiment on ombrotrophic blanket bog at Moor House National Nature Reserve (NNR), northern England, have shown that both warming and shrub removal treatments individually have strong effects on different components of the microbial community: bacterial communities are principally affected by warming, whereas fungal communities are more strongly affected by shrub removal (Ward et al., 2015). Combined with warming and drought, a change in vegetation that influences microbial community properties also has the potential to destabilise ecosystem properties such as carbon and nitrogen stocks and cycles (Wagg et al., 2021). Understanding the role of soil microbial communities in stabilising ecosystem functions is still in its infancy. In peatlands, where cold, waterlogged conditions inhibit decomposition of organic matter, and are extremely vulnerable to alleviation of these constraints, it is critical to understand how a potential change in external conditions could have effects on ecosystem functions modulated by microbes.

Here we experimentally tested how long-term warming and vegetation manipulation (i.e. dwarf shrub removal) individually and interactively modify the response of peatland microbial communities to drought. We hypothesised that 1) long-term warming causes changes in the diversity and composition of the soil microbial community, that in turn alters its resistance and resilience to drought; 2) warming-induced changes in microbial community diversity and community composition are amplified by shrub removal, which reduces the input of phenolic rich litter to soil, thus enabling increased enzymatic activity and release of nutrients (Dunn et al., 2018), and further reducing resilience of microbial communities to drought. Based on previous findings, we expected bacteria in warmed soils to be less resistant to drought but to recover more rapidly, while fungal communities, with close symbiotic and saprotrophic links with plants, were expected to have high resistance, but lower resilience to drought, especially when shrubs were removed (Ward et al., 2015); 3) that there would be strong individual and interactive effects of warming and shrub removal on the resistance and resilience of microbially driven functions which could impact the stability of globally important peatland carbon stocks. These hypotheses were tested using soils taken from a long-term (9 year) warming and vegetation manipulation experiment established in 2008 at Moor House NNR on ombrotrophic blanket bog (Ward et al., 2013, 2015), which were then subjected to an experimental dry/wet cycle under standardised laboratory conditions to quantify the resistance and resilience of microbial communities to simulated drought. Overall, our goal was to determine whether long-term warming and shrub removal affect the ability of peatland bacterial and fungal communities to resist and recover from drought, and whether this has implications for microbial-mediated functions of carbon and nutrient cycling.

2. Methods

2.1. Experimental design

The field experiment was set up in 2008 on an ombrotrophic blanket bog at Moor House National Nature Reserve (54°65′ N, 2°45′ W), northern England, and was designed to test the individual and combined effects of warming and vegetation change on ecosystem properties (Ward et al., 2013). The warming treatment involved the use of hexagonal open top chambers based on the International Tundra EXperiment design (~0.46 °C warming over 24 h during the growing season; 1 °C as an annual average (Stenström and Henry, 1997), which remained in place year-round. The vegetation manipulation treatment involved selective removal by clipping at ground level of the three dominant plant functional groups, namely dwarf shrubs (dominated by *Calluna vulgaris*), graminoids (dominated by *Eriophorum vaginatum*), and bryophytes (including *Sphagnum* spp. and *Hypnum jutlandicum*) in a full factorial design. Clipping ensured the soil was not disturbed. Shrub removal on average increased the temperature at soil level by 0.2 °C through the growing season. In total, there were eight vegetation treatments, including a control with all functional groups present, three treatments for each of the single functional groups, three treatments with two combinations of functional groups, and a treatment where all above-ground vegetation was removed. The experiment had four blocks in an RCBD factorial design and is situated 550 m above sea level, with a January mean temperature of 3.2 °C, July mean temperature of 14.8 °C, and total annual rainfall of 898.2 mm (1981–2010 average; MetOffice.gov.uk). For further details on the site and field experiment see Ward et al. (2013).

Here we focussed on the warming and shrub removal treatments (i.e., with and without *C. vulgaris*), which have previously been shown to have the strongest impact on soil microbial and faunal communities and ecosystem functions (Ward et al., 2013, 2015). Neither treatment was shown to impact water table drawdown in previous work at this experimental site (Ward et al., 2013, 2015). Specifically, five soil samples per plot were collected from the surface 10 cm of 16 plots and bulked into a plot-level composite sample (2 warmed/unwarmed, 2 shrub removal/control, 4 replicate blocks) in June 2017 within 2 h and left to equilibrate for 2 weeks at 14 °C, representing summer mean temperature at the site. The equilibration period was partly intended to allow the inevitable spike in microbial activity caused by the disturbance to pass, and to adjust the soils to the average July/August temperatures at the site. We kept some soil for baseline measurements and divided the remaining soil from each plot into two portions to be subject to drought or remain well-watered. We then subdivided again into six so that destructive harvests of each treatment could take place over six time points. At the start of the experiment, our design yielded 192 open-top 500 ml Kilner jars (2 warming x 2 vegetation x 2 drought x 4 field replicates x 6 destructive harvests), each containing 40 g of field capacity peat soil. For baseline measures, we assessed total carbon and nitrogen, soil inorganic N availability and microbial biomass (see below for full methods).

2.2. Drought procedure

First, we determined gravimetric soil moisture content (SMC) in the field soil by drying 5 g of soil for 72 h at 80 °C. This was close to saturation point, with standing water present. Next, we measured field capacity of the soil, which is the moisture content the control samples would be maintained at. The pressure point method has been validated for use with peat, as many methods are intended for use on mineral soil, which has very different hydraulic properties (Bechtold et al., 2018). Briefly, the peat was oven dried, then placed on a ceramic cup before being saturated with water. The sample was left overnight, before being placed in the pressure container and exposed to 0.33 bar of pressure. The excess water was pushed through the ceramic cup. After 24 h we measured gravimetric water content as above. Field capacity of the soil at the start of the experiment was approximately 90 % SMC. Permanent wilt point, or the point where peat is too dry for microbes or plants to extract water, was measured in a similar way, except we exposed the sample to 15 bar of pressure for 48 h. We aimed to allow the drought treatment to dry down passively to permanent wilt point (PWP), which was 26.5 % SMC, and then maintained this for five weeks to simulate an extreme drought. Passive drying was achieved by covering the jars with Parafilm, with holes, and leaving them in the dark in an incubator.

In the meantime, well-watered microcosms were maintained at 90 % SMC using a rainfall recipe mix based on chemistry data from the Centre for Ecology and Hydrology, Eskdalemuir between 2005 and 2007 (Cape et al., 2014). We arrived at five weeks using a twenty-year rainfall dataset (1995–2015) from CEDA (Office, 2025), and modelling an extreme once in a century drought event using a Gumbel distribution in the VGAM package of R (Yee, 2010). After the five weeks of extreme drought, drought applied soils were rewetted to the same moisture content as the control, and replicate pots of each treatment were destructively harvested before rewetting (day 0), to measure resistance, on the day of rewetting (day 1), and then at days 7, 14, 28 and 56 to measure post-drought recovery of microbial communities and microbial-mediated functions (Fig. S1). Weekly measurements were deemed appropriate partly because two months was considered to be an appropriate recovery period, based on previous work (Fry et al., 2023), and microbial cycles have been shown to take place over timespans of eight days and longer (Leitner et al., 2021).

2.3. Microbial community properties

2.3.1. Microbial community structure

Microbial biomass was measured using phospholipid fatty acid analysis (PLFAs). PLFAs were extracted from soil using a high-throughput protocol based on the method of Buyer and Sasser (2012). The dried PLFAs were resuspended with hexane and analysed on a gas chromatograph (GC; Agilent 7890B, Cheadle, UK). Total PLFA biomass was calculated by summing all bacterial and fungal markers.

To measure bacterial and fungal community composition and diversity, soil microbial DNA was extracted using a Qiagen PowerSoil extraction kit, with 0.25 g soil (Qiagen, Manchester, UK). DNA was amplified using PCR with 16S rRNA gene primers Eub558 and Eub518, and ITS primers ITS1F and 5.8S (Fierer et al., 2005). DNA was exported to the Next Generation Sequencing Facility at Hawkesbury Institute for the Environment (Western Sydney University, Australia), where the samples were sequenced using an Illumina MiSeq analyser. For raw pair-end 16S rDNA and ITS reads, index and primers at the beginning of each sequence were trimmed off and then dereplicated beforehand using USEARCH (Edgar, 2010). Bases with quality lower than 30 were trimmed off from the end before merging, and the maximum of expected error (ee) was set as 1.0 for merged reads filtering. A total of 4,836,980 and 3,334,910 high-quality reads were gained for bacteria and fungi, respectively. zOTUs (denoised sequences, 100 % sequence identity) were identified by denoising (error-correction) the amplicon reads using unoise3 (Edgar, 2016). Representative sequences were annotated against the RDP (https://doi.org/10.1093/nar/gkt1244) and UNITE (Kõljalg et al., 2005) database in QIIME (Caporaso et al., 2010) for bacteria and fungi, respectively. A normalization procedure was performed at 13,076 and 17,417 sequences per sample for bacteria and fungi, respectively, before diversity analysis. Resultant OTU tables were converted into the biom file and imported into QIIME for diversity calculation. Alpha diversity metrics including richness, Shannon diversity, Chao I index, and Faith's phylogenetic diversity were calculated.

2.4. Microbial-mediated properties

2.4.1. Greenhouse gas emissions

Prior to harvest, each microcosm was tested for greenhouse gas fluxes. We placed a lid containing a Suba seal on each glass Kilner jar and collected gas using a 10 ml syringe and needle immediately upon closure, then at 10-min intervals until 30 min had passed. The gas samples were injected into individual 3 ml exetainers which had been evacuated under vacuum and the gas content (carbon dioxide; CO_2, methane; CH_4 and nitrous oxide; N_2O) analysed using a gas chromatograph (GC; Agilent 7890B, Cheadle, UK; Ward et al. (2013)). Changes in pressure were included as offsets in the calculations. We could then calculate gas flux rates for each as mg m^{-2} hr^{-1} for CO_2 and CH_4, and μg m^{-2} hr^{-1} for N_2O accounting for air temperature and headspace of the Kilner jar. We used four certified gas standards to validate the data on the GC (BOC, Worsley, UK). Calculations were carried out based on those outlined by Zaman et al. (2021), which describe full methodology of static chambers.

2.4.2. Enzymes

We used fresh soil to assess enzyme activity after harvest using para-nitrophenol (pNP) based substrates. We measured five enzymes that degrade carbon, nitrogen and phosphate based molecules in the soil: β-glucosidase (GLC), which degrades the β-d-glucoside bonds in maltose and cellobiose, β-xylosidase (XYL), which degrades short chain xylan chains into xylose, β-N-acetylglucosaminidase (NAG), which catalyses hydrolysis of *N*-acetyl-beta-ᴅ-glucosaminide residues in chitin, and acid phosphatase (PHO), which mineralises organic P into phosphate (German et al., 2011). Briefly, we created a soil slurry consisting of 0.75g soil ml^{-1} acetate buffer for each sample. Each enzyme was made up to recommended concentrations by dissolving in acetate buffer (GLC: 30 mM, XYL: 30 mM, NAG: 5 mM and PHO: 40 mM), and the soil slurry, acetate buffer and enzymes were added to 96 well plates each in aliquots of 150 μl, with each sample run in triplicate and soil and reagent blanks included. The plates were sealed, centrifuged for 10 s and placed on a shaker (GLC: 90 min, XYL and NAG: 3.5 h, PHO: 30 min). We prepared fresh microplates with 200 μl 50 mM sodium hydroxide solution in each well. When shaking was complete, we centrifuged each plate at 2900×*g* for 5 min, then sampled 100 μl from each well, adding the samples to the prepared microplate. Absorbance was read at 405 nm (see (Jackson et al., 2013) for full protocol). Enzyme activity (nmol h^{-1} g^{-1}dry soil) was calculated as (pNP concentration (μM) x volume in well (0.0003L) x 1000)/(incubation time (h) x g wet soil in well (0.1125g) x (1-soil moisture %)).

2.5. Soil nutrients

After soil collection we carried out a preliminary set of measurements on field soil of the effects of warming and vegetation removal on plant available nutrients and total soil C and N. We used two extraction solutions: Milli-Q water and potassium chloride (KCl). 2.5 g soil was extracted in 25 ml water and shaken on an orbital shaker at 150 rpm for 10 min before being filtered using Whatman number 42 filter paper. Total organic carbon was measured from the water extracts using a Shimadzu TOC-L analyser, and dissolved inorganic nitrogen (ammonia- NH_4 and nitrate- NO_3) was measured using a Seal AA3 autoanalyser. These samples were not diluted. For the KCl extracts (NH_4 only), we added 2.5 g soil to 25 ml 1 M KCl and shook them at 150 rpm for 1 h. These were then filtered through Whatman number 1 filter paper and analysed on the autoanalyser at a 1:1 dilution. For total C and N, the soil was ground using a ball mill and analysed using an elemental combustion analyser (Elementar vario EL, Hanau, Germany). After harvest, the soil was immediately extracted for plant available N and C as above. We also measured the rate of N turnover by incubating a 5 g subsample of each sample at 14 °C for 14 days. The N was extracted using the KCL protocol, then rate of N mineralisation was calculated by subtracting NH_4 and NO_3 at day 0 from N at day 14, then dividing by 14 to get g N d^{-1}. Nitrification rate was calculated using the same method but only using NO_3 (Bardgett et al., 2003).

2.6. Phenolics

Phenolic concentrations in the soil were measured using the technique of Peñuelas et al. (1996), using a colourimetric assay of a water extraction. Briefly, 0.5 g of air-dried soil was shaken in 15 ml of Milli-Q water on an orbital shaker for 14 h, before being centrifuged at 3000 rpm for 2 h. The supernatant was then filtered using Whatman number 1 filter paper. To begin the reaction, we took 170 μl of the water sample and added 10 μl of Folin-Ciocalteu reagent (Folin, 1915), and 20 μl of a saturated sodium carbonate solution, to stabilise the reaction. The mixture was vortexed briefly and left in the dark for 2.5 h. We read the optical density of the samples on a microplate reader at 765 nm (Clariostar, BMG LabTech, Aylesbury UK). The samples were compared with a calibration curve created from the phenol gallic acid. The protocol does not give total phenolic concentrations per sample, so the results are expressed with reference to gallic acid.

2.7. Statistical analysis

We first tested the effect of the field treatments (warming x shrub removal) on the soil and microbial C and N as a baseline using two-way ANOVA in R4.0.3 (R Development Core Team 2020).

To assess the impact of the treatments on bacterial and fungal community structure at the end of the microcosm drought treatment, we calculated non-metric multidimensional scaling (NMDS) values based on Bray-Curtis dissimilarity generated by QIIME to assess treatment differences over time using a*donis2* in the *vegan* package of R with a Bray-Curtis distance matrix (Oksanen et al., 2010). We used all three-way interactions between timepoint, warming, drought and shrub removal and did not simplify the models. We decided not to use four-way interactions, as these are difficult to interpret and have low statistical power (Smith et al., 2002).

We carried out indicator species analysis to determine which taxa were strongly associated with each treatment. We used the *indicspecies* package in R and assessed each treatment in turn with 999 permutations and Benjamini-Hochberg correction (Benjamini and Hochberg, 1995; De Caceres and Legendre, 2009). Only taxa that achieved corrected $p <$ 0.05 were retained in the final table.

To assess the effect of the treatments on alpha diversity of the bacterial and fungal communities, we carried out linear mixed effects models with species richness, Shannon's diversity index, Chao 1 index of rarity and Faith's Phylogenetic Distance as response variables, and factors timepoint, warming, shrub removal and drought as fixed effects (*nlme* package, Pinheiro et al. (2025)). To meet the assumptions of the model, species richness and Faith's PD were log transformed because they were count data, and Shannon's diversity and Chao 1 were Gaussian values and tested for heteroscedasticity. Where appropriate they were transformed using Box-Cox transformation in the MASS package (Venables and Ripley, 2002). We included all three-way interactions between these variables. The field plot the soils were taken from was included as a random effect for each model. Species richness was log transformed, and other responses were tested for Gaussian distribution, and transformed where necessary.

To assess the effect of the treatments on resistance and resilience of the soil properties and gas fluxes, we calculated the metrics presented by Orwin and Wardle (2004). Both metrics are bounded between 1 and -1. We calculated the effect of drought on each treatment relative to its control. For day 0, before the drought was alleviated, we calculated the resistance as follows in equation (1):

$$Resistance = 1 - \frac{2\,x\,|D_0|}{C_0 + |D_0|} \qquad \text{(eq. 1)}$$

Where D_0 is the absolute difference between the control soil (C_0) and the drought applied soil on Day 0 for each treatment. For resistance, 1 means full resistance (i.e., the drought has had no effect on the variable), 0 means a difference of 100 % of the control value, and −1 is where the drought soil has changed more than 100 % relative to the control.

Resilience for each time point after the drought was alleviated was calculated as follows in equation (2):

$$Resilience = \frac{2\,x\,|D_0|}{|D_0| + |D_x|} - 1 \qquad \text{(eq.2)}$$

Where x refers to each time point after the drought was alleviated. As before, each treatment was compared with its control soil at that time point. A value of 1 indicates that the variable in the drought soil has recovered to the same level as the control, a value of 0 means that there has been no post-drought recovery, or a 100 % increase or decrease relative to the control. A negative value indicates that the variable is more different from the control on this time point than it was on day 0, usually due to a burst of microbial activity when stress is alleviated.

We then calculated soil multifunctionality based on measurements of both carbon-based properties and nitrogen-based properties. We did this by averaging the resistance or resilience values for each group of properties. For carbon-based properties we include soil carbon, carbon-cleaving enzymes, carbon dioxide and methane fluxes. This is a commonly used method for calculating multifunctionality: by standardising all functions to a single scale and then averaging these, an intuitive metric can be obtained. In our study the resistance and resilience metrics are bounded between −1 and 1, which allows for simple averaging (Byrnes et al., 2014). We did not weight any functions as we could not find adequate justification for deeming one function more important than another. For nitrogen-based properties, we included nitrogen molecules, nitrogen cleaving enzymes, mineralisation, nitrification and nitrous oxide. We then analysed these data using ANOVA, to determine whether to accept hypothesis 3. Because of the high likelihood of negative values, the data were left untransformed. We ran resistance values separately from resilience. The resistance ANOVAs had the resistance of each multifunctionality metric in turn as the response, with warming and shrub removal as interactive explanatory factors. For the resilience we ran ANOVAs, with timepoint, warming and shrub removal as factors, and included all three-way interactions. These models were not simplified.

Next, we assessed the temporal stability of each ecosystem function and its relationship with bacterial and fungal species richness, which were individually averaged over the course of the study. Temporal stability of each individual function was calculated following Wagg et al. (2021), where the inverse of the coefficient of variation was calculated for each set of mesocosms over the six time points measured. Sets of mesocosms were created by matching soils from the field plots. Using the temporal stability of the function as the response, we used linear mixed effects models with bacterial species richness, and a three-way interaction between drought, warming and shrub removal as the fixed effects. The ID of the field plot the soil was taken from was the random effects. These were then repeated with fungal species richness.

3. Results

3.1. *Effects of field treatments on soil properties before drought*

Preliminary tests from soil taken from the field indicated that while total % soil carbon was not affected by warming or shrub removal (Fig. S2A), total % soil nitrogen was significantly higher in warmed and shrub removal plots, and in plots with both treatments, compared to control plots that had not received treatments (Fig. S2B). This resulted in a lower carbon to nitrogen ratio in soils subject to both warming and/or shrub removal (Fig. S2C). This was primarily driven by the dissolved organic N, because the inorganic nitrogen showed different patterns. Inorganic nitrate concentrations were lower in soils that were both warmed and had shrubs removed (Fig. S2D). Plant available ammonium was not impacted by the field treatments (Fig. S2E), although total plant available nitrogen (dissolvable $NH_4 + NO_3$ + organic N) was impacted by warming, where warmed soils with shrubs present had higher N than control soils, while warmed soils with shrubs removed had lower inorganic nitrogen (Fig. S2F).

3.2. *Treatment effects on microbial community composition*

The resistance of the bacterial community to drought (amplitude of the change from the control) was not altered by warming or shrub removal, although warming and drought both had strong independent effects on bacterial community structure (Fig. 1; PERMANOVA: Warming: $F_{1,86} = 1.76$, $p = 0.043$, Drought: $F_{1,86} = 1.76$, $p = 0.043$). Shrub removal had no significant effect on the bacterial community structure.

Bacterial community resilience to drought, i.e. the change of NMDS values back towards the control over the post-drought recovery period, was low. Instead of becoming more similar to the control, drought soil communities became increasingly different from the control, although this response was not affected by warming or shrub removal (Fig. 1; Timepoint: $F_{3,86} = 2.26$, $p = 0.002$). Overall, bacterial communities are highly vulnerable to drought, with little recovery, and this was not modulated by warming or shrub removal.

In contrast to bacterial communities, warming and shrub removal did not alter fungal community composition, and thus did not impact the resistance or resilience of the fungal community to drought.

3.3. *Response of microbial diversity to warming, shrub removal and drought*

Bacterial richness on day 0 was strongly negatively affected by drought, indicative of low resistance, although this response was unaffected by warming or shrub removal (Table S1; control: 1654 ± 27, drought: 1410 ± 27, $F_{1,86} = 44.13$, $p < 0.001$).

While warming alone led to a slight increase in bacterial richness, Chao's index and Phylogenetic Distance, shrub removal further amplified the effect, with significantly higher values of these when warming and shrub removal were combined relative to the control (Fig. 2A–C,D; Table S1). Bacterial Shannon's Diversity Index (where high values mean higher species richness and evenness of species relative abundance) was increased by warming but not impacted by shrub removal (Fig. 2B).

In terms of the resilience metric, warming and shrub removal significantly interacted to influence the resilience to drought of bacterial species richness, Shannon's Diversity and Chao's Index (Fig. S3). In all cases, the control treatment was most resilient to drought, and shrub removal the least resilient, with an average fold change of 0.38 in warmed soils, 0.26 in soils with shrubs removed and 0.58 in soils with both warming and shrub removal. The interaction between warming and shrub removal led to higher resilience to drought than warming or shrub removal alone. These effects were not affected by the sampling date, so no significant change over the recovery period was observed.

Fungal diversity metrics showed different responses relative to those of bacteria. Fungal species richness, Shannon's diversity, Chao's index and Faiths PD all increased in response to drought, and diversity metrics kept increasing through the post-drought recovery period, with a fold change increase in species richness of 1.36 on day 56 (Table S2). The increase in response to drought was not related to the warming or shrub treatments.

Warming alone did not change any of the fungal alpha diversity metrics relative to the control (Fig. 3). Shrub removal decreased fungal diversity metrics, apart from Shannon's diversity, although when soils had a legacy of both warming and shrub removal, values were statistically similar to those of the control, in both drought applied and control treatments. Fungal richness and diversity were therefore increased by the drought independently of warming or shrub removal.

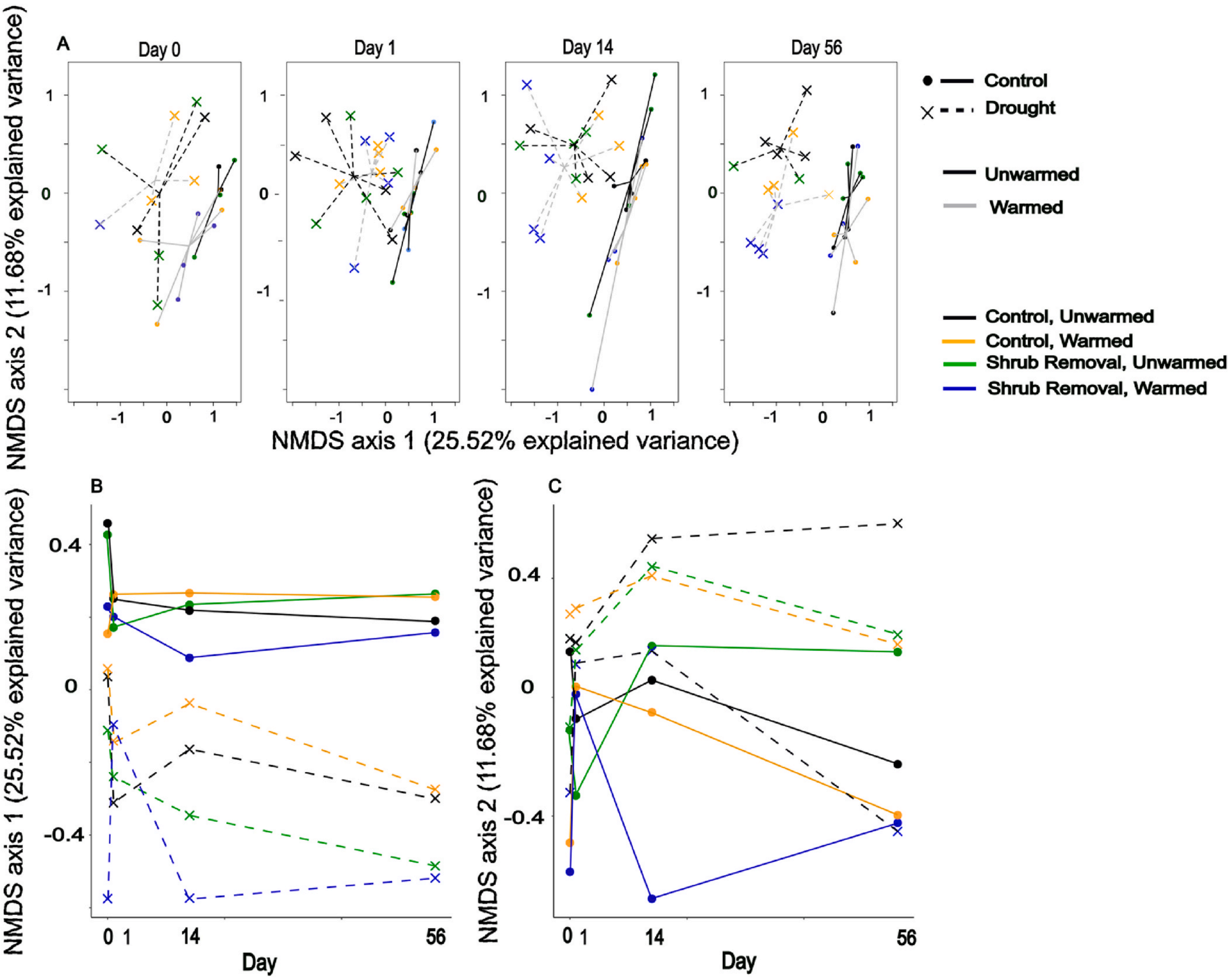

Fig. 1. NMDS of the Bray-Curtis dissimilarities of the soil bacterial community structure ($n = 4$). PERMANOVA: Timepoint: $F_{3,86} = 2.26$, $p = 0.002$, Warming: $F_{1,86} = 1.76$, $p = 0.044$, Drought: $F_{1,86} = 1.75$, $p = 0.043$). Panels B and C show the centroid of each treatment from the NMDS panel A, and the change over time.

Similarly to bacteria, there was no effect of warming or shrub removal on resistance to drought (day 0). For the resilience metrics, there was a significant interaction between warming and shrub removal. The control soils had the highest resilience, and warmed soils the lowest (average fold change 0.12). This low value occurred because the diversity in warmed soils overshot the control, resulting in negative values. The interacting warming and shrub removal treatments led to higher resilience than either treatment alone (average fold change 0.59).

3.4. *Effects on microbial taxa at the end of the study*

At the end of the drought period, almost all bacterial higher order taxa were reduced in both warmed soils and those subject to shrub removal, indicating low resistance (Table S3). At the end of the post-drought recovery period, warming did not impact the resilience to drought of any bacterial taxa. However, warming decreased the relative abundance of Bacteriodetes and Proteobacteria, and increased the relative abundance of Saccharibacteria (Table S3). The relative abundance of three bacterial taxa was altered by interactive effects of warming and shrub removal (Table S3). Chlamydiae decreased in its relative abundance in warmed soils, but when shrubs were removed from warmed soils, the negative effect of warming was no longer apparent. Planctomycetes and Candidate division WPS-1 showed higher relative abundance in warmed and warming x shrub removal soils compared with the control, although no effect of shrub removal alone. Indicator analysis of bacteria showed many species were associated with warmed soil, from a wide range of life histories (Table S5). Many of those favoured under warming were aquatic species from wetland environments. Much fewer species were associated with shrub removal, and fewer again with drought.

We observed no effects of warming or shrub removal on the relative abundances of the known fungal taxa, although there was a significant three-way interaction between timepoint, warming and shrub removal on fungal OTUs from unidentified taxa, that had been collected into one group (Table S5, $F_{1,33} = 8.01$, $p = 0.008$). However, indicator analysis showed that while there were no indicator species associated with shrub removal, indicators associated with warming were more numerous and largely made up of plant pathogens (Table S6). We observed no change in relative abundances of higher order taxa in response to drought, but four species were associated with drought, encompassing a range of life histories.

Our detailed assessment of the microbial taxa indicated that there were strong changes in relative abundances due to warming and shrub removal, but as for the broader measures, these did not alter the resistance or resilience of the communities to drought.

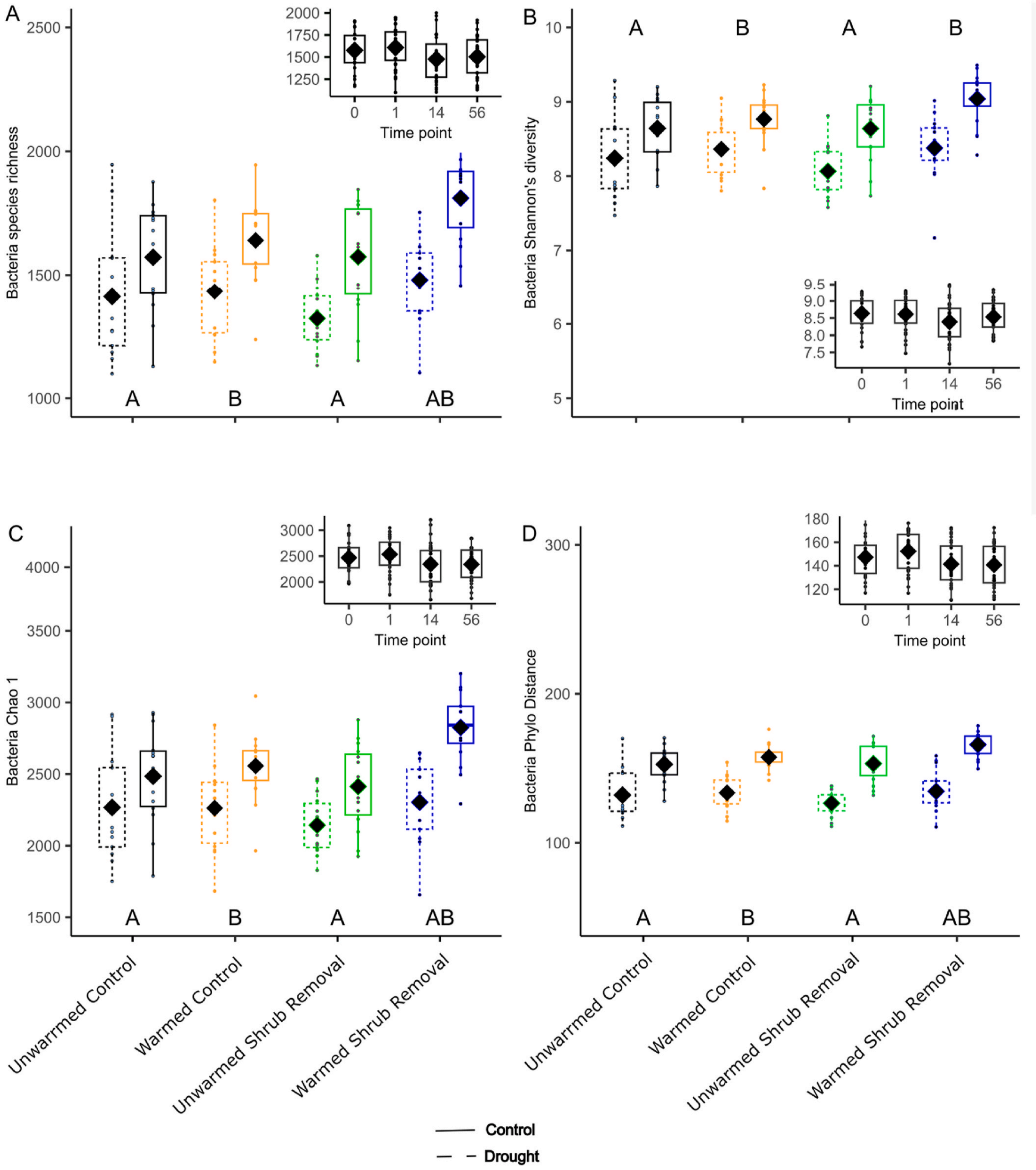

Fig. 2. Alpha diversity metrics for bacteria, showing the effect of time in inset boxes n = 4. Diamonds refer to the mean value. Significant orthogonal differences are shown using compact letter display. A) Species richness (Warming x Shrub Removal: $F_{1,86} = 5.13$, $p = 0.026$; Drought: $F_{1,86} = 44.13$, $p < 0.001$; Timepoint: $F_{3,86} = 3.04$, $p = 0.033$), B) Shannon's diversity (Warming: $F_{1,86} = 10.25$, $p = 0.002$; Drought: $F_{1,86} = 42.62$, $p < 0.001$; Timepoint: n.s)., C) Chao 1 index (Warming x Shrub Removal: $F_{1,86} = 6.23$, $p = 0.015$; Drought: $F_{1,86} = 38.90$, $p < 0.001$; Timepoint: $F_{3,86} = 3.39$, $p = 0.022$), D) Faith's Phylogenetic Distance (Warming x Shrub Removal: $F_{1,86} = 4.05$, $p = 0.047$; Drought: $F_{1,86} = 174.86$, $p < 0.001$; Timepoint: $F_{3,86} = 8.40$, $p < 0.001$).

3.5. Treatment effects on stability, resistance and resilience microbial-mediated functions

We did not find significant effects of warming or shrub removal on the resistance of any microbial-mediated functions and soil multifunctionality to drought, as measured on day 0 (Fig. 4).

Resilience of carbon functions to drought, including enzyme activities, carbon dioxide emissions and soil carbon were not affected by

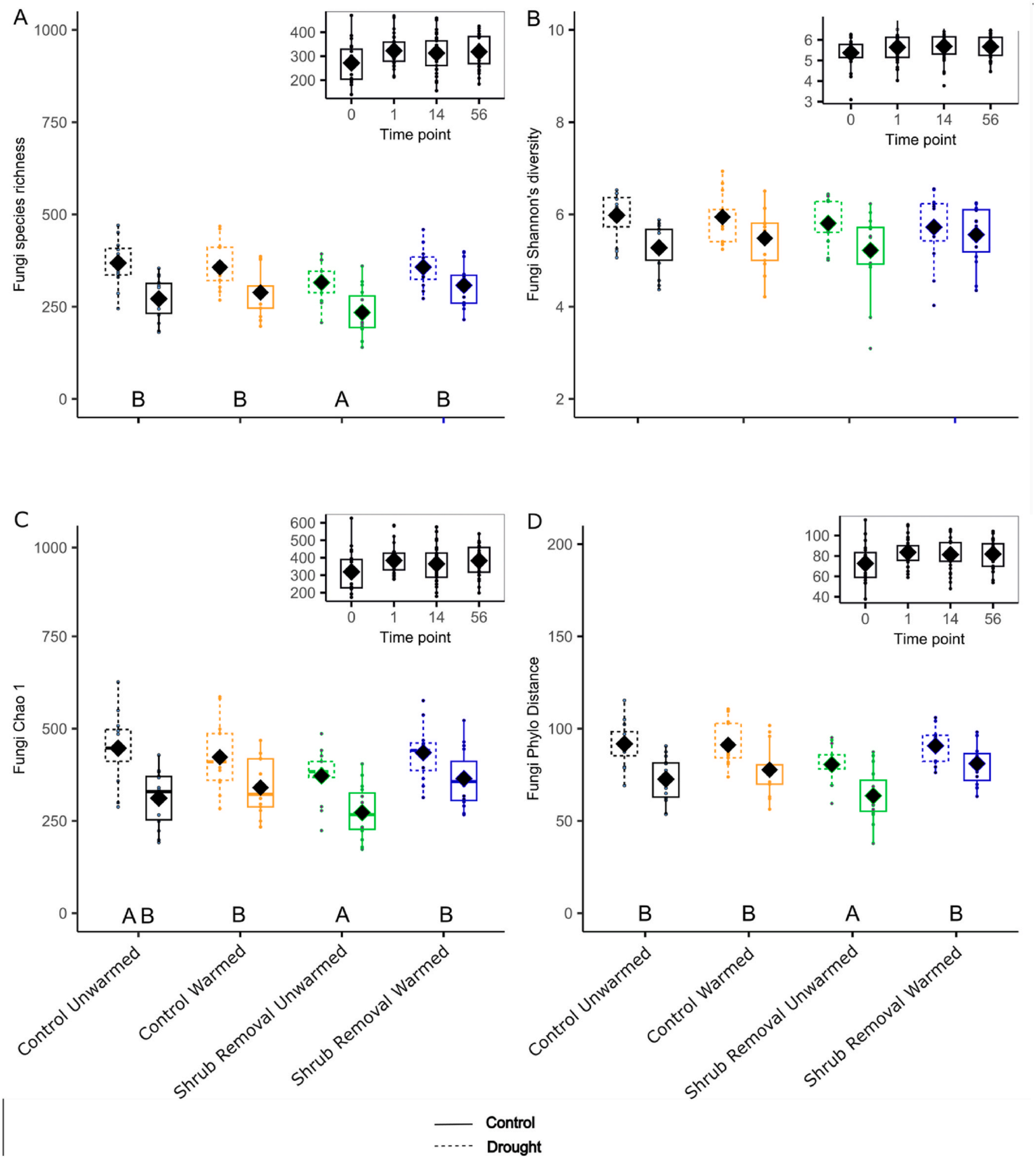

Fig. 3. Fungal alpha diversity metrics. The drought, shrub removal and warming treatments are presented, with the time effect shown in inset (n = 4). Diamonds refer to the mean value. A) Fungal species richness (Warming x Shrub Removal: $F_{1,78} = 5.21$, $p = 0.025$, time x Drought: $F_{3,78} = 3.18$, $p = 0.028$); B) Shannon's diversity index (Drought: $F_{1,78} = 14.96$, $p < 0.001$); C) Chao 1 index (Warming x Shrub Removal: $F_{1,78} = 5.58$, $p = 0.021$, Drought: $F_{1,78} = 47.04$, $p < 0.001$, time: $F_{3,78} = 4.00$, $p = 0.011$); D) Faith's phylogenetic diversity (Warming x Shrub Removal: $F_{1,78} = 5.57$, $p = 0.021$, Drought: $F_{1,78} = 44.36$, $p < 0.001$, time: $F_{3,78} = 4.13$, $p = 0.009$). Orthogonal contrasts are denoted using compact letter display.

warming, but primarily by shrub removal alone (see carbon multifunctionality, Fig. 4, and Figures S4a-c,g, S4A). However, resilience of phenolic concentrations to drought was impacted by warming, where warming reduced phenolic resilience relative to the control, which gradually increased through the post-drought recovery period, becoming more similar to the control over time (Fig. 4, S4e). Resilience of methane emissions indicated that warming increased resilience to drought compared with the unwarmed soils. We also observed interactions between warming and shrub removal in terms of the recovery of phenolics and methane emissions from drought. Resilience of

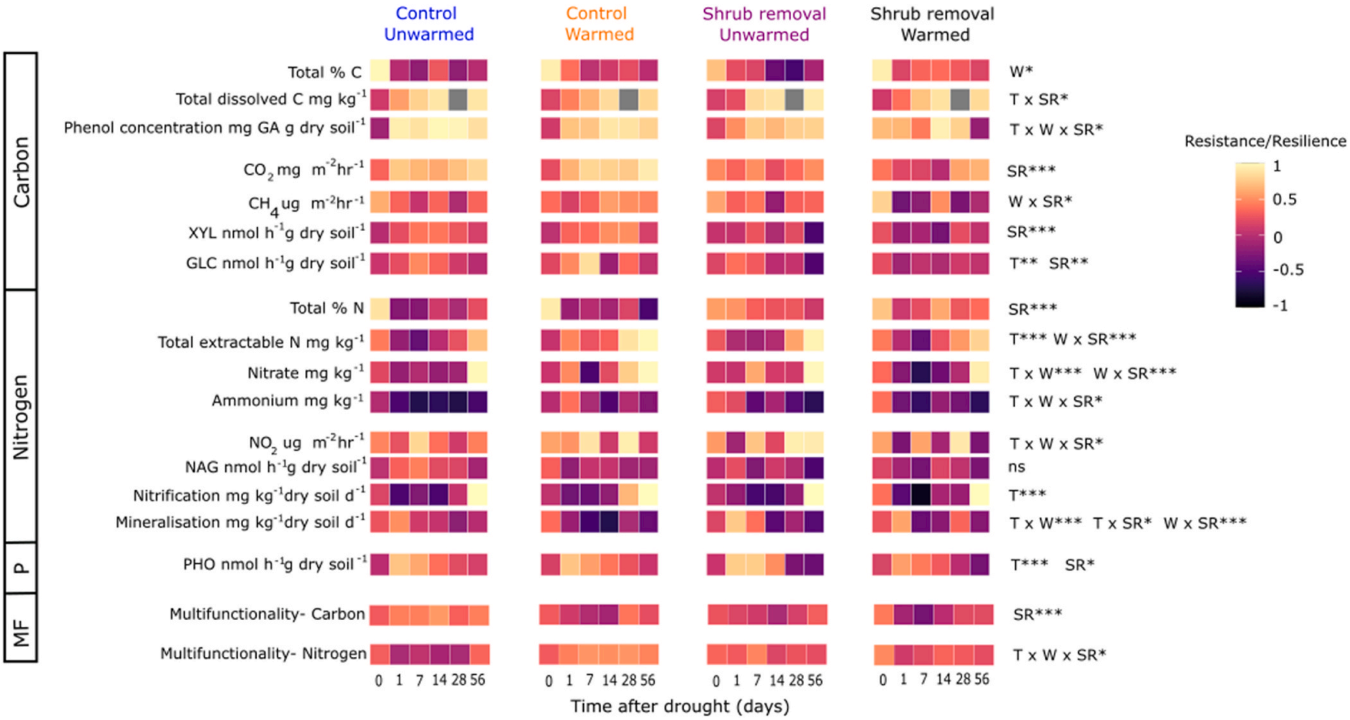

Fig. 4. Resilience of soil properties and multifunctionality metrics to drought, with the impact of prior warming and shrub removal considered during the post-drought recovery time. Day 0 represents resistance, where values were taken before rewetting. Days 1–56 are recovery with consistent watering (resilience). Data were analysed separately for resistance and resilience, but no significant treatment effects were observed on day 0. A value of 1 (pale yellow) denotes full recovery to the control. 0 (purple) represents 100 % change in value from the control, or no recovery. −1 (dark blue) represents an increase in the value relative to the control. Grey squares mean no data for this timepoint. T- time, W- warming, SR- shrub removal. Significant effects represented by significance stars refer to days 1–56 only. Significance is * $p < 0.05$ ** $p < 0.01$, *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

phenolic concentrations became much more variable over the post-drought recovery period when shrub removal altered the warming

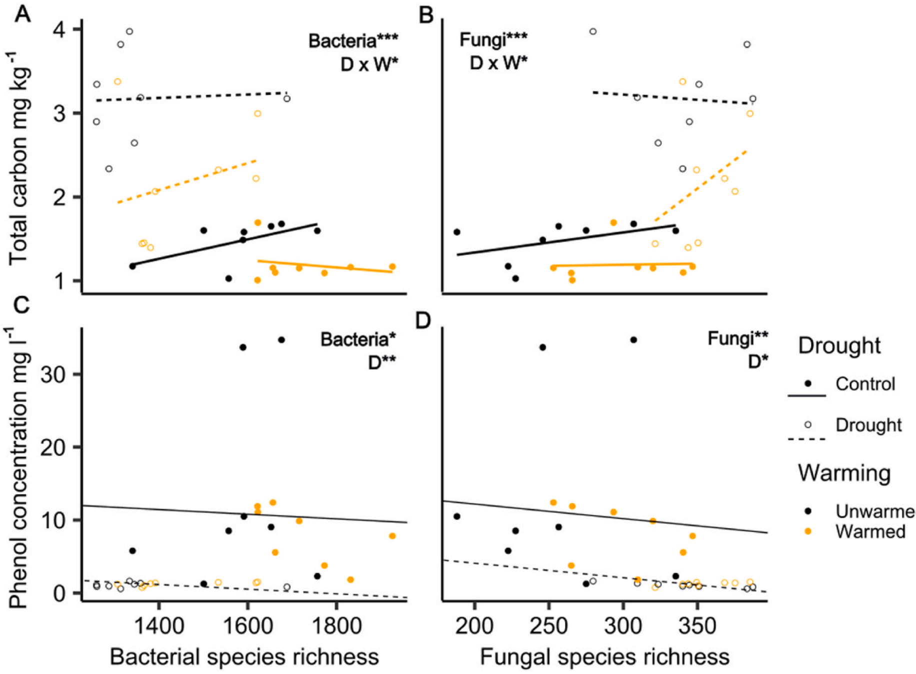

Fig. 5. Temporal stability, i.e. variation in values over time, of total dissolved organic carbon and phenolic concentration in relation to bacterial and fungal species richness. Shrub removal effects were not significant so are excluded here for clarity. A) Total organic carbon and bacterial species richness: Bacteria: $F_{1,11} = 39.53$, $p < 0.001$; W x D: $F_{1,11} = 4.87$ $p = 0.0496$. B) Total organic carbon and fungal species richness: Fungi: $F_{1,13} = 50.01$, $p < 0.001$, W x D: $F_{1,13} = 5.01$, $p = 0.043$. C) Phenols and bacterial species richness: Bacteria: $F_{1,11} = 8.53$, $p = 0.014$, D: $F_{1,11} = 9.76$, $p = 0.010$. D) Phenols and fungal species richness: Fungi: $F_{1,13} = 9.55$, $p = 0.009$, D: $F_{1,13} = 6.03$, $p = 0.029$.

effect, while resilience of methane was much lower when shrub removal altered the warming effect.

We found that warming impacted the resilience to drought of many nitrogen-based functions by increasing them relative to the control, including mineralisation, total extractable nitrogen, nitrate and ammonium, and this effect changed over the post-drought recovery period (Fig. 4, S3e, S4b,c,d). For nitrate and nitrification, resilience increased to 1 (no different from control), by the end of the study which likely indicates all nitrate in all microcosms was converted to other nitrogen forms. The other nitrogen-based processes indicated an overshoot relative to the control in warmed soils, which supports this. Warmed soils had higher extractable nitrogen in the baseline tests than the control soils, so there was more nitrogen available to cycle.

The effect of warming on resilience of individual nitrogen-based functions to drought, and multifunctionality of nitrogen-based functions, was moderated by shrub removal. Warming and shrub removal combined reduced the resilience of total extractable nitrogen, nitrification rates and nitrate concentrations to drought, as did multifunctionality of nitrogen functions.

Overall, we saw that carbon-based functions recovered more slowly from drought when shrubs were removed, and warming also had a negative effect on some functions. Recovery of nitrogen-based functions were more strongly affected by warming.

Temporal stability of total dissolved organic carbon and phenol concentration was linked with bacterial and fungal species richness (Fig. 5), while there was no relationship between temporal stability of gas fluxes, enzyme activity or soil nitrogen with microbial species richness. Temporal stability of total organic carbon increased as bacterial and fungal species richness increased (Fig. 5A and B). There was an interaction between drought and warming, where warming led to decreased temporal stability of TOC, while drought strongly increased stability of TOC. By contrast, soils with higher bacterial and fungal richness were associated with decreased temporal stability of phenol concentrations, with drought leading to further decreases in stability (Fig. 5C and D). We did not observe any modification of the warming effect by shrub removal.

4. Discussion

Peatland ecosystems are particularly vulnerable to climate change, including drought, as this can expose these globally important carbon stores to increases in decomposition and carbon loss (Austin et al., 2025). Our findings provide insight into how and why multiple interacting stresses can alter the resistance and resilience of peatland microbial communities to drought with implications for ecosystem functioning. We show that neither long-term warming nor shrub removal impacted the resistance of soil microbial communities to drought ($p > 0.05$). However, we show that warming and shrub removal individually and significantly reduced the resilience of bacterial and fungal diversity to drought, despite significantly increasing the diversity of fungal communities ($p < 0.05$). Importantly, we also found that warming strongly decreased the resilience ($p < 0.05$), but not resistance, of nitrogen-based functions to drought, although shrub removal dampened this effect. Overall, our findings demonstrate potential for long-term warming and vegetation change to modify microbial responses to extreme drought events, with wider implications for peatland carbon and nitrogen cycling and peatland management under future climate scenarios.

4.1. Warming

We first hypothesised that long-term warming modifies the composition and diversity of the soil microbial community, and that this would reduce its resistance and resilience to a simulated drought event. Accordingly, we found that long-term warming altered the composition of the bacterial community and increased its richness and diversity. While warming had no detectable impact on the resistance of bacterial communities to drought ($p > 0.05$), resilience was reduced substantially compared with the control (fold change 0.38, $p < 0.05$). Changes in bacterial community structure resulting from warming had downstream impacts on stability of carbon-based functions, which is a cause for concern (Ferguson and Lindo, 2025). Many of the microbial indicator species that increased with warming were saprotrophic or nitrogen fixing, which could mean that soil organic matter was more vulnerable to degradation in warmer soils (Tripathi et al., 2025). Moreover, irrespective of warming, drought effects on bacterial community composition became stronger over the post-drought recovery period, rather than returning to control values, indicating a prolonged impact of drought and a lack of recovery ($p < 0.05$, Cordero et al., 2023). These results indicate that drought has a generally negative effect on the bacterial community of peatland, regardless of the way that the community had been previously assembled and shaped. This finding contrasts with the results of a meta-analysis of global peatlands that found bacterial species richness to be generally unresponsive to warming (Hedges g -0.01 ± 0.27), although Shannon's diversity was marginally reduced by -0.56 ± 0.48 (Le Geay et al., 2024). Warming and drought in peatlands is of concern as studies have shown that they can trigger a positive feedback for climate change due to increased microbial activity and emissions of carbon dioxide (Kitson and Bell, 2020; Rafat et al., 2021; Quan et al., 2025). The peatland bacterial community appears constrained by the cold, waterlogged conditions in the soil, and raising ambient temperature seems to have favoured many of the species present, although these were uniformly negatively affected by drought.

Fungal diversity was not changed by warming, although there was an increase in the relative abundance of plant pathogenic fungi, which could potentially impact plant community composition over time (Casadevall, 2023). Further, fungal diversity increased following drought (up to fold change increase of 1.35, $p < 0.05$), a response that was independent of warming. Fungal communities are typically more stable in response to climate perturbations than bacterial communities (de Vries et al., 2018; Oram et al., 2025), and the weak responses and lack of change in fungal community structure observed using the NMDS are in line with findings of other studies (Roth et al., 2023). Overall, our results indicate that in peatlands, the microbial community is well adapted to wet soil conditions and drying therefore has a much stronger effect, albeit in the short-term, than changes in ambient temperature or plant community composition. Recommendations for peatland management usually focus on raising the water table, and our findings support this as a strategy for maintaining the stability of microbial communities in the face of ongoing environmental change (Strack et al., 2022).

4.2. Shrub removal modifying warming

We expected that a legacy of shrub removal would increase the effect of warming on the microbial community and further destabilise the community when exposed to drought. Shrub removal had strong effects on the microbial community, increasing bacterial species richness (fold change 1.02, $p < 0.05$), but decreasing fungal richness (fold change 0.94, $p < 0.05$). When combined with warming, bacterial richness increased further, while fungal species richness returned to the level of the control, thereby cancelling out the effect of shrub removal. However, these effects did not impact the community resistance or resilience to drought ($p > 0.05$). Microbial ability to withstand drought is driven by changes in either gene expression, physiological acclimation or community structure (Allison, 2023). While we did see changes in community structure in response to warming and shrub removal, these were mainly exploitative (such as the increase in plant pathogenic fungi under warming) and did not impact the ability of the community overall to withstand drought. The waterlogging experienced by peat soils represents a strong environmental filter on the microbial community (Robinson et al., 2023). While in our study the legacy of warming and shrub removal created a different starting point from the control soils, it is likely that the drought placed similar constraints on the microbial communities in all treatments. Therefore, when peatland dries, microbial communities are likely to become unstable and activity will increase, metabolising available organic matter (Canarini et al., 2017; Kitson and Bell, 2020). Nevertheless, interactive effects of temperature and vegetation change can have strong, long-term effects on bacterial community structure. These effects could have long-lasting effects on greenhouse gas emissions and carbon storage in peatlands and have implications for peatland management and the maintenance of shrubs (Rowland et al., 2021).

Fungal species richness was reduced by shrub removal but increased under drought. It seems likely that the lost species were mainly from rhizosphere communities closely associated with the dwarf shrub, as shown by other studies (Wang et al., 2022). There is evidence that the polyphenols released by shrubs form an important substrate for fungi, so while the indicator analysis did not highlight any species primarily associated with shrub removal, it is likely that a wide range of species that decompose polyphenols were lost in this treatment (Wang et al., 2022; Zhang et al., 2025). The negative association between temporal stability of phenolics and fungal species richness could indicate that drought, which increased fungal richness, is a major contributor to instability of phenolics via the microbial community. Therefore, stable phenolic concentrations are likely to occur when shrubs were removed, because fungal richness and phenolics were low. Overall, this may be a negative outcome for the peatland ecosystem, particularly as the shrub removal and warming benefits bacteria and could increase metabolism of nitrogen and carbon.

4.3. Function

Contrary to microbial community composition, recovery of carbon and nitrogen-based functions from drought was strongly affected by warming and shrub removal ($p < 0.05$). We found a strong negative effect of shrub removal on carbon-based variables, thereby likely destabilising carbon storage (Robinson et al., 2023). This is consistent with previous work on the experiment showing that shrub removal increased litter decomposition (Ward et al., 2013; Walker et al., 2015). It has been suggested that shrub presence could be a regulating mechanism in peatlands to buffer against abiotic change (Wang et al., 2022; Buttler et al., 2023). As well as stabilising the peat against erosion, shrubs direct a range of phenolic compounds into the soil, promoting a fungal community that can use this substrate (Zhang et al., 2025). In our study shrub removal and warming were also linked with higher total nitrogen, which could be a result of increased microbial activity ($p < 0.05$). In support of our third hypothesis that changes to the microbial community in response to a warming and shrub removal would impact the resistance and resilience to drought of microbial-mediated functions, we found that this was the case for resilience, but not resistance. Specifically, we found that there was no effect of warming, and no interaction with shrub removal on resistance of any function to drought. In a similar long-term study, it was shown that warming increased the number of active bacterial taxa, rather than the overall activity rates of the community, which mirrors what we found (Metze et al., 2024). As for the microbial community, the drought seems to have overwhelmed function, so initial response and recovery were independent of warming and shrub removal. This may suggest that warming and shrub loss should be of less concern than the severe effect of drought. However, the long lasting and complex interactions between the legacy treatments and the drought destabilise carbon and nitrogen pools and processes in unpredictable ways.

5. Conclusions

Our results show that warming and shrub removal individually have no impact on the resistance but lower the resilience of peatland microbial communities to drought, at least in terms of their diversity. These responses were dampened when warming and shrub removal were combined, although recovery of both bacterial and fungal communities was still incomplete eight weeks after rewetting, suggesting a low resilience of peatland microbial communities to severe drought. We also show that warming strongly decreased the resilience of nitrogen-based functions to drought, albeit only in the presence of shrubs. Taken together our results point to strong effects of warming and vegetation change on the resilience of peatland microbial communities and their functioning to severe drought, which could potentially undermine the carbon storage potential of peatland. Moreover, our results also underscore the potential for different global change factors to interact to shape soil microbial communities and microbial-mediated functions, with potential implications for biogeochemical processes and carbon-cycle feedback to climate change (Chomel et al., 2022; Knorr et al., 2024; Quan et al., 2025). As such, mitigating such responses should be a priority for sustainable management, for instance by maintaining the dominance of shrubs and raising the water table in peatland.

CRediT authorship contribution statement

Ellen L. Fry: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Amy L. Evans:** Writing – review & editing, Methodology, Investigation, Data curation. **Deborah Ashworth:** Writing – review & editing, Methodology, Investigation, Data curation. **Ana Soto:** Writing – review & editing, Methodology, Data curation. **Juntao Wang:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Nick Ostle:** Writing – review & editing, Resources, Investigation, Conceptualization. **Brajesh K. Singh:** Writing – review & editing, Supervision, Software, Resources, Project administration, Conceptualization. **Richard D. Bardgett:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2026.110100.

References

Allison, S.D., 2023. Microbial drought resistance may destabilise soil carbon. Trends in Microbiology 31, 780–787.

Austin, K.G., Elsen, P.R., Honorio Coronado, E.N., DeGemmis, A., Gallego-Sala, A.V., Harris, L., Kretser, H.E., Melton, J.R., et al., 2025. Mismatch between global importance of peatlands and the extent of their protection. Conservation Letters 18, e13080.

Bardgett, R.D., 2025. The Ecology of Soil: from Communities to Ecosystems. Oxford University Press.

Bardgett, R.D., Caruso, T., 2020. Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. Philosophical Transactions of the Royal Society B 375.

Bardgett, R.D., Marsden, J.H., Howard, D.C., 1995. The extent and condition of heather on moorland in the uplands of England and Wales. Biological Conservation 71, 155–161.

Bardgett, R.D.S., Streeter, T.C., Bol, R., 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84, 1277–1287.

Bechtold, M., Dettmann, U., Wohl, L., Durner, W., Piayda, A., Tiemeyer, B., 2018. Comparing methods for measuring water retention of peat near permanent wilting point. Soil Science Society of America Journal 82, 601–605.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B 57, 289–300.

Buttler, A., Bragazza, L., Laggoun-Défarge, F., Gogo, S., Toussaint, M.-L., Lamentowicz, M., Chojnicki, B.H., Słowiński, M., Słowińska, S., et al., 2023. Ericoid shrub encroachment shifts aboveground-belowground linkages in three peatlands across Europs and Western Siberia. Global Change Biology 29, 6772–6793.

Buyer, J.S.S., Sasser, M., 2012. High throughput phospholipid fatty acid analysis of soils. Applied Soil Ecology 61, 127–130.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., Cardinale, B.J., Hooper, D.U., Dee, L.E., Duffy, J.E., 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods in Ecology and Evolution 5, 111–124.

Camenzind, T., Mason-Jones, K., Mansour, I., Rillig, M.C., Lehmann, J., 2023. Formation of necromass-derived soil organic carbon determined by microbial death pathways. Nature Geoscience 16, 115–122.

Canarini, A., KiKiær, L.P., Dijkstra, F.A., 2017. Soil carbon loss regulated by drought intensity and available substrate: a meta-analysis. Soil Biology and Biochemistry 112, 90–99.

Cape, J.N., Smith, R.I., Leaver, D.S., 2014. In: Centre, N.E.I.D. (Ed.), Cleaned UK Rainfall Chemistry Data (1986-2011).

Caporaso, J.G., Kuczynski, J., Stombaugh, J., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Casadevall, A., 2023. Global warming could drive the emergence of new fungal pathogens. Nature Microbiology 8, 2217–2219.

Chen, Y., Qin, W., Zhang, Q., et al., 2024. Whole-soil warming leads to substantial soil carbon emission in an alpine grassland. Nature Communications 15, 4489.

Chiang, F., Mazdiyasni, O., AghaKouchak, A., 2021. Evidence of anthropogenic impacts on global drought frequency, duration, and intensity. Nature Communications 12, 2754.

Chomel, M., Lavallee, J.M., Alvarez-Segura, N., Baggs, E.M., Caruso, T., de Castro, F., Emmerson, M.C., Magilton, M., Rhymes, J.M., de Vries, F.T., Johnson, D., Bardgett, R.D., 2022. Intensive grassland management disrupts below-ground multi-trophic resource transfer in response to drought. Nature Communications 13, 6991.

Cordero, I., Leizaga, A., Hicks, L.C., Rousk, J., Bardgett, R.D., 2023. High intensity perturbations induce an abrupt shift in soil microbial state. The ISME Journal 17, 2190–2199.

Dang, P., Chang, S.X., Chen, J., Delgado-Baquerizo, M., Bahram, M., Eisenhauer, N., Xu, C., Li, X., Wang, S., Mikryukov, V., Zhao, X., Wang, X., Xue, J., Qin, X., Ros, G.H., Tedersoo, L., 2025. Long-term climate warming substantially reduces global soil microbial richness. One Earth, 101511 (in press).

De Caceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., 2018. Soil bacterial networks are lesss table under drought than fungal networks. Nature Communications 9, 3033.

Dunn, C., Zielinski, P., Ken, M., Freeman, C., 2018. Investigating whether light intensity can modify decomposition rates in peatlands through control of the 'enzymic latch'. Ecological Engineering 114, 167–172.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Edgar, R.C., 2016. UNOISE2: Improved error-correction for Illumina 16S and ITS Amplicon Sequencing. bioRxiv.

Ferguson, P., Lindo, Z., 2025. Disentangling the effect of temperature and moisture on boreal peatland microbial activity and function. Pedobiologia 111, 151063.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71.

Folin, O.D., 1915. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. Journal of Biological Chemistry 22, 305–308.

Fry, E.L., Ashworth, D., Allen, K.A.J., Chardon, N.I., Rixen, C., Bjorkmann, M.P., Bjork, R. G., Stalhandske, T., Molau, M., Locke-King, B., Cantillon, I., McDonald, C., Liu, H., De Vries, F.T., Ostle, N.J., Singh, B.K., Bardgett, R.D., 2023. Vegetation type, not the legacy of warming, modifies the response of microbial functional genes and greenhouse gas fluxes to drought in oro-arctic and alpine regions. FEMS Microbiology Ecology 99 fiad145.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimisation of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397.

Hernandez, D.J., David, A.S., Menges, E.S., Searcy, C.A., Afkhami, M.E., 2021. Environmental stress destabilizes microbial networks. The ISME Journal 15, 1722–1734.

Jackson, C.R., Tyler, H.L., Millar, J.J., 2013. Determination of microbial extracellular enzyme activity in waters, soils, and sediments using high throughput microplate assays. Journal of Visualized Experiments 80, 1–9.

Kitson, E., Bell, N.G.A., 2020. The response of microbial communities to peatland drainage and rewetting. A review. Frontiers in Microbiology 11.

Knorr, M.A., Contosta, A.R., Morrison, E.W., Muratore, T.J., Anthony, M.A., Stoica, I., Geyer, K.M., Simpson, M.J., Frey, S.D., 2024. Unexpected sustained soil carbon flux in response to simultaneous warming and nitrogen enrichment compared with single factors alone. Nature Ecology & Evolution. https://doi.org/10.1038/s41559-024-02546-x.

Kõljalg, U., Larsson, K.H., Abarenkov, K., 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166, 1063–1068.

Le Geay, M., Lauga, B., Walcker, R., Jassey, V.E.J., 2024. A meta-analysis of peatland microbial diversity and function responses to climate change. Soil Biology and Biochemistry 189, 109287.

Leitner, Z.R., Daigh, A.L.M., DeJong-Hughes, J., 2021. Temporal fluctuations of microbial communities within the crop growing season. Geoderma 391, 114951.

Loreau, M., De Mazacourt, C., 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecology Letters 16, 106–115.

Melillo, J.M., Frey, S.D., DeAngelis, K.M., et al., 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. Science 358, 101–105.

Meng, C., Tian, D., Zeng, H., Li, Z., Chen, H.Y.H., Niu, S., 2020. Global meta-analysis on the responses of soil extracellular enzyme activities to warming. Science of the Total Environment 705, 135992.

Metze, D., Schnecker, J., Le Noir De Carlan, C., Bhattarai, B., Verbruggen, E., Ostenen, I., Janssens, I.A., Sigursson, B.D., Hausmann, B., 2024. Soil warming increases the number of growing bacterial taxa but not their growth rates. Science Advances 10.

Office, M., 2025. MIDAS open: UK daily weather observation data, v202507. In: NERC EDS Centre for Environmental Data Analysis.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2010. Vegan: community ecology package. https://cran.r-project.org/package=vegan.

Oram, N.J., Praeg, N., Bardgett, R.D., Brennan, F., Caruso, T., Illmer, P., Ingrisch, J., Bahn, M., 2025. Drought intensity shapes soil legacy effects on grassland plant and soil microbial communities and their responses to future drought. Global Change Biology 31, e70495.

Orwin, K.H., Wardle, D.A., 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology and Biochemistry 36, 1907–1912.

Peñuelas, J., Estiarte, M., Kimball, B.A., Idso, S.B., Pinter, P.J., Wall, G.W., Garcia, R.L., Hansaker, D.J., LaMorte, R.L., Hendrix, D.L., 1996. Variety of responses of plant phenolic concentration to CO2 enrichment. Journal of Experimental Botany 47, 1463–1467.

Philippot, L., Griffiths, B.S., Langenheder, S., 2021. Microbial community resilience across ecosystems and multiple disturbances. Microbiology and Molecular Biology Reviews 85 (mmbr), 00026-00020.

Pinheiro, J., Bates, D., R Core Team, 2025. nlme: Linear and Nonlinear Mixed Effects Models.

Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Penuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biology and Biochemistry 131, 28–39.

Quan, Q., Zhou, J., Hanson, P.J., Ricciuto, D., Sebestyen, S.D., Weston, D.J., Chanton, J. P., Wilson, R.M., Kotka, J.E., et al., 2025. Drought-induced peatland carbon loss exacerbated by elevated CO$_2$ and warming. Science 390, 367–370.

Rafat, A., Rezanezhad, F., Quinton, W.L., Humphreys, E.R., Webster, K., Van, Cappellen, 2021. Non-growing season carbon emissions i a northern peatlands are projected to increase under global warming. Communications Earth & Environment 2, 111.

Robinson, C.H., Ritson, J.P., Alderson, D.M., Malik, A.A., Griffiths, R.I., Heinemeyer, A., Gallego-Sala, A.V., et al., 2023. Aspects of microbial communities in peatland carbon cycling under changing climate and land use pressures. Mires & Peat 29, 2.

Roth, S.W., Griffiths, N.A., Kolka, R.K., Oleheiser, K.C., Carrell, A.A., Klingeman, D.M., Seibert, A., Chanton, J.P., Hanson, P.J., Schadt, C.W., 2023. Elevated temperature alters microbial communities, but not decomposition rates, during 3 years of *in situ* peat decomposition. mSystems 8. https://doi.org/10.1128/msystems.00337-00323.

Rowland, J.A., Bracey, C., Moore, J.L., Cook, C.N., Bragge, P., Walsh, J.C., 2021. Effectiveness of conservation interventions globally for degraded peatlandsin cool-climate regions. Biological Conservation 263–109327.

Seneca, J., Sollinger, A., Herbold, C.W., Pjevac, P., Prommer, J., Verbruggen, E., Sigurdsson, B.D., Penuelas, J., Janssens, I.A., Urich, T., Tveit, A.T., Richter, A., 2021. Increased microbial expression of organic nitrogen cycling genes in long-term warmed grassland soils. ISME Communications 1.

Simon, E., Canarini, A., Martin, V., Séneca, J., Böckle, T., Reinthaler, D., Pötsch, E.M., Piepho, H.-P., Bahn, M., Wanek, W., Richter, A., 2020. Microbial growth and carbon use efficiency show seasonal responses in a multifactorial climate change experiment. Communications Biology 584.

Smith, R.A., Levine, T.R., Lachlan, K.A., Fediuk, T.A., 2002. The high cost of complexity in experimental design and data analysis: type I and type II error rates in multiway ANOVA. Human Communication Research 28, 515–530.

Soong, J.L., Castanha, C., Hicks Pries, C.E., et al., 2021. Five years of whole-soil warming led to loss of subsoil carbon stocks and increased CO2 efflux. Science Advances 7, eabd1343.

Sorenson, J.W., Shade, A., 2020. Dormancy dynamics and dispersal contribute to soil microbiome resilience. Philosophical Transactions of the Royal Society B 375.

Stenström, M.G.F., Henry, G.H.R., 1997. Response of *Saxifraga oppositifolia* L., to simulated climate change at three contrasting latitudes. Global Change Biology 3, 44–54.

Strack, M., Davidson, S.J., Hirano, T., Dunn, C., 2022. The potential of peatlands as nature-based climate solutions. Current Climate Change Reports 8, 71–82.

Tripathi, B.M., Pineiro, J., Dang, C., Brzostek, E., Morrissey, E.M., 2025. Mycorrhiza-saprotroph interacions and carbon cycling in the rhizosphere. Global Change Biology, e70173.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, fourth ed. Springer, New York.

Wagg, C., Hautier, Y., Pellkofer, S., Banerjee, S., Schmid, B., van der Heijden, M.G.A., 2021. Diversity and asynchrony in soil microbial communities stabilizes ecosystem functioning. eLife 62813.

Walker, T.W.N., Ward, S.E., Ostle, N.J., Bardgett, R.D., 2015. Contrasting growth responses of dominant peatland plants to warming and vegetation composition. Oecologia 178, 141–151.

Walker, T.W.N., Kaiser, C., Strasser, F., 2018. Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. Nature Climate Change 8, 885–889.

Wang, H., Richardson, C.J., Ho, M., 2015. Dual controls on carbon loss during drought in peatlands. Nature Climate Change 5, 584–587.

Wang, X., Li, Y., Yan, Z., Hao, Y., Kang, E., Zhang, X., Li, M., Zhang, K., Yan, L., Yang, A., Niu, Y., Kang, X., 2022. The divergent vertical pattern and assembly of soil bacterial and fungal communities in response to short-term warming in an alpine peatland. Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.986034.

Ward, S.E., Ostle, N.J., Oakley, S., Quirk, H., Henrys, P.A., Bardgett, R.D., 2013. Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. Ecology Letters 16, 1285–1293.

Ward, S.E., Orwin, K.H., Ostle, N.J., Briones, M.J.I., Thomson, B.C., Griffiths, R.I., Oakley, S., Quirk, H., Bardgett, R.D., 2015. Vegetation exerts a greater control on litter decomposition than climate warming in peatlands. Ecology 96, 113–123.

Xiao, W., Chen, X., Jing, X., Zhu, B., 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. Soil Biology and Biochemistry 123, 21–32.

Yee, T.W., 2010. The VGAM package for categorical data analysis. Journal of Statistical Software 32, 1–34.

Zaman, M., et al., 2021. Methodology for measuring greenhouse gas emissions from agricultural soils using non-isotopic techniques. In: Zaman, M., Heng, L., Müller, C. (Eds.), Measuring Emission of Agricultural Greenhouse Gases and Developing Mitigation Options Using Nuclear and Related Techniques. Springer, Cham.

Zhang, Y., Huang, X., Zhao, B., Yan, C., Zhao, H., Zhang, H., Halamka, T.A., Peel, R.H., et al., 2025. Microbial responses to changing plant community protect peatland carbon stores during Holocene drying. Nature Communications 16, 6912.