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Soil microbial community structure and function mainly respond to indirect effects in a multifactorial climate manipulation experiment

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ABSTRACT

Future climate change scenarios predict increases in surface temperature as well as atmospheric CO_2 concentration. In this study we simultaneously addressed individual and combined effects of these factors on the soil microbial community structure and function. We tested linear as well as non-linear responses in a multifactorial climate manipulation experiment.

After two years of climate change manipulations on a pre-Alpine managed grassland, topsoil samples were taken for analysis of functional enzyme activities, as well as microbial community structure. Besides, soil and vegetation parameters were measured to allow evaluation of direct and indirect effects.

Pronounced and statistically significant spatial effects were observed on our field site for some variables. It is assumed that the history of site preparation could provide an explanation for the observed differences. Elevation of temperature or atmospheric CO_2 did not induce strong shifts of soil fungal or bacterial communities. Only the inclusion of the spatial effects in the response surface regression model allowed the detection of subtle microbial responses to climate change scenarios. *Mucor globulifera* responded to temperature and CO_2 in a pattern similar to soil water content. An increase in the relative abundance of coprophilous white rot fungi was observed upon warming, and this might be attributed to preferences of macrofauna for warmer plots. Specific extracellular enzyme activities were positively correlated with each other, especially within two groups of enzymes, which were involved in C-acquisition and in N-mining. The latter group responded positively to elevated CO_2 concentrations. Chitinolytic activity increased with the relative abundance of the nematophagous and entomopathogenic ascomycete *Purpureocillium lilacinum*.

We conclude that the indirect effects of future climate change scenarios prevail over direct effects on soil microbial community composition and function. Soil water content, nutrient pools, atmospheric CO_2 and plant root identity were identified as drivers of the observed changes after removal of unintended spatial effects. Application of advanced statistical tools, which take spatial variability into account, was necessary to detect these effects. Minor changes in the fungal community occurred already after a short period of climate manipulation. More pronounced effects of elevated atmospheric CO_2 concentration and surface warming on soil microbial community structure and function are expected on the longer-term, but indirect effects will most likely remain the dominant drivers.

1. Introduction

Global surface temperatures are projected to increase in response to rising atmospheric concentrations of CO_2 (IPCC, 2014). There is considerable evidence that climate change will affect soil microbial organisms, the direction and magnitude of the response are, however, still uncertain (Castro et al., 2010; Xiao et al., 2018). Soil microbial organisms represent the driving force of a large number of ecosystem processes (Xiong et al., 2014), some of them being central for the response of the ecosystem to climate change (Austin et al., 2009). The

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primary mechanism by which soil microbial organisms affect carbon (C) and nutrient cycling is the production of extracellular enzymes, which catalyse degradation, transformation and mineralization of organic molecules in soil organic matter (Sinsabaugh, 2010; Kelley et al., 2011; Xiao et al., 2018) and alter microbial function, soil quality (Marx et al., 2001) and ecosystem productivity (Sayer et al., 2013). A major challenge is to understand the underlying mechanisms of how climate change will affect soil and ecosystem function (Gutknecht et al., 2010). Temperature cannot only directly influence soil microbial life, but can also exert indirect effects through changes in evapotranspiration, plant physiology, root exudation and vegetation (Dieleman et al., 2012; Lange et al., 2014). An increase in atmospheric CO_2 does not directly influence soil CO_2 since concentrations in soil are orders of magnitude higher than concentrations in the air (Kuzyakov et al., 2019). Atmospheric CO_2 has, however, strong effects on plants, which can translate into changes in soil processes (Phillips et al., 2011; Engineer et al., 2016; Piepho et al., 2017). Generally, it is assumed that direct effects are stronger than indirect ones (Kuzyakov et al., 2019). However, this does not always apply to soil, since indirect effects, including soil moisture and substrate availability, can mask direct effects on soil organisms (Zhou et al., 2008; Selsted et al., 2012; de Menezes et al., 2016; Jansen-Willems et al., 2016; Brenzinger et al., 2017). Further, microorganisms are unequally distributed in a soil system (Mills and Franklin, 2003), and about half of the variation in the community could be attributed to the effects of habitat and geographical distance (Vos et al., 2013). As it is hardly possible to measure all direct and indirect biotic and abiotic variables that constitute 'the environment', the multiple effects of climate change on microbial community structure and function remain obscure.

Warming (elevated temperature, eT) can stimulate microbial abundance, activity and nutrient cycling if water and nutrient availability are not limiting growth (Mosier, 1998; Pilegaard et al., 2006; Castro et al., 2010). In the long term, adaptations through shifts in the community composition are expected. Elevated atmospheric CO_2 (eCO_2) is well known for its variable effects on soil properties and microbes. Few studies suggest lower nitrogen (N) availability accompanied with eCO_2 tends to favour fungi over bacteria, due to a more efficient nitrogen uptake of fungal hyphae (Janus et al., 2005; Carney et al., 2007). Many other studies, however, have found that eCO_2 has little or no detectable effects on soil microbial community (Grüter et al., 2006; Austin et al., 2009; de Menezes et al., 2016; Brenzinger et al., 2017). As described by Brenzinger et al. (2017) it is likely that altered soil conditions (e.g., soil moisture, substrate availability) rather than eCO_2 affect the soil microbial community.

Similarly, effects of eT and eCO_2 on soil extracellular enzyme activities are mainly mediated through changes in soil moisture and nutrient availability, which are transmitted through the vegetation cover (Brockett et al., 2012; Henry, 2012; Xiao et al., 2018). If soil moisture is not limiting, warming can increase plant-derived substrate C input (Baldrian et al., 2013; Meier et al., 2015). Moreover, biomass input can also be enhanced by eCO_2 (Drissner et al., 2007; de Menezes et al., 2016; Brenzinger et al., 2017), which in turn increases the substrate availability for C and N acquiring enzymes (Meier et al., 2015).

Previous studies discussed that (i) studying multifactor experiments can help optimizing ecosystem models, since climate change factors might not be just additive, but also synergistic and antagonistic and (ii) effects of warming and eCO_2 are typically quantitative in nature (Dieleman et al., 2012; Piepho et al., 2017). This is the first study, to our knowledge, that addresses simultaneously individual and combined climate change factors with varying levels of warming and CO_2 fumigation on soil microbial community structure and function. In order to master the complexity of the experimental setup, a second-order response surface regression approach was used to disentangle quantitative multifactorial manipulation effects. Generally this statistical approach is applied in experiments for process optimization in industrial branches (Piepho et al., 2017). Actually, response surface regression is an attractive approach to optimize statistical power also in ecology. Up to now, only few ecosystem experiments have recognized the need to account for non-linear responses and these experiments were largely limited to single factors.

The effects of climate change on soil microbial community structure and function were investigated by high-throughput sequencing of bacterial and fungal phylogenetic markers and by soil extracellular enzyme activity measurements. The climate manipulation site ClimGrass is installed in a pre-Alpine managed grassland at Gumpenstein, Austria (Piepho et al., 2017; Groh et al., 2018; Deltedesco et al., 2019; Pötsch et al., 2019).

2. Material and methods

2.1. Site description

The research was conducted at the long-term multifactorial climate manipulation experiment "ClimGrass", established at the Agricultural Research and Education Centre (AREC) Raumberg-Gumpenstein in Austria as described in Deltedesco et al. (2019). Briefly, the experimental site is located at an altitude of 710 m a.s.l. and the soil is classified as Cambisol with loamy texture (IUSS Working Group WRB, 2015). The study year 2016 was characterized by a mean annual air temperature of 9.1 °C and mean annual precipitation of 1142.6 mm (data from the ZAMG weather station, located at the experimental site).

The grassland vegetation (nutrient-rich meadow) was established in the year 2007. The soil of the experimental site was ploughed up to a depth of 0.25 m, totally levelled with a curry-comb and sown with a seed mixture for permanent grassland using a seed density of 27 kg ha^{-1}. This mixture contained tall oat-grass (*Arrhenatherum elatius L.*), Kentucky bluegrass (*Poa pratensis L.*), meadow fescue (*Festuca pratensis L.*), orchard grass (*Dactylis glomerata L.*), meadow foxtail (*Alopecurus pratensis L.*), red fescue (*Festuca rubra L.*), perennial ryegrass (*Lolium perenne L.*), Timothy (*Phleum pratense L.*), golden oat grass (*Trisetum flavescens L.*), white clover (*Trifolium repens L.*) and bird's-foot trefoil (*Lotus corniculatus L.*). In the year 2010 the east part of the experimental site (east, plots no. 1–24) and in 2012 the west part (west, plots no. 25–54) was equipped with vertically adjustable metal constructions to carry the warming and fumigation devices on the plot scale, 16 m^2 each (Pötsch et al., 2019). For more details about experimental design and plot distances see Supplementary Fig. S1.

The experiment was designed by a response surface regression approach (Piepho et al., 2017) and treatments were completely randomized. Treatments (Table 1 and Fig. S1) are based on combinations of three levels of air temperature (ambient, + 1.5 °C and +3 °C) and atmospheric CO_2 concentrations (ambient, + 150 ppm, and +300 ppm). The all-out operation (warming and fumigation) of the ClimGrass site started in May 2014 after the first harvest. The grassland is harvested three times during the growing season from April to the end of October, and mineral fertilizer is applied in three batches (total load of 90 kg N ha^{-1} y^{-1}, 65 kg P ha^{-1} y^{-1}, 170 kg K ha^{-1} y^{-1}). For more details about the treatment realization and experimental set-up see Deltedesco et al. (2019).

Table 1
A 3 × 3 response surface regression design with treatment-codes (C = atmospheric CO_2, T = Temperature) for a total of 27 plots, the number of replicates are given in parenthesis (Deltedesco et al., 2019).

eT (C°)	eCO_2 (ppm)		
	0	150	300
0	C0T0 (7)	C1T0 (3)	C2T0 (3)
1.5	C0T1 (3)	C1T1 (2)	
3	C0T2 (3)		C2T2 (6)

2.2. Harvest of aboveground biomass and soil sampling

The total aboveground biomass (TAGB) of the third growth in 2016 was harvested on the 4th of October using a hedge trimmer. Right after harvesting the plots, soil sampling was conducted using a soil auger with a 1.9 cm inner diameter and a height of 10 cm. Sampling was repeated (min. 5 times $plot^{-1}$) on various positions within the treatment area of the plots to obtain representative soil samples and to account for in plot heterogeneity. The sub-samples were pooled into one soil sample per plot and homogenized via sieving (<2 mm). To account for confounding influences of roots from different plant species on soil microbial communities, the determination of root identities in soil cores taken for microbial community analysis was included. Plant species data were, however, not used to deduce influences of climate change scenarios on vegetation composition. During the sieving procedure, root biomass was separated and washed immediately. Aliquots (2 g) of sieved soil were suspended in extraction buffer (provided in the RNA PowerSoil® MO BIO kit) to minimize nucleic acid degradation. Remaining soil and root samples were placed in plastic bags and stored at 4 °C for further processing.

2.3. Laboratory analysis

Soil moisture content was determined by oven drying for 24 h at 105 °C. Soil pH was determined in 0.01 M $CaCl_2$ according to Horn et al. (2010). Soil organic carbon (SOC) and N_{tot} were determined according to ÖNORM L1095. Chloroform fumigation extraction (CFE) was applied as described by Schinner et al. (1996) to determine extractable organic carbon (EOC), microbial biomass carbon (MBC), extractable total nitrogen (ETN) and dissolved organic nitrogen (DON). Therefore, fumigated and non-fumigated samples were measured with an automated TOC/TN analyzer (TOC-V CPHE200V, linked with a TN-unit TNM^{-1} 220 V, Shimadzu Corporation, Kyoto, Japan) according to Ferretti et al. (2018). Ammonium (NH_4+) was determined by Berthelot reaction as described in Schinner et al. (1996) and nitrate (NO_3-) was measured according to Hood-Nowotny et al. (2010). SOC and N_{tot} were determined from separate soil cores collected at the same time for laboratory trace gas flux experiments. Values are means from duplicate determinations from each of two separate soil cores, i.e. from four measurements (Deltedesco et al., 2019). The dry weight of TAGB was determined by drying the samples first at a temperature of 55 °C for 48 h in a drying cabinet and then using the Brabender-technique (105 °C, 4 h) according to VDLUFA (1976). All data were calculated on soil dry matter basis (DM).

Soil DNA was extracted using a commercial kit (RNA PowerSoil® MO BIO) with minor modifications. In brief, to extract DNA, after removal of RNA from the column 2 ml of washing Buffer (Buffer QC, Qiagen) was added to wash residual debris and inhibiting substances. By using 100 µl of elution buffer (Buffer QF, Qiagen), pure DNA could be eluted. Washed roots were lyophilized at - 50 °C and ground to a fine powder (Retsch MM200, Germany) for homogenization. Root DNA was extracted from 0.1 g of the lyophilized powder with the Qiagen DNeasy Plant Mini Kit according to the manufacturer's instruction. The fungal ITS2-region was amplified from soil DNA with primer pair ITSMix/ITS4Mix, which was originally described by Tedersoo et al. (2014) and modified by Keiblinger et al. (2018), to obtain good coverage of the total fungal community (Tedersoo et al., 2015). The bacterial 16S V3–V4 region was amplified by primer pair Illumina_16S_341F/Illumina_16S_805R according to Klindworth et al. (2013). Library preparation for Illumina MiSeq Sequencing followed the protocols as described in Keiblinger et al. (2018) for fungal communities and in Leitner et al. (2017) for bacterial communities from soil samples. Plant community composition from root samples was determined by amplification of the plant ITS2-region with primer pair ITSS2F_NeXTf(TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGCGATACTTGGTGTGAAT) and ITSS3R_NeXTr (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACGCTTCTCCAGACTACAAT) (adapted from Chen et al., 2010). Indexed samples were pooled and purified with the PureLink PCR Purification Kit using Binding Buffer High-Cuttoff B3 (Thermo Fischer Scientific). Pooled and purified samples were sent to the sequencing core facility at the Vienna Biocenter (VBCF-NGS, Vienna, Austria).

The potential hydrolytic enzyme activity of cellobiohydrolase (CBH), acid phosphatase (PHO), β-1,4-glucosidase (BGL), ß-*N*-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) was measured on a spectrophotometer (PerkinElmer® type 2300 EnSpire™) at an emission wavelength of 450 nm and an extinction wavelength of 364 nm according to Ameur et al. (2018). To determine potential oxidative activities, i.e., phenoloxidase activity (PHE) and peroxidase activity (PER), L-3,4-dihydroxyphenylalanine (L-DOPA; CAS. No. 59-92-7) served as substrate, and the absorbance was measured at 450 nm on a plate reader (PerkinElmer® type 2300 EnSpire) according to Ameur et al. (2018). Briefly, 1 g of soil was homogenized in 100 ml 100 mM sodium acetate buffer (pH 6.5), 200 µl of the homogenate and 50 µl fluorogenic substrate was added into black microplates. The absolute hydrolytic and oxidative enzyme activities were calculated based on previous literature (German et al., 2011). The aminohydrolase activity (urease, URE) was measured according to Sinsabaugh et al. (2000) with minor modifications. An amount of 1 ml soil homogenate (as described above) was pipetted into two 1.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany), respectively. One of the tubes received urea (20 mM) as substrate. After incubation at 20 °C and 18 h tubes were centrifuged for 5 min at 5000 rpm. Supernatants were transferred into microplates to measure the background ammonium concentration from samples without urea; while the formation of NH_4^+ from samples after the addition of urea was determined to calculate potential amidohydrolase activity (Shand et al., 2008). The absolute urease activity was calculated according to Sinsabaugh et al. (2000). All extracellular enzyme activities (EAA) are expressed in µmol μg^{-1} h^{-1} DM. The specific enzyme activities (sEAA) were calculated by dividing total enzyme activities by the MBC determined by CFE in order to normalize activity to the size of the MBC. These data are shown in µmol μg^{-1} MBC h^{-1} DM.

2.4. Sequencing data processing

Raw data quality was checked in FastQC and reads were screened for PhiX contamination using Bowtie 2.2.6 (Langmead and Salzberg, 2012). A Bayesian clustering for error correction was applied (Nikolenko et al., 2013; Schirmer et al., 2015) before merging the PE reads using PEAR 0.9.8 ($p < 0.001$) (Zhang et al., 2013). Forward and reverse primers were then stripped from merged reads employing Cutadapt 1.8.3 (Martin, 2011) and quality filtering performed in VSEARCH v.2.8.5 (maximum expected error = 0.5) (Rognes et al., 2016). METAXA2 v.2.2 was used to extract SSU ribosomal reads and to verify the 16S rRNA V5–V7 region of the sequences (Bengtsson-Palme et al., 2015). Similarly, ITSx v.1.1 was used to target the extraction of the ITS2 region from both fungal and plant amplicon-related ITS sequences (Bengtsson-Palme et al., 2013). Targeted reads were labelled according to the sample name of origin and combined in QIIME 1.9.1 (Caporaso et al., 2010). Sequences were dereplicated, sorted and clustered at 97% of similarity using VSEARCH. Chimeras were checked adopting a *de-novo*-based approach, as a routine of the above-mentioned tool. An optimal global alignment was applied afterward in VSEARCH and a BIOM table generated. Taxonomy assignment was performed employing the naïve Bayesian RDP classifier v2.10 (Wang et al., 2007) in QIIME using SILVA release 132 (Quast et al., 2013) and UNITE 8.0 (Nilsson et al., 2018) as reference databases for archaeal/bacterial and fungal sequences, respectively. A dedicated ITS-based database for plant taxonomic assignment was built after retrieving nucleotide sequences from GenBank using the NCBI's E-utilities tools and the entrez_qiime python utility (https://github.com/bakerccm/entrez_qiime/blob/master/entrez_qiime.py). Fungal OTUs (FOTUs) were additionally mapped to ecological guilds based on available scientific literature. At low

resolution, FOTUs were grouped into saprotrophic (SAP), symbiotic (SYM) and potentially plant pathogenic (PAT) fungi (see LS1 in sheet "FOTU" Supplementary Information). Especially OTUs, which could not be correctly classified at the genus level, could often not be categorized and were thus not assigned (NA) to any group. For a better resolution of ecological guilds, FOTUs were further divided into a total of 18 different groups (see LS2 in sheet "FOTU" Supplementary Information and the explanations of the abbreviations in a separate sheet).

2.5. Nucleotide sequence accession number

Sequence data are available at NCBI database under BioProject number PRJNA542595, BioSamples SAMN11774061-SAMN11774087 and GenBank accession numbers KCZZ01000001-KCZZ01000529 for bacterial OTUs (BOTUs), MK951058-MK951645 for fungal OTUs (FOTUs) and MK950948-MK951003 for plant OTUs (POTUs). Taxonomic affiliation and relative abundances of BOTUs, FOTUs and POTUs are summarized in the Supplementary Information.

2.6. Statistical analysis

Analysis of the experiment was done using linear mixed models. Treatment effects were modelled using linear and quadratic polynomial regression terms for both quantitative treatment factors, as well as their interaction. This model represents a response surface. The main advantage over simple analysis of variance, followed by mean comparisons, is to facilitate interpolation between observed treatment levels and drawing up graphs of the response function in two or three-dimensional plots (Box and Draper, 2007; Piepho and Edmondson, 2018). A further advantage is that treatment effects can be modelled more parsimoniously, i.e. using fewer parameters than when treatment means are compared, thus providing a more efficient analysis. Response surface regression is a popular and well established methodology in many fields, including engineering, but as yet is not as often used as it could in soil ecology. A separate intercept was fitted for the east (plots 1–24) and west (plots 24–54) part of the ClimGrass site, in order to account for spatial effects, along with a second-order response surface regression model. An anisotropic power model for residual error was fitted to spatial row and column coordinates (expressed in meters). In addition, an independent random plot error was fitted to allow for a nugget effect. Type I significance tests were done using the second-order Kenward-Roger method (Kenward and Roger, 2009). First, the second-order terms of the response-surface were inspected, and the model was reduced for non-significant terms ($\alpha = 5$ %). At this stage, significance tests for the first-order (linear) terms were disregarded. Next, if all second-order terms were non-significant, the first-order terms were also inspected, and only the significant ones were kept in the model. When reducing the model, the marginality principle was observed (Piepho and Edmondson, 2018). When the interaction term of both covariates was significant, the two linear main effects were kept. The fitted model was used to obtain predicted values for all treatments. These were corrected for the east-versus-west contrast to obtain a smooth surface, averaging the east and west halves of the field. The fitted predictions were graphed as contour plots using the GLM procedure. All analyses were done using the MIXED procedure of SAS.

Two indices for β-diversity, Morisita-Horn (MH) and Bray-Curtis (BC) (Jost et al., 2010), were calculated with the software EstimateS, v.9.1 (Colwell, 2013) for fungal and bacterial communities. MH is the β-diversity counterpart of Simpson's Index for α-diversity and is therefore mainly determined by dominant species (Jost et al., 2010). The Bray-Curtis Distance (BC), on the other hand, is less dependent on dominant species. Regression analyses for correlation of β-diversity to geographic distance were conducted with Daniel's XL Toolbox add-in for Excel, v.7.3.4 (Kraus, 2014). NMDS-plots were calculated in R (R Core Team, 2015) with the package 'vegan' (Oksanen et al., 2018) from BC-distances of the fungal and bacterial communities, respectively.

3. Results

3.1. General soil parameters and total aboveground biomass

Statistically significant field-scale spatial effects, which were independent of the increase in temperature or CO_2, were observed for soil microbial biomass carbon (MBC) and soil organic carbon (SOC). Correlation between the two variables was weak and statistically not significant (Table S7). The spatial effects were mainly caused by differences between the east part (left, plots 1–24) and the west part (right, plots 25–54) of the ClimGrass site (Deltedesco et al., 2019; Fig. S1). MBC increased from 161.6 ± 37.2 μg g^{-1} in the east part to 205.6 ± 75.2 μg g^{-1} in the west part and SOC increased from 2.65 ± 0.12 % in the east part to 2.79 ± 0.14 % in the west part (Fig. 1). Differences between the north part (front) and the south part (back) were less pronounced. Standard physical and chemical soil parameters including pH-value, total nitrogen (N_{tot}) and C:N-ratio were not significantly different between the east and the west part. All values are given in the Supplementary Information. Results from Wald-type F-test and parameter estimates are summarized in Supplementary Tables (Table S1 and Table S2). To account for the spatial effect, separate intercepts for the east and west part of the experimental field and an anisotropic power model were included in the response surface regression (see above section 2.6).

By this approach, treatment effects could be observed for soil water content (WC) and extractable total nitrogen (ETN). The WC and ETN ranged from 19.28 to 26.66 % and 36.08 and 56.81 μg g^{-1}, respectively. Both increased with elevated CO_2 and decreased with elevated temperature (Fig. 2). All other measured soil parameters showed only weak or no treatment effects (Table S1 and Table S2). Total aboveground biomass (TAGB) ranged from 1532 to 3963 kg DM ha^{-1} and showed an opposite trend to WC and ETN, as it increased with elevated temperature but decreased with elevated CO_2 (Fig. 3).

3.2. Soil microbial communities

The soil fungal communities at the ClimGrass site were dominated by Ascomycota, followed by Basidiomycota, Mortierellomycota, Mucoromycota, Chytridiomycota, and Glomeromycota. The most abundant orders were Hypocreales, Mortierellales, Tremellales, Chaetosphaeriales and Mucorales (Fig. 4, left panel). FOTU_1 (*Purpureocillium lilacinum*) was the most prevalent FOTU over the whole ClimGrass site with an average abundance of 10.1 % explaining the dominance of the order Hypocreales. The majority of the soil fungi could be classified as saprotrophic, while on average less than 15 % are potentially plant pathogenic. Only a small fraction – approx. 2 % – was symbiotic and this group consists mainly of Glomeromycota. A substantial fraction could, however, not be classified into any ecological group as many FOTUs could only be categorized into higher taxonomic groups like order or class.

Soil bacterial communities were dominated by the phyla Proteobacteria, Verrucomicrobia, Actinobacteria and Acidobacteria (Fig. 4, right panel). BOTU_1 (Spartobacteria) was the most abundant BOTU over the whole ClimGrass site with an average abundance of 6.4 %.

Two different indices of β-diversity were calculated for the microbial communities, the Morisita-Horn (MH) and the Bray-Curtis Index (BC). Both indices were higher for the fungal community than the bacterial community and BC was higher than MH (Fig. 5). This difference was more pronounced for bacteria. β-diversity of the fungal community increased significantly with the distance between the plots (Fig. 5A). Differences in abundance were observed for selected fungal groups, especially for dominant groups such as *Purpureocillium lilacinum* and *Fusarium* spp. between the east part (plots 1–24) and the west part (plots 25–54) of the ClimGrass site: Potentially plant pathogenic fungi (including *Fusarium* spp.) and coprophilous fungi were more prevalent in the right part, saprotrophic fungi were more prevalent in the east part

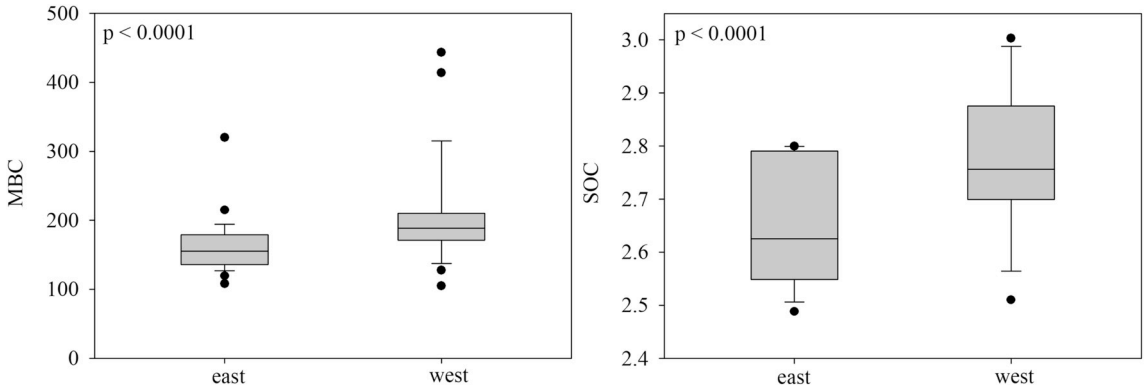

Fig. 1. Microbial biomass C (MBC, µg g^{-1}, left panel) and soil organic carbon (SOC, %, right panel). East (plots 1–24) and west part (plots 25–54) of the ClimGrass site are shown separately, the p-values indicate significant differences in Wald Type *t*-test.

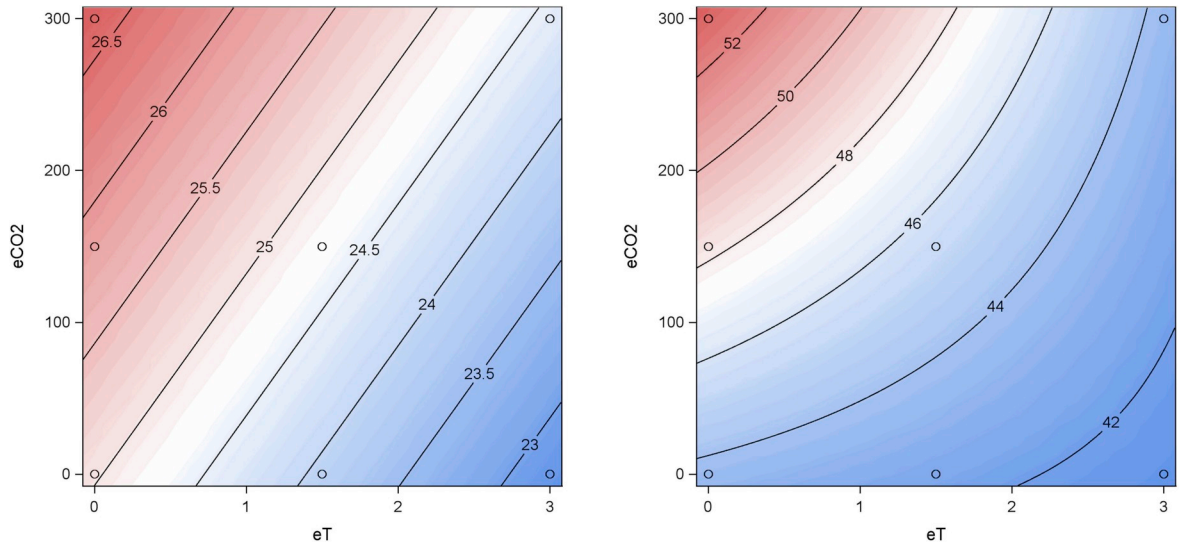

Fig. 2. Contour plots for soil water content (WC, %, left panel) and extractable total nitrogen (ETN, µg g^{-1}, right panel). Elevated temperature in °C (eT) and CO_2 in ppm (eCO_2) above ambient are indicated. Treatment combinations, from which data were available for modelling, are indicated by circles. Amounts of WC and ETN increase from blue to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Table S3).

Correlations between certain plant roots and selected FOTUs were identified. The basidiomycetous yeast *Solicoccozyma terricola* (FOTU_17) increased in abundance with *Arrhenatherum elatius* (POTU_2 and POTU_54) (Fig. S3). Additionally, a relatively high abundance of roots from common sorrel (*Rumex acetosa*) was found in the sample from plot 23 (C1T0), which was mirrored by the dominance of *Mucor hiemalis* (FOTU_2) in the same sample. *Mucor hiemalis* (FOTU_2) had relative abundances of max. 12.5 % on all other plots but accounted for 38.2 % of the total community on plot 23.

The BC for bacterial community showed a weak but statistically significant increase with geographic distance, while no significant change of MH was observed (Fig. 5B). The same as for FOTUs, selected BOTUs showed a significant difference in abundance between the east part and the west part of the ClimGrass site (Table S3). Besides, plot 35 (C2T2) strongly deviated from all other plots due to an unusually high abundance of 16.3 % for *Providencia* sp. (BOTU_58), which was on most other plots well below 1 %. Community distances to plot 35 are highlighted in Fig. 5B.

With standard statistical procedures, only weak treatment effects could be detected for the microbial communities. In NMDS plots, climate change scenarios with elevated CO_2 and/or temperature did not separate fungal or bacterial communities from the controls (Fig. S2). Inclusion of spatial effects into response surface modelling allowed, however, the identification of fungal taxa with a treatment-dependent distribution. Coprophilous white rot fungi, which mainly consisted of *Coprinopsis cordispora* (FOTU_141) and *Sphaerobolus stellatus* (FOTU_158), increased in abundance upon eT but were not affected by eCO_2 (r^2 = 0.508) (Fig. 6). *Mortierella globulifera* (FOTU_43) responded to both environmental changes, resulting in a reduction of abundance with elevated temperature and an increase with eCO_2. An increase in both climate change factors counterbalanced each other with little effect on the relative abundance of FOTU_43 (Fig. 7). Due to the similarity to the distribution pattern of WC and ETN (Fig. 2), the relative abundance of FOTU_43 was correlated with both. A significant positive correlation was found for the abundance of FOTU_43 and WC (r^2 = 0.280; Fig. S4) but not for ETN.

Responses of bacterial taxa to climate change scenarios were weaker and only affected BOTUs with a maximal abundance below 1 %.

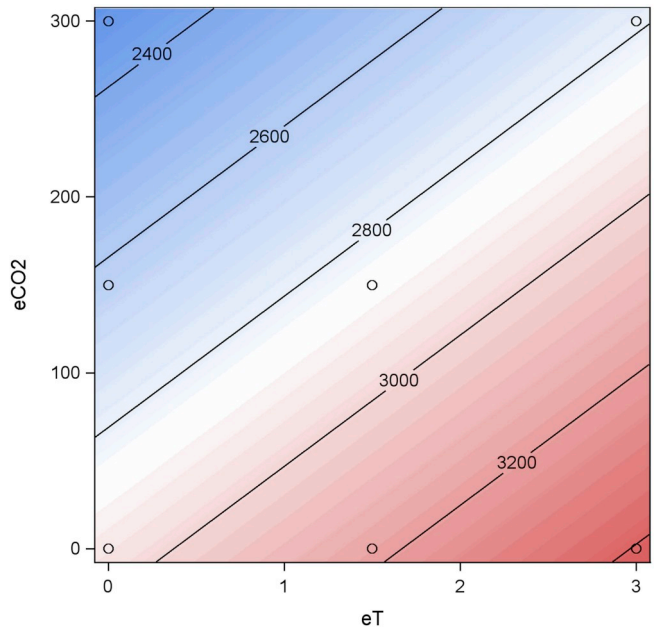

Fig. 3. Contour plot for total aboveground biomass (TAGB, kg dry matter ha^{-1}). Elevated temperature in °C (eT) and CO_2 in ppm (eCO_2) above ambient are indicated. Treatment combinations, from which data were available for modelling, are indicated by circles. TAGB increases from blue to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Enzyme activity

All specific extracellular enzyme activities (sEEA) were positively correlated to each other and responded negatively to soil NH_4–N content, although not all correlations were statistically significant. Highly significant correlations were observed within two groups of sEEA (Table S7). Group 1 consisted of sCBH, sBGL, sNAG, sLAP and sPHO, enzymes which are mostly involved in breakdown of polymers for C-supply. Group 2 consisted of sNAG, sLAP, sURE, sPHE, sPER and sPHO, enzymes which are mostly involved in acquisition of N from organic compounds and in the breakdown of recalcitrant compounds like lignin. The decrease in activity with increasing NH_4–N was especially pronounced for sLAP and for sPHO (Table S7). The activity of sNAG showed a good correlation with the relative abundance of the nematophagous and entomopathogenic fungus *Purpureocillium lilacinum* (FOTU_1) (r^2 = 0.501; Fig. S5).

Spatial effects, caused by the east part (plots 1–24) and the west part (plots 25–54) could be observed for a set of absolute and specific EEA (absolute: NAG and CBH, specific: sLAP and sPHE; Table S5). The updated response surface regression model (see above in section 2.6) allowed to detect treatment effects on some specific EEA. Enzymes involved in N-cycling – sLAP and sURE – and in breakdown of lignin – sPER and sPHE – responded negatively to an increase in atmospheric CO_2. Only PHE showed additional interaction with temperature. NAG, which is also involved in the acquisition of organically bound N, showed no treatment effect. sCBH decreased weakly with eCO_2 and showed an increase with warming (Fig. 8).

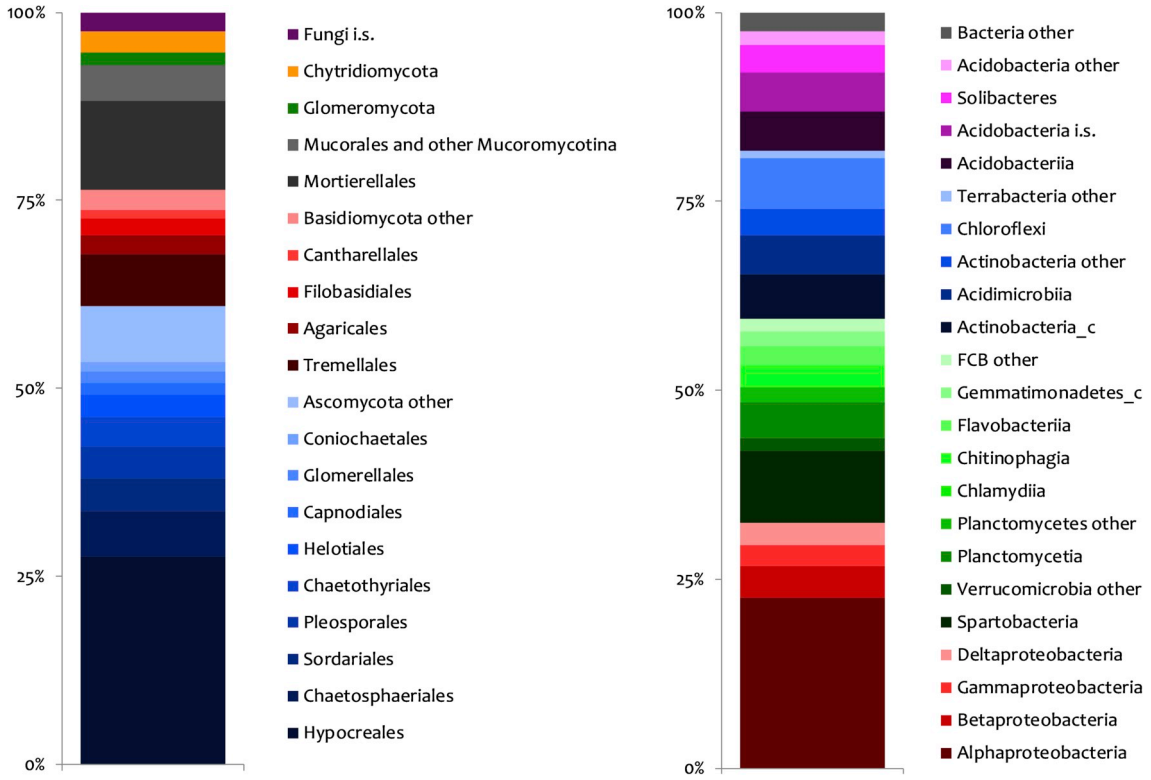

Fig. 4. Fungal (left panel) and bacterial (right panel) community structure at the ClimGrass experimental field in October 2016. Data from 27 plots were combined, OTUs were clustered at the appropriate level, and taxa with abundances below 1% were grouped. Fungi were grouped at the ordinal level. Ascomycota: blue; Basidiomycota: red; Mortierellomycota and Mucoromycota: grey; Glomeromycota: green; Chytridiomycota: gold; Fungi i. s.: violet. Bacteria were mainly grouped at the class level. Proteobacteria: red; PVC and FCB group: green; Terrabacteria: blue; Acidobacteria: purple; other bacteria: grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

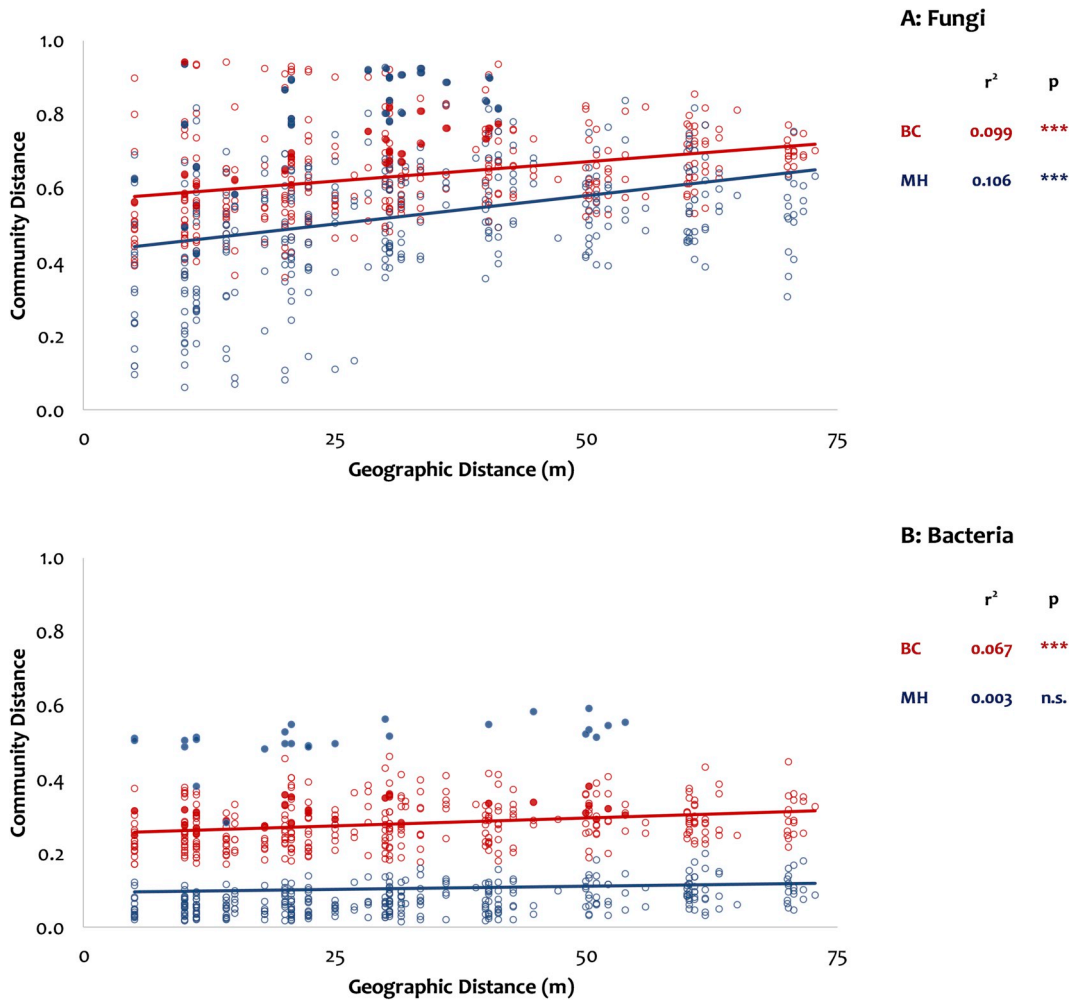

Fig. 5. β-Diversity of fungal (A) and bacterial (B) communities at the ClimGrass experimental field in October 2016. Two indices of β-diversity – Bray-Curtis (BC) and Morisita-Horn (MH) – were plotted against geographic distance between plots. Filled circles for the fungal community highlight distances to plot 23. The sample from plot 23 showed strong differences in the plant root composition (high levels of *Rumex acetosa L.*) and the fungal community (dominance of FOTU_2 – *Mucor hiemalis*). Similarly, distances to plot 35 are highlighted for bacterial communities by filled circles, as this plot is characterized by an exceptionally high abundance of BOTU_58 (*Providencia* sp.).

4. Discussion

Statistically significant spatial effects, which were independent of climate change manipulations, were observed for certain measured parameters in this study at the ClimGrass site in Gumpenstein. MBC, SOC, several dominant fungal taxa, some bacterial taxa and selected EEA showed uneven distribution between the west and east parts of the experimental field, i.e. at an intermediate scale between 5 and 70 m (Fig. 1, Table S1, Table S2 and Table S5). Large-scale spatial variability of microbial communities is mainly driven by differences in soil properties, topography and vegetation. At the other end of the scale, factors like soil aggregates, plant debris and roots can influence composition of the microbial community and the associated processes. Effects of individual plants and plant communities as well as from burrowing animals were suggested to affect soil microbial communities at the intermediate scale (Ettema and Wardle, 2002). Apart from MBC and SOC, no spatial patterns were observed for general soil parameters and for plant biomass (Table S1). The two halves of the experimental field have been prepared for site construction and installations in different years. The east part was equipped with the experimental infrastructure in 2010, and the west part was added in 2012. However, vegetation establishment (2007) and the start of climate change manipulations (May 2014 after the first harvest) was the same for the whole field. It is thus assumed that currently unknown legacy effects from historical site management are the basis for the observed spatial effects.

4.1. General soil parameters and total aboveground biomass

The imbalance between the two halves of the field were integrated into the response surface regression models (Piepho et al., 2017) and consequently improved the elucidation of treatment effects on soil, plant and microbial variables. By this approach it was possible to detect pronounced changes in soil water content (WC), extractable total nitrogen (ETN) and total aboveground biomass (TAGB) upon eCO_2 and eT (Figs. 2 and 3). WC followed the previously observed pattern, where sustained warming treatment increases evapotranspiration and decreases therefore WC, while higher CO_2 levels reduce stomatal aperture and abundance and consequently transpiration, which leads to higher WC (Morgan et al., 2004, 2011; Engineer et al., 2016). Response of ETN to climate change scenarios was similar to WC, a correlation between the two could be detected (Table S7). TAGB, on the other hand, showed an opposite trend at the ClimGrass site: a reduction upon eCO_2 and an increase upon eT (Fig. 3). This is in contrast to most studies, which describe a positive effect of eCO_2 on plant biomass through varying

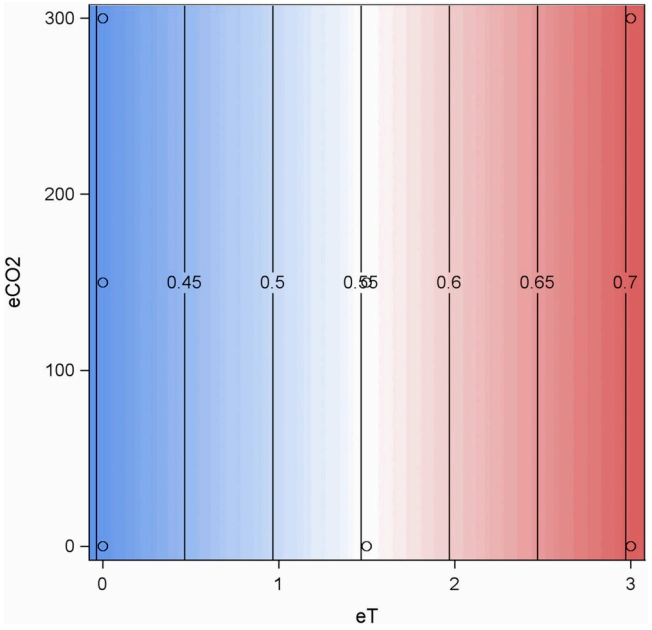

Fig. 6. Contour plot for abundance distribution of coprophilous white rot fungi. Relative abundances of FOTUs categorized as coprophilous white rot fungi were square root transformed and corrected for unequal distribution between the east and west halves of the experimental field. Elevated temperature in °C (eT) and CO_2 in ppm (eCO_2) above ambient are indicated. Treatment combinations, from which data were available for modelling, are indicated by circles. Relative abundance increases from blue to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

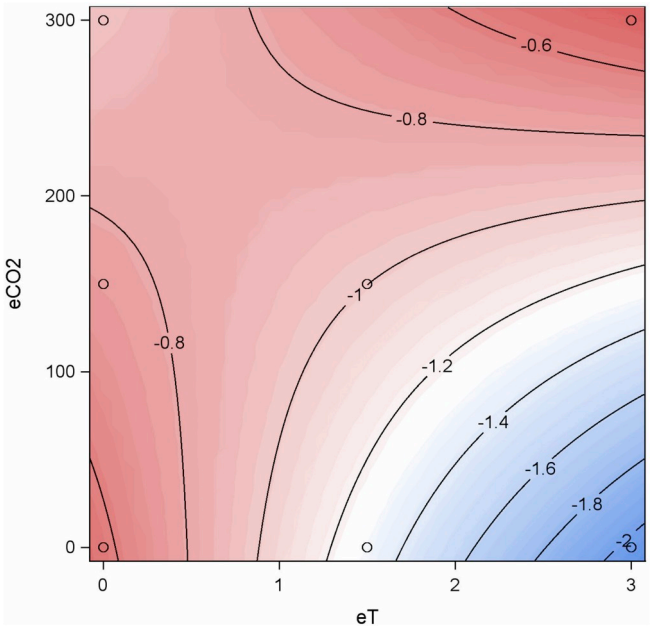

Fig. 7. Response of FOTU_43 (*Mortierella globulifera*) to environmental factors. Relative abundances of FOTU_43 were log transformed and corrected for unequal distribution between the east and west halves of the experimental field. Elevated temperature in °C (eT) and CO_2 in ppm (eCO_2) above ambient are indicated. Treatment combinations, from which data were available for modelling, are indicated by circles. Relative abundance increases from blue to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

combinations of direct effects on photosynthesis and indirect effects on WC (Zak et al., 2000; Morgan et al., 2004; Kuzyakov et al., 2019). Warming, on the other hand, often has a negative impact on plant biomass production through enhancement of water deficiency (De Boeck et al., 2008). We assume that in the investigated Alpine grassland with moderate temperatures during the summer and an annual precipitation of over 1000 mm, water is not a limiting factor. In this environment an increase in eT can therefore exert a direct positive effect on TAGB production. A CO_2-induced increase in WC, on the other hand, has no positive effect on plant growth. Reduction of stomatal aperture and abundance and reduced transpiration can limit mineral nutrient uptake from the soil as reflected by an increase in ETN with higher WC. Available data of plant species from root sequencing do not indicate strong effects from climate change scenarios on species composition in 2016, but roots from a soil core with a diameter of 1.9 cm may not provide a representative picture of the plant species composition at the plot scale. Effects of climate change drivers on plant species composition and physiology are still under investigation and were not the focus of our study, which targeted effects on soil microbial community structure and function.

4.2. Soil microbial communities

The bacterial communities (Fig. 4, right panel) in the grassland soils from the ClimGrass site were dominated by the phyla Proteobacteria, Acidobacteria, Actinobacteria and Verrucomicrobia i.e., taxa which are frequently found in agricultural and grassland soils (Bergmann et al., 2011; Hartmann et al., 2015). Domination of the fungal communities (Fig. 4, left panel) by Ascomycota as seen in the ClimGrass site is also a commonly observed feature in agricultural and grassland soils (Klaubauf et al., 2010; Hartmann et al., 2015; Moll et al., 2016). Plant root associated fungi from Glomeromycota, which can form arbuscular mycorrhiza, and from the Sebacinales, which interact with roots in highly diverse manners (forming distinct mycorrhizae and endophytic interactions; Weiss et al., 2016), were only of minor abundance. The percentage of Glomeromycota was, however, in a similar range as found in a global study for grasslands and shrublands: 1.4 % (Tedersoo et al., 2015) vs. 1.8 % on average at the ClimGrass site. The strong dominance of *Purpureocillium lilacinum*, on the other hand, has not been previously observed in soils. *P. lilacinum* is a nematophagous and entomopathogenic fungus, which has been isolated from decaying vegetation, insects, nematodes and soil (Domsch et al., 1993; Luangsa-Ard et al., 2011).

For the bacterial communities, a low β-diversity and consequently minor spatial effects were observed (Fig. 5B). The fungal community, on the other hand, exhibited a high β-diversity with distinct spatial effects (Fig. 5A). In addition, several bacterial and fungal OTUs showed highly uneven distribution over the ClimGrass site with pronounced differences between the east and west part of the experimental site (see above). Plant identity and diversity can additionally modulate soil microbial communities via root characteristics (Huang et al., 2014; Lange et al., 2014). In autumn 2016, when soil samples for microbial community analyses were collected, no significant effects of almost 2 years of climate change manipulation on aboveground plant community composition were observed (Pötsch, unpublished). Single soil cores were generally found to be dominated by roots from a single plant species, which was in nearly all cases either of the grasses *Dactylis glomerata*, *Arrhenatherum elatius* and *Poa trivialis*. The basidiomycetous yeast *Solicoccozyma terricola* (FOTU_17) increased in abundance with oat-grass (*Arrhenatherum elatius*; POTU_2 and POTU_54) (Fig. S3). Plot 23 (C1T0) additionally showed a relatively high abundance of roots from common sorrel - *Rumex acetosa* L. (Polygonaceae), and contained an unusually high abundance of *Mucor hiemalis* (FOTU_2; Fig. 5). It is therefore assumed that plant identity shapes the soil microbial communities – especial the fungal communities – at the ClimGrass site at scales below the plot level.

Generally, future climate change factors had no strong effect on the

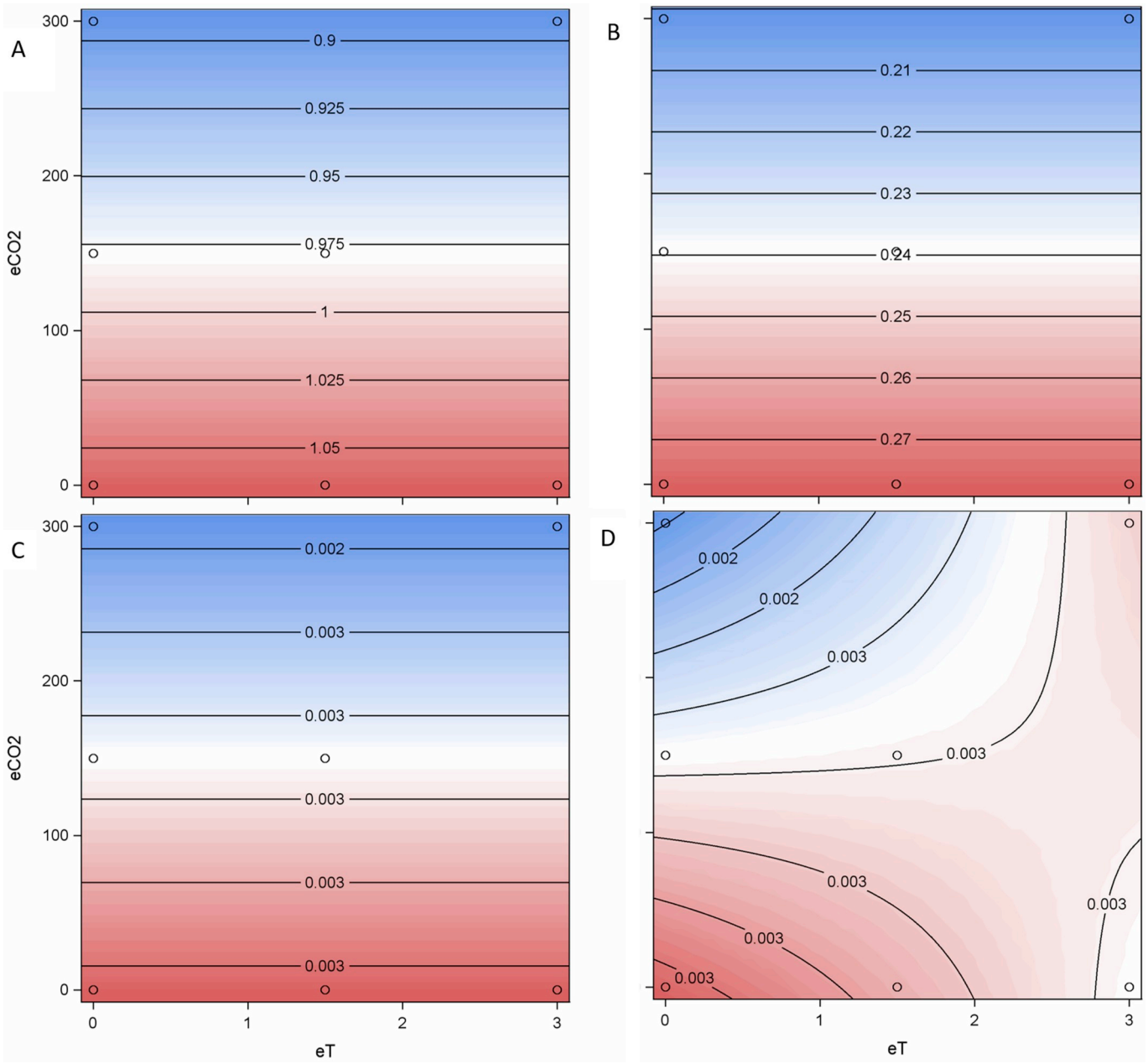

Fig. 8. Contour plots for specific EEA (A) sLAP, (B) sURE, (C) sPER and (D) sPHE to environmental factors (µmol µg^{-1} MBC h^{-1}). Data were corrected for unequal distribution between the east and west halves of the experimental field. Elevated temperature in °C (eT) and CO_2 in ppm (eCO_2) above ambient are indicated. Treatment combinations, from which data were available for modelling, are indicated by circles. Specific activities increase from blue to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

entire microbial community (Fig. S2), only a few fungal taxa showed a response to treatments (Table S4). Previous studies discussed mostly microbial community structure at the phylum level (Austin et al., 2009; Castro et al., 2010; Oliverio et al., 2017) or the OTU levels of bacterial community structure (Lesaulnier et al., 2008; de Menezes et al., 2016). In the latter case, experiments were focused on the individual effect of eCO_2. At the ClimGrass site *Mortierella globulifera* (FOTU_43) responded to both, eCO_2 and eT (Fig. 7), in a pattern similar to soil WC (Fig. 2). It is likely that the soil WC was the main driver for the observed effect, since the relative abundance of FOTU_43 and the WC correlated positively. Furthermore, coprophilous white rot fungi were found to respond positively to eT (Fig. 6). Higher soil perforation with numerous holes at warming plots, as observed during soil sampling, may hint to a preference of burrowing macrofauna (e.g., field mice, moles, voles) for eT, which in turn might have led to the increase in abundance of coprophilic white rot fungi. In agreement with this hypothesis is the high abundacnes of *Providencia* sp., a bacterium commonly found in the intestines of several animals (e.g. The NIH HMP Working Group et al., 2009), which was found in plot 35, a warming treatment elevated by + 3 °C (Fig. 5). Addition of herbivore dung to soil was previously shown to increase coprophilous fungal populations (Hartmann et al., 2015) and animal responses to climate change scenarios in experimental fields (e.g., to eT: gastropods, insects; to eCO_2: herbivores) is a widely observed phenomenon (Moise and Henry, 2010). Highly uneven distributions of individual animals are directly related to the experimental settings with pronounced temperature differences at small scales. These effects can,

however, not generally be translated to warming at the global scale.

4.3. Enzyme activity

Besides the taxonomy based functional categorization of the microbial communities into saprotrophic, symbiotic, potentially plant pathogenic and white rot fungi, extracellular enzyme activities (EEA) were measured as proxies for selected microbial processes. Determination of specific EAA (sEAA) by normalization to MBC as suggested by Sinsabaugh et al. (2008) could remove some of the spatial effects. In our study, MBC was preferred over SOC as a reference, as EAA and MBC were determined from the same soil cores, while SOC was determined from separate soil cores. The remaining spatial effects in sEAA were accounted for by the improved response surface regression model. Coregulation of enzymes involved in the breakdown of organic matter for C-acquisition, i.e. for sCBH, sBGL, sNAG and sLAP was observed. Similarly, coregulation of enzymes involved in N-acquisition from organic matter, i.e. sNAG, sLAP, sURE, and of ligninolytic enzymes sPHE and sPER was observed. sPHO activity was related to both, and all together showed a negative response to soil NH_4–N, albeit to varying degrees (Table S7). It is therefore concluded that sEAA in general, and more specifically for the breakdown of organic N-compounds and recalcitrant lignin, are induced upon N-limitation (Leatham and Kirk, 1983). With the exception of sNAG, N-cycling and ligninolytic enzymes responded negatively to eCO_2. An additional effect of temperature was observed for sPHE (Fig. 8). As CO_2 has been recently shown to have no strong direct effect on EAA (Xiao et al., 2018), indirect effects, such as described in the "resource allocation theory" are more likely (Henry, 2012; Xiao et al., 2018). ETN increased with eCO_2 at the experimental site and can probably partially explain the observed effects on EAAs. The increased ETN concentration in soil seemed to be related to the reduced aboveground biomass. More N remains in the soil and, hence, less N acquiring enzymes have to be produced to cover the N requirement for microorganisms. A significant correlation of EEA with ETN was, however, only found for sPHE and sLAP (see Table S7).

sNAG, which is not only involved in N-cycling but also in C-cycling, showed no response to eCO_2. Instead, sNAG was correlated positively with the relative abundance of the nematophagous and entomopathogenic fungus *P. lilacinum* (FOTU_1; Fig. S5). NAG, the extracellular chitinase, is involved in the degradation of chitinous polymers found in the exoskeleton of arthropods, in nematode egg shells and in fungal cell walls (Merzendorfer and Zimoch, 2003). There is extensive evidence that parasitic nematophagous fungi produce extracellular hydrolytic enzymes such as proteases, collagenase, and chitinase (Khan et al., 2003; Yang et al., 2007). It would be interesting to relate these results to soil mesofauna at the ClimGrass site in future investigations.

5. Conclusion

Climate change scenarios with stepwise increases in atmospheric CO_2 and surface temperature had only minor effects on soil microbial community composition and function after two years of operation. Site specific spatial effects strongly influenced several soil and microbial parameters. Inclusion of these imbalances into the response surface model allowed the detection of subtle changes in the fungal population and in sEAA after only two years of warming and CO_2 fumigation. It is, however, advised for future climate manipulation field experiments to collect soil, plant, animal and microbial parameters after site preparation but before start of the treatment implementation to detect and to correct for field-scale spatial imbalances. At the ClimGrass site future climate change scenarios mainly induced changes in WC, ETN and TAGB. Changes in soil microbial community composition and functioning could be related to CO_2, WC, NH_4–N, plant root identity and assumed presence of animals, while eT only had minor effects. As it is not expected that an increase in atmospheric CO_2 directly influences CO_2 levels in soil (Kuzyakov et al., 2019), it is concluded that indirect effects of future climate change scenarios are of higher importance for soil microbial community composition and functioning than direct effects. Furthermore, it is highly desirable to also collect data on fauna, to better understand the observed phenomena. The behaviour of animals including soil inhabiting insects and mammals is certainly influenced by climate manipulations.

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.2020.107704.

References

Ameur, D., Zehetner, F., Johnen, S., Jöchlinger, L., Pardeller, G., Wimmer, B., Rosner, F., Faber, F., Dersch, G., Zechmeister-Boltenstern, S., 2018. Activated biochar alters activities of carbon and nitrogen acquiring soil enzymes. Pedobiologia 69, 1–10.

Austin, E.E., Castro, H.F., Sides, K.E., Schadt, C.W., Classen, A.T., 2009. Assessment of 10 years of CO_2 fumigation on soil microbial communities and function in a sweetgum plantation. Soil Biology and Biochemistry 41, 514–520.

Baldrian, P., Šnajdr, J., Merhautová, V., Dobiášová, P., Cajthaml, T., Valášková, V., 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biology and Biochemistry 56, 60–68.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4, 914–919.

Bengtsson-Palme, J., Hartmann, M., Eriksson, K.M., Pal, C., Thorell, K., Larsson, D.G.J., Nilsson, R.H., 2015. METAXA2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Molecular Ecology Resources 15, 1403–1414.

Bergmann, G.T., Bates, S.T., Eilers, K.G., Lauber, C.L., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biology and Biochemistry 43, 1450–1455.

Box, G.E., Draper, N.R., 2007. Response Surfaces, Mixtures, and Ridge Analyses. John Wiley & Sons.

Brenzinger, K., Kujala, K., Horn, M.A., Moser, G., Guillet, C., Kammann, C., Müller, C., Braker, G., 2017. Soil conditions rather than long-term exposure to elevated CO_2 affect soil microbial communities associated with N-cycling. Frontiers in Microbiology 8, 1976.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9–20.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335.

Carney, K.M., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2007. Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proceedings of the National Academy of Sciences 104, 4990–4995.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76, 999–1007.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., Leon, C., 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS One 5, e8613.

Colwell, R.K., 2013. EstimateS: statistical estimation of species richness and shared species from samples. And Earlier. User's Guide and Application, Version 9. http://purl.oclc.org/estimates.

De Boeck, H.J., Lemmens, C.M.H.M., Zavalloni, C., Gielen, B., Malchair, S., Carnol, M., Merckx, R., Van Den Berge, J., Ceulemans, R., Nijs, I., 2008. Biomass production in experimental grasslands of different species richness during three years of climate warming. Biogeosciences 5, 585–594.

de Menezes, A.B., Müller, C., Clipson, N., Doyle, E., 2016. The soil microbiome at the Gi-FACE experiment responds to a moisture gradient but not to CO_2 enrichment. Microbiology 162, 1572–1582.

Deltedesco, E., Keiblinger, K.M., Naynar, M., Piepho, H.-P., Gorfer, M., Herndl, M., Bahn, M., Pötsch, E.M., Zechmeister-Boltenstern, S., 2019. Trace gas fluxes from managed grassland soil subject to multifactorial climate change manipulation. Applied Soil Ecology 137, 1–11.

Dieleman, W.I.J., Vicca, S., Dijkstra, F.A., Hagedorn, F., Hovenden, M.J., Larsen, K.S., Morgan, J.A., Volder, A., Beier, C., Dukes, J.S., King, J., Leuzinger, S., Linder, S., Luo, Y., Oren, R., De Angelis, P., Tingey, D., Hoosbeek, M.R., Janssens, I.A., 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. Global Change Biology 18, 2681–2693.

Domsch, K.H., Gams, W., Anderson, T.-H., 1993. Compendium of Soil Fungi. IHW-Verlag, Eching, Germany.

Drissner, D., Blum, H., Tscherko, D., Kandeler, E., 2007. Nine years of enriched CO_2 changes the function and structural diversity of soil microorganisms in a grassland. European Journal of Soil Science 58, 260–269.

Engineer, C.B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordström, M., Azoulay-Shemer, T., Rappel, W.-J., Iba, K., Schroeder, J.I., 2016. CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. Trends in Plant Science 21, 16–30.

Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. Trends in Ecology & Evolution 17, 177–183.

Ferretti, G., Keiblinger, K.M., Di Giuseppe, D., Faccini, B., Colombani, N., Zechmeister-Boltenstern, S., Coltorti, M., Mastrocicco, M., 2018. Short-term response of soil microbial biomass to different chabazite zeolite amendments. Pedosphere 28, 277–287.

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

Groh, J., Slawitsch, V., Herndl, M., Graf, A., Vereecken, H., Pütz, T., 2018. Determining dew and hoar frost formation for a low mountain range and alpine grassland site by weighable lysimeter. Journal of Hydrology 563, 372–381.

Grüter, D., Schmid, B., Brandl, H., 2006. Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. BMC Microbiology 6, 68.

Gutknecht, J.L., Henry, H.A., Balser, T.C., 2010. Inter-annual variation in soil extracellular enzyme activity in response to simulated global change and fire disturbance. Pedobiologia 53, 283–293.

Hartmann, M., Frey, B., Mayer, J., Mader, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. The ISME Journal 9, 1177–1194.

Henry, H.A.L., 2012. Soil extracellular enzyme dynamics in a changing climate. Soil Biology and Biochemistry 47, 53–59.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald- Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Science Society of America Journal 74, 1018–1027.

Horn, R., Brümmer, G.W., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Wilke, B.-M., 2010. Scheffer/schachtschabel: Lehrbuch der bodenkunde. Springer-Verlag.

Huang, X.-F., Chaparro, J.M., Reardon, K.F., Zhang, R., Shen, Q., Vivanco, J.M., 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92, 267–275.

IPCC, 2014. Climate Change 2014: Synthesis Report. Cointribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel in Climate Change.

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, Update 2015, International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106 FAO, Rome.

Janus, L.R., Angeloni, N.L., McCormack, J., Rier, S.T., Tuchman, N.C., Kelly, J.J., 2005. Elevated atmospheric CO 2 alters soil microbial communities associated with trembling aspen (Populus tremuloides) roots. Microbial Ecology 50, 102–109.

Jost, L., Chao, A., Chazdon, R.L., 2010. Compositional similarity and beta diversity. In: Magurran, A.E., McGill, B.J. (Eds.), Biological Diversity: Frontiers in Measurement and Assessment. Oxford University Press, USA.

Jansen Willems, A.B., Lanigan, G.J., Grünhage, L., Müller, C., 2016. Carbon cycling in temperate grassland under elevated temperature. Ecology and Evolution 6, 7856–7868.

Keiblinger, K.M., Schneider, M., Gorfer, M., Paumann, M., Deltedesco, E., Berger, H., Jochlinger, L., Mentler, A., Zechmeister-Boltenstern, S., Soja, G., Zehetner, F., 2018. Assessment of Cu applications in two contrasting soils-effects on soil microbial activity and the fungal community structure. Ecotoxicology 27, 217–233.

Kelley, A.M., Fay, P.A., Polley, H.W., Gill, R.A., Jackson, R.B., 2011. Atmospheric CO_2 and soil extracellular enzyme activity: a meta-analysis and CO_2 gradient experiment. Ecosphere 2, 1–20.

Kenward, M.G., Roger, J.H., 2009. An improved approximation to the precision of fixed effects from restricted maximum likelihood. Computational Statistics & Data Analysis 53, 2583–2595.

Khan, A., Williams, K., Molloy, M.P., Nevalainen, H., 2003. Purification and characterization of a serine protease and chitinases from *Paecilomyces lilacinus* and detection of chitinase activity on 2D gels. Protein Expression and Purification 32, 210–220.

Klaubauf, S., Inselsbacher, E., Zechmeister-Boltenstern, S., Wanek, W., Gottsberger, R., Strauss, J., Gorfer, M., 2010. Molecular diversity of fungal communities in agricultural soils from Lower Austria. Fungal Diversity 44, 65–75.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research 41, e1.

Kraus, D., 2014. Consolidated data analysis and presentation using an open-source add-in for the Microsoft Excel® spreadsheet software. Medical Writing 23, 25–28.

Kuzyakov, Y., Horwath, W.R., Dorodnikov, M., Blagodatskaya, E., 2019. Review and synthesis of the effects of elevated atmospheric CO_2 on soil processes: No changes in pools, but increased fluxes and accelerated cycles. Soil Biology and Biochemistry 128, 66–78.

Lange, M., Habekost, M., Eisenhauer, N., Roscher, C., Bessler, H., Engels, C., Oelmann, Y., Scheu, S., Wilcke, W., Schulze, E.-D., Gleixner, G., 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. PLoS One 9, e96182.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357.

Leatham, G.F., Kirk, T.K., 1983. Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. FEMS Microbiology Letters 16, 65–67.

Leitner, S., Berger, H., Gorfer, M., Reichenauer, T.G., Watzinger, A., 2017. Isotopic effects of PCE induced by organohalide-respiring bacteria. Environmental Science and Pollution Research 24, 24803–24815.

Lesaulnier, C., Papamichail, D., McCorkle, S., Ollivier, B., Skiena, S., Taghavi, S., Zak, D., van der Lelie, D., 2008. Elevated atmospheric CO_2 affects soil microbial diversity associated with trembling aspen. Environmental Microbiology 10, 926–941.

Luangsa-Ard, J., Houbraken, J., van Doorn, T., Hong, S.B., Borman, A.M., Hywel-Jones, N.L., Samson, R.A., 2011. *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiology Letters 321, 141–149.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17, 10–12.

Marx, M.-C., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33, 1633–1640.

Meier, I.C., Pritchard, S.G., Brzostek, E.R., McCormack, M.L., Phillips, R.P., 2015. The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO_2. New Phytologist 205, 1164–1174.

Merzendorfer, H., Zimoch, L., 2003. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. Journal of Experimental Biology 206, 4393–4412.

Mills, A.L., Franklin, R.B., 2003. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. FEMS Microbiology Ecology 44, 335–346.

Moise, E.R.D., Henry, H.A.L., 2010. Like moths to a street lamp: exaggerated animal densities in plot-level global change field experiments. Oikos 119, 791–795.

Moll, J., Hoppe, B., König, S., Wubet, T., Buscot, F., Krüger, D., 2016. Spatial distribution of fungal communities in an arable soil. PLoS One 11, e0148130.

Morgan, J.A., Pataki, D.E., Körner, C., Clark, H., Del Grosso, S.J., Grünzweig, J.M., Knapp, A.K., Mosier, A.R., Newton, P.C.D., Niklaus, P.A., Nippert, J.B., Nowak, R.S., Parton, W.J., Polley, H.W., Shaw, M.R., 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. Oecologia 140, 11–25.

Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., Williams, D.G., Heisler-White, J., Dijkstra, F.A., West, M., 2011. C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. Nature 476, 202.

Mosier, A.R., 1998. Soil processes and global change. Biology and Fertility of Soils 27, 221–229.

Nikolenko, S.I., Korobeynikov, A.I., Alekseyev, M.A., 2013. BayesHammer: Bayesian Clustering for Error Correction in Single-Cell Sequencing. BMC Genomics, BioMed Central, p. S7.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47, D259–D264.

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., 2018. Package "vegan". R Packag ver 20–8. https://cran.r-project.org/web/packages/vegan/vegan.pdf.

Oliverio, A.M., Bradford, M.A., Fierer, N., 2017. Identifying the microbial taxa that consistently respond to soil warming across time and space. Global Change Biology 23, 2117–2129.

The NIH HMP Working Group, Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J.A., Bonazzi, V., McEwen, J.E., Wetterstrand, K.A., Deal, C., Baker, C.C., Di Francesco, V., Howcroft, T.K., Karp, R.W., Lunsford, R.D., Wellington, C.R., Belachew, T., Wright, M., Giblin, C., David, H., Mills, M., Salomon, R., Mullins, C., Akolkar, B., Begg, L., Davis, C., Grandison, L., Humble, M., Khalsa, J., Little, A.R., Peavy, H., Pontzer, C., Portnoy, M., Sayre, M.H., Starke-Reed, P., Zakhari, S., Read, J., Watson, B., Guyer, M., 2009. The NIH human microbiome project. Genome Research 19, 2317–2323.

Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters 14, 187–194.

Piepho, H., Edmondson, R., 2018. A tutorial on the statistical analysis of factorial experiments with qualitative and quantitative treatment factor levels. Journal of Agronomy and Crop Science 204, 429–455.

Piepho, H.P., Herndl, M., Pötsch, E.M., Bahn, M., 2017. Designing an experiment with quantitative treatment factors to study the effects of climate change. Journal of Agronomy and Crop Science 203, 584–592.

Pilegaard, K., Skiba, U., Ambus, P., Beier, C., Brüggemann, N., Butterbach-Bahl, K., Dick, J., Dorsey, J., Duyzer, J., Gallagher, M., Gasche, R., Horvath, L., Kitzler, B., Leip, A., Pihlatie, M.K., Rosenkranz, P., Seufert, G., Vesala, T., Westrate, H., Zechmeister-Boltenstern, S., 2006. Factors controlling regional differences in forest soil emission of nitrogen oxides (NO and N_2O). Biogeosciences 3, 651–661.

Pötsch, E.M., Herndl, M., Bahn, M., Schaumberger, A., Schweiger, M., Kandolf, M., Reinthaler, D., Schink, M., Adelwöhrer, M., 2019. Climgrass - ein innovatives Freilandexperiment zur Klimafolgenforschung im Grünland, 21. Alpenländisches Expertenforum, Klimawandel im Alpenraum - Auswirkungen auf das Ökosystem Grünland und dessen Bewirtschaftung. HBLFA Raumberg-Gumpenstein, Irdning-Donnersbachtal.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner Oliver, F., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Redearch 41 (Database issue). https://doi.org/10.1093/nar/gks1219. In this issue.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.

Sayer, E.J., Wagner, M., Oliver, A.E., Pywell, R.F., James, P., Whiteley, A.S., Heard, M.S., 2013. Grassland management influences spatial patterns of soil microbial communities. Soil Biology and Biochemistry 61, 61–68.

Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R., 1996. Methods in Soil Biology. Springer Science & business Media.

Schirmer, M., Ijaz, U.Z., D'Amore, R., Hall, N., Sloan, W.T., Quince, C., 2015. Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. Nucleic Acids Research 43 e37-e37.

Selsted, M.B., Linden, L., Ibrom, A., Michelsen, A., Larsen, K.S., Pedersen, J.K., Mikkelsen, T.N., Pilegaard, K., Beier, C., Ambus, P., 2012. Soil respiration is stimulated by elevated CO_2 and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). Global Change Biology 18, 1216–1230.

Shand, C.A., Williams, B.L., Coutts, G., 2008. Determination of N-species in soil extracts using microplate techniques. Talanta 74, 648–654.

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404.

Sinsabaugh, R.L., Reynolds, H., Long, T.M., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biology and Biochemistry 32, 2095–2097.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T. W., Harend, H., Guo, L.D., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Fungal biogeography. Global diversity and geography of soil fungi. Science 346, 1256688.

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, H., Hildebrand, F., Bork, P., Abarenkov, K., 2015. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys 10.

VDLUFA, 1976. Methodenbuch Band III - Die chemische Untersuchung von Futtermitteln, inkl. Ergänzungsblätter 1983, 1988, 1993. VDLUFA-Verlag, Darmstadt, p. 1997.

Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants of bacterial diversity in soil. FEMS Microbiology Reviews 37, 936–954.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261–5267.

Weiss, M., Waller, F., Zuccaro, A., Selosse, M.A., 2016. Sebacinales - one thousand and one interactions with land plants. New Phytologist 211, 20–40.

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.

Xiong, J., Sun, H., Peng, F., Zhang, H., Xue, X., Gibbons, S.M., Gilbert, J.A., Chu, H., 2014. Characterizing changes in soil bacterial community structure in response to short-term warming. FEMS Microbiology Ecology 89, 281–292.

Yang, J., Tian, B., Liang, L., Zhang, K.-Q., 2007. Extracellular enzymes and the pathogenesis of nematophagous fungi. Applied Microbiology and Biotechnology 75, 21–31.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Vogel, C.S., Holmes, W.E., Lussenhop, J., 2000. Atmospheric CO_2, soil-N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. Ecological Applications 10, 34–46.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2013. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620.

Zhou, J., Kang, S., Schadt, C.W., Garten, C.T., 2008. Spatial scaling of functional gene diversity across various microbial taxa. Proceedings of the National Academy of Sciences 105, 7768–7773.