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„Seasonal responses of microbial growth and respiration to multiple climate change drivers"

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List of abbreviations

ANPP	Aboveground net primary production
BNPP	Belowground net primary production
C	Carbon
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
CUE	Carbon Use Efficiency
DM	Dry mass
eCO_2	Elevated CO_2
eT	Elevated temperature
G	Growth
GLS	Generalised Least Square
G_m	Biomass-specific growth
KCl	Potassium chloride
MBC	Microbial biomass carbon
miniFACE	Mini Free Air Carbon dioxide Enrichment
O	Oxygen
ppm	Parts-per-million
R	Respiration rate
R_m	Biomass-specific respiration rate
RSM	Response Surface Model
SWC	Soil water content
TukeyHSD	Tukey Honest Significant Difference

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PART 1

General Introduction

Atmospheric CO_2 concentrations are ever increasing and are now more than 40 percent above pre-industrial levels (IPCC 2013). Man-caused emissions, mostly from fossil fuel combustion, land use change and agriculture, substantially contribute to the current concentration of about 410 parts-per-million (ppm) CO_2 (2019a) and of 1.85 ppm CH_4 in the atmosphere (2019b). Following the trend of greenhouse gas concentrations in the atmosphere, the global average air temperature has been rising about 1 degree Celsius above pre-industrial levels (IPCC 2018). Continuous emissions of greenhouse gases will cause further warming in the future (Rustad et al. 2001; Lal 2008; IPCC 2013). Depending on the current and future human behaviour, humankind will face global surface warming between plus 1.5 and 4.5 degree Celsius (according to different Representative Concentration Pathways (RCPs) of the IPCC) relative to the average from 1850 to 1900 until the end of the 21st century. Climate warming will persist for many centuries after emissions were stopped (IPCC 2013), unless significant amounts of carbon dioxide will be removed from the atmosphere. Warming causes alterations in the water cycle, which lead to region-specific changes in precipitation regimes (IPCC 2013). Thus, intensity and frequency of droughts are likely to increase in some regions (IPCC 2013).

Until now both oceans and the land system act as sinks for atmospheric CO_2. Oceans will continue taking up CO_2 and sequestering carbon with high confidence under all RCP scenarios (IPCC 2013). The fate of the land carbon sink is less certain and still under discussion (Melillo et al. 2002; IPCC 2013; Bradford et al. 2016; Crowther et al. 2016; Jackson et al. 2017; Marañón-Jiménez et al. 2018).
Models predict that warming will stimulate losses of soil carbon to the atmosphere (Davidson and Janssens 2006; Crowther et al. 2016; Alvarez et al. 2018) by enhancing soil microbial respiration (Rustad et al. 2001). A positive soil carbon-

climate feedback would further accelerate climate change (Rustad et al. 2001; Bradford 2013; Crowther et al. 2016; Alvarez et al. 2018). On the other hand, acclimatization of soil respiration to elevated temperature could offset further CO_2 emissions from soils (Luo et al. 2001). Increased carbon sequestration in soils could even mitigate climate change (Yuan et al. 2018).

By far the largest part of terrestrial organic carbon is stored in soil organic matter (Lal 2008; Lehmann and Kleber 2015; Jackson et al. 2017), which is also the biggest reservoir of organic carbon globally. Evidence is accumulating that microbial remnants (necromass) are major constituents of stable soil organic matter (Simpson et al. 2007; Liang and Balser 2011; Miltner et al. 2012; Kallenbach et al. 2015, 2016). This implies that most of the plant organic matter input into soil has been transformed by microorganisms before becoming soil organic matter (Cotrufo et al. 2013; Liang et al. 2017), which underlines the special importance for microorganism in the formation of the largest persistent pool of organic carbon on earth.

Grasslands cover approximately one quarter of the ice-free land surface area (Bai et al. 2010; Foley et al. 2011) and contain and sequester substantial amounts of carbon (Hui and Jackson 2006; Abberton et al. 2010; Ingrisch et al. 2018).

Regarding organic carbon, plants are the connecting link between the atmosphere and the soil (Drigo et al. 2008). On a global scale, about 60 gigatons of photosynthesized carbon (i.e., almost the whole net primary productivity of terrestrial systems) are yearly transferred to the non-living terrestrial carbon pool by aboveground and belowground plant litter production and rhizodeposition (Lal 2008). On the other hand, approximately 60 gigatons of carbon are annually respired back to the atmosphere (Lal 2008) at least in a steady state. Currently, however, about 3 gigatons of carbon per year are additionally stored in terrestrial systems globally (Ontl and Schulte 2012).

Plant inputs are the primary source of organic carbon in soils (Drigo et al. 2008). The fate of plant carbon inputs is strongly dependent on heterotrophic microorganisms. Microorganisms differ in their ability of degrading substrates of wide-ranging complexity (Schimel and Schaeffer 2012). Depending on the molecular weight of a carbon substrate, heterotrophic microorganisms can either consume it directly or need to excrete extracellular enzymes, which depolymerise plant polymers to small oligomers or monomers, which microorganisms can directly take up. Microorganisms allocate substrates taken up to respiration, growth, synthesis of extracellular enzymes, EPS and stress response compounds as osmolytes, etc. (Schimel and Schaeffer 2012). The amount of carbon, which is allocated to build-up of microbial biomass, largely controls the amount of carbon, which can potentially be stabilized in soil in the long-term (Manzoni et al. 2012; Kallenbach et al. 2016; Liang et al. 2017). Carbon allocation to growth over carbon uptake is termed microbial carbon use efficiency (CUE) (Dijkstra et al. 2011; Sinsabaugh et al. 2013; Hagerty et al. 2018; Manzoni et al. 2018). CUE is believed to be a valuable parameter in predicting the soil carbon-climate feedback (Geyer et al. 2016) and potential carbon sequestration in soils (Schimel and Schaeffer 2012; Sinsabaugh et al. 2013; Jackson et al. 2017). Consequently, CUE is a key parameter in soil carbon models, which incorporate microbial controls and increasingly also in Earth System Models (Dijkstra et al. 2011; Manzoni et al. 2012; Sinsabaugh et al. 2013; Hagerty et al. 2018).
CUE ultimately is a function of the intercellular metabolism, that is of the balance between anabolic and catabolic processes or between growth and energy production (Manzoni et al. 2012; Hagerty et al. 2018). Thus, CUE depends on the life history strategy (Kallenbach et al. 2015) and stress tolerance of members of the microbial community (Schimel and Schaeffer 2012).

Environmental factors (Williams and Rice 2007; Regan et al. 2014) as soil temperature and soil moisture together with the availability of nutrients shape microbial communities (Schmidt et al. 2007; Kaiser et al. 2010; Schimel and Schaeffer 2012; Fierer 2017). Besides, plant carbon inputs were suggested to have a major impact on microbial community composition and physiology (Wan et al.

2007; Classen et al. 2015; Eisenhauer et al. 2017). Consequently, alterations of microbial community composition are linked to vegetation activity and composition (Fierer 2017). Physiological acclimation of plant species to given soil water content, temperature and atmospheric CO_2 concentration might cause varying carbon inputs into soil (Bardgett et al. 2014).

The magnitude of belowground assimilate allocation and rhizodeposition depends on plant species identity (Van Der Krift et al. 2001; Sanaullah et al. 2012; Pausch and Kuzyakov 2018), plant developmental stage (Nguyen 2003; Badri and Vivanco 2009; Pritchard 2011; Philippot et al. 2013; Calvo et al. 2017) and environmental conditions (Nguyen 2003; Ainsworth and Long 2005) such as water availability and temperature (Pausch et al. 2013). Studies conducted on herbaceous species and grasses observed highest root exudation during active root growth, before plants started to flower (Nguyen 2003; Badri and Vivanco 2009). At the end of the vegetation period (i.e., in autumn) plants exude relatively small amounts (Badri and Vivanco 2009) as roots grow older and plants allocate less carbon belowground (Nguyen 2003). Root growth declines along with photosynthesis when the days grow shorter and cooler (Dumbrell et al. 2011). Reductions of exudation might be partly compensated by increased rhizodeposition in form of dead fine roots (Leigh et al. 2002; Edwards et al. 2004) due to senescence (Regan et al. 2014) at the end of the growing season.

Microorganisms have the ability to adapt fast to changes in environmental conditions and quantity and quality of substrate (Tecon and Or 2017) as they have short turnover times (Schmidt et al. 2007). Consequently, seasonal variations of habitat variables result in varying microbial activity and a succession of the active microbial communities composition over the course of a year (Blume et al. 2002; Schmidt et al. 2007; Bardgett and Van Der Putten 2014; Regan et al. 2014; Classen et al. 2015).

Climate change is thought to alter microbial carbon cycling both directly, i.e. by affecting microbial physiology and community composition, and indirectly via

vegetation (Castro et al. 2010; Bardgett and Van Der Putten 2014), i.e. by altering plant primary production (Bardgett et al. 2008), quality and quantity of plant litter and rhizodeposition (Drigo et al. 2008; Pritchard 2011; Classen et al. 2015; Calvo et al. 2017; Sayer et al. 2017), plant phenology (Rustad et al. 2001), plant physiology and vegetation composition (Classen et al. 2015; Sayer et al. 2017). Alterations of physiological processes of heterotrophic microorganisms in response to varying environmental conditions and substrate availability across spatial and temporal scales might result in changed CUE (Geyer et al. 2016; Manzoni et al. 2018).

Air and soil warming are expected to affect plants and soil microorganisms directly (Pritchard 2011) as chemical reactions are generally temperature sensitive (Davidson and Janssens 2006; Bradford 2013; Alster et al. 2016a; Moinet et al. 2017). Warming usually stimulates microbial respiration (DeAngelis et al. 2015) and growth rate (Suseela and Dukes 2013; Classen et al. 2015) and accelerates microbial activity in general (Blume et al. 2002; Siles et al. 2016). Thus, microbial process rates are expected to be highest in the warm season (Blume et al. 2002; Siles et al. 2016). Increased rhizodeposition, which might result from accelerated root turnover due to increased activity of soil pathogens might additionally boost saprotrophic microbial activity at elevated temperature (Gill and Jackson 2000). However, at high temperatures, i.e., after reaction rates have reached their optimal temperature, reaction rates may not further increase with temperature or even decline again (Alster et al. 2016b, a).

Although the direct response of processes to temperature is easy to predict based on thermodynamics, several studies found contrasting responses of heterotrophic respiration rates to warming in the long-term (Classen et al. 2015), which makes the long-term effect of rising temperatures on respiration highly uncertain (Alster 2019). For example, increases in respiration rate were found to often be ephemeral (Rustad et al. 2001; Kirschbaum 2004; Hartley et al. 2007; Bradford 2013; Tucker et al. 2013) and to return to control levels after a few years of warming (Luo et al. 2001; Melillo et al. 2002, 2017; Eliasson et al. 2005; Conant et al. 2011).

Ephemerality of enhanced respiration rates is usually attributed to either thermal acclimation or adaptation or to depletion of substrates. For example, was the adaptation of microorganisms due to evolutionary trade-offs in enzyme and membrane structure put forward as a mechanism by which heterotrophic respiration acclimates to higher temperatures with time (Bradford 2013; Dacal et al. 2019). On the other hand, attenuation of the temperature-dependent stimulation of microbial respiration could result from shifts in microbial community composition and structure (Bradford 2013; Luo et al. 2014; Bradford et al. 2016; Pold et al. 2017). However, changes in microbial community composition were only found after long-term soil warming (Luo et al. 2014; DeAngelis et al. 2015).

Other scientists argued that substrate depletion causes the attenuation of the positive response of respiration to warming as microbial activity decreases (Kirschbaum 2004; Hartley et al. 2007; Pold et al. 2017; Liu et al. 2018; Walker et al. 2018). Specifically, declined microbial biomass as a result of insufficient substrate availability was reported to cause the apparent acclimation of respiration under warming (Walker et al. 2018). Consequently, respiration rates per gram soil were found to return to control values, while biomass-specific respiration rates were still accelerated under warming (Marañón-Jiménez et al. 2018; Walker et al. 2018) due to the inherent temperature sensitivity of enzymatic reactions (Liu et al. 2018).

Besides substrate depletion, substrate inaccessibility due to low soil moisture under warming could as well explain microbial respiration rates, which are lower than expected from their temperature sensitivity (Schimel 2018). This is supported by the finding that seasonal changes in soil moisture modified the apparent temperature sensitivity of microbial respiration and growth (Rustad et al. 2001). Warming only triggered increased respiration at optimal soil water content, whereas rates were low when soil water was limiting in summer (Suseela and Dukes 2013) as microorganisms could not access enough resources (Bradford 2013). Other studies confirmed decreased microbial respiration rate under water-limited conditions (Canarini et al. 2017; Schwarz et al. 2017; Fuchslueger et al. 2019).

Soil water availability has a strong control over soil microorganisms as water acts as a metabolic resource, a solvent and transport medium of substrates and extracellular enzymes, and a medium for the motility of bacteria (Tecon and Or 2017; Schimel 2018). Soil microorganisms generally rely on the diffusion of extracellular enzymes to reach the substrates and diffusion of products back to the cell, as almost all substrates, which microorganisms take up, are water soluble (Schimel 2018). Soil moisture regulates the connectivity of the pore space and thereby determines if substrates are accessible to soil microorganisms (Schimel and Schaeffer 2012). If soils dry, the water film might be reduced or even disrupted (Schimel et al. 2007). Reduced connectivity may cause constrained substrate accessibility to microbes (Davidson and Janssens 2006; Schimel et al. 2007; Bradford 2013) as bacteria might become immobile and depend on the diffusion of extracellular enzymes and degraded molecules (Schimel and Schaeffer 2012). Consequently, solute availability might become the limiting factor of microbial activity (Manzoni et al. 2011). Drought might not only cause lower substrate accessibility, but poses a direct physiological stressor of microorganism in soils (Schimel 2018). Reductions in soil water potential force microbes to allocate resources from growth to survival pathways as synthesis of molecules for osmotic adjustment or of protective molecules (such as chaperons) in order to withstand the environmental stress (Schimel et al. 2007; Karlowsky et al. 2018).

Drought likely also results in changes in the composition of the active microbial community (Poll et al. 2013; Fuchslueger et al. 2019) as microorganisms have different resistance against dry conditions according to their life history. If drought becomes too severe microorganisms either become dormant (Schimel et al. 2007; Salazar et al. 2018) or die (Schimel et al. 2007). Although growth rates are thought to decrease under drought, microbial biomass was observed to stay unaltered (Fuchslueger et al. 2019) or even increase (Parker and Schimel 2011; Sanaullah et al. 2012; Schaeffer et al. 2017; Karlowsky et al. 2018). This apparent contradiction might be the result of decreased death rates due to reduced predation pressure on microorganisms during dry periods (Parker and Schimel 2011; Schaeffer et al. 2017;

Schimel 2018). As relatively larger soil pores dry first, movement of microbial predators through the soil matrix may be constrained before microbes are affected (Schimel 2018).

Elevated CO_2 concentrations were found to stimulate plant carbon inputs into soil (Drigo et al. 2008; Pritchard 2011; Nie et al. 2013), although studies are known, where nutrient limitation dampened the CO_2 fertilization effect in the long-term (Reich and Hobbie 2013; Wieder et al. 2015; Terrer et al. 2016). The CO_2 fertilization effect on plants summarises beneficial effects of elevated CO_2 on belowground (Van Der Krift et al. 2001; Pausch et al. 2013; Barré et al. 2018) and aboveground plant net primary production (Yuhui et al. 2017; Andresen et al. 2018; Parvin et al. 2019) due to stimulated photosynthesis (Ainsworth and Long 2005; Bishop et al. 2014; Yuhui et al. 2017).
Rhizodeposits fuel microbial life in the vicinity of plant roots and are quickly consumed by microorganisms (Pausch et al. 2013; Bardgett and Van Der Putten 2014; Bardgett et al. 2014). Thereby CO_2-induced changes in plant photosynthesis and carbon allocation patterns strongly impact on heterotrophic microbial communities in the rhizosphere (Drigo et al. 2008). Increased rhizodeposition at enhanced CO_2 concentrations could remove carbon limitation from microorganisms (Drigo et al. 2008; Eisenhauer et al. 2012). Increased microbial biomass build-up might promote activity and abundances of predators (Pritchard 2011) and thereby accelerate microbial turnover under elevated CO_2 concentration (Drigo et al. 2008).

Another indirect effect of elevated CO_2 is not related to carbon availability but to the availability of water. Elevated CO_2 also leads to reduced leaf-level transpiration (Yuhui et al. 2017; Jin et al. 2018) and stomatal conductance (Ainsworth and Long 2005; Terashima et al. 2014; Parvin et al. 2019), which results in increased plant water use efficiency (Wan et al. 2007; Eisenhauer et al. 2012; Madhu and Hatfeld 2013; Yuhui et al. 2017; Andresen et al. 2018; Parvin et al. 2019), increasing the soil moisture content (Eisenhauer et al. 2012; Mueller et al. 2016). This may be specifically important when warming and/or drought occurs, as increased plant water

use efficiency is one mechanisms by which elevated CO_2 may offset the effects of reduced soil water content (Jin et al. 2018; Carrillo et al. 2018; Parvin et al. 2019).
The positive effect of elevated CO_2 concentration on soil moisture was found to be especially pronounced under dry conditions (Bishop et al. 2014). However, a severe drought was found to significantly limit the beneficial effect of elevated atmospheric CO_2 on plants (Obermeier et al. 2017; Jin et al. 2018). It was suggested that if drought gets too severe reduced enzymatic activity and rising respiratory costs of plants will result in a down-regulation of the positive effect of CO_2 fertilization on plants (Yuan et al. 2018).

Several studies have investigated effects of climate change on microbial physiology, specifically, on CUE due to its relevance for the carbon sequestration potential of soils. The great majority of studies looked into the effects of warming on this metric. However, so far, no consensus has been reached in which direction warming affects CUE:
A majority of studies reported a decrease of CUE with warming, which was explained by an increase in costs for maintenance respiration (Manzoni et al. 2012; Sinsabaugh et al. 2013) or by greater temperature sensitivity of respiration relative to growth (Allison et al. 2010; Lehmeier et al. 2016; Fuchslueger et al. 2019). Others found CUE to be invariant to warming, as growth and respiration rates were both consistently accelerated (Hagerty et al. 2014; Walker et al. 2018). And yet others found an increased CUE in the short term, as growth rates exhibited greater temperature sensitivity than respiration rates (Zheng et al. 2019). Such differences in the response of CUE to warming might derive from differences in terminology, methodology and procedures (Sinsabaugh et al. 2013) or from differences in microbial communities, soil types, plant communities or different initial states of the soils under investigation.
Fewer studies examined responses of CUE to other climate change drivers.
CUE was expected to decline under drought, if microorganisms invest resources, which were formerly allocated to growth, into synthesis of protective substances, which would lower biomass build-up, while maintenance respiration stays unaltered

(Manzoni et al. 2012). However, CUE was found to stay constant under drought as both growth and respiration declined (Fuchslueger et al. 2019).

Although, rising temperature, elevated CO_2 concentration and drought events will occur in concert in the future, most experiments focus on the effects of single climate change factors on belowground processes (Castro et al. 2010; Eisenhauer et al. 2012). However, joint occurrence of multiple climate change factors may have interactive effects on soil microbial carbon cycling rather than additive ones (Eisenhauer et al. 2012; Carrillo et al. 2018; Castro et al. 2010). Additionally, we need studies that vary treatment levels, so that possible non-linear effects can be analysed. Thus, studies that combine different climate change treatments at different levels are urgently needed.

We here report on a study that aims at understanding the combined effects of elevated temperatures, elevated CO_2 concentrations and drought on microbial growth and carbon use efficiency, a research that is completely missing so far. In the following manuscript, I will describe and discuss: (1) responses of microbial physiology metrics (growth, respiration and CUE) to various combinations of three climate change drivers and (2) the relevance of seasonality for microbial activity and effects of climate change.

PART 2

MANUSCRIPT

“Seasonal responses of microbial growth and respiration to multiple climate change drivers“

Introduction

Microbial remnants are major constituents of soil organic matter (Simpson et al. 2007; Liang and Balser 2011; Miltner et al. 2012), which contains more organic carbon than the atmosphere and biosphere combined (Lehmann and Kleber 2015). Consequently, the efficiency by which microorganisms allocate the organic carbon taken up to growth (Dijkstra et al. 2011; Sinsabaugh et al. 2013; Hagerty et al. 2018) exerts a strong control over carbon sequestration in soils (Manzoni et al. 2012; Sinsabaugh et al. 2013; Allison 2014; Kallenbach et al. 2015; Geyer et al. 2019). Hence this efficiency, called CUE or microbial carbon use efficiency is included in most carbon cycling models (Dijkstra et al. 2011; Sinsabaugh et al. 2013; Hagerty et al. 2018). The magnitude of CUE is determined by the balance between anabolic and catabolic processes (Manzoni et al. 2012; Hagerty et al. 2018), which depend on the life history strategies of the active members of the microbial communities (Schimel and Schaeffer 2012). It is widely recognized that microbial physiology and community composition are strongly affected by environmental factors such as temperature and soil moisture (Williams and Rice 2007; Manzoni et al. 2011, 2012; Sinsabaugh et al. 2013; Zheng et al. 2019). Besides abiotic conditions, substrate availability plays a crucial role in shaping microbial activity (Hartley et al. 2007; Classen et al. 2015). Thus both environmental variables and substrate availability may affect microbial CUE (Manzoni et al. 2012; Sinsabaugh et al. 2013).

Seasonal variations in environmental factors and plant carbon inputs result in a succession of active microbial communities throughout the year (Blume et al. 2002; Schmidt et al. 2007; Regan et al. 2014; Classen et al. 2015). Depending on the time of the year, different factors may limit microbial activity (Bradford 2013). For example, during the cold seasons temperature is thought to be a major limiting factor, whereas water availability might restrict microbial activity during summer, when temperature constraints are relaxed (Bradford 2013).

It is widely acknowledged that climate change will have pronounced effects on microbial carbon cycling (Manzoni et al. 2012). Rising atmospheric CO_2 concentration, warming and consequently altered precipitation regimes directly and indirectly affect microbial physiology and community composition (Bardgett et al. 2008; Rousk et al. 2012; Classen et al. 2015) by leading to altered plant carbon inputs, soil temperature and moisture (Fig. 1).

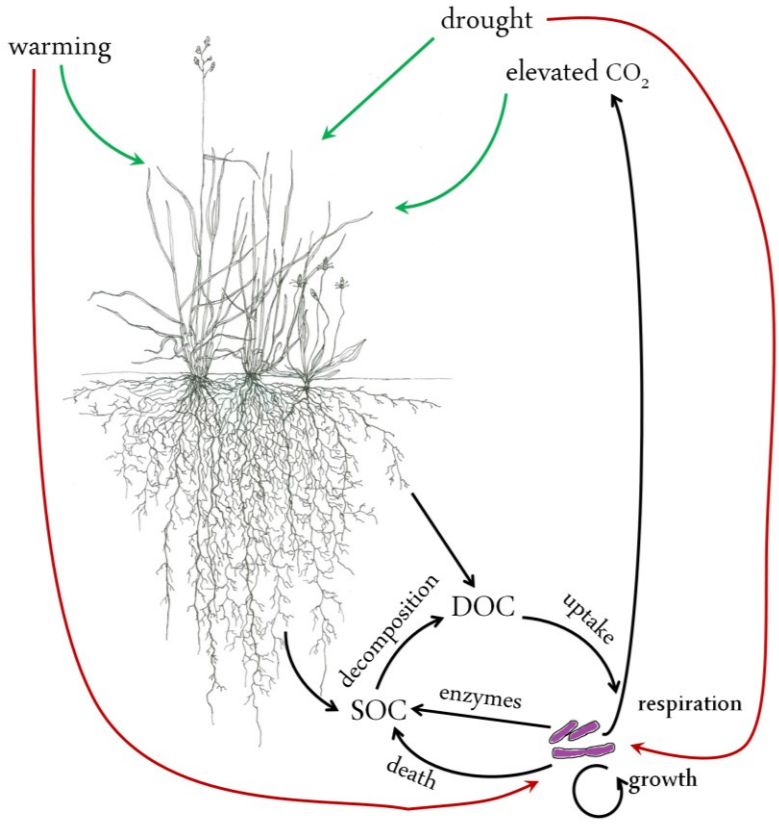

Figure 1 | **Direct and indirect effects of climate change drivers on microbial carbon cycling in soils.** Red arrows indicate direct - and green arrows indicate indirect effects of warming, elevated atmospheric CO_2 concentration and drought on microbial carbon cycling.

A range of studies showed that elevated temperatures have a stimulating effect on microbial activity, as enzymatic reactions are generally temperature sensitive (Davidson and Janssens 2006; Bradford 2013; Classen et al. 2015; Alster et al. 2016a; Moinet et al. 2017). Reaction rates at least increase until a certain temperature, the so-called optimum temperature, at which microbial activity is

declining again (Bárcenas-Moreno et al. 2009; Alster et al. 2016b, a). The thermal optimum of microbial communities however shifts seasonally with changing soil temperature (Fenner et al. 2005) and mostly lies above in-situ soil temperatures (Bárcenas-Moreno et al. 2009; Liu et al. 2018).
Stimulation of microbial activity through warming was found to attenuate over time (Luo et al. 2001; Melillo et al. 2002), which can be either ascribed to thermal adaptation of microbial communities via physiological adjustments or community shifts (Bradford 2013) or to reduced substrate accessibility (Kirschbaum 2004; Eliasson et al. 2005; Davidson and Janssens 2006; Yvon-Durocher et al. 2012).
Additionally, warming may also reduce the soil water content (Sierra et al. 2015), which possibly might mask the inherently positive response of microbial growth and respiration to elevated temperature (Bradford 2013; Schimel 2018), as reduced soil moisture can lead to a disruption of the microorganism-substrate-connecting water film, which might ultimately cause substrate inaccessibility (Davidson & Janssens, 2006; J. P. Schimel, 2018; Wan et al., 2007, Xu et al., 2018).

Drought, similar to temperature, can directly affect microorganisms (Schimel et al. 2007). Even dry soil contains some water, in which microorganisms may live. The water potential of this remaining water is very low, however. To maintain their intracellular water potential and prevent cell damage, microorganisms need to synthesize organic osmolytes, which is costly and might reduce microbial growth (Schimel et al. 2007). Thus, drought may uncouple growth and respiration (Sinsabaugh et al. 2013) as maintenance respiration may be unchanged, but microorganisms may grow less in dry soils compared to soils with sufficient water content (Manzoni et al. 2012).
At the same time, soil drying might result in lower microbial activity due to reduced diffusion of extracellular enzymes and substrates (Schimel et al. 2007; Manzoni et al. 2011; Schimel 2018).

In contrast to warming and drought, elevated atmospheric CO_2 concentration only indirectly affects heterotrophic microbial communities. It was observed, for example,

that elevated CO_2 caused increased soil water availability for microbes by increasing plant water use efficiency (Wan et al. 2007; Eisenhauer et al. 2012; Madhu and Hatfeld 2013; Yuhui et al. 2017; Andresen et al. 2018; Parvin et al. 2019), which could offset water limitation caused by warming and drought. CO_2 enrichment was also found to lead to increased plant biomass production (Bardgett et al. 2008; Yuhui et al. 2017; Andresen et al. 2018; Parvin et al. 2019) and higher plant carbon inputs into soil (Bardgett et al. 2008; Eisenhauer et al. 2012; Nie et al. 2013; Yuhui et al. 2017), which may possibly improve soil carbon availability and may counteract substrate depletion (Tucker et al. 2013).

A multitude of studies also assessed the responses of CUE to warming, and reported seemingly contradictory findings: While some authors found CUE invariant to warming (Hagerty et al. 2014; Walker et al. 2018), others observed declined CUE (Allison et al. 2010; Dijkstra et al. 2011; Manzoni et al. 2012; Sinsabaugh et al. 2013; Tucker et al. 2013; Allison 2014; Lehmeier et al. 2016; Alvarez et al. 2018; Fuchslueger et al. 2019), and yet others reported increased CUE as temperatures raised (Zheng et al. 2019). However, these studies used a range of different approaches to estimate CUE (Manzoni et al. 2012), which may not allow to directly compare them (Sinsabaugh et al. 2013; Geyer et al. 2016; Manzoni et al. 2018).
Studies investigating effects of other climate change factors on CUE are scarce. One could expect reduced CUE due to carbon investment into synthesis of osmolytes under drought (Manzoni et al. 2012). On the other hand, it was reported that a high moisture level reduces CUE due to strong decreases of microbial growth under anoxia (Zheng et al. 2019).

While more and more studies are available that research into the effect of single climate change drivers on microbial physiology, almost no studies exist up to date that look into the combined effect of elevated air temperatures, elevated atmospheric CO_2 concentrations and drought. This is surprising given that we know that multiple climate change drivers will occur in concert and that effects of climate change on

microorganisms may be interactive rather than additive (Bardgett et al. 2008; Castro et al. 2010; Yuhui et al. 2017; Carrillo et al. 2018).

In my master thesis, I intended to answer two main research questions:
(1) How do single or combined climate change drivers affect microbial growth, respiration and carbon use efficiency? and
(2) Are there seasonal difference in the response of microbial communities to climate change?
In order to answer these questions, I made use of a climate change simulation experiment (ClimGrass) in Styria, Austria, in which various combinations of three levels of temperature and three levels of atmospheric CO_2 concentration were applied in a surface response design and in which drought was additionally superimposed on the high CO_2/high temperature treatment and ambient controls. In order to assess relevance of seasonality for microbial activity and for microbial responses to climate change, I sampled soil at three time points throughout the vegetation period (May, July and October) in 2017. In the lab, I determined microbial growth using a novel technique, based on measuring the incorporation of ^{18}O from labelled water into dsDNA. Furthermore, I measured CO_2 production during a 24-hours incubation to ultimately calculate microbial CUE.
I specifically set out to test the following hypotheses:
(1) Elevated temperatures and CO_2 concentrations alone will lead to increased microbial activity, while drought overall will reduce microbial activity. However, combined effects of elevated temperatures and CO_2 concentration will be interactive, rather than additive.
(2) The responses of microbial growth, respiration and carbon use efficiency to the climate change treatments will differ across seasons, as for example, temperature would rather be limiting microbial activity in the cold season (autumn) than in summer and increased plant carbon inputs into soils under elevated CO_2 are expected to occur to a larger extend in spring and summer than in autumn.

Material and Methods

Field site and soil sampling

This study was conducted at a multifactorial climate change experiment, named "ClimGrass", which is located at the AREC (Agricultural Research and Education Centre) Raumberg-Gumpenstein, Austria. This experiment has unique experimental facilities, which allow assessing responses of microbial physiological metrices to multiple climate change factors, either individually or in combination. Specifically, the experiment is set out to study non-linear and non-additive interactions between elevated temperatures and elevated atmospheric CO_2 concentration, which were applied in three levels each. In addition, a severe summer drought is simulated in plots, which were subjected to high CO_2 levels in combination with high temperatures and to ambient controls.

The study site is located in Styria, Austria (49°29´37´´N, 14°06´10´´E), 710 meters above the sea level and was established in a managed sub-montane grassland in 2013. The soil is classified as Cambisol with loamy texture (Deltedesco et al. 2019) with a pH ($CaCl_2$) of 5 in the upper 10 centimetres. The overall goal of the project is to quantify responses of productivity of a managed grassland in a future climate.

The experimental design (the number of replicates per treatment) was based on a response surface regression approach (Piepho et al. 2017). The experiment comprises in total 54 plots (4 x 4 meters each) showing various combinations of three different levels of temperature (ambient, +1.5 °C, +3°C) and atmospheric CO_2 concentration (ambient, +150 ppm, +300 ppm), and is provided with automated rain-out shelters to simulate summer drought (only on specific plots, which are circled in red in Fig. 3). Within this experiment we chose a subset of 34 plots for our experiment (see Fig. 2 and Table 1 for (replicate number of) different treatments).

Plots were sown with a local mixture of seeds for the establishment of permanent grasslands ("Dauerwiese B" of the HBLFA Raumberg-Gumpenstein).

Aboveground biomass was mown and removed three times a year. Plots were regularly, but modestly amended with mineral fertilizer (spring: 30 kg N, 32.5 kg P, 85 kg K, after first harvest: 30 kg N, after second harvest: 30 kg N).

Figure 2 | **Experimental setup**: a miniFACE ring for CO_2 fumigation and infrared heaters fixed on a triangular frame to manipulate air temperature.

Potential future climate scenarios are simulated through the combination of an infrared heating system to regulate temperature and a mini-FACE, a Free Air CO_2 Enrichment system for fumigation with CO_2 (Fig. 2). Treatments were launched in 2014. Infrared heaters are switched on all year round except when snow cover exceeded a height of ten centimetres. CO_2 fumigation is only realized from beginning of April until end of November during daytime. Plots which are not heated or fumigated were equipped with not-functioning heaters and/or miniFACE rings with same shape and size to be able to account for possible disturbances.

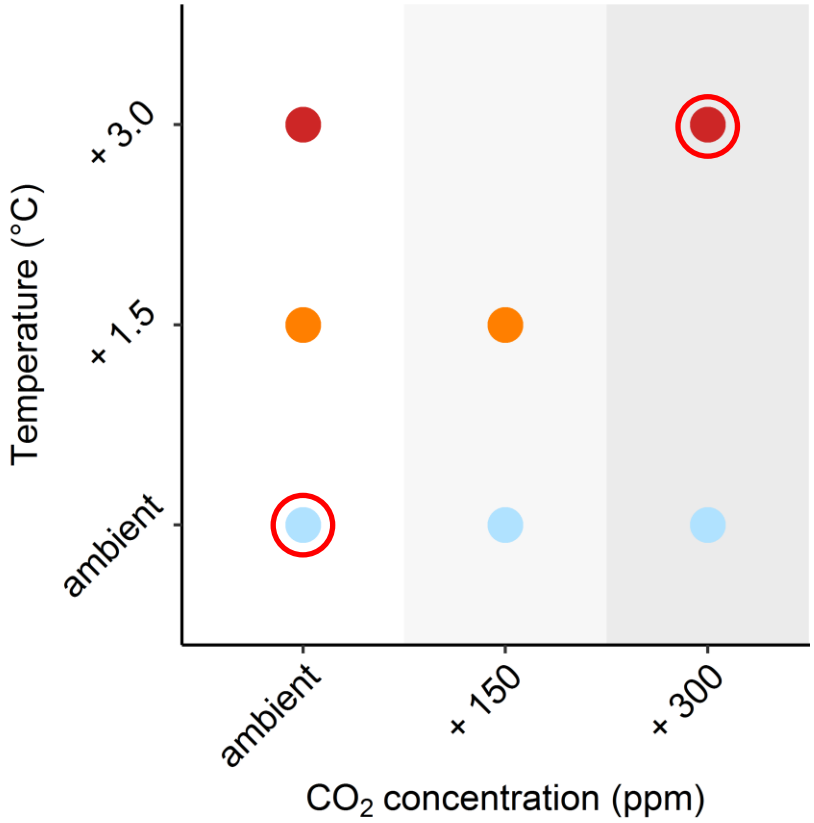

Figure 3 | **Different combinations of three temperature levels:** ambient (blue), +1.5 °C (orange), +3 °C (red); **three CO_2 levels:** ambient (white), +150 ppm (light grey), +300 ppm (dark grey); **and drought** (red circled dots).

Table 1 | **Number of replicates (replicated plots in the surface response design) of the nine selected treatments.**

Treatment	Number of plots
ambient climate	8
ambient CO_2, +1.5°C	3
ambient CO_2, +3°C	3
+150 ppm, ambient temperature	3
+150 ppm, +1.5°C	2
+300 ppm, ambient temperature	3
future climate (+300 ppm, +3°C)	4
ambient climate + drought	4
future climate + drought	4

Table 2 | **Incubation temperatures according to field temperature treatments.**

Temperature treatment	May	July	October
ambient	16	17	13
+1.5 °C	17.5	18.5	14.5
+3 °C	19	20	16

Incubation temperatures were chosen according to field temperatures of the three temperature treatments in May, July and October.

In 2017 automated rain-out shelters were installed above four ambient plots (ambient CO_2 concentration and ambient temperature) and four future climate plots (receiving a combination of +3 °C and +300 ppm above ambient levels) (Fig. 3).
Automatic rain-out shelters were first operated in the fourth year of the study, i.e. for two months in the year 2017, starting from end of May (23rd of May) until end of July (27th of July). During the first month, precipitation was only temporarily blocked in order to start decreasing water levels slowly to avoid too severe soil drying. After the second harvest (July 27th), plots were rewetted simulating 40 mm of rain fall. Following soil rewetting the rain-out shelters were switched off.
We collected soil samples at three time points during the vegetation period in 2017: in early summer (30th and 31st of May), midsummer (25th and 26th of July) and beginning of autumn (3rd and 4th of October). Regarding the drought experiment early summer represented summer drought (midsummer) and autumn the recovery period after rewetting.
Three to eleven soil cores of two centimetres in diameter were taken from the upper ten centimetres of the soil profile in the centre of the 34 plots within one hour after aboveground biomass was harvested. The soil cores were pooled to obtain one mixed sample per plot.
We removed stones, roots and shoots from our soil samples by sieving with two-millimetre mesh size directly after soil cores were taken. The samples, then, were transported to the laboratory of the University of Vienna. All experiments and assays were done at respective field temperatures.

Soil parameters and microbial biomass carbon

Soil water content was determined gravimetrically by weighing 5 grams of fresh soil and drying at 95 °C for 24 hours in a drying oven. This was done once, one day before the start of the incubation experiment in order to calculate, how much ^{18}O-labelled water/DNAse-free water could be added to the soils and a second time on the day of the incubation start to determine the precise soil water content of the incubated samples for further calculation of CUE.

Also, the volumetric soil water content (SWC, Vol%) was recorded at minute interval with soil moisture sensors (SM150T, DeltaT) throughout the vegetation period in a subset of our plots at three and nine centimetres depth. We averaged the two measurements (in 3 and 9 centimetres depth) to represent changes in soil moisture throughout the season.

Soil pH was determined in a 1:5 mix of dry soil and 0.01M $CaCl_2$ solution with a pH meter (SENTRON pH meter).

Microbial biomass carbon (MBC) was measured via the chloroform-fumigation extraction (CFE) method (Vance et al. 1987). Briefly, one subset of samples was directly extracted in 1M KCl (7.5 ml per 1 g of fresh soil), representing the extractable organic carbon (EOC). The other subset was extracted after 24-hours chloroform fumigation in a desiccator (which was started on the same date as the soil incubation with ^{18}O-labelled water). The fumigated samples were extracted in the same way as the un-fumigated samples. Microbial biomass carbon is calculated as the difference between carbon in the fumigated samples minus the carbon in the un-fumigated samples. The extracts were filtered and stored at -20 °C until analysis of total organic carbon (TOC) on a TOC/N Analyzer (TOC- VCPH/CPNTNM-1, Shimadzu, Japan).

Microbial physiology metrics

To understand combined effects of temperature increase and CO_2 enrichment on microbial physiology, we measured microbial growth and respiration and calculated community-level CUE.

To assess growth rates and CUE of the microbial community, we determined the incorporation of ^{18}O into microbial genomic DNA via a substrate-independent method (Spohn et al. 2016). For this, two subsets of 400 mg for each sample were weighed into 1.2 ml-cryovials. The open vial was placed in a headspace vial (27 ml) which then was sealed with a rubber sea and crimp-capped.

One subset of soil samples was amended with ^{18}O-labelled water (Campro Scientific) of various concentrations (at% ^{18}O) with a syringe, in order to reach approximately 25 at% of ^{18}O in the final soil solution and to concurrently maintain

differences in the soil water content of samples. Control samples were amended with the same volume of DNAse-free water instead of the ^{18}O-labelled water. After the amendment, vials were incubated for 24 hours at temperatures (Table 2) corresponding to field temperature at the time of harvest. After the incubation the cryovials containing the soils were closed, shock-frozen in liquid nitrogen and subsequently stored at -80 °C until DNA extraction.

DNA was extracted from the entire soil sample from labelled soil samples and natural abundance controls using a DNA extraction kit (FastDNA™ SPIN Kit for Soil, MO Biomedicals). Extraction was carried out according to the protocol provided by the producer, except that the initial centrifuge step was prolonged to 15 minutes to gain a larger proportion of the cell debris from the supernatant and that the entire matrix containing the DNA was loaded on the SPIN™ filter. DNA extracts were stored at -80 °C and the dsDNA concentration of a 5 µl aliquot of each extract was measured fluorimetrically by the picogreen assay (Quant-iT™ PicoGreen ® dsDNA Reagent, Life Technologies).

To determine isotopic ratio of ^{18}O to ^{16}O of the DNA, 50 µl of the DNA extract were dried in a silver capsule at 60 °C for 24 hours to remove all water. Hereafter, the ^{18}O abundance and total oxygen content were measured using a Thermochemical elemental analyser (TC/EA Thermo Fisher) coupled via a Conflo III open split system (Thermo Fisher) to an Isotope Ratio Mass Spectrometer (IRMS, Delta V Advantage, Thermo Fisher).

To assess microbial respiration rates during the incubation period 5 ml gas samples were taken twice from each headspace vial and were transferred to an evacuated 3 ml exetainer. The first gas sample was obtained directly after amendment of the soil and the other at the end of the incubation. CO_2 concentration and isotopic composition were determined using a Gasbench II (Thermo Fisher) coupled to an Isotope Ratio Mass Spectrometer (IRMS, Delta V Advantage, Thermo Fisher).

Calculations

Microbial biomass carbon (MBC, µg C per g soil dry mass) was calculated as the difference between the organic carbon content (TOC, µg C per g soil dry mass) of

the fumigated (fum) and the non-fumigated (non-fum) samples using an extraction factor of 0.45 (Vance et al. 1987):

$$MBC = \frac{TOC_{fum} - TOC_{non-fum}}{extraction\ factor}$$

Microbial respiration rate (R, µg C per hour per g soil dry mass) was identified as the amount of CO_2 being produced per hour and gram soil dry mass during the 24-hour-lasting incubation.

To determine microbial community growth (G, µg C per hour per g soil dry mass) and carbon use efficiency (CUE), we calculated the amount of DNA, which was produced during the incubation period (DNA_p, µg DNA per hour per g soil dry mass). Based on the DNA concentration and microbial biomass carbon that we determined for each sample, we calculated growth as:

$$G = DNA_p\ x\ \frac{MBC}{DNA}$$

In order to obtain microbial community CUE, we divided microbial growth through total carbon uptake (U, µg C per hour per g soil dry mass), which was calculated as the sum of microbial growth and respiration:

$$CUE = \frac{G}{U} = \frac{G}{G + R}$$

Data analysis and statistics

We conducted all statistical analyses and graphs in R (3.4.2). The significance threshold was set to alpha < 0.05 for all statistical tests. Data was statistically analysed following three complementary approaches:

1) Effects of season, temperature and CO_2 concentration were analysed together through generalised least square models.
2) Effects of temperature and CO_2 were also analysed within each season by using response surface models, in order to reduce the complexity of the model within each season and represent results in a way which is easier to understand.

3) Effects of drought and climate treatment were analysed through a Two-way ANOVA approach, as we used a reduced number of plots within the general experiment.

1) Effects of season, elevated temperature and CO_2 concentration

We performed Generalised Least Square (GLS) models to test for the effect of sampling time point and overall effect of elevated temperature and atmospheric CO_2 concentration on microbial physiology parameters, using the function *gls* of the R package "nlme" (Pinheiro et al. 2019).

We built four different models varying in complexity. As "design" (differentiating between left and right side of the experimental site) did not significantly explain variations of microbial physiology metrics at the single time points according to the RSMs, we included sampling time point, elevated temperature and elevated CO_2 concentration as predictors of microbial growth, respiration and CUE in our GLSs.

Model 1 presented the simplest model by considering sampling time point, elevated temperature and CO_2 level as factors in a linear GLS model, which included all three levels of temperature and CO_2 (ambient, +1.5 °C and +3 °C, ambient, +150 ppm and +300 ppm). Model 2 included a *weights* function (to account for variance heterogeneity between dates). Model 3 fitted a quadratic non-linear response to multiple levels of temperature and CO_2 concentration. Model 4 fitted a quadratic model with an additional *weights* function.

Among the four models, we selected the best-fitting model that minimized the second-order Akaike information criterion (AIC). To account for potential autocorrelation between sampling dates, we integrated different autocorrelation corrections (functions *corCAR1, corAR1, corSymm, corCompSymm*) in our selected model. To inspect for possible autocorrelation presence, we fitted an autocorrelation function (ACF) and inspected the resulting plots. ACF is estimated by calculating the correlation between pairs of log-transformed population densities, between time lags or time delay in the feedback response. The autocorrelation coefficients were then plotted against the lags to give the ACF. ACF reveals periodic patterns more clearly

than the time plot and also provides an objective estimate of the cycle period (Box and Jenkins 1976; Finerty 1980; Nisbet and Gurney 1982; Turchin and Taylor 1992; Davies and Chatfield 2007). When the best fitting model was chosen, we tested for homogeneity of variances (Levene´s test), normality of residuals (histogram, QQ-plot, Shapiro test) and outliers based on Cook´s distance. In the results section we display the output of an *anova* of the chosen model, which displays the significance of explanatory factors.

Microbial biomass-specific growth rate, respiration rate and biomass-specific respiration rate were log-transformed to meet the assumption of normality and homogeneity of variances required by the Generalised Least Square (GLS) model.

2) Effects of temperature and CO_2 within each season

In order to determine, how microbial growth, respiration and community-level CUE were affected by climate change drivers within a specific season, we performed multiple Response Surface Models (RSMs) with increasing complexity, using the function *rsm* of the R package “rsm” (Lenth 2012).

As left and right side of the field site were set up in two different years, we included “design” besides elevated temperature and CO_2 concentration as an additional predictor variable to check for its potential effect on microbial physiology metrics.

Model 1 fitted a linear function to the data. In Model 2 we added a two-way interaction term between CO_2 and temperature. In Model 3 a quadratic term for CO_2 and temperature was included. The *rsm* function automatically generated an ANOVA table to assess the significant contribution for each part added to the model (two-way interaction and quadratic terms).

On the basis of this output, we decided which model to keep. Furthermore, we examined the overall model performance by means of R^2 and p value of the F statistic for the whole model. When the best fitting model was chosen, we tested for homogeneity of variances, normality of residuals and outliers, based on Cook´s distance.

In the results section we display the output of an *anova* of the chosen model, which show the significances of explanatory factors by displaying the t and p values.

3) Effects of drought and climate treatment

To test the effect of drought on microbial physiology in ambient climate and future climate plots (+3°C, +300 ppm) we performed a Two-way ANOVA with climate treatment and drought set as factors using the *aov*. Subsequently, we tested for homogeneity of variances, normality of residuals and potential outliers (also using the Cook's test). If all assumptions were met, we performed a Tukey´s HSD (function *TukeyHSD*) as post-hoc test for each date to check for significant differences between treatments.

Results

In the ClimGrass experiment infrared heaters and miniFACE rings were installed above treatment plots to manipulate air temperature (1.5 °C and 3 °C above ambient temperatures) and atmospheric CO_2 concentration (150 ppm and 300 ppm above ambient concentration) in the plant canopy. Additionally, summer drought was simulated with rain-out shelters, which were closed to exclude precipitation for two months. Warming, CO_2 enrichment and drought all affected soil moisture. Reductions in soil water content in warmed plots were evident throughout the plant vegetation period (see Supplementary Material, Fig. S2a-h). Elevated CO_2 concentration had a minor positive effect on soil moisture. Exclusion of precipitation through rain-out shelters strongly reduced soil water content irrespective of climate treatment (current or future climate conditions) (see Supplementary Material, Fig. S3c-e).

To gain a better understanding on how multiple climate change drivers affect microbial physiology, we assessed biomass-specific growth and respiration rates as well as CUE of microbial communities in soil exposed to various combinations of three temperature and three CO_2 levels at three time points throughout the plant growing season (May, July, and October).

Elevated temperature significantly affected biomass-specific growth (G_m, mgC h^{-1} g^{-1} MBC, Fig. 4a-c) and biomass-specific respiration rates (R_m, mgC h^{-1} g^{-1} MBC, Fig. 4d-f) at all time points (May, July and October).

In May, elevated temperature enhanced biomass-specific growth (Fig. 4a, Table 3) and biomass-specific respiration (Fig. 4d, Table 3) under elevated CO_2 concentration. Growth was relatively reduced in warmed plots (+1.5 °C) compared to ambient temperature at intermediate elevated CO_2 level (+150 ppm). In contrast, the microbial community grew faster in the three degrees warmed plots in comparison to ambient temperature at the highest CO_2 concentration (+300 ppm). Biomass-specific

respiration was also increased in warmed plots at both CO_2 levels enrichments (Fig. 4d, Table 3).

We observed lower biomass-specific growth (Fig. 4b) and respiration (Fig. 4e) in warmed plots in July, which was also evident from the output of the response surface models (RSMs) (Table 3). Reductions occurred irrespective of atmospheric CO_2 level (Table 3).

Overall, metabolic rates were lowest in October compared to the other two sampling dates (Fig. 4). Responses of biomass-specific growth and respiration to warming were reversed in October compared to July (Fig. 4c and f). Microbial communities respired more (Fig. 4f, Table 3) and grew more in warmed field plots compared to ambient temperature plots (Fig. 4c, Table 3).

CO_2 fumigation did not have an influence on the magnitude of microbial growth, respiration or CUE in autumn (Table 3).

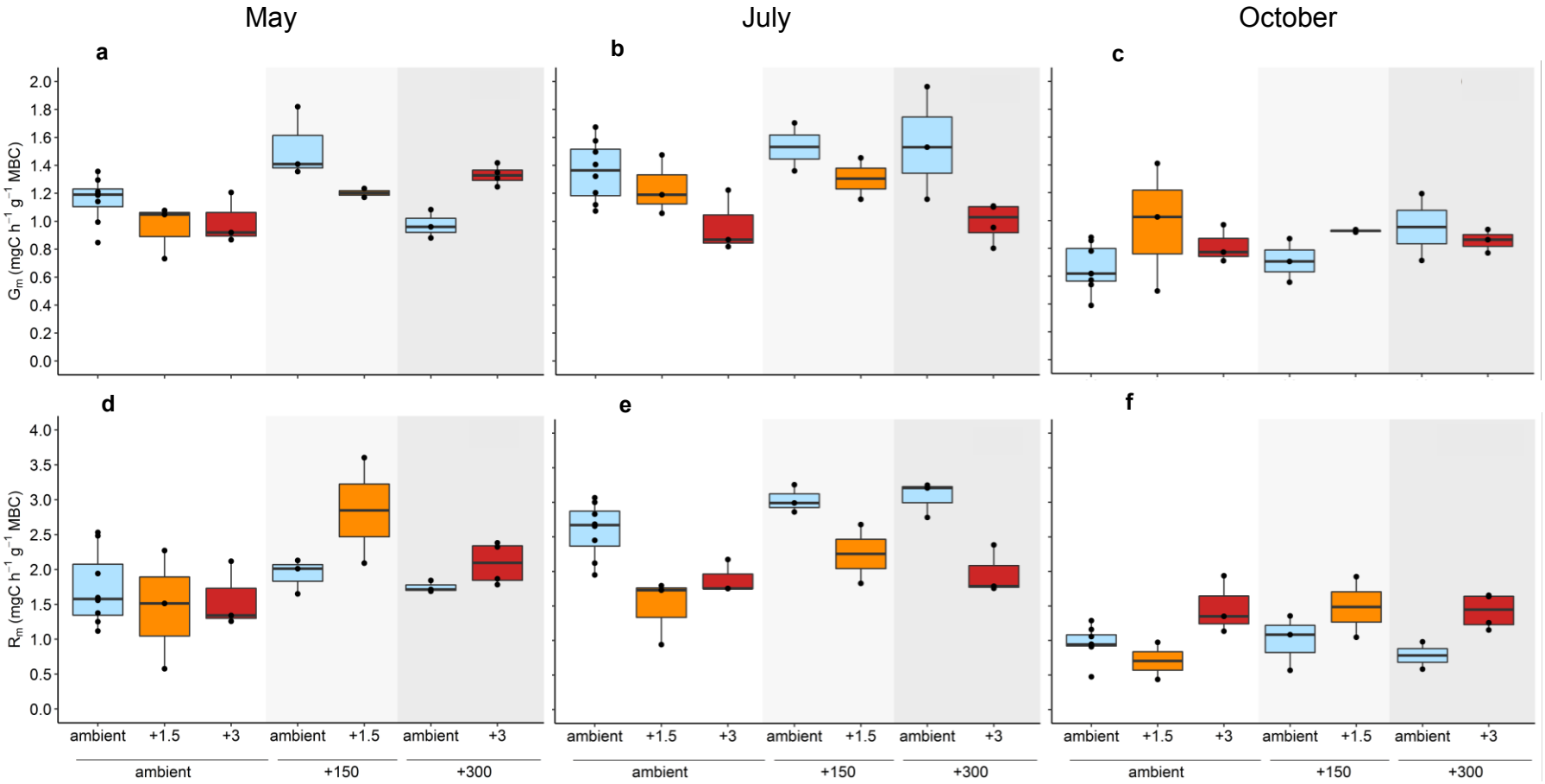

Figure 4 | **Responses of microbial biomass-specific growth and respiration to elevated temperature and CO_2 concentration. a-i,** Microbial biomass-specific growth (G_m, mgC h^{-1} g^{-1} MBC) in May **(a)**, July **(b)** and October **(c)** and microbial biomass-specific respiration (R_m, mgC h^{-1} g^{-1} MBC) in May **(d)**, July **(e)** and October **(f)** under various combinations of three temperature - and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C warmed (+1.5, orange), 3 °C above ambient air temperature (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm CO_2 above ambient level (+150, light grey), additional 300 ppm CO_2 (+300, dark grey). MBC = microbial biomass carbon. For details see Material and Methods.

Table 3 | **Effect of climate change drivers on microbial biomass-specific growth (G_m), biomass-specific respiration (R_m) and carbon use efficiency (CUE).**

	May		July		October	
G_m	*t*	*p*	*t*	*p*	*t*	*p*
eCO_2	**2.35**	**0.0294**	1.01	0.3229	0.15	0.8791
eT	**-3.04**	**0.0066**	**-4.26**	**<0.001**	**2.77**	**0.012**
eCO_2^2	**-2.61**	**0.0171**				
eT^2	**2.53**	**0.0202**				
eCO_2:eT	**2.81**	**0.0109**				
R_m						
eCO_2	1.16	0.1566	**2.51**	**0.0200**	0.16	0.8737
eT	0.45	0.6566	**-4.87**	**<0.001**	**3.05**	**0.0062**
eCO_2^2						
eT^2						
eCO_2:eT						
CUE						
eCO_2	-0.68	0.5034	-0.41	0.685	0.444	0.6612
eT	-0.23	0.8155	**3.07**	**0.0068**	-0.45	0.652
eCO_2^2			0.29	0.7715		
eT^2			**-3.01**	**0.0077**		
eCO_2:eT			-0.0007	0.9994		

Significances of elevated atmospheric CO_2 level (eCO_2) and increased air temperature (eT) as predictors of biomass-specific growth rate (G_m, mgC h^{-1} g^{-1} MBC), biomass-specific respiration rate (R_m, mgC h^{-1} g^{-1} MBC) and carbon use efficiency (CUE) at each sampling time point (May, July and October). Values are derived from RSM models. eCO_2^2 & eT^2 – quadratic function of elevated CO_2 and temperature, eCO_2:T interaction of elevated CO_2 concentration and temperature. p-values < 0.05 are given in bold.

In those plots, where a future climate was simulated (+ 3 °C, +300 ppm above ambient climate conditions) summer drought significantly increased biomass-specific growth and respiration rates (Fig. 5a and b, Table 4). In contrast, biomass-specific growth was slightly decreased in ambient plots (ambient temperature, ambient CO_2 concentration) under drought (Fig. 5a). Microbial respiration was equally high in un-manipulated precipitation and drought-exposed plots under ambient climate conditions (Fig. 5b).

Two months after rewetting we found equally high biomass-specific growth and respiration rates in previously drought-exposed plots and their controls (Fig. 6a and b, Table 4).

Table 4 | **Biomass-specific growth (G_m), biomass-specific respiration (R_m) and microbial carbon use efficiency (CUE) during a summer drought and after a 2-month rewetting period.**

	G_m		R_m		CUE	
	F	*p*	*F*	*p*	*F*	*p*
Drought						
(eCO_2 + eT)	1.44	0.2463	**9.67**	**0.007159**	0.97	0.3402
drought	2.09	0.1669	**18.3**	**<0.0001**	2.32	0.147
(eCO_2 + eT): drought	**27.94**	**<0.0001**	**32.64**	**<0.0001**	1.68	0.2135
Rewetting						
(eCO_2 + eT)	**4.81**	**0.04453**	**38.05**	**<0.0001**	1.72	0.20852
drought	0.03	0.86084	0.86	0.36667	0.16	0.69495
(eCO_2 + eT): drought	0.06	0.80762	**6.3**	**0.02315**	0.89	0.06619

Statistical significances of drought, climate change treatments (eCO_2 + eT) and their interaction ((eCO_2 + eT): drought) during drought and after a 2-month rewetting period as explanatory variables of biomass-specific growth rate (G_m, mgC h^{-1} g^{-1} MBC), biomass-specific respiration rate (R_m, mgC h^{-1} g^{-1} MBC) and carbon use efficiency (CUE). Values are derived from Two-way ANOVAs for each sampling date. p-values < 0.05 are given in bold.

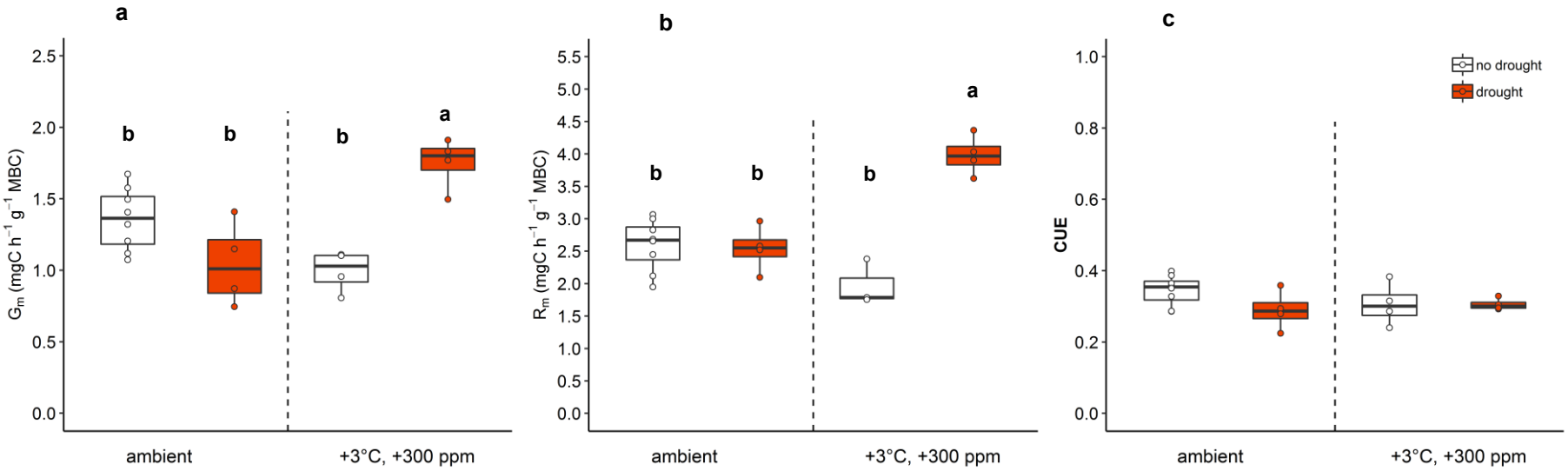

Figure 5 | **Microbial responses to summer drought. a-c,** Microbial biomass-specific growth rates (G_m, mgC h^{-1} g^{-1} MBC) **(a)**, biomass-specific respiration rates (R_m, mgC h^{-1} g^{-1} MBC) **(b)** and microbial carbon use efficiency (CUE) **(c)** under ambient precipitation (white) or under rain exclusion (drought, red) at ambient - (ambient) or future climate conditions (+3 °C, +300 ppm). Letters above box-whiskers indicate significant differences between groups ($p < 0.05$, Tukey's HSD test). MBC = microbial biomass carbon.

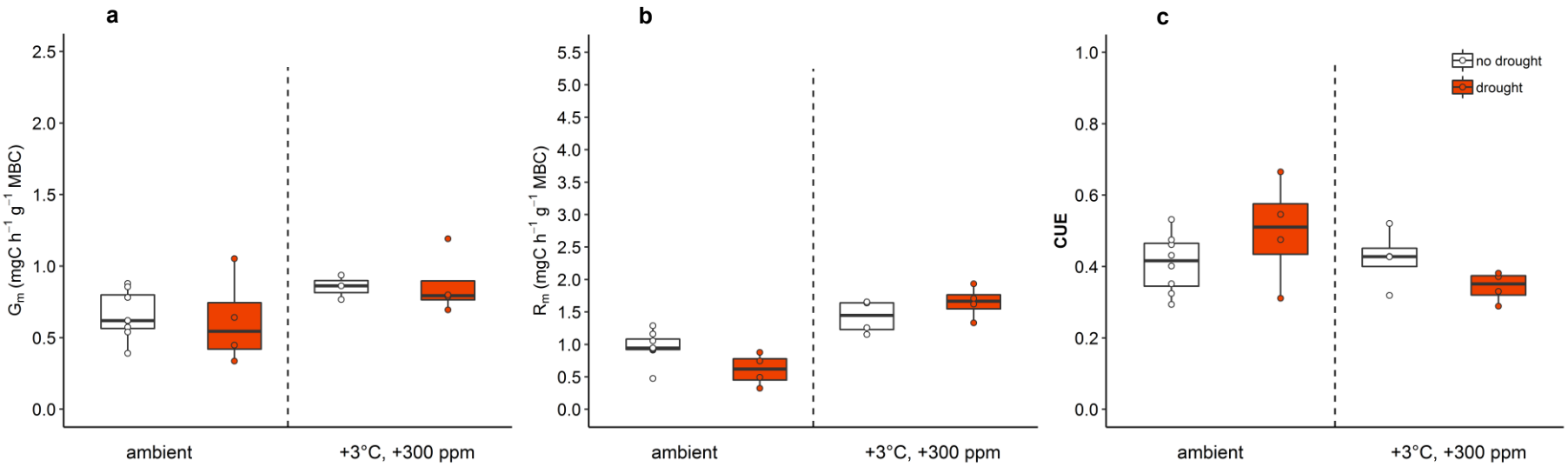

Figure 6 | **Recovery of microbial physiology metrics from drought after a 2-month rewetting period. a-c,** Microbial biomass-specific growth rate (G_m, mgC h^{-1} g^{-1} MBC) **(a)**, microbial biomass-specific respiration (R_m, mgC h^{-1} g^{-1} MBC) **(b)** and microbial carbon use efficiency (CUE) **(c)** in former to drought-exposed plots (drought) and ambient precipitation plots (white) at ambient - (ambient) or future climate conditions (+3 °C, +300 ppm) after a 2-month rewetting period of. MBC = microbial biomass carbon.

Microbial community-level carbon use efficiency (CUE) did not significantly respond to combined or single elevated temperature and CO_2 level in May and October (Fig. 7a and c, Table 3). In July, we found the highest proportional carbon allocation to growth relative to total uptake at intermediate temperature increase (+ 1.5 °C) (Fig. 7b). Over the three sampling time points, CUE ranged between 0.26 and 0.59 and was not significantly different between sampling time points. A summer drought over two months also did not alter CUE (Fig. 5c, Table 4).

Microbial biomass carbon (MBC, µgC g^{-1} DM) was neither significantly altered through single or combined warming and elevated CO_2 levels in any season (Fig. 8a-c, Table 5) nor affected by summer drought (Fig. 9a, Table 6).
Consequently, growth and respiration rates per gram soil (see Supplementary Material, Fig. S1a-f) approximately followed the patterns of the intrinsic microbial rates (calculated per gram microbial biomass carbon). Over all seasons, microbial biomass did not vary drastically (Fig. 8a-c, Table 5).

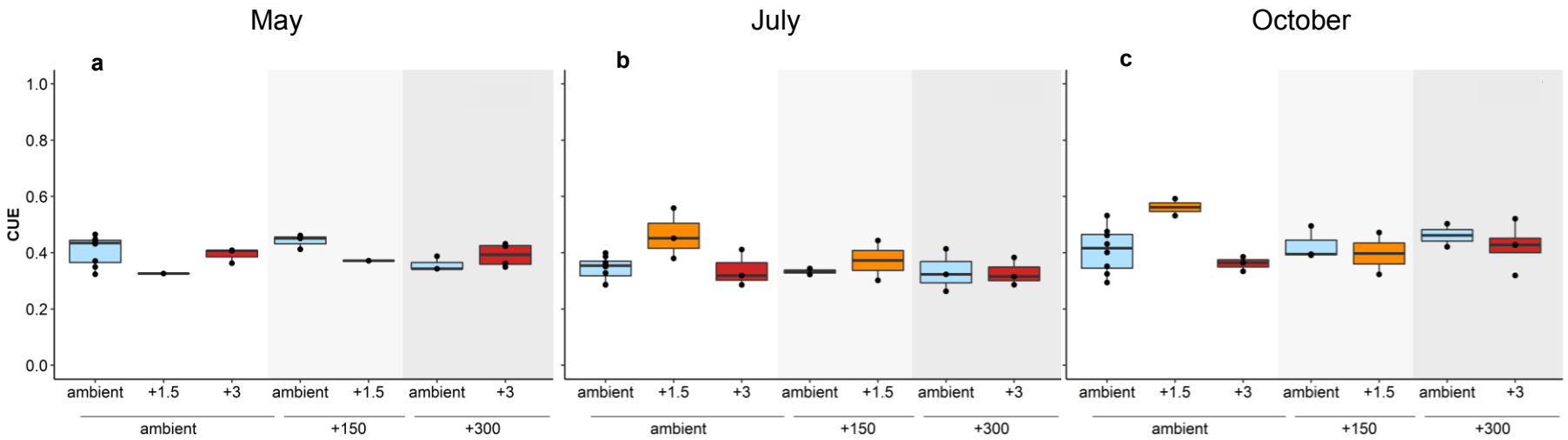

Figure 7 | **Community-level carbon use efficiency under climate change. a-c,** Carbon use efficiency (CUE) in May **(a)**, July **(b)** and October **(c)** under various combinations of three temperature - and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C above ambient air temperature (+1.5, orange), 3 °C above ambient air temperature (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm above ambient levels (+150, light grey), additional 300 ppm (+300, dark grey).

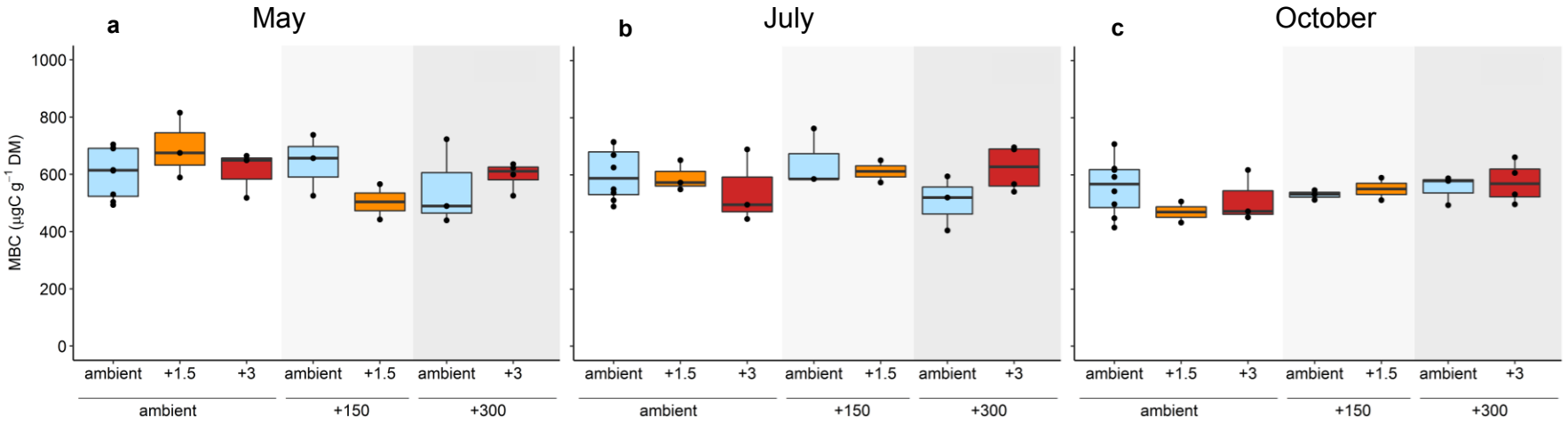

Figure 8 | **Constant microbial biomass across different climate change treatments. a-c,** Microbial biomass carbon per gram dry soil (MBC, µgC g^{-1} DM) at the three sampling dates: May **(a)**, July **(b)** and October **(c)** under various combinations of three temperature and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C warmed (+1.5, orange), 3 °C above ambient air temperature heated (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm above current levels (+150, light grey), additional 300 ppm (+300, dark grey). DM = dry mass.

Table 5 | **No significant effects of climate change treatments on microbial biomass carbon throughout the vegetation period.**

	May		**July**		**October**	
MBC	*t*	*p*	*t*	*p*	*t*	*p*
eCO_2	-1.16	0.2561	-0.13	0.8909	0.93	0.3623
eT	0.27	0.7853	0.13	0.8938	-0.41	0.6843
eCO_2^2						
eT^2						
eCO_2:eT						

Significances of elevated atmospheric CO_2 level (eCO_2) and increased air temperature (eT) as predictors of microbial biomass carbon (MBC, µgC g^{-1} DM) at each sampling time point (May, July and October). Values are derived from RSM models. eCO_2^2 & eT^2 – quadratic function of elevated CO_2 and temperature. eCO_2:T interaction of elevated CO_2 concentration and temperature.

Table 6 | **Microbial biomass carbon during drought and after a 2-month rewetting period.**

	MBC	
	F	*p*
Drought		
(eCO_2 + eT)	3.47	0.081
drought	3.42	0.0831
(eCO_2 + eT): drought	1.19	0.2907
Rewetting		
(eCO_2 + eT)	0.09	0.7586
drought	0.74	0.4026
(eCO_2 + eT): drought	0.0007	0.9799

Statistical significances of drought (drought) and climate change treatment (eCO_2 + eT), respectively significances of legacy effect of former drought plots (drought) and future climate plots (eCO_2 + eT) as explanatory variables of microbial biomass carbon (MBC, µgC g^{-1} DM) after a 2-month rewetting period. Values are derived from a Two-way ANOVA. (eCO_2 + eT): drought interaction between climate treatment and drought.

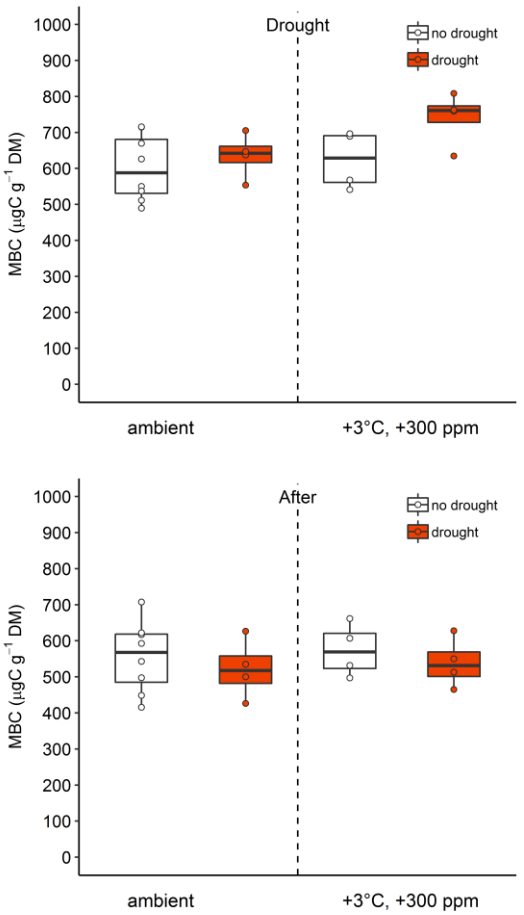

Figure 9 | **Microbial biomass during drought and after a 2-month rewetting period. a+b**, Microbial biomass carbon (MBC, µgC g^{-1} DM) in drought (red) and non-drought (white) plots **(a)**, microbial biomass (MBC, µgC g^{-1} DM) in former drought (red) and in former non-drought (white) plots after a 2-month rewetting period **(b)**, under ambient and future climate conditions (+3 °C, +300 ppm). DM = dry mass.

In order to assess the temporal dynamics of abiotic variables and plant carbon inputs on microbial physiology, we sampled soils of all treatments after the three biomass harvests in this hay meadow. The samplings represent distinct parts of the plant growing season (i.e., early summer, midsummer, autumn). Subsequently, we constructed Generalized Least Square (GLS) models to assess, how well season and climate change drivers can explain patterns of microbial physiology metrics.

Table 7 | **Seasonality as a driver of microbial physiology.**

	MBC		**G_m**		**R_m**		**CUE**	
	F	*p*	*F*	*F*	*F*	*p*	*F*	*p*
date	**4.17**	**0.045**	**15.44**	**0.0002**	**20.17**	**<0.0001**	1.51	0.2224
eCO_2	0.36	0.549	**4.5**	**0.0376**	3.2	0.073	0.37	0.5414
eT	0.03	0.8506	1.28	0.2618	0.76	0.5141	0.5	0.4792
eCO_2^2	0.12	0.7246			2.89	0.703	0.2	0.6494
eT^2	0.01	0.8881			2.65	0.0807	0.18	0.6673
date:eCO_2	1.14	0.2889	0.09	0.7553	0.26	0.6422	0.12	0.7237
date:eT	0.07	0.7795	0.04	0.8411	2.18	0.2545	0.03	0.8579
eCO_2:eT	2.25	0.1376	1.83	0.1803	0.77	0.1012	0.4	0.5269
date:eCO_2: eT	0.1	0.7426	**5.25**	**0.025**	0.05	0.5396	0.43	0.514

Statistical significances of the effect of seasonality (date), elevated CO_2 level (eCO_2) and increased temperature (eT) on microbial biomass carbon (MBC, µgC g^{-1} DM), biomass-specific growth rate (G_m, mgC h^{-1} g^{-1} MBC), microbial biomass-specific respiration (R_m, mgC h^{-1} g^{-1} MBC) and microbial carbon use efficiency (CUE). Values are derived from GLS models. eCO_2^2 & eT^2 quadratic functions, : indicate interaction of two or three predictors. p-value < 0.05 are given in bold.

The GLS models elucidated sampling date as the most significant explanatory factor of changes in microbial growth and respiration rates across the seasons (Table 7). Furthermore, elevated CO_2 levels explained some of the variation in physiological rates across seasons (Table 7).

Discussion

It is widely assumed that climate change critically affects microbial communities and biogeochemical processes in soils. While much is known about how each climate change driver is affecting soils, our knowledge on how elevated temperature, elevated atmospheric CO_2 concentration and altered precipitation regime in concert will alter soil carbon cycling is still inconclusive. Therefore, we conducted a field experiment, where we not only manipulated temperature, but also atmospheric CO_2 concentration and soil moisture. As it is not well understood if effects of elevated temperature and CO_2 concentration are interactive or additive, we exposed field plots to various combinations of three temperature levels and three CO_2 levels. Because we, additionally, were interested on how summer drought affects microbial growth, respiration and CUE under elevated temperature and enhanced CO_2 level, we excluded rain from some ambient plots and plots that were heated and received CO_2 fumigation during summer. We assumed that season-specific temperature, soil water content and carbon inputs would play an important role in determining microbial activity and, therefore, studied effects of single and combined climate change drivers in three different seasons during the vegetation period.

Warming had most pronounced, but season-specific effects on microbial physiology. Compared to warming, elevated CO_2 concentration, which is believed to impact microbial communities mainly via its effects on plant productivity, had only minor effects on microbial growth and respiration rate. Drought led to a pronounced acceleration of microbial physiology, but only under elevated temperature and enriched CO_2. Seasonal dynamics in soil temperature, water content and carbon availability seemed to have strongly impacted microbial activity and to have modulated microbial responses to climate change drivers.

In our study, microbial communities had not acclimated to warming, as biomass-specific respiration and growth were still accelerated in October after four years of

warming (Fig. 4c and f). Observed absence of thermal adaptation corresponded to findings of other studies (Marañón-Jiménez et al. 2018; Walker et al. 2018).

Enhanced biomass-specific growth and respiration rates in October (Table 3), reflected the widely recognized temperature sensitivity of chemical and biological processes (Davidson and Janssens 2006; Bradford 2013; Alster et al. 2016a). Our observations confirmed the findings of many studies, which found a positive relationship between elevated temperature and microbial activity (Blume et al. 2002; Classen et al. 2015; Siles et al. 2016), as well as heterotrophic respiration rate (Melillo et al. 2002; Bradford 2013; DeAngelis et al. 2015; Fuchslueger et al. 2019).

Low temperatures in autumn might have been the primary limiting factor of microbial activity. An increase of 1.5 degrees or 3 degrees respectively might have posed a substantial rise in temperature in autumn, whereas the same magnitude might have weighed comparatively little in summer, during which temperatures are highest and temperature limitation of microbial activity is not expected. We found support for temperature limitation of microbial activity in autumn as biomass-specific growth and respiration rates were overall lowest in October (Fig. 4a-f). Our findings were consistent with earlier studies, which observed lower microbial activity at low temperatures (Blume et al. 2002) and argued that temperature dominates enzyme activity at cold conditions (Liu et al. 2018). It was argued that with rising temperatures microorganisms get closer to the optimal temperature of their enzymes, where reaction rate is greatest (Alster et al. 2016a) and consequently, exhibit higher metabolic rates (Liu et al. 2018).

Observed enhanced growth and respiration rates per gram soil (see Supplementary Material, Fig. S1c and f) were driven by the inherent temperature sensitivity of biomass-specific growth and respiration rate as microbial biomass was equally high across different temperature treatments (Fig. 8c, Table 5).

As we dealt with soil samples from a sufficiently fertilized and thus productive grassland, microbial communities most likely did not experience substrate depletion in warmed plots at any time of the year. Consequently, the amount of microbial biomass was not changed under climate warming, which contrasted to what Walker et al. (2018) observed as a result of substrate depletion. Equally high microbial

biomass across treatments, although biomass-specific growth was accelerated (Fig. 4c, Table 3) might be explained by accelerated microbial turnover under warming as was argued by others (Hagerty et al. 2014).

Warming-mediated acceleration of microbial growth and respiration rates might have been supported by enhanced root-derived substrate supply. Rhizodeposition might have been increased due to dying of roots as a result of senescence (Pausch et al. 2013) or due to accelerated root turnover, which might have resulted from enhanced maintenance costs of roots or increased densities of pathogens and herbivores under warming (Gill and Jackson 2000; Wang et al. 2019).

While warming stimulated microbial activity in autumn (October) (Fig. 4c and f), biomass-specific growth and respiration decreased under elevated temperatures in midsummer (July) and microbial responses to warming depended on atmospheric CO_2 level in early summer (May).

The positive effect of elevated temperature on growth and respiration in October was reversed in July. Irrespective of atmospheric CO_2 concentration, microorganisms grew and respired less in warmed plots in midsummer (July) (Fig. 4b and e, Table 3), which stands in contrast with a range of previous findings (Hagerty et al. 2014; Walker et al. 2018). The negative response of microbial activity to warming suggests that other environmental factors might have masked the inherent temperature sensitivity of microbial activity. Growth and respiration rates per gram soil also decreased (see Supplementary Material, Table S1) as microbial biomass was not affected by climate treatment (Fig. 8b, Table 5).

Low soil moisture, specifically in warmed plots during midsummer, might have been the leading cause of decreased biomass-specific growth and respiration and have dampened the positive temperature response. We observed decreased volumetric soil water content in heated plots at all three sampling time points (see Supplementary Material, Fig. S2a-h). Reduction of soil water content in midsummer, where soils were overall driest, might have caused inaccessibility of substrates as the water film within the pore space, which allows for enzyme and substrate diffusion, may have been disrupted as was suggested by Schimel (2018). Other

studies also argued that apparent absence or a negative response of microbial activity to warming was derived from low soil water content (Rustad et al. 2001; Schwarz et al. 2017) and consequent low substrate availability (Hartley et al. 2007). Factors as soil moisture and substrate availability were suggested to become limiting if temperature limitation of enzymatic activity is removed (Rustad et al. 2001; Davidson and Janssens 2006; Schwarz et al. 2017; Liu et al. 2018).
While reduced connectivity may have restricted the accessibility of soil carbon for microbes, soil carbon inputs by plants may have also been reduced in midsummer.

Unlike the situation in July, insufficient plant carbon inputs rather than low soil moisture might have limited the positive response of microbial activity to warming in May. Initial acceleration of microbial activity under warming might have caused insufficient substrate availability. This was suggested as biomass-specific growth was relatively lower in warmed plots compared to ambient temperature plots at ambient CO_2 concentration and intermediate CO_2 enrichment. Only at highest CO_2 enrichment microbial communities grew faster in warmed plots (Fig. 4a, Table 3), possibly because only at the highest CO_2 concentration plants deposited sufficient amounts of substrates. In contrast to growth, substrate availability seemed sufficient at both CO_2 enrichments to support higher respiration in warmed plots (Fig. 4d, Table 3). The interdependence between the effect of warming and atmospheric CO_2 level was already observed by others (Carrillo et al. 2018) and it was argued that plant carbon inputs into soil exert a considerable control over the temperature sensitivity of microbial decomposition (Classen et al. 2015). Accelerated microbial activity in warmed, fumigated plots might have resulted from greater rhizodeposition due to the stimulating effect of elevated CO_2 on plant productivity or from slightly increased soil moisture due to improved water use efficiency of plants under elevated CO_2.
The co-occurrence of relatively lower growth in warmed compared to ambient plots at 150 ppm CO_2 (Fig. 4a) with relatively lower below- (see Supplementary Material, Fig. S5a) and aboveground plant net primary production during the peak-growing season (see Supplementary Material, Fig. S4a) gave further indication that varying

plant carbon inputs might have shaped the response of microbial communities to warming in May.

Equal below- and aboveground plant primary production across climate treatments in midsummer and autumn might indicate that rhizodeposition were not altered by CO_2 fumigation. Thus, unaltered amount of rhizodeposits might explain the lack of an effect of elevated CO_2 on biomass-specific growth and respiration in July and October.

When a summer drought was imposed to future climate plots (+3 °C, +300 ppm), we observed pronouncedly enhanced microbial biomass-specific growth and respiration rates (Fig. 5a and b). Although, ambient plots fell equally dry after rain exclusion over two months (see Supplementary Material, Fig. S3e) as plots that have been subjected to drought and elevated temperature and CO_2 level, microbial activity was equally low in drought-exposed and ambient precipitation plots (Fig. 5a and b). The discrepancy between the effects of drought on microbial physiology is displayed by the significant interaction term (Table 4).

Increased biomass-specific growth and respiration under drought in warmed plots might have resulted from shifts of microbial community composition towards an active community, which could deal better with reduced soil water content as was deduced by another study (Adair et al. 2019).

Alternatively, water addition in the course of the microbial growth measurement might have made formerly spatially inaccessible substrates available to microorganisms as pore connectivity was re-established. Substrate availability might have been higher in drought-exposed plots than in plots, where rain was not excluded, as root exudates might have accumulated during drought due to low microbial uptake. Former studies observed accumulation of root exudates during dry periods as exudates were consistently released, while substrate uptake by microorganisms was impaired (Karlowsky et al. 2018). As water was added microorganisms might have grown and respired relatively more in warmed plots than in ambient climate plots (Fig. 5a and b) due to the stimulating effect of elevated temperatures on microbial activity.

In the long run, drought did not seem to cause legacy effects on growth and respiration as two months after rewetting microbial communities showed similar biomass-specific growth and respiration rates in formerly to drought exposed plots and their controls (Fig. 6a and b, Table 4). This confirms past studies, which found that microbial activities no longer varied between different precipitation treatments within days after rewetting (Karlowsky et al. 2018).

While microbial growth and respiration both changed in response to warming in all investigated seasons (Fig. 4a-f), we found microbial CUE, i.e. the allocation of carbon to growth, insensitive to warming (Fig. 7a-c, Table 3). This observation, albeit somewhat in contrast with theoretical considerations (see Manzoni et al., 2012), is consistent with several recent studies (Hagerty et al. 2014; Walker et al. 2018). However, the positive effect of warming on microbial CUE at 1.5 °C warming in midsummer (Fig. 7b) and autumn (Fig. 7c) highlights the importance of multilevel climate change experiments that allow addressing questions to non-linear responses to climate change factors. Apart from warming, neither elevated CO_2 nor drought led to significant changes in microbial CUE at the community-level (Table 3, Table 4).

Examining growth and respiration over all sampling time points, season was the best predictor of variations in microbial growth and respiration rate (Table 7, see Supplementary Material, Table S2). Microbial communities responded differentially to climate change drivers across seasons, which suggested that the main limiting factor of microbial activity was season-specific. Other studies as well found that time had a pronounced effect on microbial physiology (Wan et al. 2007) and that treatment effects varied across seasons (Williams and Rice 2007).

We may conclude that season-specific alterations in temperature, precipitation and plant carbon inputs or changing microbial community composition seemed to have evocated bigger effects on microbial activity than climate change treatments themselves. To which climate change drivers and how microbial growth and respiration responded depended on sampling time point. Besides warming, which by

directly stimulating microbial activity and by indirectly resulting in decreased soil water content had the most pronounced effects on microbial physiology across all sampling time points, substrate availability seemed to have posed a key factor controlling microbial activity. However, community-level CUE was not altered by elevated temperature, CO_2 concentration and summer drought and was identified as a robust parameter.

Season-specific effects of elevated temperature and CO_2 level on microbial growth and respiration highlight the need to assess physiological responses to climate change in different seasons. Additionally, interactive effects of elevated temperature and CO_2 concentration on microbial growth and respiration underpin the importance of conducting further multifactorial climate change simulation experiments with different levels of climate change factors to gain a comprehensive understanding, on how climate change will alter microbial carbon cycling.

Supplementary Material

Table S1 | **Effects of climate change drivers on microbial growth and respiration throughout the vegetation period.**

	May		July		October	
G	*t*	*p*	*t*	*p*	*t*	*p*
eCO_2	1.11	0.2798	0.28	0.7803	1.08	0.2896
eT	-1.61	0.1237	**-2.565**	**0.01804**	1.71	0.1011
eCO_2^2	-1.33	0.1983				
eT^2	1.36	0.189				
eCO_2:eT	1.39	0.179				
R						
eCO_2	0.94	0.3573	1.95	0.6652	0.36	0.7222
eT	0.43	0.666	**-3.27**	**0.0041**	**2.66**	**0.0149**
eCO_2^2			-1.87	0.0764		
eT^2			**2.34**	**0.0309**		
eCO_2:eT			0.53	0.5987		

Significances of elevated atmospheric CO_2 level (eCO_2) and increased air temperature (eT) as predictors of growth rate per gram soil (G, µgC h^{-1} g^{-1} DM) and respiration rate per gram soil (R, µgC h^{-1} g^{-1} DM) at each sampling time point (May, July and October). Values are derived from RSM models. eCO_2^2 & eT^2 quadratic function of elevated CO_2 and temperature, eCO_2:T interaction of elevated CO_2 concentration and temperature. p-value < 0.05 are given in bold.

Table S2 | **Effects of treatment and seasonality on microbial growth and respiration.**

	G		R	
	F	*p*	*F*	*F*
date	**19.02**	**<0.0001**	**46.74**	**<0.0001**
eCO_2	0.92	0.3402	3.32	0.073
eT	0.04	0.8306	0.43	0.5141
eCO_2^2	0.51	0.4742	0.14	0.703
eT^2	1.85	0.1773	3.14	0.0807
date:eCO_2	1.29	0.2588	0.21	0.6422
date:eT	0.36	0.5503	1.32	0.2545
eCO_2:eT	**19.02**	**<0.0001**	2.76	0.1012
date:eCO_2:eT	0.92	0.3402	0.38	0.5396

Statistical significances of the effect of seasonality (date), elevated CO_2 level (eCO_2) and increased temperature (eT) on growth rate per gram soil (G, µgC h^{-1} g^{-1} DM) and respiration rate per gram soil (R, µgC h^{-1} g^{-1} DM). Values are derived from GLS models. eCO_2^2 & eT^2 quadratic functions, : indicate interaction of two or three predictors. p-value < 0.05 are given in bold.

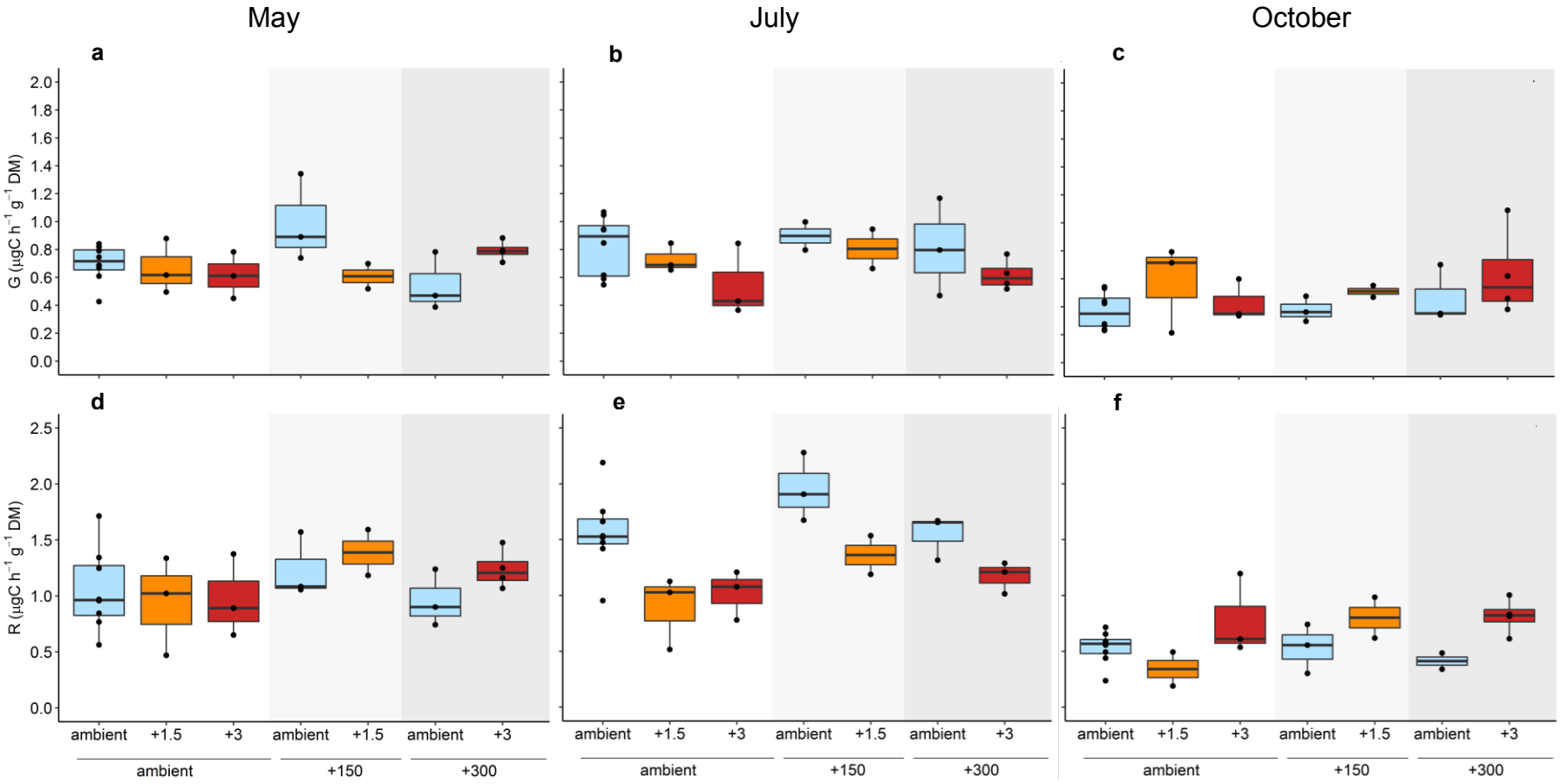

Figure S1 | **Response of microbial growth and respiration to single and combined elevated temperatures and CO_2 concentrations. a-f,** Growth per gram soil (G, µgC h^{-1} g^{-1} DM) in May **(a)**, July **(b)** and October **(c)** and respiration per gram soil (R, µgC h^{-1} g^{-1} DM) in May **(d)**, July **(e)** and October **(f)** under various combinations of three temperature - and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C warmed (+1.5, orange), 3 °C above ambient air temperature (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm CO_2 above ambient levels (+150, light grey), additional 300 ppm CO_2 (+300, dark grey). DM = dry mass.

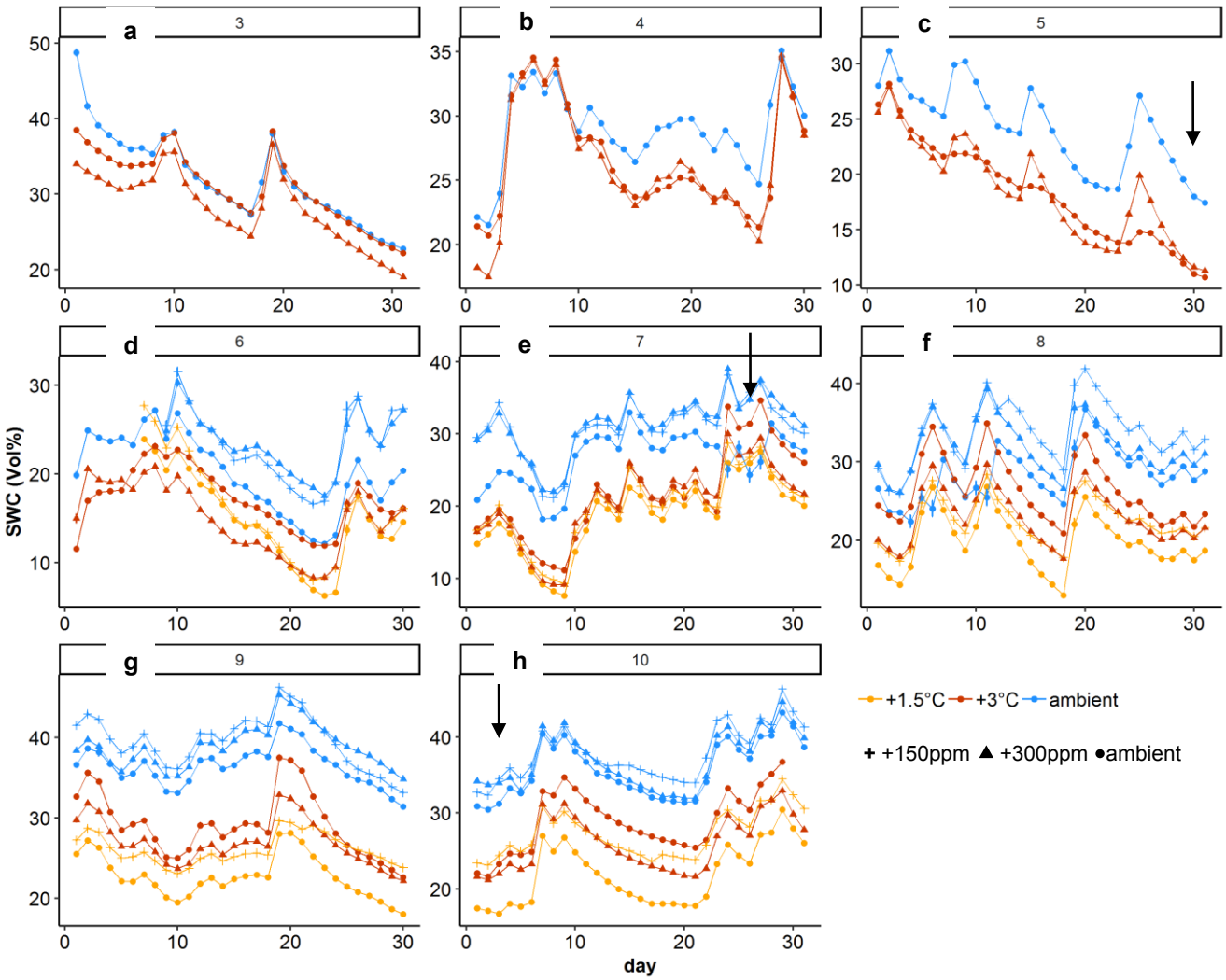

Figure S2 | **Soil water content under single and combined elevated temperature and CO_2 concentration.** Volumetric soil water content (SWC, Vol%) in March **(a)**, April **(b)**, May **(c)**, June **(d)**, July **(e)**, August **(f)**, September **(g)** and October **(h)** under various combinations of three levels of temperature: ambient (sky blue), 1.5°C above ambient air temperature (orange), 3°C heated (red) and three levels of atmospheric CO_2 concentration: ambient (circular symbols), with additional 150 ppm fumigated (plus symbol), 300 ppm enriched CO_2 concentration (triangular symbols). black arrows indicate sampling time points. Vol% = volumetric %. Data provided by David Reinthaler (University of Innsbruck).

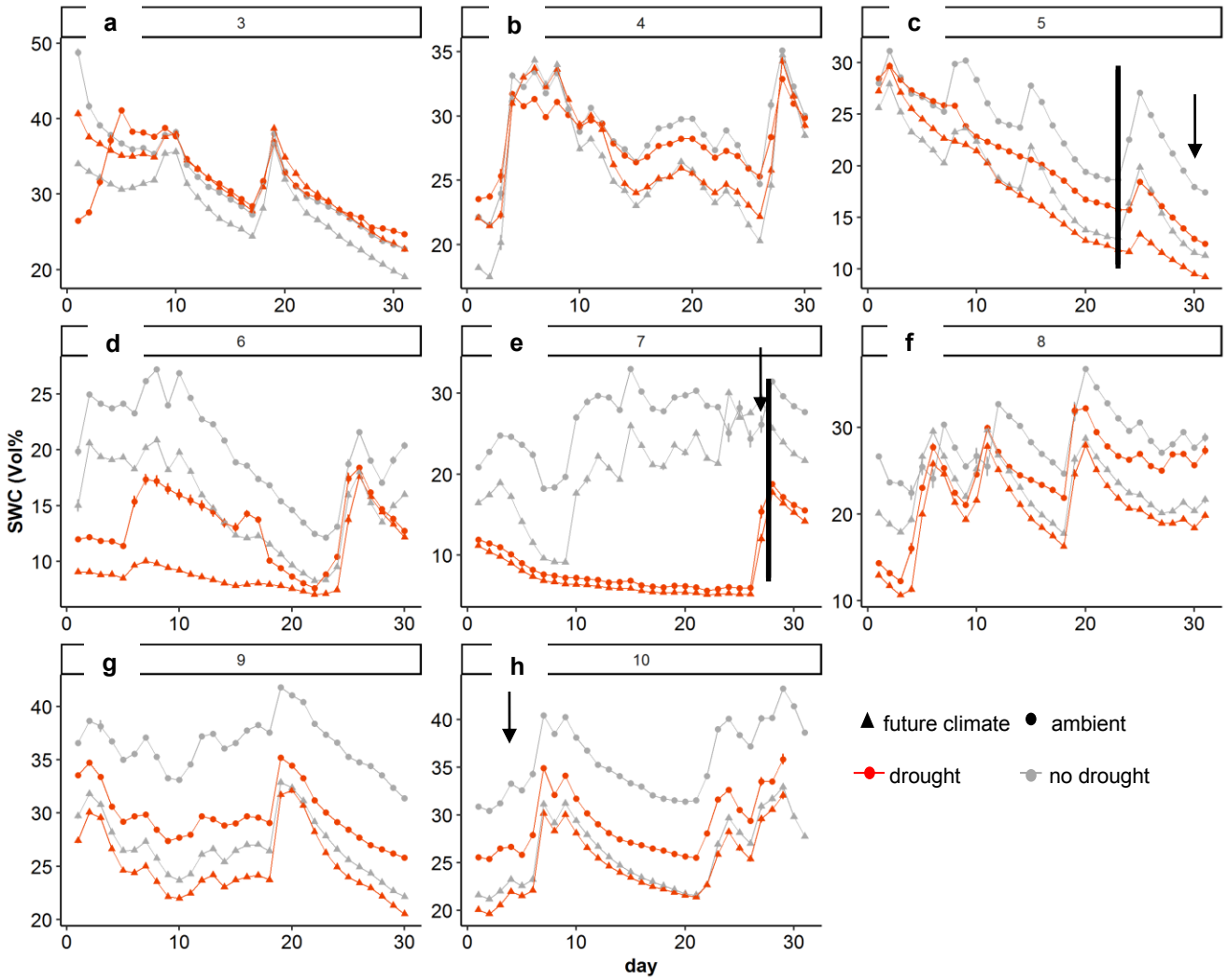

Figure S3 | **Soil water content before -, during drought and after rewetting.** Volumetric soil water content (SWC, Vol%) of future climate - (triangular symbol, +3 °C, +300 ppm) and ambient plots (circular symbols), which were exposed to drought (red) or were left unmanipulated (grey) in March **(a)**, April **(b)**, May **(c)**, June **(d)**, July **(e)**, August **(f)**, September **(g)** and October **(h)**. first vertical line represents launch of rain-out shelters, second line indicates rewetting of the drought plots, black arrows indicate sampling time points. Vol% = volumetric %. Data provided by David Reinthaler (University of Innsbruck).

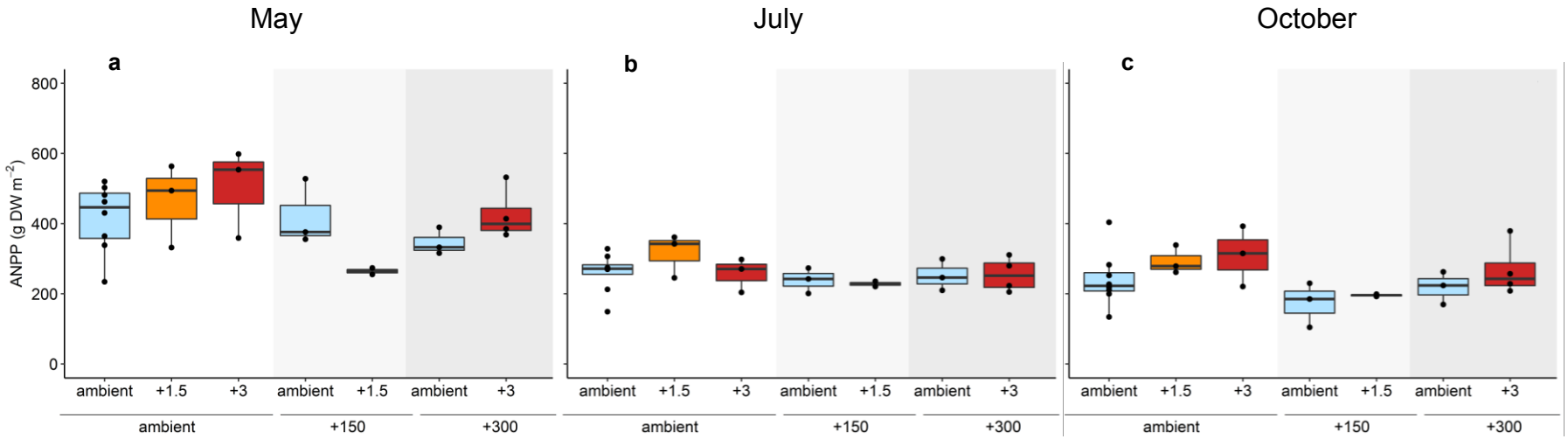

Figure S4 | **Response of plant aboveground net primary production to single and combined elevated temperature and CO_2 concentration. a-c,** Aboveground net primary production (ANPP, g DW m^{-2}) in May **(a)**, July **(b)** and October **(c)** under various combinations of three temperature - and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C warmed (+1.5, orange), 3 °C above ambient air temperature (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm CO_2 above ambient levels (+150, light grey), additional 300 ppm CO_2 (+300, dark grey). DW = dry weight. Data provided by the staff of AREC Raumberg-Gumpenstein.

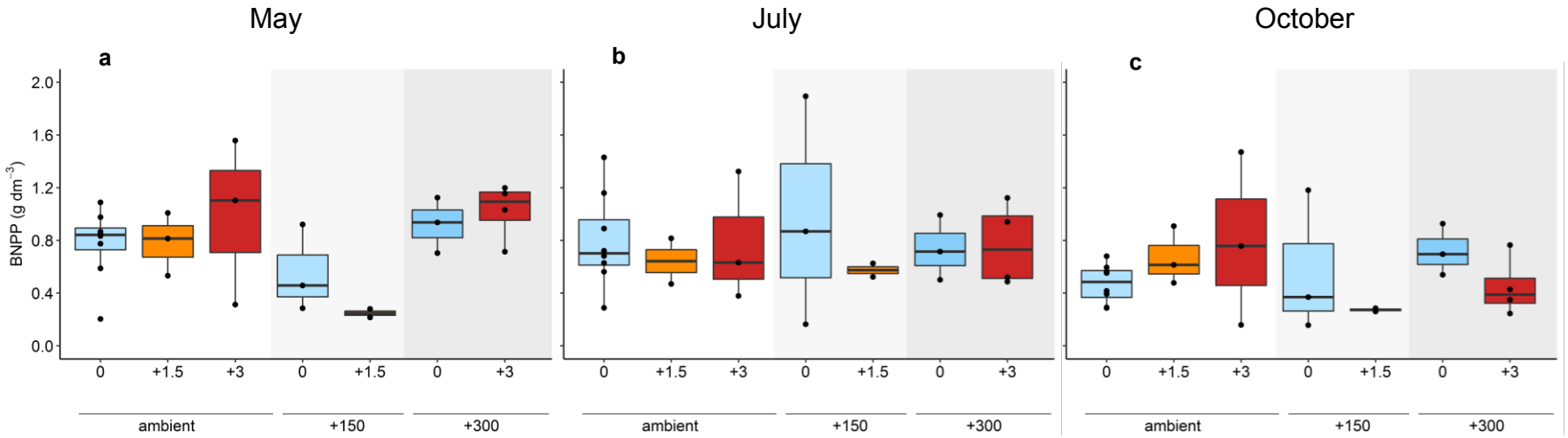

Figure S5 | **Response of plant belowground net primary production to single and combined elevated temperature and CO_2 concentration. a-c,** Belowground net primary production (BNPP, g dm^{-3}) in May **(a)**, July **(b)** and October **(c)** under various combinations of three temperature - and three CO_2 levels: ambient air temperature (ambient, blue), 1.5 °C warmed (+1.5, orange), 3 °C above ambient air temperature (+3, red); ambient atmospheric CO_2 concentration (ambient, white), 150 ppm CO_2 above current levels (+150, light grey), additional 300 ppm CO_2 (+300, dark grey). Data provided by Sarah Geiger (University of Innsbruck).

PART 3

Summary

Soil organic matter is the largest pool of organic carbon globally and is comprised to a large extent of microbial remnants. Consequently, microbial growth and carbon use efficiency (CUE), i.e. the proportion of organic uptake carbon which microbial communities allocate to growth, determine how much carbon can potentially be stabilized in soils. In order to assess how environmental factors and climate change may influence soil carbon storage, this study explicitly focuses on microbial growth, respiration and CUE. Soil temperature, soil moisture and substrate availability mainly control microbial activity in the short term and may vary in their importance between seasons. Changes in the terrestrial carbon cycle have the potential to aggravate or attenuate climate change by altering the amount of greenhouse gases such as CO_2 and CH_4 in the atmosphere. It is widely recognized that climate change affects biogeochemical processes; however, it is less recognized that these effects can only be explained mechanistically through understanding the effect of climate change on microbial physiology and community composition. Thus, multifactorial experiments, which assess the combined effects of multiple climate change drivers on microbial physiology metrics, are scarce, and the consequences of direct and indirect effects of combined climate warming, elevated CO_2 concentration and altered precipitation regime on microbial physiology remain obscure.

In this study, we aimed to assess (1) the relevance of seasonality on microbial growth, respiration and CUE, and (2) the season-specific effects of combined elevated temperature, CO_2 concentration and drought on microbial growth, respiration and CUE. Towards this end, we took soil samples from various climate treatments of a climate change experiment in a grassland ecosystem (hay meadow) in central Austria (ClimGrass experiment) at three time points during the plant growing period. We measured microbial biomass and microbial respiration and determined microbial growth and CUE by assessing the incorporation of ^{18}O from

labelled water into microbial dsDNA. To gain a better understanding of the responses of microbial physiology to climate change, we express the measured rates per unit of microbial biomass (i.e., biomass-specific growth and respiration rates).

Different combinations of three air temperatures and three atmospheric CO_2 levels in a response surface design enabled us to dissect the effects of single and combined effects of warming and CO_2 concentration on microbial activity. Our models revealed seasonality as the main driver of alterations of microbial physiology metrics across different seasons. Climate change drivers affected microbial growth and respiration equally within each sampling date. The physiological response to elevated temperature and CO_2 however differed across seasons, suggesting that different factors might have limited microbial activity throughout the plant growing season. Temperature, soil moisture and carbon availability seemed to have had the most pronounced effects on the extent of microbial growth and respiration. CUE proved to be a robust metric throughout the plant growing season and was not affected by the climate change treatments.

In summary, we found that (1) simulation of climate change altered microbial growth and respiration across all seasons; (2) the direction of the effect depended on the season; and (3) that CUE was invariant and did not respond to elevated temperature, drought or changed plant carbon inputs.

Zusammenfassung

Organisches Material im Boden stellt den weltweit größten Pool an organischem Kohlenstoff dar und setzt sich zu einem großen Teil aus mikrobiellen Überresten zusammen. Folglich bestimmen mikrobielles Wachstum und Kohlenstoff-nutzungseffizienz (CUE), das heißt der Anteil an aufgenommenen organischen Kohlenstoff, den Mikroorganismen in ihr Wachstum investieren, wie viel Kohlenstoff im Boden potentiell stabilisiert werden kann.
Um feststellen zu können, inwieweit Umweltfaktoren und der Klimawandel die Kohlenstoffspeicherung im Boden beeinflussen, liegt der Fokus dieser Arbeit auf dem mikrobiellen Wachstum, der mikrobiellen Atmung und der Kohlenstoffnutzungseffizienz. Auf kurze Sicht gesehen bestimmen Bodentemperatur, Bodenfeuchte und Substratverfügbarkeit die mikrobielle Aktivität. Ihre jeweilige Bedeutung ist abhängig von der Jahreszeit. Da Veränderungen des terrestrischen Kohlenstoffkreislaufes zu einer Änderung der Konzentration von Treibhausgasen wie CO_2 oder CH_4 in der Atmosphäre führen können, haben sie das Potential den Klimawandel entweder zu verstärken oder abzuschwächen. Obwohl weithin anerkannt ist, dass der Klimawandel biogeochemische Kreisläufe beeinflusst, wurde bislang wenig zur Auswirkungen des Klimawandels auf mikrobielle Physiologie und mikrobielle Gemeinschaftszusammensetzung geforscht. Multifaktorielle Experimente, welche gemeinsame Auswirkungen von unterschiedlichen Klimawandeltreibern auf Kenngrößen der mikrobiellen Physiologie erfassen, sind ebenfalls rar. Somit sind direkte und indirekte Auswirkungen von Klimaerwärmung, erhöhter Kohlendioxidkonzentration und veränderter Niederschlagsregime auf mikrobielle Physiologie noch weitgehend ungeklärt.

Die wichtigsten Ziele unserer Studie waren daher: (1) die Bedeutung von Saisonalität für mikrobielles Wachstum, Atmung und CUE besser zu verstehen, und (2) die saisonabhängigen Auswirkungen von gemeinsam auftretender Temperatur-erhöhung, steigender CO_2 Konzentration und Trockenheit auf mikrobielles Wachstum, Atmung und Kohlenstoffnutzungseffizienz zu beleuchten. Hierfür

nahmen wir zu drei Zeitpunkten während der Vegetationsperiode Bodenproben von unterschiedlichen Klimawandelbehandlungen in einem Klimawandelexperiment in einem genutzen Grünland in Zentralösterreich (ClimGrass Experiment). Wir erhoben mikrobielle Biomasse und Atmungsraten und erfassten mikrobielles Wachstum und CUE, indem wir den Einbau von ^{18}O aus markiertem Wasser in mikrobielle dsDNA bestimmten. Wir legen das Hauptaugenmerk auf die Erfassung von Raten pro Einheit mikrobieller Biomasse (Biomasse-spezifisches Wachstums- und Atmungsraten), um physiologische Antworten der Mikroorganismen auf den Klimawandel verstehen zu können.

Unterschiedliche Kombinationen von je drei Lufttemperaturen und atmosphärischen CO_2 Konzentrationen, die experimentell in einem *Response Surface Design* arrangiert wurden, machten es uns möglich die Auswirkungen von Erwärmung und erhöhter CO_2 Konzentration einzeln von denen in Kombination zu trennen. Unsere statistischen Modelle zeigten Saisonalität als Haupttreiber von Veränderungen der physiologischen Kenngrößen. Treiber des Klimawandels beeinflussten mikrobielles Wachstum und Atmung in ähnlicher Weise zu den jeweiligen Zeitpunkten der Probennahmen. Unterschiedlich geartete physiologische Antworten auf erhöhte Temperatur und CO_2 in den drei beprobten Jahreszeiten legten nahe, dass die mikrobielle Aktivität von unterschiedlichen Faktoren während der Vegetationsperiode limitiert ist. Temperatur, Bodenfeuchte und Kohlenstoffverfügbarkeit schienen die ausgeprägtesten Auswirkungen auf die Höhe von mikrobiellem Wachstum und Atmung gehabt zu haben. Die Kohlenstoffnutzungseffizienz erwies sich als robuste Kenngröße während der gesamten Pflanzenwachstumsperiode und wurde nicht von Klimawandelbehandlungen beeinflusst.

Zusammengefasst fanden wir: (1) dass sich der simulierte Klimawandel in allen Jahreszeiten auf mikrobielles Wachstum und Atmung auswirkte; (2) dass die Richtung dieser Auswirkungen von der Jahreszeit abhing; und (3) dass die Kohlenstoffnutzungseffizienz ein robuster Parameter war und keine Veränderung

ihrer durch erhöhte Temperatur, Trockenheit oder veränderte Kohlenstoffeinträge eintrat.

PART 4

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