

Long-term manipulation of mean climatic conditions alters drought effects on C- and N-cycling in an arable soil

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Funding information

Anton and Petra Ehrmann-Stiftung

Abstract

Climate is changing and predicted future scenarios include both changes in long-term mean climatic conditions and intensification of extreme events such as drought. Drought can have a major impact on soil functional processes; soil microorganisms, key to these processes, depend on water and temperature dynamics. Consequently, feedback mechanisms regarding microbially mediated carbon and nitrogen cycling in soils may be affected. There are indications that microbial exposure to increasingly unfavorable environmental conditions influences their stress responses. Here, the long-term field experiment Hohenheim Climate Change (HoCC) provided a research platform to explore how microbial exposure to long-term reduced water availability and soil warming modifies microbially driven soil processes, especially gas fluxes from soil, both during drought and after rewetting. The HoCC experiment is an agroecosystem in which the soil microbiome has been exposed to reduced annual mean precipitation and elevated temperature since 2008. Treatment levels were chosen based on a realistic future climate scenario. In June 2019, we exposed this system to a drought period of four weeks. We found that even after 11 years, warming remained a driver of CO₂ and N₂O fluxes across the different soil moisture conditions in our drought experiment. Importantly, however, microbial exposure to long-term reduced water availability limited the stimulatory effect of warming on gas fluxes during drought and after rewetting. Our results were neither related to a legacy effect within overall microbial biomass carbon levels nor a shift towards enhanced fungal abundance. We found no indications that extracellular enzyme activities or microbial substrate availability explained the gas flux dynamics observed in our drought experiment. Our study indicates that soil warming promotes gaseous C and N loss even under extreme drought conditions. We suspect, however, that a shift in microbial function following long-term water limitation can hamper the enhancing effect of warming on soil gas fluxes.

KEY WORDS

climate change, drought stress, legacy effect, soil gas fluxes, soil microorganisms, temperate agroecosystem

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1 | INTRODUCTION

Soil microorganisms are the drivers of relevant biogeochemical cycles and thus control soil ecosystem functions. The rate of microbially driven matter cycling and the species involved depend on water availability and temperature conditions (Frindte et al., 2019; Zhang et al., 2019). Consequently, changes in mean climatic conditions pose an evolutionary challenge for soil microorganisms as they must optimize their resource use to survive in an evolving microenvironment (Cavicchioli et al., 2019). In addition to ongoing changes in long-term mean climatic conditions, extreme events such as droughts are predicted to intensify in frequency and duration in the future (Grillakis, 2019). Droughts can have a major impact on soil functional processes; water films in soil pores serve as microbial habitat as well as solvent and transport media for microbial resources. Furthermore, drought requires that soil microorganisms reallocate resources from growth to cell protection with feedback mechanisms that affect carbon (C) and nitrogen (N) cycling in soils (Schimel, 2018; Schimel et al., 2007). While intensifying drought periods undoubtedly affect soil ecosystem processes, it is unclear how changing mean climatic conditions influence the response of soil microorganisms to a discrete drought period.

According to future climate scenarios, soil microorganisms in the temperate climate zone will be challenged by changing precipitation regimes during the summer months. Changes include a reduction in total precipitation as well as prolonged dry periods followed by heavier rainfall events (Frei et al., 2006). In addition, increasing air temperatures will result in rising average soil temperatures (Bradford et al., 2019). Besides directly affecting the soils' microclimate, temperature will interact with soil moisture (Zheng et al., 2016), most notably by amplifying evaporation and thus desiccation of the topsoil. Temperature and precipitation form an associated site-specific soil moisture history, a parameter that is becoming increasingly important in research on climate change-induced water stress (Cuddington, 2011). Soil moisture history may have a lasting effect, imparting a 'legacy' to soil microbial abundance and functioning, significantly influencing microbial responses to future disturbances such as drought. Long-term studies targeting this issue are rare, however. To date, in studies on multiple heathland, grassland, and forest-steppe sites across Europe, neither long-term water limitation nor temperature elevation had a legacy effect on soil microbial community structure as determined by growth and respiration rates in samples that were equally incubated in the lab (Rousk et al., 2013). In contrast, multiyear precipitation reductions at a heathland site lowered the ratio of respiration to bacterial growth during subsequent drying and rewetting cycles (Nijs et al., 2019). In the same approach in a tallgrass prairie, long-term water limitation led to lower respiration rates following initial soil rewetting (Evans & Wallenstein, 2012). These studies indicate that within natural ecosystems, microbial exposure to one stressor (long-term precipitation limitation) can have a legacy effect on the outcome of another, in this case in the form of a change in functional response within cycles of extreme

drying and rewetting. However, drought and rewetting stress were simulated within laboratory incubations in those studies and not under field conditions. Furthermore, in the context of climate change scenarios, knowledge is lacking on factorial combinations of reduced precipitation and elevated temperatures. This could be a critical factor in determining the importance of water limitation legacies. For example, elevated temperatures have been shown to enhance heterotrophic respiration activity (Rustad et al., 2001) as well as N mineralization and transformation (Dai et al., 2020). However, below a threshold in moisture level, soil warming can lose its stimulatory effect on microbial activity (Li et al., 2017; Liu et al., 2009; Noh et al., 2016). Hence, assigning legacy effects to either moisture or temperature alone seems inadequate when these parameters are mutually dependent. In addition, to date only natural ecosystems have been investigated, which, in contrast to arable land agroecosystems, experience significantly less disturbance throughout the year. Soil management practices in agroecosystems, such as tillage, crop rotation, etc., create a multi-stress environment, requiring increased microbial tolerance to disturbance. This suggests there are ecosystem-specific characteristics of microbial stress responses in arable land.

Soil respiration is often used as a bioindicator to detect changes in microbially mediated processes, as it integrates most belowground microbial activity. Much of this activity depends on the activity of extracellular enzymes (EEA), which are considered to be the functional link between organic matter and respiration, with EEA as the rate-limiting step in decomposition (Bengtson & Bengtsson, 2007; Schimel & Bennett, 2004). Overall, the sensitivity of enzyme activities to changing moisture and temperature patterns is not yet thoroughly understood, in part because synthesis and secretion of extracellular enzymes, as well as their turnover, are not solely dependent on direct effects of moisture and temperature. Rather, the interacting effects of climatic factors on microbial abundance and community structure influence EEA within soil, where extracellular enzymes are present in various immobilized and diffusible forms (Burns et al., 2013). Still, warming in particular has been shown to increase mass-specific EEA; models suggest that enhanced microbial cell maintenance costs induced by warming demand increased the allocation of nutrients to enzyme production (Steinweg et al., 2013). This is consistent with a global study across different types of ecosystems in which soil warming enhanced EEA (Meng et al., 2020). Accordingly, warmed soils may contain a surplus of microbially available C and N. This would not only influence gaseous C flux dynamics from soil but also N flux dynamics; nitrifiers and denitrifiers require readily available C for oxidation of ammonium (NH_4^+) and reduction of nitrate (NO_3^-) (Wang et al., 2021).

During periods of matric and osmotic stress such as drought, the production of enzymes is in direct resource competition with the microbial need to adapt physiologically for cell protection by accumulating osmolytes and extracellular polymeric substances (Chowdhury et al., 2011; Schimel et al., 2007). Accordingly, it has been shown that drought can significantly reduce EEA (Singh et al.,

2021). However, a recent study simulating repeated droughts revealed mechanisms of an 'ecological memory': EEA in soils which were annually exposed to drought periods for 10 years remained at the same potential activity levels as the untreated controls, while exposure to only one drought significantly decreased EEA (Canarini et al., 2021). The repeated droughts in this study were rather extreme; therefore, it is unclear whether moderate annual precipitation reduction based on a change in mean climatic conditions can trigger the same mechanism.

Functional shifts in microbial responses to different stressors have repeatedly been associated with shifts in microbial communities toward greater relative fungal abundance. In theory, fungal traits, such as hyphal networks and thick cell walls, enable fungi to maintain physiological activity even when water films in drying soils diminish (Treseder et al., 2018). However, despite theoretical advantages, results on fungal drought resistance have been contradictory (Barnard et al., 2013; Kaisermann et al., 2017; Manzoni et al., 2012; Meisner et al., 2013, 2018; Vries et al., 2018). Nevertheless, the legacy of multiple years of water limitation in forests, grasslands, and shrublands has been shown to favor fungal abundance (Haugwitz et al., 2016; Yuste et al., 2011), especially when combined with elevated temperatures. Moreover, enhanced fungal abundance also led to the highest cumulative CO₂ emissions from these soils (Haugwitz et al., 2016), emphasizing the central role of soil fungi in litter decomposition and therefore C cycling in soils. As fungal abundance in arable soils is generally lower than in natural ecosystems, moisture and temperature induced legacy effects that increase relative fungal abundance could contribute to fundamental changes in soil ecosystem functioning in arable soils.

Literature indicates that microbial exposure to changes in mean climatic conditions could have a legacy effect on microbial responses to subsequent stress events such as drought. However, it remains unclear how microbial abundances, resource availability, and extracellular enzyme dynamics in soil evolve along a change in long-term mean climatic conditions, thus affecting the outcome of a discrete drought period.

We hypothesized that long-term drier and warmer conditions increase gas fluxes from soils during drought as a result of ongoing higher microbial activity compared to ambient soils. This may be even more likely when soils were exposed to a combination of elevated temperature and altered precipitation. Increased gas fluxes during drought may derive from long-term warming induced increases in potential EEA in these soils, providing easily degradable substrates for microbial metabolic activity. Finally, we assumed that observed higher gas fluxes in warmer and drier soils can derive from a shift in microbial community structure toward fungi, which are capable of remaining physiologically active under drought conditions. To test these hypotheses, we applied an extreme drought event to the long-term field experiment 'Hohenheim Climate Change' (HoCC). In the HoCC experiment, in a factorial design on an arable field, soil temperature has been elevated by 2.5°C since 2008, while both precipitation amount and frequency have been reduced.

2 | MATERIALS AND METHODS

2.1 | Field experiment

The research platform for this study is the 'Hohenheim Climate Change Experiment'. This is an arable field located at the experimental station of the University of Hohenheim (Stuttgart, Germany, 48°42'50"N, 9°11'26"E, 395 m a.s.l.). The site is characterized by mean annual precipitation of 689 mm and temperature of 9.1°C (1990–2020). The soil is a loess-derived stagnic Luvisol with silty loam-texture (9% sand, 69% silt, and 22% clay).

The HoCC experiment is set up in a split-plot design with four blocks, each block consisting of four main plots. Every main plot is divided into four sub-plots (1 m × 1 m). Mean precipitation and temperature patterns have been manipulated since 2008. The simulated changes are based on predictions for a climate change scenario for Germany until the year 2100 (Umweltbundesamt, 2006). In half the main plots, the soil is warmed by 2.5°C with heating cables placed on the soil surface and temperature measurement at 4 cm soil depth (T_a : ambient temperature; T_e : elevated temperature) throughout the year. Using foil roof constructions, precipitation has been manipulated annually from the beginning of June to the end of August. The first treatment reduced total precipitation by 25% (A_a : ambient amount and A_r : reduced amount). The second treatment reduced the precipitation frequency, where at every second precipitation event, soils received the cumulative rainfall (F_a : ambient frequency and F_r : reduced frequency). The third treatment was a combination of reduced precipitation amount and frequency. For further detailed information about the site and the experimental setup, see Poll et al. (2013). The precipitation treatments described above were applied for the last time in summer 2018 (early June to late August). The drought experiment of our study started 1 year later, i.e., 9 months after the last precipitation manipulation, and, therefore, no differences in soil moisture between precipitation treatments were detected before the start of our drought experiment.

In June 2019, this agroecosystem was exposed to a drought period of 30 days by excluding all precipitation via the above-mentioned foil roof constructions. As a rewetting event, we applied 20 mm of water to each sub-plot. After the rewetting event, we let the soil dry again for 18 days and afterwards proceeded with the regular HoCC precipitation treatments of reduced amount and frequency as described above. The crop was spring barley (*Hordeum vulgare*; Type RGT Planet), which was sown in April and harvested in August, with manual weed control (Table S1). Plant biomass and grain yield data were calculated per hectare.

2.2 | Soil sampling and properties, soil temperature and moisture monitoring

Soil sampling was carried out at five time points (D1–R3) within the three phases of the drought period: before drought (status quo after

11 years of the HoCC experiment; D1; 06–03–2019), at the end of the drought period (just before rewetting; D2; 07–03–2019), and 2 h (R1; 07–03–2019), 5 days (R2; 07–08–2019), and 14 days (R3; 07–17–2019) after rewetting. Samples were taken with a soil corer at a depth of 0–15 cm at five to seven randomized positions within each sub-plot and were pooled per sub-plot afterwards. All soil samples were sieved (<2 mm), gravimetric water content was determined, and due to the large number of samples, they were stored at –20°C. In order to prevent a freeze-thawing dynamic influencing our analyzed parameters, we did not defrost our samples but instead immediately started the respective analysis with the frozen soils after water adjustment. We therefore think that this procedure minimized the interference of either thawing or water adjustment on microbial biomass, ergosterol contents, and enzyme activities.

In order to account for a gradient in soil organic carbon content (SOC) on our experimental field site, sub-plot specific measures from 2008 were used as a covariate. SOC content was determined on an elemental analyzer (Elementar vario MACRO CNS) from soil samples taken before starting the HoCC experiment in June 2008.

Throughout the year, soil temperature and moisture were monitored via temperature probes at 4, 15, and 30 cm soil depths and time domain reflectometry (TDR) probes at depths of 0–15 and 15–30 cm.

2.3 | Gas fluxes from soil

Gas fluxes (CO_2 and N_2O) were measured weekly until the beginning of the drought period of 30 days. Within the drought period, five gas samplings were carried out (2, 10, 14, 24, and 28 days after the beginning of the drought). After rewetting, gas samples were taken after 1, 8, 24, 32, 48, 120, and 176 h.

Gas samples were taken within each subplot via closed chambers with inner volumes of 4850 cm^3 , each covering an area of 270 cm^2 . Gas samples (20 ml) were taken 0, 10, 20, and 30 min after closure with a syringe via three-way stopcock and injected into pre-evacuated 12 ml exetainers (Labco Ltd.). Concentrations of CO_2 and N_2O were determined on a gas chromatograph (Agilent Technologies) equipped with a methanizer and FID for CO_2 and an ECD for N_2O measurements. Three external standards per gas were used for calibration by linear regression. Cumulative fluxes of CO_2 and N_2O were calculated by linear interpolation between two successive gas samplings.

At every gas sampling date, we calculated Q_{10} values for each precipitation treatment according to the following equation:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\left(\frac{10}{T_2 - T_1} \right)}.$$

R_1 and R_2 are the CO_2 flux rates at ambient temperature (T_1) and elevated temperature (T_2) of the respective precipitation treatment. We excluded Q_{10} values smaller than 1 or higher than 6 for plausibility reasons (Fierer et al., 2006).

2.4 | Soil enzyme activities

Potential activities of the enzymes β -glucosidase, N-acetylglucosaminidase, and leucine-aminopeptidase were determined according to Marx et al. (2001) using buffer solutions (MES buffer 2-[N-morpholino]ethane sulfonic acid, TRIZMA buffer, a mixture of α, α, α -Tris-(hydroxymethyl)-methylamine and Tris-(hydroxymethyl)-aminomethane-hydrochloride), substrate solutions, and the fluorescent compounds 4-methylumbellifluorone (MUF) and 7-amino-4-methylcoumarin (AMC), all from Sigma-Aldrich. The MES buffer (pH 6.1) had a concentration of 0.1 M, and the TRIZMA buffer (pH 7.8) a concentration of 0.05 M. To prepare the standard solutions, MUF or AMC were dissolved in $\text{H}_2\text{O}_{\text{deion}}$ and methanol (ratio 1:1) and diluted with the corresponding buffer solution to a concentration of 10 μM . The substrates were dissolved in 300 μl dimethyl sulfoxide (DMSO) and filled to 10 ml with sterile water. From these standard solutions, the working solutions (1 mM) with autoclaved buffer solutions were prepared (MES buffer for MUF substrates, TRIZMA buffer for AMC substrates). For the analyses, 1 g field-fresh soil was dispensed into 50 ml $\text{H}_2\text{O}_{\text{deion}}$ by an ultrasonic disaggregator (50 J s^{-1} for 120 s) to break up soil aggregates. For each sample, 50 μl soil suspension, 50 μl buffer, and 100 μl substrate solution were pipetted into microplates (PP microplate, black 96 well, Greiner Bio-One GmbH) in triplicate. Standard series were prepared for each sample and buffer. For this, 50 μl soil suspension and MUF or AMC working solution were pipetted in the order of 0, 10, 20, 50, 80, 120 μl and combined with MES or TRIZMA buffer to a total volume of 200 μl . All plates were then pre-incubated at 30°C for 30 min. Fluorescence was then measured using a microplate reader (Synergy HTX, Multi-Mode Reader, BioTek Instruments Inc.) after 0, 30, 60, 120, and 180 min at a wavelength of 360 nm for excitation and 460 nm for emission. Between measurements, the plates were kept in the incubator.

2.5 | Microbial biomass carbon and nitrogen, extractable organic carbon, ammonium, and nitrate

Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) contents were determined via chloroform-fumigation-extraction (CFE) as described by Vance et al. (1987). Here, 10 g soil were adjusted to a water content of 25% relative to dry matter in order to account for the prerequisites of the CFE method in terms of soil moisture (Oren et al., 2018). Samples were then fumigated with ethanol-free chloroform for 24 h in a desiccator. Samples were then extracted in 40 ml 0.5 M K_2SO_4 on a horizontal shaker at 250 rpm for 30 min and centrifuged at 4400 g for 30 min. As a non-fumigated control and for the estimation of extractable organic carbon (EOC) from soil, another 10 g were directly extracted as described above but without the fumigation step. The supernatants were filtered through 20 μm filters and diluted 1:4 with $\text{H}_2\text{O}_{\text{deion}}$ to avoid high salinity, which could interfere with detection. C and N contents of the extracts were measured on a total organic carbon analyzer

(multi-N/C 2100S, Analytic Jena AG). C_{mic} and N_{mic} were calculated from the differences in concentrations between fumigated and non-fumigated samples with correction by the k_{EC} factor of 0.45 (Joergensen & Mueller, 1996) for C_{mic} and the k_{EN} factor of 0.54 for N_{mic} (Brookes et al., 1985).

The non-diluted extracts of the controls were used to determine NH_4^+ -N and NO_3^- -N concentrations in the soil samples. NH_4^+ -N and NO_3^- -N concentrations were measured colorimetrically with a continuous flow analyzer (CFA; Bran + Luebbe Autoanalyzer 3, SEAL Analytical).

2.6 | Ergosterol

Ergosterol is a component of fungal cell membranes, used as a quantitative biomarker of fungal biomass; it was determined according to Djajakirana et al. (1996). In brief, 1 g field-moist soils were weighed into 100 ml amber glass wide-neck bottles. Samples from time points D2, R2 and R3 were adjusted to a water content of 15% relative to dry matter as required by the method. Each sample was extracted with 25 ml ethanol on a horizontal shaker for 30 min at 250 rpm. After extraction, the suspensions were transferred to 50 ml Falcon tubes and centrifuged for 30 min at 4400 g. Ten ml of the extracts were then transferred to 10 ml centrifuge tubes and concentrated to dryness in a rotary vacuum concentrator at 50°C and 1 bar vacuum. Subsequently, the dried extracts were dissolved in 1 ml methanol and transferred through syringe filters (0.45 µm) into amber glass HPLC vials. For each extraction, two standard soil samples as well as blanks (without soil) were included. The measurements were performed via HPLC (Agilent 1260 Infinity series, Agilent Technologies).

2.7 | Statistical analysis

Data were analyzed using a mixed model approach. The design is a split plot with main plot factor temperature (T_i with levels T_a : ambient temperature and T_e : elevated temperature) and a two-factorial subplot factor. The two subplot factors were the amount of precipitation (A_j with levels A_a : ambient amount and A_r : reduced amount) and the frequency of precipitation (F_k with levels F_a : ambient frequency and F_r : reduced frequency). As data were taken repeatedly over time, the day of measurement (D_l with five levels D1 to R3) was the repeated measure factor and correlations between corresponding random and error effects of all time points were assumed. The model can be described as follows:

$$\begin{aligned} y_{hijkl} = & \mu + b_h + \beta c_{hijk} + T_i + A_j + F_k + D_l + (TD)_{ijl} \\ & + (AD)_{jl} + (FD)_{kl} + (TA)_{ij} + (TF)_{ik} + (AF)_{jk} + (TAD)_{ijl} \\ & + (TFD)_{ikl} + (AFD)_{jkl} + (TAF)_{ijk} + (TAFD)_{ijkl} + p_{hi} + e_{hijkl} \end{aligned}$$

where y_{hijkl} is the observation of the i th temperature, j th precipitation level, k th precipitation frequency of the h th block at day l , μ is the

intercept, b_h is the random effect of the h th complete block, β is the fixed slope for SOC c_{hijk} measured in subplots in h th block treated with i th temperature, j th precipitation level, k th precipitation frequency, T_i , A_j , F_k , and D_l are the fixed main effects for the i th temperature, j th precipitation level, k th precipitation frequency and l th day, respectively, $(TD)_{ijl}$, $(AD)_{jl}$, $(FD)_{kl}$, $(TA)_{ij}$, $(TF)_{ik}$, $(AF)_{jk}$, $(TAD)_{ijl}$, $(TFD)_{ikl}$, $(AFD)_{jkl}$, $(TAF)_{ijk}$, and $(TAFD)_{ijkl}$ are the fixed two-, three-, and four-way interactions of the corresponding factors involved, and p_{hi} and e_{hijkl} are the main plot and subplot errors, respectively. To account for the potential correlation of errors, a first-order autoregressive variance-covariance matrix with homogeneous and heterogeneous day-specific variances was fitted. The best fitting model was selected via AIC (Wolfinger, 1993). The covariate was kept in the model only if it was significant. Thus, means were adjusted for the covariate only if the covariate was significant via F test. For analyzing the data of cumulative gas fluxes, the model was reduced by the repeated measures factor. To account for the assumptions of the mixed model analysis, we checked residuals graphically for normal distribution and deviations from variance assumptions made within the model (Kozak et al., 2018). For data of 'EOC', 'Ergosterol', 'Ammonium', 'Nitrate', 'N-acetylglucosaminidase' and 'cumulative N_2O emissions', a logarithmic transformation was used to fulfill the assumptions mentioned above. Afterward, the model was reduced by dropping non-significant fixed effects starting with the highest-level interaction term $(TAFD)_{ijkl}$. Backward selection with the p -values of the corresponding F tests were performed until all effects remaining in the model were significant. Note that main effects and lower-level interactions were not considered if a higher-level interaction existed in the model. Further note that block effects of block, main plot, and error were not selected and thus remained in the final model. In case of a logarithmic transformation, estimated means on the transformed scale were back-transformed for presentation purposes. In this case, standard errors were back-transformed using the delta method. Note that back-transformed means estimated the medians on the original scale and were therefore denoted as median. Data were analyzed using the package 'asrem' (version 4.1.0.106) for R (version 3.6.3, R Core Team 2020).

3 | RESULTS

3.1 | Soil moisture and temperature; Plant data

All soils continuously dried during the experimental drought; volumetric soil moisture showed a small peak after rewetting, followed by decreasing moisture content due to the recurring drying (Figure 1a). Warming reduced gravimetric soil moisture content at every sampling date ($p < .01$; D1: -10%; D2: -12%; R1: -8%; R2: -10%; R3: -7%). Neither at the start of the drought experiment (D1) nor at peak drought conditions (D2) and after rewetting (R1-R3) did the long-term precipitation manipulations influence gravimetric soil moisture contents. During the experiment, warming increased soil temperature by around 2.3°C at 4 cm, 1.5°C at 15 cm, and 1°C at 30 cm depth (Figure 1b).

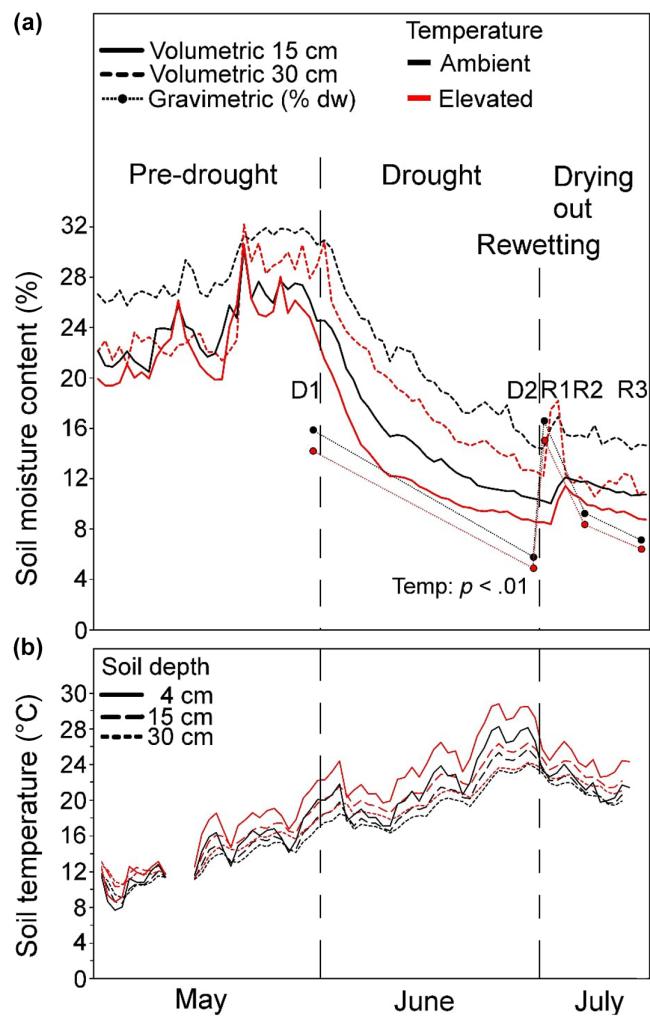


FIGURE 1 (a) Soil moisture content during the experiment at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 h, R2: 5 days, R3: 14 days after rewetting) in ambient and elevated soil temperature plots at a soil depth of 0–15 cm (volumetric (lines) and gravimetric (dots)) and 15–30 cm (volumetric)). Volumetric soil moisture data are presented as mean values (0–15 cm; $n = 16$; 15–30 cm; $n = 4$). Gravimetric soil moisture content data are presented as mean values including all precipitation treatments with standard errors ($n = 16$). Gravimetric soil moisture contents of long-term precipitation manipulated soils did not show any difference and are, therefore, not shown individually but as a mean. Declarations on statistics include all five timepoints. (b) Soil temperature during the experiment at 4 cm (solid line), 15 cm (dashed line) and 30 cm (dotted line), soil depth for ambient (black) and elevated (red) temperature treatments

Aboveground total plant biomass was 7.1 t ha^{-1} (± 1.1) and grain yield 4.6 t ha^{-1} (± 0.7) without significant differences between the treatments.

3.2 | Gas fluxes from soil

During the pre-drought period, CO_2 emission rates from warmed soils were for the most part higher than rates from ambient soils

(Figure 2a). As drought proceeded, soil CO_2 emission rates decreased, and differences between treatments diminished. Shortly after rewetting, CO_2 emission rates from warmed soils spiked to above pre-drought levels (+50% related to pre-drought, +95% related to peak drought conditions), while emission rates at ambient temperature increased only slightly (no increase related to pre-drought, +40% related to peak drought conditions) and peaked after 24 h. CO_2 emission rates decreased overall, though rates from warmed soils remained above ambient soils. Forty-eight hours after rewetting, emission rates from warmed and ambient soils had returned to pre-drought levels.

A Pearson correlation coefficient (Figure S4) of gravimetric soil moisture content at peak drought and the increase in CO_2 flux rate after rewetting was calculated to test for a possible link between the degree of drying and the size of the CO_2 pulse. No correlation was found between the two variables.

In the period before the drought event, cumulative CO_2 emissions increased with soil warming on average about +35% ($p < .01$), except for the soils exposed to both reduced precipitation amount and ambient precipitation frequency (Figure 2b). During the drought period, warming increased cumulative CO_2 emissions only in soils that were not exposed to reduced precipitation amount (+35%; $p < .01$). After rewetting, warming overall increased cumulative CO_2 emissions ($p < .01$), and the effect was, similar to that observed during drought, more pronounced in treatments with ambient (+50%) than with reduced precipitation amount (+20%).

Before drought, warming increased microbial biomass-specific CO_2 emission rates by about 35% ($p < .01$; Figure 2c). At peak drought (D2), specific rates were lower overall than before drought (-45%; $p < .01$) and warming increased rates in soils with ambient precipitation amount (+35%; $p < .05$) but had no effect in soils with reduced precipitation amount. Shortly after rewetting (R1), rates tripled compared to peak drought conditions ($p < .01$) and warming also increased rates in ambient precipitation amount soils (+45%; $p < .01$), however, this tendency was only observed in soils with reduced precipitation amount (+15%; $p = 0.1$). Five days after rewetting (R2), microbial biomass-specific rates were back to peak drought conditions and again, warming increased rates from ambient precipitation amount plots only (+55%; $p < .01$), but had no effect in soils with reduced precipitation amount.

During the course of our drought experiment (D1 to R3), long-term reduced precipitation amount significantly lowered average Q_{10} from about 3.8 in ambient precipitation amount soils to about 2.3 in soils with reduced precipitation amount ($p < .05$).

During the pre-drought period, N_2O emission rates were similar across all treatments. They decreased during drought in plots at ambient temperature and remained constant or increased in warmed plots (Figure 3a). Shortly after rewetting, N_2O emission rates from warmed soils spiked (+170% related to pre-drought, +450% related to peak drought conditions), whereas in ambient temperature soils only pre-drought emission levels were reached (no increase related to pre-drought, +450% related to peak drought conditions). Rates from all treatments declined to pre-drought levels within 24 h. In

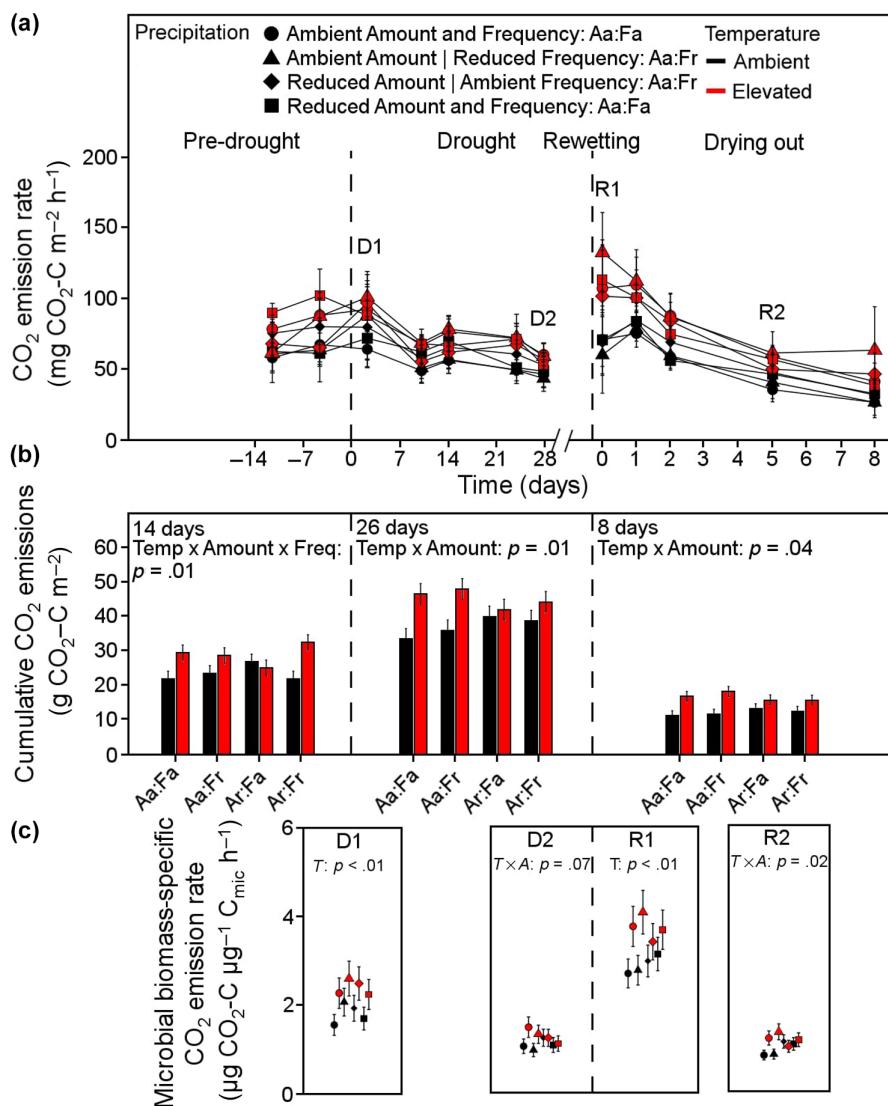


FIGURE 2 (a) CO_2 emission rates within the different phases 'Pre-drought', 'Drought' and 'Drying out after rewetting'; (b) Cumulative CO_2 emissions. The number of days indicate the length of the integrated period; (c) Microbial biomass specific CO_2 emission rates within specific timepoints D1: before drought, D2: just before rewetting, R1: 1 h, and R2: 5 days after rewetting. Precipitation amount (A; ambient; reduced), precipitation frequency (F; ambient; reduced) and temperature (T; ambient; elevated) indicate the long-term precipitation and warming treatments. CO_2 emission rates and cumulative CO_2 emissions data are presented as mean values including each precipitation and temperature treatment with standard errors ($n = 4$). Microbial biomass-specific emission rates are presented as back-transformed medians on the original scale including each precipitation and temperature treatment with standard errors ($n = 4$). Data points at the different dates are displayed with an offset for better visualization

the following 7 days of recurring drought, all N_2O emission rates decreased, with rates from warmed soils slightly higher than rates in ambient temperature soils.

Before drought, warming enhanced cumulative N_2O emissions on average by about 40% ($p < .05$). During drought, warming increased cumulative N_2O emissions tenfold from ambient precipitation soils ($p < .01$), while in soils with reduced precipitation, the increase was only 2.5- to 5-fold ($p < .01$; Figure 3b). After rewetting, warming increased cumulative N_2O emissions and this was more pronounced in ambient precipitation amount soils (+115%) than in soils with reduced precipitation (+75% to 85%; $p < .01$).

3.3 | Microbial biomass carbon and CN ratios

Before drought, warming tended to decrease C_{mic} content by about 10% ($p = .07$; Figure 4). As peak drought conditions were reached (D1–D2), C_{mic} levels remained stable overall, then increased by about 15% ($p < .01$) in response to rewetting (R1). Five days after rewetting

(R2), C_{mic} levels were still above pre-drought conditions ($p = .01$) but had returned to pre-drought levels 14 days after rewetting (R3).

Microbial CN ratios were similar at the onset of drought but differed significantly, although not consistently, at peak drought conditions and shortly after rewetting (Figure 5). The combination of reduced precipitation frequency and warming led to a significant increase in the microbial CN ratio from about 6.7 before drought (D1) to 8.4 at peak drought ($p < .01$; D2). Under reduced precipitation frequency alone, however, the lowest microbial CN ratio in the dry soil (D2) resulted from the significantly highest microbial biomass N content under this treatment (Figure S3). Shortly after rewetting (R1), warming tended to lower the microbial CN ratio compared to ambient temperature ($p = .1$).

3.4 | Soil enzyme activities

Potential β -Glucosidase activity remained stable overall during drought (D1 to D2; Figure 6a). In the freshly rewetted soil (R1),

FIGURE 3 (a) N_2O emission rates from soil; (b) Cumulative N_2O emissions within the different phases 'Predrought', 'Drought' and 'Rewetting' and 'Drying out after rewetting'. The number of days indicate within which timeframe the data points of the respective phase are integrated. Precipitation amount (A ; ambient; reduced), precipitation frequency (F ; ambient; reduced), and temperature (T ; ambient; elevated) indicate the long-term precipitation and warming treatments. N_2O emission rates are presented as mean values and cumulative N_2O emissions are presented as back-transformed medians on the original scale including each precipitation and temperature treatment with standard errors ($n = 4$)

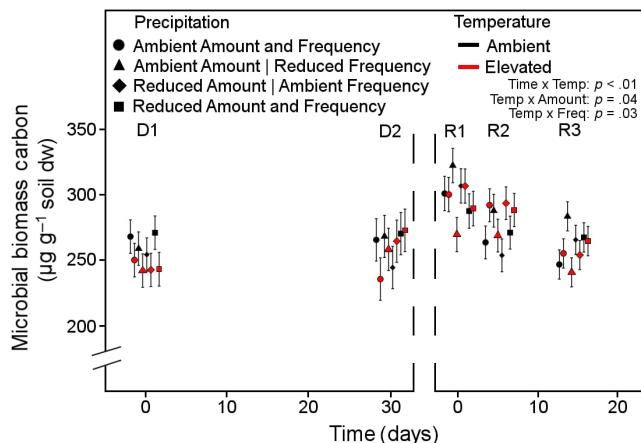
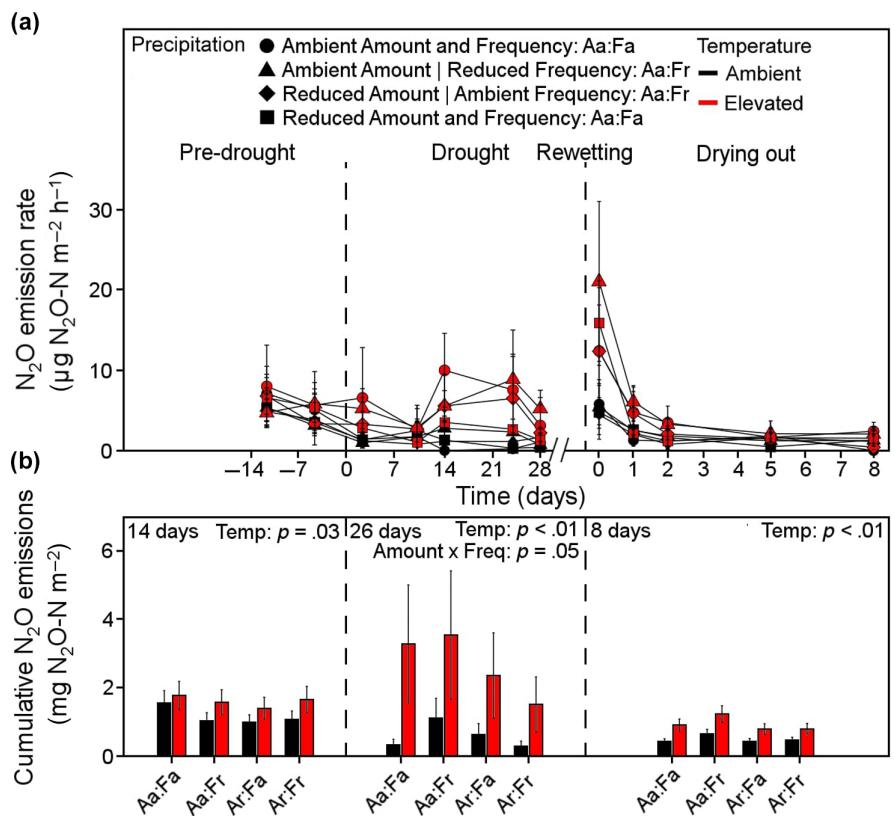


FIGURE 4 Microbial biomass carbon content at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 h, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation amount (A ; ambient; reduced), precipitation frequency (F ; ambient; reduced) and temperature (T ; ambient; elevated) indicate the long-term precipitation and warming treatments. Data are presented as mean values including each precipitation and temperature treatment with standard errors ($n = 4$). Declarations on statistics include all five timepoints. Data points at the different dates are displayed with an offset for better visualization

activity levels remained for the most part at peak drought levels, except in soils with ambient precipitation, where levels decreased about 20% ($p = .05$). Five days after rewetting (R2), all levels were

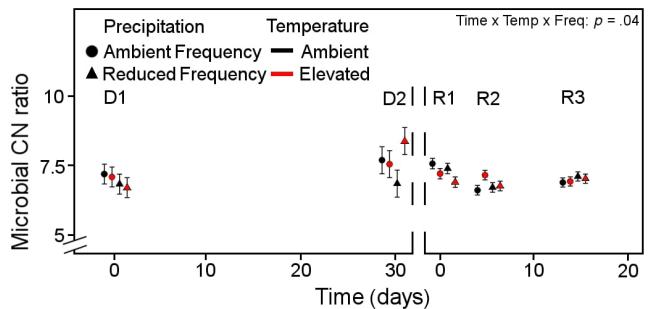


FIGURE 5 Microbial CN ratios at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 h, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation frequency (F ; ambient; reduced) and temperature (T ; ambient; elevated) indicate the long-term precipitation and warming treatments. Data are presented as mean values including the precipitation frequency and temperature treatments with standard errors ($n = 8$). Declarations on statistics include all five timepoints. Datapoints at the different dates are displayed with an offset for better visualization

back at around peak drought conditions and remained at these levels until 14 days after rewetting (R3).

Potential leucine-aminopeptidase activity increased during drought (D1–D2), on average by about 40% ($p = .05$) in ambient temperature soils, remaining stable in warmed soils (Figure 6b). In response to rewetting (R1), activity levels decreased in ambient temperature soils (~35%; $p < .01$) compared to peak drought conditions,

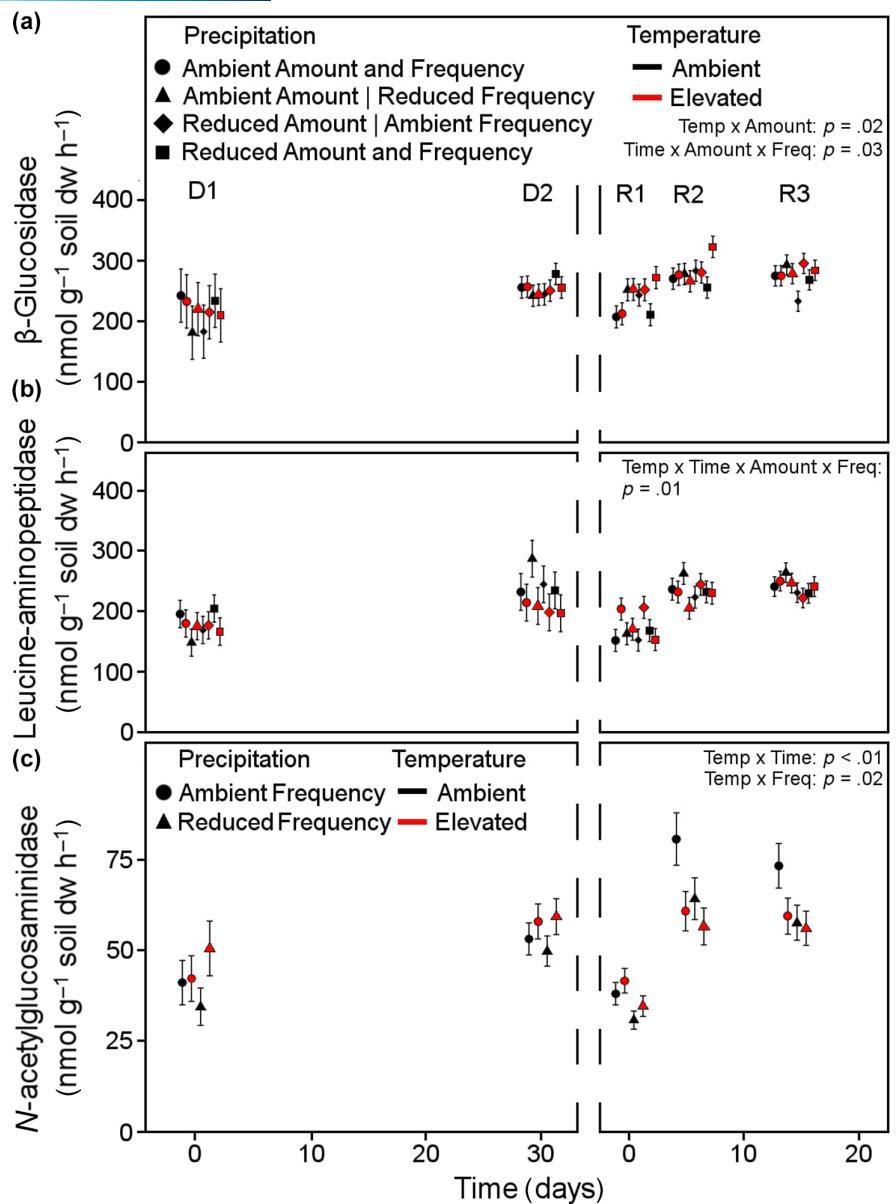


FIGURE 6 (a) Potential extracellular enzyme activities of β -glucosidase, (b) leucine-aminopeptidase, and (c) N-acetylglucosaminidase at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 hour, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation amount (A; ambient; reduced), precipitation frequency (F; ambient; reduced), and temperature (T; ambient; elevated) indicate the long-term precipitation and warming treatments. β -glucosidase and leucine-aminopeptidase data are presented as mean values including each precipitation and temperature treatment with standard errors ($n = 4$). N-acetylglucosaminidase data are presented as back-transformed medians on the original scale including precipitation frequency and temperature treatments with standard errors ($n = 8$). Declarations on statistics include all five timepoints. Datapoints at the different dates are displayed with an offset for better visualization

while levels in warmed soils remained stable. During recurring drought (R2–R3), levels increased back to peak drought conditions ($p < .01$) without any treatment effects.

Potential N-acetylglucosaminidase activity levels tended to increase during drought (D1–D2) on average by about 30% ($p = .1$; Figure 6c), then responded to rewetting with a decrease of about -45% ($p < .01$; R1). In the recurring drought (R2–R3), the levels returned to peak drought conditions ($p < .01$), with soils at ambient temperature and precipitation exhibiting the significantly highest

activity levels among all treatment groups ($p < .01$), surpassing peak drought levels.

3.5 | Ergosterol

Absolute ergosterol levels (Figure 7) as well as ergosterol levels related to C_{mic} (Figure S2) showed no major changes during the studied period. Before drought (D1), ergosterol levels related to C_{mic}

were significantly higher in soils with reduced precipitation (+25%; $p = .05$; Figure S2). During drought (D1–D2), after rewetting (R1), and during recurring drought (R2–R3), absolute and relative ergosterol levels remained stable.

3.6 | Extractable organic carbon, ammonium and nitrate

EOC levels increased significantly during drought (D1–D2) by about 30% ($p < .01$), independent of the long-term treatments (Figure 8). Shortly after rewetting (R1), the EOC content remained at peak drought levels and then decreased slightly in the recurring drying of the soil (R2–R3). Fourteen days after rewetting (R3), the EOC content was still above levels under pre-drought conditions (+20%; $p < .01$).

Before drought, the NH_4^+ content was about 40% lower in warmed than in ambient soils ($p < 0.01$) and levels remained more or less stable overall during drought (D1–D2; Figure 9a). Shortly after rewetting (R1), the NH_4^+ content increased on average by about 90% ($p < .01$). Five days after rewetting (R2), levels were back at around peak drought conditions and remained there during recurring drought (R2–R3).

Before drought (D1), the NO_3^- content in warmed soils was about 30% lower than in ambient temperature soils ($p = .02$; Figure 9b). Drought (D1–D2) reduced the NO_3^- content by 65% to 75% ($p < .01$). Rewetting increased the NO_3^- content more than threefold compared to peak drought conditions ($p < .01$). Within 5 days after rewetting (R2), the NO_3^- content in warmed soils remained at levels

significantly above levels in ambient soils (+55%; $p < .01$), as content from ambient soils decreased.

4 | DISCUSSION

Soil microorganisms play a key role in many soil functions; understanding their response to climatic extremes is essential for understanding soil functioning under climate change. We were interested in how microbial exposure to changing mean climatic conditions would influence the responses of microbial abundance and activity during an extreme drought period and after rewetting. Our results demonstrated an enhancing effect of warming on soil CO_2 fluxes throughout our drought experiment. However, more importantly, we found evidence for a legacy effect of reduced mean precipitation amount that limited the enhancing effect of warming.

4.1 | Impact of soil warming during drought and after rewetting

Typically, microbial activities and thus CO_2 fluxes from soil decrease with the severity of water limitation (Manzoni et al., 2012; Schimel, 2018; Wang et al., 2014), because the fraction of the microbial community maintaining an active state decreases. In contrast, when soil moisture content is not limited, it is widely recognized that soil warming stimulates microbial physiological processes and thus increases CO_2 fluxes from soil (Zhou et al., 2016). In our experiment, warming enhanced soil respiration even at the end of the drought when the soil was very dry and even drier in warmed than in control plots. Different mechanisms may explain this observation. First is the availability of substrates, i.e., resources fueling respiration. However, under drought conditions, the link between EOC production and consumption may be decoupled. Although the general decrease in soil respiration during drought in conjunction with an increase in EOC points in this direction and is in agreement with the study by Homayak et al. (2018), we did not find differences in EOC pool sizes between warmed and ambient soils (Figure 8), which could explain our observed differences in CO_2 gas fluxes. Second, we expected that increased gas fluxes due to warming would be connected to a warming induced increase in EEA, due to high intracellular costs to microbial communities in warmed soils (Pold et al., 2017). Yet, neither before drought nor throughout drought and after rewetting, EEA patterns were consistently increased in warmed soils. Third, higher respiration rates from warmed soils may simply derive from greater microbial abundance (with similar specific microbial activities under ambient and elevated soil temperature). However, no enhancing effect of warming on microbial abundance was detected during drought. In fact, warmed soils tended to have lower microbial biomass in the beginning of our drought experiment (Figure 4). Finally, the lack of significant differences in our plant biomass data with respect to the abovementioned parameters gives us

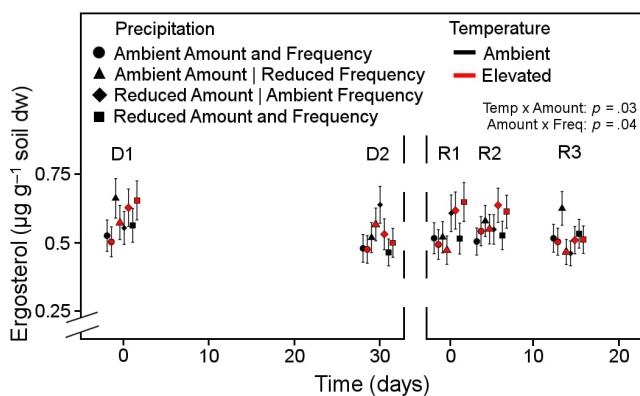


FIGURE 7 Ergosterol content at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 hour, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation amount (A; ambient; reduced), precipitation frequency (F; ambient; reduced) and temperature (T; ambient; elevated) indicate the long-term precipitation and warming treatments. Data are presented as back-transformed medians on the original scale including each precipitation and temperature treatment with standard errors ($n = 4$). Declarations on statistics include all five timepoints. Datapoints at the different dates are displayed with an offset for better visualization

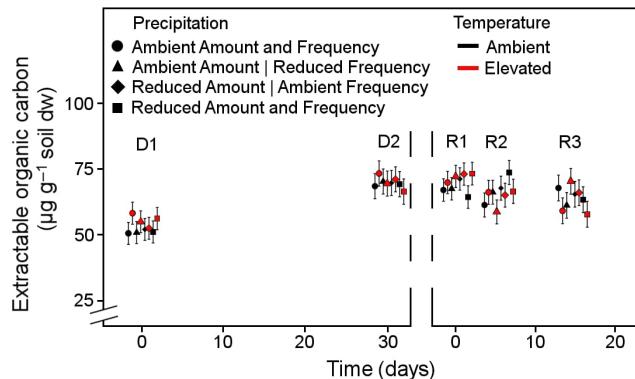


FIGURE 8 Extractable organic carbon content at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 hour, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation amount (A; ambient; reduced), precipitation frequency (F; ambient; reduced) and temperature (T; ambient; elevated) indicate the long-term precipitation and warming treatments. Data are presented as mean values including each precipitation and temperature treatment with standard errors ($n = 4$). Data points at the different dates are displayed with an offset for better visualization

no reason to suspect that plant C input controlled our observations on CO_2 fluxes.

Based on our results, we therefore exclude substrate availability, microbial abundance, extracellular enzymes, plant biomass, or root respiration as explanations for the observed soil respiration response. Rather, we suggest changes in microbial physiology as the main driver of our observed increases in CO_2 fluxes from warmed soils even during drought. For example, in a C pool modeling approach, long-term warming shifted the community function toward increased C decomposition (Stuble et al., 2019). The authors were able to show that this microbial functional shift was profound in that it even overcame the short-term stimulative effects of warming on respiration in laboratory incubations. Additionally, the results of a metaproteomic analysis showed that long-term warming increased the content of specific proteins involved in microbial energy production and conversion, leading to increased CO_2 fluxes (Liu et al., 2019). Such a shift in microbial metabolism would result in reduced carbon use efficiency, which has often been observed in warmed soils (Manzoni et al., 2012). Accordingly, less efficient C utilization corresponds with our observed increase in specific microbial respiration in warmed soils (Figure 2c) under very different soil moisture conditions over the course of our experiment. This contrasts with previous findings, in which warming was shown to further reduce soil respiration during drought due to intensified drying-out of the soil (Selsted et al., 2012). It also has been speculated that drought may possibly even offset warming-induced C loss from soils (Schindlbacher et al., 2012). However, we cannot confirm this with our study. The time immediately after rewetting of the dry soils is particularly stressful to microorganisms (Schimel et al., 2007) and is characterized by

a typical respiration pulse. Previous studies have shown that the size of the pulse depends on the degree of drying and rewetting (Carbone et al., 2011). The pronounced respiration pulse might, therefore, be explained by the significantly drier soils in warmed plots at the end of the drought. However, the missing correlation between soil moisture at peak drought and size of the respiration pulse contradicts this explanation (Figure S4).

We assume that the abovementioned mechanisms occur mainly in topsoil. In addition to that, the warming by 1°C at 30 cm depth may have substantially contributed to our observed warming effects on soil respiration. At this depth, volumetric water content was still in the range of 15% at the end of the drought (Figure 1a), thus likely not limiting microbial respiration. This ongoing CO_2 production in the deeper soil may additionally explain the consistently higher CO_2 fluxes from warmed soil even though microbial activity may have been water limited in the topsoil.

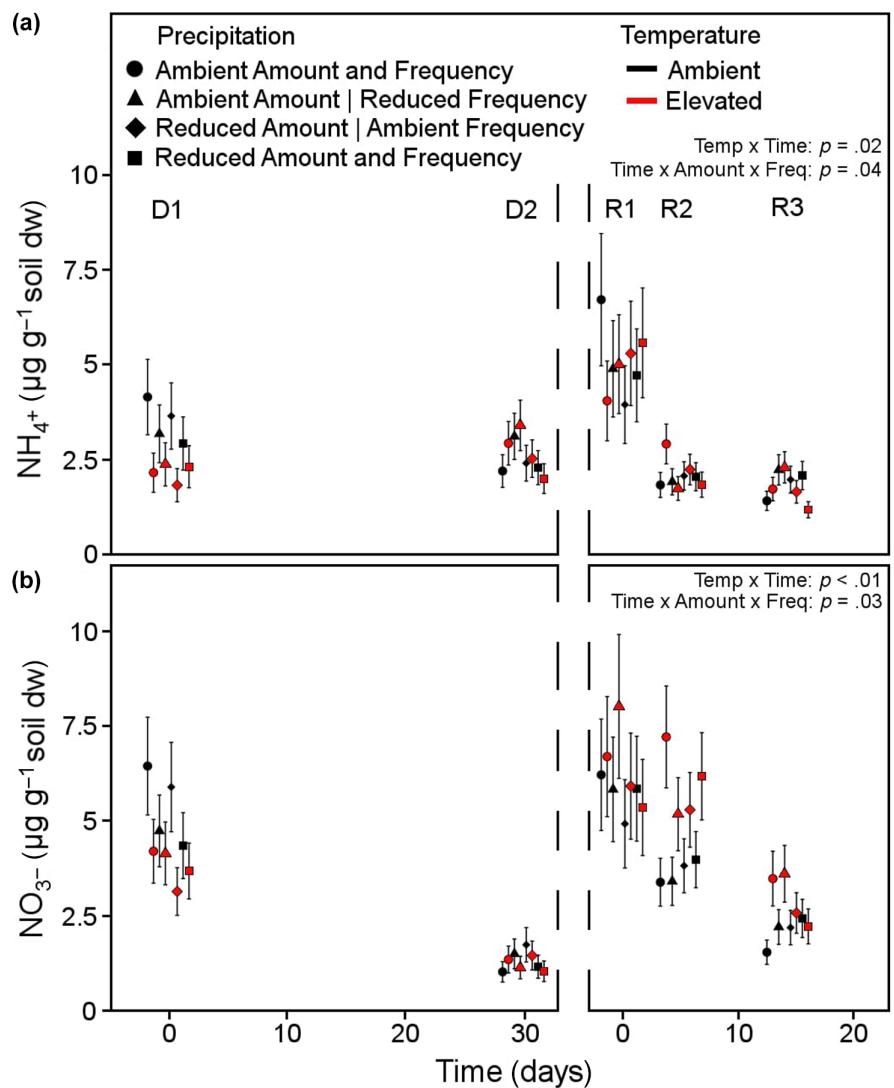
4.2 | Legacy effects of long-term precipitation treatments during drought and after rewetting

In our drought experiment, prior long-term precipitation treatments changed the CO_2 flux dynamics mainly by limiting the enhancing effect of warming (Figure 2b,c). Within the HoCC experiment, reduction in the precipitation frequency had no effect on gravimetric soil moisture content between 2008 and 2018 since the ambient net amount of water had been applied each year. In addition, the relatively wet summers of the temperate climate of southwest Germany with its frequent precipitation events prevented this treatment from resulting in repeated drought conditions. Consequently, the reduced precipitation frequency treatment did not change the effects of warming on CO_2 fluxes during the drought experiment (Figure 2b). In contrast, reducing the precipitation amount during the summer months for the years between 2008 and 2018 decreased gravimetric soil moisture content at the end of each summer by on average two percentage points (Poll, unpublished data). To put this in perspective, this decrease outweighed the effect of continuous soil warming on soil moisture content in the long-term HoCC experiment.

The effect of the long-term reduction in precipitation amount on CO_2 flux dynamics observed during our drought experiment provides an indication of a legacy effect. Our data on cumulative (Figure 2b) as well as microbial biomass-specific CO_2 fluxes (Figure 2c) showed that an annually reduced precipitation amount can limit the enhancing effect of warming on CO_2 fluxes during drought and after rewetting. As the last manipulation of summer precipitation was completed nine months before the start of our drought experiment, our observed fluxes may be explained by long-term changes in microbial traits within the HoCC experiment.

One key microbial trait with consistent effects on CO_2 flux dynamics in modeling is microbial biomass (Čapek et al., 2019). However, the long-term precipitation manipulations did not affect microbial abundance during our drought experiment. Indeed, microbial biomass seems to be a relatively stable C pool in soil (Salazar-Villegas

FIGURE 9 (a) Ammonium (NH_4^+ -N) and (b) nitrate (NO_3^- -N) content at different sampling dates (D1: before drought, D2: just before rewetting, R1: 1 hour, R2: 5 days, R3: 14 days after rewetting) at a soil depth of 0–15 cm. Precipitation amount (A; ambient; reduced), precipitation frequency (F; ambient; reduced) and temperature (T; ambient; elevated) indicate the long-term precipitation and warming treatments. Data are presented as back-transformed medians on the original scale including each precipitation and temperature treatment with standard errors ($n = 4$). Declarations on statistics include all five timepoints. Datapoints at the different dates are displayed with an offset for better visualization



et al., 2016), which is unaffected by extreme drought (Homyak et al., 2017; Siebert et al., 2019). Still, we were surprised to find that microbial biomass had moderately increased 2 h after rewetting. We would rather have expected that osmotic shock and subsequent cell lysis after rewetting (Schimel et al., 2007) would have decreased the microbial biomass pool shortly after rewetting. In accord with this, Meisner et al. (2015) suggested that when drought periods last longer than two weeks, microbial growth after rewetting resumes only after a lag-phase with almost no growth. Additionally, Göransson et al. (2013) found that microbial growth required up to 12 h to recover from rewetting after extreme drought. However, laboratory incubations simulating drying and rewetting cycles have demonstrated that microbial growth can start immediately after rewetting (Nijs et al., 2019). Our data suggests that the observed increase in microbial biomass shortly after rewetting was likely driven by bacterial growth, as the relative fungal abundance (Figure S2) remained stable.

We expected that long-term reduced precipitation would have promoted the relative abundance of soil fungi and that this would contribute to the CO_2 flux dynamics in our experiment. Indeed,

before drought, fungal abundance relative to the overall microbial biomass pool was greater in soils with long-term water limitation treatment (Figure S2). This supports previous studies in which a history of repeated drought has been shown to enhance the abundance of soil fungi, leading to increased litter decomposition rates and thus higher CO_2 fluxes (Haugwitz et al., 2014, 2016). However, neither the greater relative fungal abundance in soils with reduced precipitation before drought nor the pattern of absolute fungal abundance throughout our drought experiment corresponded with our observed differences in cumulative CO_2 fluxes. Still, our finding of largely unaffected fungal abundance during either drought or after rewetting is in agreement with Barnard et al. (2013).

In addition to the minor effect that reducing mean precipitation had on total microbial and fungal abundance, our results further showed that EOC pool size and EEA levels were also unaffected by reducing mean precipitation over the long-term. Thus, these variables cannot explain the observations of CO_2 fluxes within our drought experiment. We assume that the historical precipitation regime may have induced a functional shift within the microbial community. The estimation of Q_{10} values support this speculation;

long-term reduction of the precipitation amount significantly decreased Q_{10} within our soils throughout the drought experiment. This is in line with Yan et al. (2020), who also observed lower Q_{10} as a response to reduced precipitation in the previous year before sampling. However, more field studies targeting legacy effects on microbial C allocation during drought and after rewetting are needed to improve our mechanistic understanding of historical precipitation regimes' influence on the effects of soil warming.

4.3 | Effect of long-term climate change on N-cycling during drought and after rewetting

Soil warming increased N_2O fluxes during our drought experiment, particularly during drought and shortly after rewetting.

The microbial production of N_2O may have depended on the availability of mineral N substrates. Before drought, we found NH_4^+ and NO_3^- levels to be lower in warmed soils (Figure 9a,b). When soils are relatively moist, which applies here as more than 100 mm of precipitation occurred during the two weeks prior to our drought (Figure S1), warming has widely been shown to increase both nitrification and denitrification rates (Dai et al., 2020). Besides, warming can have a strong positive impact on plant nitrogen uptake induced by enhanced pre-anthesis root growth (Hou et al., 2018). This agrees with the results of a previous study made within our HoCC experiment, where warming accelerated plant development of barley (Drebenstedt et al., 2020). Accordingly, we suspect that enhanced gaseous loss in the form of N_2O and plant uptake explain decreased NH_4^+ and NO_3^- pools in warmed soils before initiation of drought.

During drought, NH_4^+ levels were stable in our experiment (Figure 9a), which contrasts with studies that have suggested that NH_4^+ content increases due to ongoing microbial N mineralization/ammonification (Homyak et al., 2017). However, there have been other studies in which drought decreased N mineralization at least during summer (Novem Auyeung et al., 2013). NH_4^+ oxidizing bacteria and archaea, which govern the rate limiting step in nitrification (Lehtovirta-Morley, 2018) have been shown to be drought resistant within grassland ecosystems (Fuchsleger et al., 2014; Séneca et al., 2020). Accordingly, even in our arable system, ongoing nitrification might explain why NH_4^+ did not accumulate during the drought phase of our experiment. However, the drought resistance of nitrifying taxa may be a grassland specific phenomenon (Deng et al., 2021) as other studies have found decreasing nitrification rates in response to drought (Novem Auyeung et al., 2013). Ongoing nitrification during drought should have increased or at least stabilized the NO_3^- contents in our soils according to those studies, but this was not the case (Figure 9b). This suggests that plant uptake of NO_3^- , the generally preferred plant N source in aerobic soils (Rubio-Asensio et al., 2014), may not have been limited by drought. Indeed, barley, in particular, has been shown to be largely unresponsive to drought in terms of NO_3^- uptake (Gloser et al., 2020), and we thus expect ongoing inorganic N uptake by plants during drought in our experiment.

While the inorganic N pool size appeared connected to the differences in N_2O emissions before drought, during drought, it was not. Inorganic N pools in warmed and ambient soils were about the same at peak drought. In well-oxygenated soils such as under conditions during drought, all N_2O fluxes should result from nitrification processes (Inatomi et al., 2019), as denitrification requires anaerobic conditions (Rohe et al., 2021). We thus assume that in warmed soils observed, increases in N_2O emission rates are based on higher nitrifier activity corresponding with overall increased microbial activity in warmed soils during drought. This may not only apply to fluxes from the topsoil but also from subsoil, where, as previously discussed for CO_2 production, moisture may not limit microbial activity. A meta-analysis by Dai et al. (2020) showed that the effect size of warming on N_2O fluxes was positively related to the effect size of warming on soil CO_2 fluxes, emphasizing the parallels between both processes in their responses to warming in our experiment. However, even denitrification may have contributed to N_2O fluxes during drought, as was shown in a recent study for well-aerated soils with very low water content (Harris et al., 2021). In their study, up to 70% of N_2O fluxes during a drought originated from denitrification. One might speculate that anoxic microsites can remain even in our dry soil, meaning that reactive N loss by denitrification continued throughout our drought period. However, we suggest that the main contribution of denitrification to N_2O fluxes took place immediately after rewetting, when quick resuscitation of microbial cells and thus respiratory activity induced O_2 deficiency and thus anaerobic conditions for denitrifiers.

Rewetting created an NH_4^+ but even more pronounced NO_3^- flush (Figure 9a,b), consistent with studies investigating the rewetting of dry soils (Leitner et al., 2017). As microbial biomass levels did not decrease in response to rewetting, we conclude that this inorganic N pulse does not have its origin in microbial cell lysis but rather is a consequence of re-mobilization and quick resuscitation of microbial mineralization processes. However, in the following days after rewetting, NH_4^+ content rapidly decreased while NO_3^- levels remained relatively high. This is in agreement with a previous study by Leitner et al. (2017) and confirms high nitrifier activity and thus nitrification rates following the rewetting of dry soil (Barnard et al., 2013; Placella & Firestone, 2013). Accordingly, a decrease in NO_3^- pool size was slowed due to quick return to aerobic and thus unfavorable conditions for denitrifiers in the soil, as it quickly dried out after rewetting. Furthermore, NO_3^- uptake by plants should have been very low to non-existent after rewetting; during our drought period of four weeks, there was a considerable heat wave in southern Germany (Figure S1). Consequently, plants were extremely stressed toward the end of drought, and phenology suggests that plant physiological processes could have been irreversibly damaged.

In line with our observations on CO_2 flux dynamics, long-term reduced precipitation seemed to also decrease (to a lesser degree, however) the temperature sensitivity of nitrification, which was the putative main driver of N_2O fluxes during drought and the predominant driver after rewetting. This observation is likely not

connected to inorganic N pools since, as mentioned above, levels were equal across all treatment groups. While there have been studies indicating that soils exposed to warming or drought can lower the Q_{10} of nitrification (Novem Auyeung et al., 2013), we are not aware of studies explicitly targeting effects of soil moisture history on N transformations, which thus remain a future challenge.

5 | CONCLUSION

The occurrence of drought periods will increase in the future, impacting microbially mediated soil C- and N-cycling processes in ways we do not yet understand. Our study showed that during drought and after rewetting, soil CO₂ and N₂O fluxes can depend on the respective mean climatic conditions to which the soil microbiome had been exposed to historically. We observed a legacy effect of reduced summer precipitation amount limiting the enhancing effect of warming on soil gas fluxes both during drought and after rewetting. However, this was not connected to overall microbial or specific fungal abundances. Also, EEA levels as well as microbial substrate availability did not appear to be relevant factors in explaining our gas flux data in terms of the legacy effect (Figures S2–S4). Instead, microbial exposure to long term reduced precipitation amount may have resulted in a shift in microbial physiology that is not necessarily connected to C and N pools in soil. This should be considered in future studies by targeting physiological measures such as carbon use efficiency, growth rate, or accumulation of cell protective compounds.

ACKNOWLEDGEMENTS

We thank Sabine Rudolph and Heike Haslwimmer for the technical support in the lab and Kathleen Regan for linguistic editing. V.L. gratefully thanks the Anton & Petra Ehrmann Stiftung, Research Training Group 'Water-People-Agriculture (WPA)' for financial support of this study as a part of his PhD scholarship, as well as Prof. Ellen Kandeler for the overall support. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

C.P. and S.M. conceived the study. V.L. and in part M.P. performed the experiment and collected data. J.H. provided the statistical approach and final model. V.L. analyzed the data and wrote the manuscript. S.M. and C.P. supervised the project from data acquisition to manuscript editing. All authors contributed to the final draft of the manuscript and gave approval for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo repository at <https://doi.org/10.5281/zenodo.6375716>.

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How to cite this article: Leyrer, V., Patulla, M., Hartung, J., Marhan, S., & Poll, C. (2022). Long-term manipulation of mean climatic conditions alters drought effects on C- and N-cycling in an arable soil. *Global Change Biology*, 28, 3974–3990. <https://doi.org/10.1111/gcb.16173>