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„Assessing the effect of drought and future climate conditions on microbial phospholipid fatty acid synthesis under field conditions."

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Nathalie Friedl, BSc

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Summary

English Abstract

Future climate (elevated CO_2 (eCO_2) and warming) changes soil microbial community composition and activity. Drought alters soil microbial community composition and reduces microbial biomass. Heavy rainfalls ending droughts are having far-reaching effects on soil ecosystems.

To analyze the effects of climate change in response to rewetting in the field, we used Deuterium-labeling (D-labeling) within an experimental setup in a managed grassland ('GlimGrass') in Syria, Austria. Plots of 6 treatments (eCO_2: +300 ppm, +0 °C (n = 3), future climate: +300 ppm, +3 °C (n = 3), future climate drought: +300 ppm, +3 °C, drought (n = 4), ambient: +0 ppm, +0 °C (n = 3), warming: +0 ppm, +3 °C (n = 3), ambient drought: +0 ppm, +0 °C, drought (n = 3)) were rewetted directly in the field with 40 mm (16 mm h^{-1}) 3300 ‰ D_2O. Samples were collected at peak drought, 2 hours, 1 day, 2 days and 4 days after rewetting. We measured total PLFAs (phospholipid fatty acids) and D-uptake as a proxy for microbial activity.

Elevated CO_2 and future climate increased total PLFAs and changed community composition. Drought significantly influenced community composition and decreased total PLFAs. Rewetting changed soil microbial community composition. Drought significantly increased D-uptake of all microbial groups. Future climate decreased D-uptake of bacteria.

Our findings are in line with past research, pointing out that eCO_2 and future climate can change soil microbial community composition. We also focus on differences between past and current findings from this paper within the same experimental setup.

German Abstract

Zukunftsklima (erhöhter CO_2-Wert (eCO_2) und Erwärmung) verändert die mikrobielle Gemeinschaft und Aktivität im Boden. Trockenheit verändert die mikrobielle Zusammensetzung und reduziert ihre Biomasse. Starkregen nach Trockenheit hat weitreichende Folgen für Bodenökosysteme.

Um die Effekte des Klimawandels in Folge einer Wiederbewässerung im Feld zu untersuchen, benutzten wir Deuterium-labeling (D-labeling) in einem auf den Klimawandel fokussierten Versuchsaufbau (‚ClimGrass') in der Steiermark, Österreich. Plots mit 6 verschiedenen Kombinationen an künstlichen Klimawandelfaktoren (eCO_2: +300 ppm, +0 °C (n = 3), Zukunftsklima: +300 ppm, +3 °C (n = 3), trockenes Zukunftsklima: +300 ppm, +3 °C, Trockenperiode (n = 4), Kontrolle: +0 ppm, +0 °C (n = 3), Erwärmung: +0 ppm, +3 °C (n = 3), trockene Kontrolle: +0 ppm, +0 °C, Trockenheit (n = 3)) wurden direkt im Feld mit 40 mm (16 mm h^{-1}) 3300 ‰ D_2O bewässert. Die Probenentnahme erfolgte vor der Bewässerung, zwei Stunden, einen Tag, zwei Tage und vier Tage nach Bewässerung. Gesamte PLFAs (Phospholipid Fatty Acids) sowie die D-Aufnahme als Einheit für mikrobielle Aktivität wurden gemessen.

Erhöhte CO_2-Werte und Zukunftsklima erhöhten die PLFA Biomasse und veränderten die mikrobielle Zusammensetzung. Trockenheit veränderte die mikrobielle Zusammensetzung und reduzierte die PLFA Biomasse. Die Bewässerung sorgte für Veränderungen in der mikrobiellen Zusammensetzung. Die Trockenheit erhöhte die D-Aufnahme signifikant in allen mikrobiellen Gruppen. Zukunftsklima reduzierte die D-Aufnahme aller Bakterien.

Unsere Resultate wurden von jenen vergangener Forschungsarbeiten bestätigt. Wir konnten zeigen, dass eCO_2 und Zukunftsklima die mikrobielle Zusammensetzung im Boden verändern können. Weiters gehen wir auf unterschiedliche Resultate ein, die im selben Experiment erzielt wurden.

General Introduction

A problematic background

Anthropogenic activities have by far exceeded the level of influence that could still be considered as unproblematic for natural ecosystems. In fact, some scientists even claim that our current epoch should be named the 'anthropocene' as human populations are having immense, mostly negative, impacts on planet earth (Crutzen 2002, Lewis and Maslin 2015, Zalasiewicz et al. 2010). Even if this nomenclature might be, to some extent, a matter of taste, climate change is simply not. Greenhouse gas emissions are still increasing and are going to reach even higher levels in the near future if not immediate action is taken (IPCC 2022). The accumulation of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gasses in the atmosphere is the main cause for climate warming (IPCC 2007). Besides temperature, the gasses themselves can modify processes. For example, elevated CO_2 (eCO_2) changes plant's water use efficiency (Eamus 1991) and increases plant biomass production (Terrer et al. 2019). Rising surface temperatures trigger a cascade of events. Extreme weather events are likely to become more frequent (IPCC 2022). For example, drought periods will increase in duration and intensity (Gobiet et al. 2014, Sherwood and Fu 2014). Yet, the pattern is not that simple in this case. Soil moisture and atmospheric humidity might counteract drought to some extent (Sippel et al. 2018). However, drought is often accompanied by heat waves which could intensify drought effects (IPCC 2007).

Climate change triggers changes in the water cycle (Austin et al. 2004). Based on a modeling approach, Hanaski et al. (2013) came to the conclusion that precipitation will increase in temperate regions but decrease in others. In consequence, we must expect that water scarcity will become more of an issue - not only for human populations (Hanaski et al. 2013) but also for different ecosystems (Gobiet et al. 2014, Müller and Bahn 2022).

A sensitive ecosystem

Although soils are the foundation of plant and microbial biomass growth, their reaction to climate change is not taken into account. Most people will, first of all, picture wilting plants, rising sea levels and animals being forced to shift their distribution ranges polewards. Nevertheless, soils play a crucial role in the functioning of many ecosystem processes (Barrios 2007, Soong et al. 2020). On a global scale, soils contain more carbon (C) than the entire vegetation and the atmosphere together (Lehmann and Kleber 2015). In particular, soil microbial growth is a major factor in assessing processes such as soil organic matter (SOM) formation (Miltner et al. 2012, Sokol et al. 2019), decomposition and nutrient turnover

(Barrios 2007). SOM represents the balance between plant inputs and C mineralization (Sokol et al. 2022). Considering possible positive feedbacks to climate change, soils might be capable of releasing additional CO_2 and CH_4 to the atmosphere (Davidson and Janssens 2006).

Elevated levels of CO_2 influence soil ecosystems and promote plant growth and can therefore increase primary production (Terrer et al. 2019) and priming (Dijkstra et al. 2013). This can result in higher C inputs from roots to soils which could stimulate microbial activity. Higher biomass production, however, requires more nutrients from the soil. Hence, biomass production, microbial activity and decomposition increase at a similar rate, which might cause net effects of eCO_2 to seem rather small.

Additionally, an increase in temperature increases enzymatic activity and therefore facilitates processes like microbial respiration and decomposition (Alster et al. 2016, Fuchslueger et al. 2019, Lloyd and Taylor 1994). The positive response of soil microorganisms to long term warming is, however, not that simple. In some ecosystems soil microbial communities adapted to elevated temperatures after several months to years (Bradford et al. 2008, Carey et al. 2016, Melillo et al. 2002), whereas in others there was no evidence (Hartley et al. 2008, Jing et al. 2014). It is therefore likely that the responses of soil communities to long term warming are highly ecosystem-specific and not as clear as in short term warming experiments.

A clearer pattern appears for drought. Reduced water availability not only increases the amount of dormant microbial colonies (Schimel 2018) but can also change the composition of the active soil microbial community (Fuchslueger et al. 2014, Metze et al. 2023). Fungi, especially mycorrhizal fungi, have an advantage in dry soils as they can still obtain C from plants. Additionally, fungi are capable of exploring larger soil volumes compared to single microbial cells (Moyano et al. 2013). This might shift communities to a fungi-dominated ecosystem during drought periods (Evans and Wallenstein 2012).

Not only the drought response, but also the response to rewetting is specific for different soil microbial groups. The so called “Birch effect” describes the respiration peak after rewetting of dry soil (Birch 1985). Water acts as primer for microbial activity. It makes nutrients accessible again and resuscitates microbes from dormancy.

Hicks et al. (2019) found that bacteria contribute more to the Birch effect than fungi. Yet, the responses have mostly been measured under laboratory conditions and in single factor experiments investigating the effects of warming, eCO_2 and drought individually. Studying this process under field conditions could give insights into feedback mechanisms between different climate change factors and their interaction, elucidate soil microbial responses and processes during extreme drought as well as the recovery and response of microbial communities after intense rainfalls.

A promising method

Stable isotope probing (SIP) is a widely used method in ecology to trace a certain label into biomarkers and to assess the contribution of different microbial groups to a process. The type of physiological process that can be studied depends on the tracer that is used (Geyer et al. 2019). To investigate soil microbial activity and growth, a range of stable isotope tracing methods can be applied. For instance, ^{13}C can be traced into microbial biomass C (Fuchslueger et al 2014), nucleic acids (e.g. Martinović et al. 2022) or phospholipid fatty acids (PLFAs, e.g. Bahn et al. 2013, Boschker and Middelburg 2002, Dungait et al. 2010). ^{18}O can be used for DNA-labeling (e.g. Morris et al. 2022, Spohn et al. 2016). Even though microbial growth can be calculated from DNA-SIP and PLFA-SIP data, the results will depend on the biomarker and, as a result, the tracer. As DNA is only replicated during mitosis, the DNA-SIP approach only takes reproductive growth into account.

In contrast, lipids are a major component of cell membranes (PLFAs) or storage compounds (neutral lipid fatty acids, NLFAs, Frostegård et al. 2011). They are also built up during maintenance and cell growth processes, not only during mitosis as in DNA. In addition, in soils PLFAs degrade quickly after the death of microbes and the PLFA composition mainly reflects the active community (Frostegård et al. 2011). Whilst PLFAs can only fingerprint major microbial groups, DNA and RNA based methods are capable of providing by far more taxonomic and phylogenetic information.

But there in one drawback that should be kept in mind: A priming effect by label addition can occur. For example, the addition of ^{13}C in form of glucose triggers microbial activity and respiration (Kuzyakov et al. 2000), making it only possible to evaluate processes resulting from glucose addition. The method can only depict the C uptake from the substrate rather than soil organic matter (Spohn et al. 2016). This priming effect can be reduced, but not completely avoided, using alternative methods like $^{13}CO_2$ pulse labeling (e.g. Bahn et al. 2013) or by selecting tracers without a C source to reduce stimulating microbial activity, such as ^{18}O-labeled water (Spohn et al 2016).

In particular, under drought conditions or in dry environments, liquid tracer additions can stimulate microbial activity (Fuchslueger et al 2019) and results might therefore over-estimate microbial activity rates. There are possibilities to avoid this effect (e.g. water vapor method, Canarini et al 2020). As this manuscript focuses on the priming effect of rewetting, we will not go into more detail regarding the priming effect by label addition.

A suitable tracer

An alternative tracer to ^{18}O-labeled water is water labeled with Deuterium (D or ^{2}H), a stable hydrogen isotope. The water vapor method has successfully been tested for D-labeling at

the department for Terrestrial Ecosystem Research (TER, University of Vienna) by A. Richter, A. Canarini, L. Fuchlueger and colleagues. However, D is not stable in DNA and RNA. In nucleic acids, protons are constantly being exchanged between the molecule and the matrix (Englander and Kallenbach 1983), making it impossible to account for actual DNA-replication after D-labeling. Deuterium is stable in PLFAs (Sessions et al. 1999, Karhu et al. 2022). It is therefore perfectly suitable to measure microbial growth as PLFA production in soil samples.

A characteristic biomarker

PLFAs are phospholipid fatty acids. In phospholipids, two fatty acid chains are bound to one polar head, consisting of glycerol and a phosphate group. In PLFA analysis, only the fatty acids are extracted and measured. As they differ in chain length, branching and saturation, they act as biomarkers for microbial taxa (Willers et al. 2015). The biosynthesis of fatty acids is rather simple as it consists of numerous equal cycles. In each cycle, the chain is elongated until the final length is reached (Magnuson et al. 1993) and hydrogen atoms are bound to the C atoms within the chain. This way, hydrogen becomes stable within the molecule and proton exchange (as described above for nucleic acids) does not occur. Hence, tracing D into PLFAs can be used to monitor recent PLFA synthesis. Moreover, PLFAs can be used to fingerprint community composition on a coarse group-level. The major groups that can be distinguished with this method are general, gram+, gram-, actinobacterial, fungal and bacterial markers (Frostegård et al. 2011, Schnecker et al. 2012). Additional to the phospholipid fatty acids (PLFAs), neutral fatty acids (NLFAs) can be derived during fractionation (Buyer and Sasser 2012). NLFAs contain additional information about fungi (Frostegård et al. 2011). A classification of all analyzed makers and their assigned microbial groups can be found in the supplement.

A field-labeling experiment

The aim of this thesis was to investigate microbial activity in using a D-SIP approach ‘in situ’ in a large multifactorial field experiment simulating different climate change scenarios including eCO_2, warming and drought and several combined treatments. The overarching question of this study was: How do different climate change factors, in particular eCO_2, warming and drought, alone and in combination, affect group-specific soil microbial activity and community composition. More specifically, we tested the following hypothesis: Both eCO_2 and warming would increase microbial activity, whereas drought would have detrimental effects on both soil microbial activity and biomass and would have a strong impact on community composition.

The main questions, about to be answered in the following manuscript, were:

I. How active are soil microbes under different climate scenarios?
    A. Does eCO_2 increase soil microbial activity?
    B. Does warming increase soil microbial activity?
    C. Does drought decrease soil microbial activity?
    D. Does soil microbial community composition differ between treatments?
    E. Do the three treatments (eCO_2, warming and drought) have interactive effects on soil microbial activity and community composition?

II. How do soil microbial groups of different treatments react to rewetting after drought?

III. Is the D-labeling PLFA method in the field suitable to study soil microbial activity rates?

Manuscript

Introduction

The functioning of many ecosystem processes depends on soils. As a result of soil organic matter (SOM) formation, soils act as carbon (C) sinks (Miltner et al. 2012). Contrary, decomposition of SOM releases C to the atmosphere (Barrios 2007). Hence, soils act as C sink and source and play a crucial role in nutrient turnover. Atmospheric CO_2 partial pressure and subsequently global temperature are likely to continue rising (IPCC 2021), leading to the question: How will climate change influence soil ecosystems?

More specifically, in this study, we wanted to study the influence of climate change on soil microbial communities in a temperate grassland ecosystem. Our goal was to identify the effects and interactive effects of elevated CO_2 (eCO_2, +300 ppm), warming (+3 °C) and drought as well as rewetting on soil microbial communities.

Elevated levels of CO_2 can change soil microbial community composition (Jin and Evans 2010, Phillips et al. 2002) and increase soil respiration (Reinthaler et al. 2021). This effect can be explained by the capability of eCO_2 to increase plant primary production (Terrer et al. 2019) and rhizosphere priming (Cros et al. 2019, Dijkstra et al. 2013).

Warming affects soil microbial communities in upper soil layers (Reinthaler et al. 2021). Studies found that the enzymatic activity of soil microbes increases with temperature (Alster et al. 2016, Fuchslueger et al. 2019). This falls in line with the Arrhenius equation, which points out that enzymatic activity increases with temperature (e.g. Alster et al. 2016, Lloyd and Taylor 1994). However, microbial growth is a by far more complex process that is not solely governed by thermodynamics. In connection with this, different soil microbial groups react differently to warming, causing community composition shifts (DeAngelis et al. 2015). Hence, responses to warming of different soil microbial groups are diverse and ecosystem specific.

Extreme weather events like heat waves or intense droughts are likely to occur at higher frequencies in the future as a result of climate change (Sippel et al. 2018). Soil microorganisms are sensitive to drought, as they need to be in contact with soil pore water to keep their physiological processes running (Tecon and Or 2017). Dry conditions disconnect aqueous patches from each other and prevent the diffusion of essential nutrients to microorganisms (Moyano et al. 2013) which could negatively impact soil microbial processes and reduce microbial activity (Schimel et al. 1999). Not all groups of microorganisms react similarly to long periods of drought (Evans and Wallenstein 2012) and many taxa remain active (Metze et al 2023). For example, some gram positive bacteria (gram+, Fuchslueger et al. 2014) and fungal groups have been found to be more resistant to lower soil moisture as they have different cell membrane structures and can access aqueous patches via hyphae

(Yuste et al. 2011). In many temperate grassland ecosystems, long periods of drought are not part of the typical characteristics of the ecosystem's climate (Gilliam 2016), but are projected to become more frequent in the future.

Heavy rainfalls that end periods of long drought trigger activity in dormant soil microbial colonies (Blagodatskaya and Kuzyakov 2013, Huntington 2006). Rewetting after drought causes a peak in soil respiration (Birch 1985) as surviving or dormant microorganisms will start metabolizing dead ones, and other accumulated organic substrate, as soon as water is available (Clein and Schimel 1994). This so-called “Birch Effect” was found in several experiments (e.g. Canarini et al. 2020, Hagedorn et al. 2016, Hicks et al. 2019, Göransson et al. 2013). One consequence of repeated drying-rewetting is that C is continuously lost from soils as a result of this respiration peak (Zhang et al. 2020).

Summarizing it can be said that soil ecosystems ale likely to undergo changes in their community composition as a result of climate change. The aim of this study was to identify the single and interactive effects of eCO_2, warming, drought and rewetting on soil microbes (community composition and activity) in a temperate grassland ecosystem. Yet, how can we measure a soil community’s reaction to climate change and rewetting under field conditions? Stable isotope probing (SIP) might do the trick.

Isotopic labeling allows analyzing physiological processes in soil communities (Dumont and Murrell 2005). Two popular isotopes to evaluate microbial growth are ^{13}C and ^{18}O (Geyer et al. 2019). ^{13}C has often been added in laboratory-based soil incubations in the form of labeled glucose or other organic compounds to trace organic matter metabolism. Organic substrates can be taken up by the microbial community and be incorporated into biomass or trigger microbial activity (Kuzyakov et al. 2000). On the downside, organic C addition artificially increases substrate availability for heterotrophic microbes, and increases both growth and respiration (e.g. substrate induced respiration and glucose metabolism) of a soil community (Geyer et al. 2019). ^{13}C has previously been used in field labeling experiments (e.g. Zhou et al. 2022). Alternative, substrate independent methods, for example the addition of water labeled with ^{18}O, allows tracing the label into DNA of active microbes without stimulating C metabolism (Spohn et al 2016). This method is, however, rather applicable under laboratory conditions as ^{18}O is expensive and needs to be applied at quite high isotope ratios to be traceable into microbial DNA, making the financing of large scale rewetting experiments challenging. Alternatively, Deuterium (D or ^{2}H) labeled water can be produced in larger quantities as pure D_2O is easily affordable and changes in isotope signal are detectable at very low label concentrations already (Wegener et al. 2016). It has been shown that it is stable and measurable in fatty acids (Neubauer et al. 2018, Sessions et al.

1999, Zhang et al. 2009). PLFA (phospholipid fatty acid) extraction is suitable to evaluate soil microbial activity by tracing D into PLFAs (Buyer and Sasser 2012).
As we added D_2O directly to the field plots, this method only allowed to measure substrate dependent activity in soil microbes. Hence, we will mainly focus on drought effects after rewetting.

In the following, we would like to address and answer the following questions: Will eCO_2 and/or warming increase soil microbial biomass and activity? How do eCO_2 and/or warming change soil microbial community composition? Does the community composition and microbial biomass of drought plots differ from the community composition of ambient rainfall plots? Will rewetting after drought cause an increase in soil microbial activity? And finally, will future climate drought (eCO_2, warming and drought) reduce soil microbial activity and biomass and will rewetting cause a particularly steep increase in activity in those plots?

In order to answer those questions, we joined the multifactorial long term climate change experiment 'ClimGrass' in Austria. In the field, grassland plots were exposed to different climate change treatments (+300 ppm CO_2, +3 °C temperature, 7 weeks artificial drought) alone and in combination, stimulating future climate conditions (+300 pm CO_2 and +3 °C) relative to ambient controls (no CO_2, no warming, no drought). Plots were amended with D-labeled water in the field to trace microbial activity in response to rewetting in particular after drought conditions.

Methods

Field site description

This study was conducted as part of the multifactorial long-term climate change experiment 'ClimGrass'. The site is located in Raumberg-Gumpenstein, Styria, Austria (47°29′37″N, 14°06′0″E). It is characterized by a mean annual temperature of 7.2°C and a mean annual precipitation of about 1000 mm. According to the WRB classification, the soil type in this region is a Cambisol with a pH of 5 (Deltedesco et al. 2019).

The multifactorial experiment 'ClimGrass' is part of the research of the local Agricultural Research and Education Center (AREC) responsible for all on-site management and maintenance measures. These included mowing and removal of aboveground biomass three times per year, as well as fertilizer application to account for biomass removal (spring: 30 kg N, 32.5 kg P, 85 kg K; after first harvest: 30 kg N; after second harvest: 30 kg N; Simon et al. 2020).

Experimental setup - in situ rewetting with D-labeled water

The experimental design of the 'ClimGrass' experiment was originally based on a response surface approach to study the effects of eCO_2 and elevated temperature on a managed grassland ecosystem (Piepho et al. 2017). For each of the two factors, three different treatments were established on overall 54 plots.

The increase in temperature was achieved by using three infrared-lights, positioned in a triangular shape around each plot. Control plots were provided with empty lamp sockets following the exact same setup. The levels of CO_2 were increased via fumigation, using a circular tube with holes and an automatic gas pump. The control plots were provided with the same setup but without any fumigation treatment. The conditions were monitored with a CO_2 sensor in the middle of each plot.

For this study we focused only on the second levels of warming and eCO_2 (+3 °C and +300 ppm) as well as the 'ambient' control plots (+0 °C and +0 ppm). In addition, four 'future climate' (+300 ppm and +3 °C) and three drought controls (+0 ppm, +0 °C) were kept under rainout shelters for seven weeks (June 15th 2021 to August 2nd 2021) to simulate an extreme summer drought before the start of the rewetting and D-labeling. In summary, we selected 19 plots with several different treatment combinations in a multifactorial long term climate change experiment (Fig. 1).

Soil water content and soil temperature of all plots were constantly monitored at all sampling times by an automatic system provided by AREC (one value per hour, 3 cm and 9 cm depth, moisture sensor: USDA-ARS Hydraprobe Analog (2.5V)).

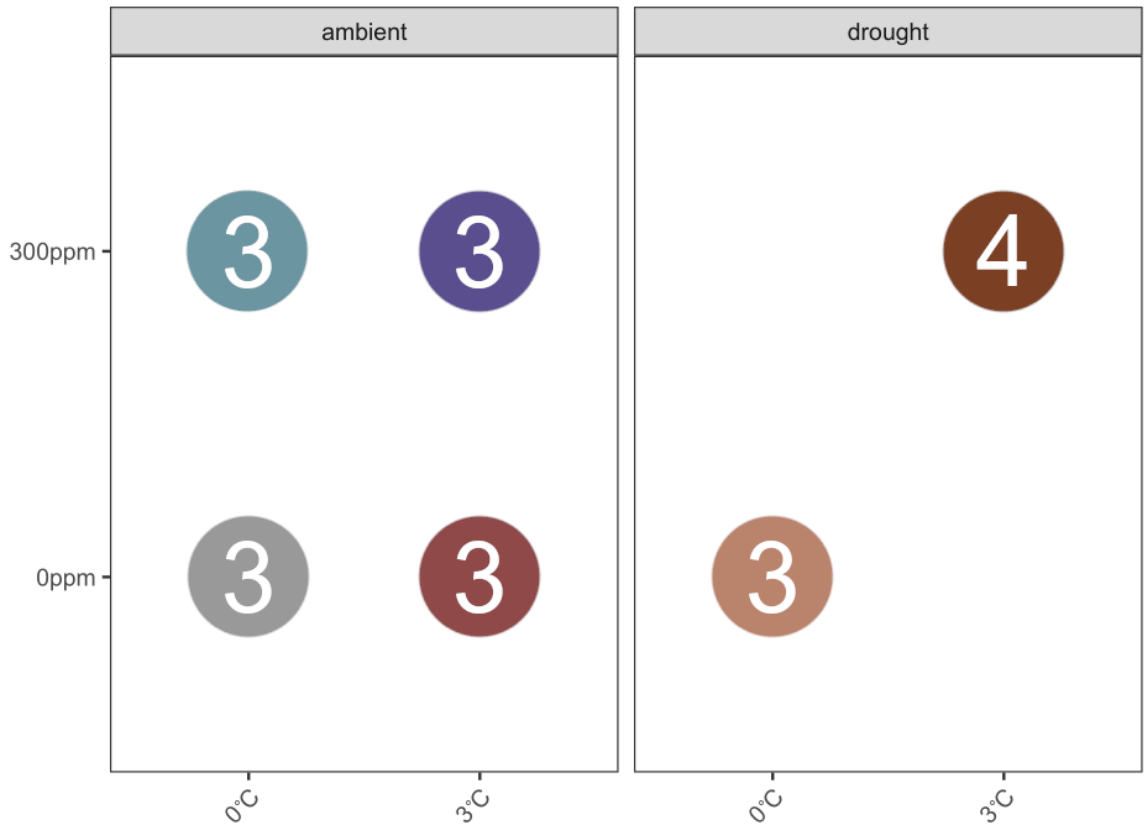

Figure 1: All sampled treatment combinations. The numbers in the circles indicate the number of sampled plots. (from left to right: eCO_2: +300 ppm, +0 °C (n = 3), future climate: +300 ppm, +3 °C (n = 3), future climate drought: +300 ppm, +3 °C, drought (n = 4), ambient: +0 ppm, +0 °C (n = 3), warming: +0 ppm, +3 °C (n = 3), ambient drought: +0 ppm, +0 °C, drought (n = 3))

External Data

All plots, including non-drought treatments, were rewetted with in total 40 mm (16 mm h^{-1}) of 3300 ‰ D_2O (mixture of rainwater and industrial D_2O) on August 2nd starting at 8am. The steps were performed on each plot individually by the personnel of the AREC and a team from the Institute of Ecology, University of Innsbruck, within 6 hours. Additionally, the liquid soil pore water isotope signature of all plots was obtained twice per day (*n = 1*) by Jesse Radolinski and his team (Institute of Ecology, University of Innsbruck). It was calculated from the soil temperature and the vapor isotope signature at 3 and 9 cm depth (measured in situ) using standard fractionation equations by Horita and Weslowski 1994 and standard correction to determine the liquid soil pore water isotope signature.

Sample Collection

From all plots the initial soil sampling (0 hours) was conducted at peak drought, right before the rewetting, and then following a time course from 2 hours, 1 day, 2 days and 4 days after rewetting (2 cm diameter, 10 cm depth soil corer). The samples were brought to the lab immediately after collection, sieved (2 mm), shock-frozen in liquid N_2 and stored at -20 °C for further analysis and PLFA-extractions.

Phospholipid Fatty Acid extractions

At each harvest, 4 g of all samples were immediately shock-frozen and stored at -20 °C for PLFA analysis. The samples were freeze-dried (Freeze Dryer Chris Alpha 1-4 LSC) and frozen again at -20 °C until further proceeding. PLFA extraction was done following a modified high throughput method of Buyer and Sasser (2012). Total lipids were extracted with a 4:10:5 (v/v/v) CMB-buffer (0.15 M citrate buffer/methanol/chloroform) solution. The separation of NLFAs and PLFAs was done using a 96 well silica column plate (Strata SI-1 Silica 55µm 70A 50mg 96 well plate) and methanol, ethanol, chloroform, water and acetone in different mixtures as eluents (eluent 1 for NLFAs: chloroform:ethanol (v/v = 98:2), eluent 2 as washing step: acetone, eluent 3 for PLFAs: methanol:chloroform:H_2O (v/v/v = 5:5:1)). As internal standard methyl-nonadecanoate (19:0) was added, then the samples were transesterified by alkaline methanolysis. Samples were analyzed for H quantity and ^2H incorporation using a Trace GC Ultra, connected to an IRMS (Delta-V-Advantage) via a GC IsoLink II™ (all Thermo Fischer). Samples were injected in splitless mode (injector temperature 300 °C) and separated on a DB5 column (60 m × 0.25 mm × 0.25 µm; Agilent, Vienna, Austria) with 1.2 ml He min^{-1} as the carrier gas (GC program: 1 min at 80 °C, 30 °C min^{-1} to 150 °C, 1 min at 150 °C, 4 °C min^{-1} to 230 °C, 30 min at 230 °C, 30 °C min^{-1} to 280 °C and 19 min at 280 °C). The H^3 factor (correction for abundance of the trihydrogen ion H^{3+}) was defined based on multiple injections of reference hydrogen gas. Fatty Acid Methyl Esters (FAMEs) were identified using mixtures of bacterial and fungal FAMEs (Bacterial Acid Methyl Ester CP Mixture (Matreya LLC, State College, PA, USA) and 37 Comp. FAME Mix (Supelco, Bellefonte, PA, USA)), and quantified against the internal standard (19:0). In addition, H concentration and ^2H isotope composition of individual and fatty acid mixes was measured before and after the measurements, as described above, and used for correcting linearity based on H area and ^2H signals using the standards USGS70 and USGS72 (Arndt Schimmelmann, Indiana University). Memory effect corrections were not applied; the maximal potential offset ranges around 4% (Wang and Sessions 2008) and analysis of methane reference standards that follow analytical peaks suggested that memory effect should be at around 0.2 % (Kopf et al. 2016).

Calculations

All calculations were done in Microsoft Excel Professional Plus 2019. Peaks were identified using the default software of the measuring unit (IsoDat, Thermo Fisher Scientific).
The previously added internal standard (methyl-nonadecanoate, 19:0) was used to calculate the concentration of PLFAs in each sample. As all measurements were based on hydrogen and not absolute fatty acid biomass, total PLFA biomass based on H will further be described as total PLFAs (*H-PLFA*, in nmol H g^{-1} dry soil).

The percentual label uptake (atom percent excess, *APE*) was calculated for each individual fatty acid (Tab. 1) as following:

$$APE = at\%_{tn} - at\%_{t0}$$

with $at\%_{tn}$ being the D-concentration in the sample collected at different time points in at%, and $at\%_{t0}$ is the natural abundance D-concentration in the sample at peak drought (before rewetting) in at%.

Additionally, the D-label taken up from the added water-tracer into PLFAs (*D-PLFA* in nmol D g^{-1} dry soil) was calculated. It will be further described as D-label incorporation. The calculation was done using the following formula:

$$D\text{-}PLFA = (H\text{-}PLFA * APE / at\%D_{soil})$$

where *H-PLFA* is the calculated concentration of total PLFAs in nmol H g^{-1} dry soil, $at\%D_{soil}$ depicts the soil water D-label concentration (in at%D). As the liquid soil pore water isotope signature (see Methods section 'External Data', Data obtained by Jesse Radolinski) was not stable over time, we used the average value between the respective harvest and the harvest before to account for potential temporal changes within the sampling period.
Both total PLFAs and PLFAs from label were calculated individually for all fatty acids. This allowed not only to analyze total PLFA biomass and total D-uptake as an indicator for total microbial activity, but also to disentangle microbial group specific differences in abundance and activity. The assignment of fatty acids to microbial groups (Supplement Tab. 10) is based on literature (e.g. Kaiser et al. 2010, Schnecker et al. 2012).
The same calculations were used for NLFA data.

Statstics

All statistical analyses were performed in R and RStudio (Version 2022.07.1). The plots were created using the package ggplot2 (Version 3.3.6). For the statistical analysis, linear mixed models were run using the package nlme (Version 3.1.158). As fixed factors, warming,

eCO_2, and drought were used, and to account for repeated samplings over time, plot number and harvest (0 h, 2 h, 1d, 2 d or 4 d after rewetting) were added as a random factors.
The effect of climate change factors on environmental variables, relative PLFA abundance (as proxy for microbial community composition) and D-label incorporation (as proxy for microbial activity) were displayed as a non-metric multidimensional scaling plot based on a Bray-Curtis similarity matrix. Relative fatty acid abundance was used to display species. Environmental factors (eCO_2, warming, control, future climate, control drought and future climate drought) were used to display sites. The NMDS plot was designed using the package 'vegan' (Version 2.6.2, Oksanen et al. 2013) and ggplot2 (Version 3.3.6). For the environmental factors, the scores of the sites were extracted. For the fatty acid distribution, the species scores were extracted. Additionally, a PERMANOVA (adonis2, vegan package) was performed, using the factors warming, eCO_2, drought and harvest as predictors.

Results

Microclimate during the experiment period

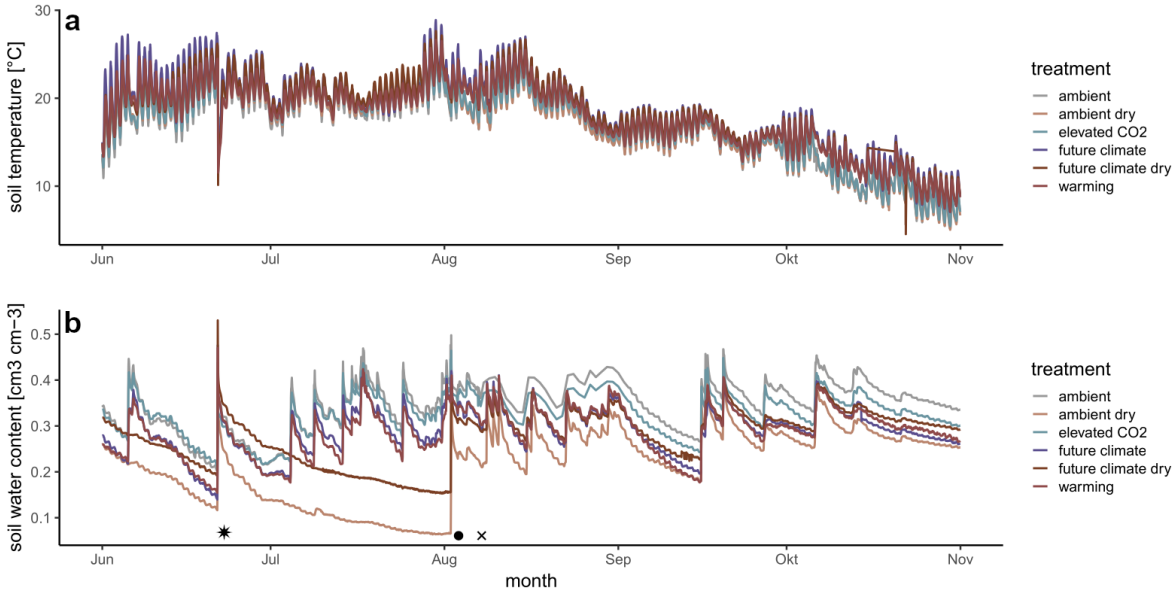

Figure 2: Soil microclimate (mean of data from 3 cm and 9 cm soil depth) from June 1st 2021 to October 31st 2021. Line colors indicate climate change treatments. a) Soil temperature in °C. (*n = 1*) b) Volumetric soil water content in cm^3 water cm^{-3} fresh soil. (*n = 1*) ✶ marks the beginning of the artificial drought period, ● rewetting, × sampling period. The data was provided by Andreas Schaumberger (AREC in Raumberg-Gumpenstein).

Soil moisture and temperature were constantly (hourly) monitored at the 'ClimGrass' field site. Here, microclimate data between June 1st 2021 (before the rainout shelters were set up) and October 31st, 2021 are shown. Soil temperature (average of soil depths 3 and 9 cm) was higher (109.1%) in all warmed plots (warmed, future and future-drought) over the course of the sampling period (August 2nd 2021 to August 6th 2021, Fig. 2a).

Plots without drought treatment showed overall higher soil moisture content over the course of the experimental period than drought treated plots. Fluctuations were caused by precipitation events. Ambient and eCO_2 plots showed similar soil moisture dynamics, while plots with increased temperature (warming and future climate) showed, relative to ambient conditions, decreased soil moisture (62.6%). The drought treated plots (both ambient and future climate) had lower soil moisture values almost immediately after the setup of the rainout shelters (marked with an ✶ in the graph), drought and future climate in combination reduced the decrease in soil moisture. The steep increase in soil moisture in early August marks the rewetting experiment (Fig. 2b).

Microbial community composition in response to climate change factors

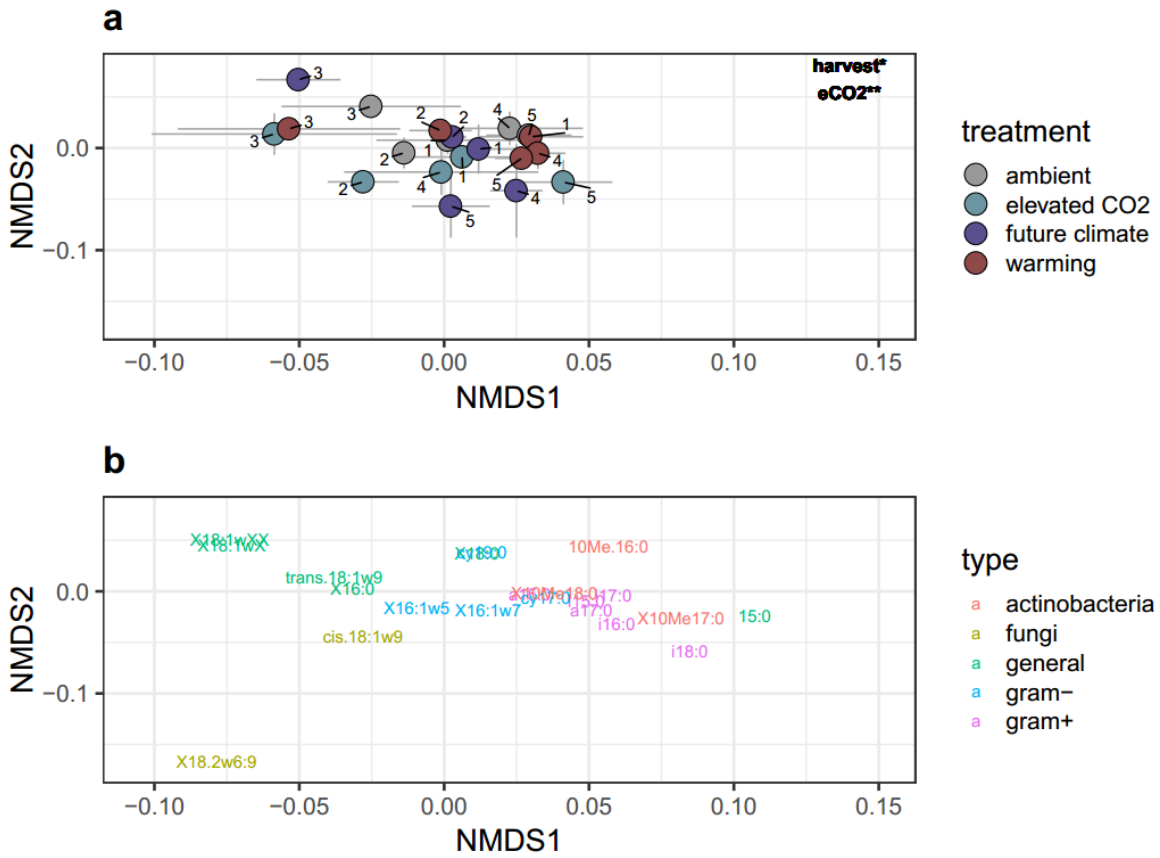

Figure 3: Effects of eCO_2 and warming treatments (individually and in combination as future climate) on the relative abundance of PLFA marker distribution (total PLFAs) displayed as NMDS plot based on a Bray-Curtis distance matrix after a simulated 40 mm rainfall experiment. a) Displays the site scores. Numbers 1-4 specify harvests (1 = 0 h (before rewetting), 2 = 2 h, 3 = 1 d, 4 = 2 d, 5 = 4 d (after rewetting)). Colors indicate climate change treatment (mean ± SE, n = 12). The effect of the treatments ambient, eCO_2, future climate and warming was evaluated using a permanova (adonis2 in vegan, *stress = 0.15*). See supplementary material for p-values. b) Species scores of PLFA markers are shown, text colors indicate the assignment to different functional groups.

The climate change treatments, both individually and independently, significantly affected soil microbial community composition. The factorial comparison of eCO_2 and warming showed that eCO_2 caused a significant shift within the soil microbial community composition (*p = 0.001,* Fig. 3a), that was driven by a higher abundance of gram negative (gram-) and fungal markers (Fig. 3b), Gram+ and actinobacterial makers were more abundant on plots with ambient CO_2 controls (+0 ppm CO_2). General markers were unaffected.

In contrast, warming had no significant influence on soil microbial community composition and there was no clear pattern in the distribution of soil microbial group markers in response to warming. No interactive effects of eCO_2 and warming were detectable. However, harvest

(time after rewetting), had a significant effect on soil community composition (*p = 0.019*). Hence, different microbial groups reacted differently to rewetting (Fig. 3).

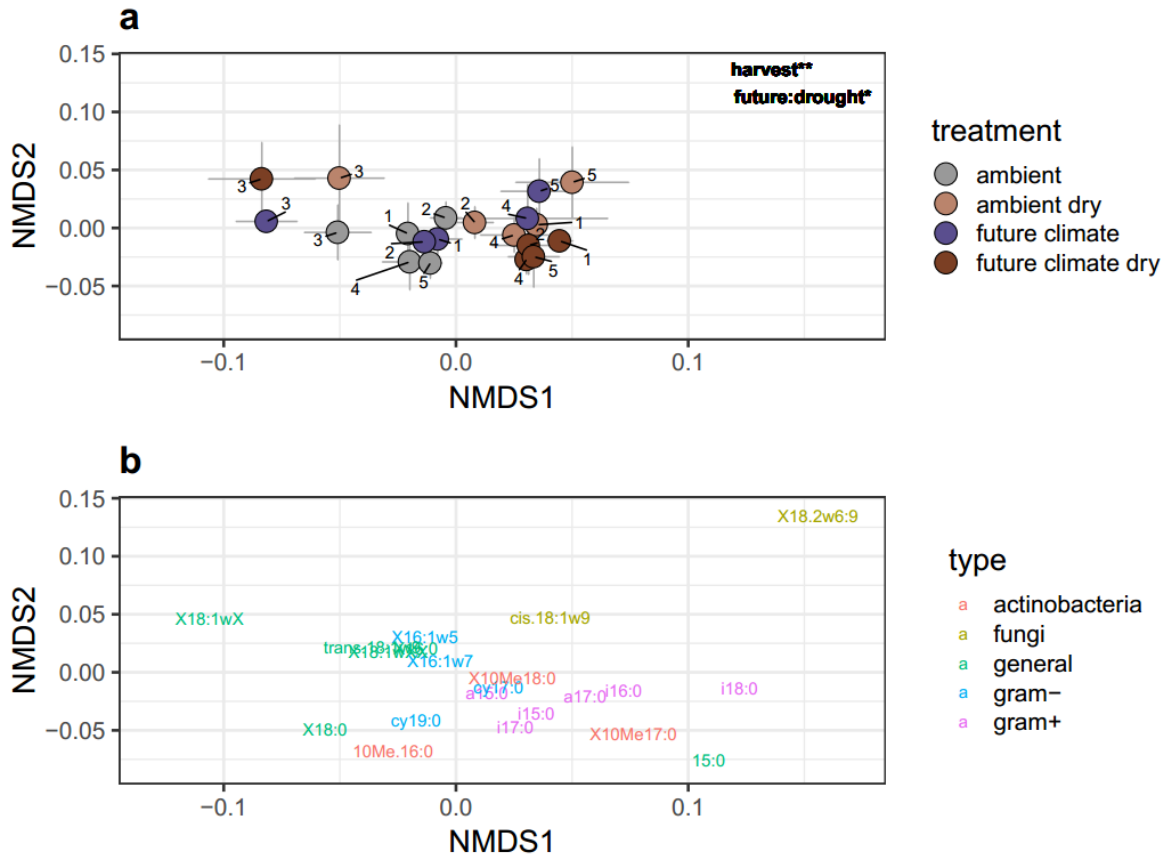

Figure 4: Effects of drought and future climate drought treatments and respective controls on the relative abundance of PLFA marker distribution (total PLFAs) displayed as NMDS plot based on a Bray-Curtis distance matrix after a simulated 40 mm rainfall experiment. a) Displays the site scores. Numbers 1-4 specify harvests (1 = 0 h (before rewetting), 2 = 2 h, 3 = 1 d, 4 = 2 d, 5 = 4 d (after rewetting)). Colors indicate climate change treatment (mean ± SE and n = 13). The effect of the treatments ambient, ambient dry, future climate and future climate dry was evaluated using a permanova (adonis2 in vegan, *stress = 0.15*). See supplementary material for p-values. b) Species scores of PLFA markers are shown, text colors indicate the assignment to different functional groups.

In addition, factorial comparisons of future climate and drought treatments were done (Fig. 4). Both, future climate and drought had a significant effect on soil community composition (*p = 0.038*), which was characterized by higher relative abundances of actinobacterial and gram+ markers, and decreased abundances of gram- and general markers in soils exposed to drought compared to non-drought treated soils. Additionally, fungal markers were more abundant in ambient climate drought plots. Future climate and ambient climate treatments with no drought had no significant influence on soil community composition. Harvest (time after rewetting) significantly changed the soil microbial community composition over the

course of the sampling period ($p = 0.001$). Data derived from samples that were collected one day after rewetting (time point 3) differs from all other harvests. Note that data focuses on drought effects after rewetting (Fig. 4).

The bacteria:fungi ratio at peak drought based on total PLFAs was significantly lower in drought treatments than in ambient ones ($p = 0.0010$).

Climate change treatment effects on total PLFA biomass

Total PLFAs significantly differed among climate change treatments. Interestingly, across all treatments total PLFAs decreased within the first two hours after rewetting and then increased again. In the factorial comparison of eCO_2 and warming (and their combination, i.e. future climate), total PLFAs were significantly higher under eCO_2 compared to +0 ppm CO_2 plots ($p = 0.001$), while warming did not have a significant effect. There were no interactive effects found, indicating that eCO_2 was the major factor increasing total PLFAs (Fig. 5a).

In the factorial comparison of drought and future climate, total PLFAs were not significantly different between drought and non-drought plots, but total PLFAs increased in response to rewetting. Additionally, future climate and future climate drought significantly influenced total PLFAs. Future climate increased total PLFAs but future climate drought did not (Fig. 5b). The strongest increase was found on eCO_2 plots (Fig 5).

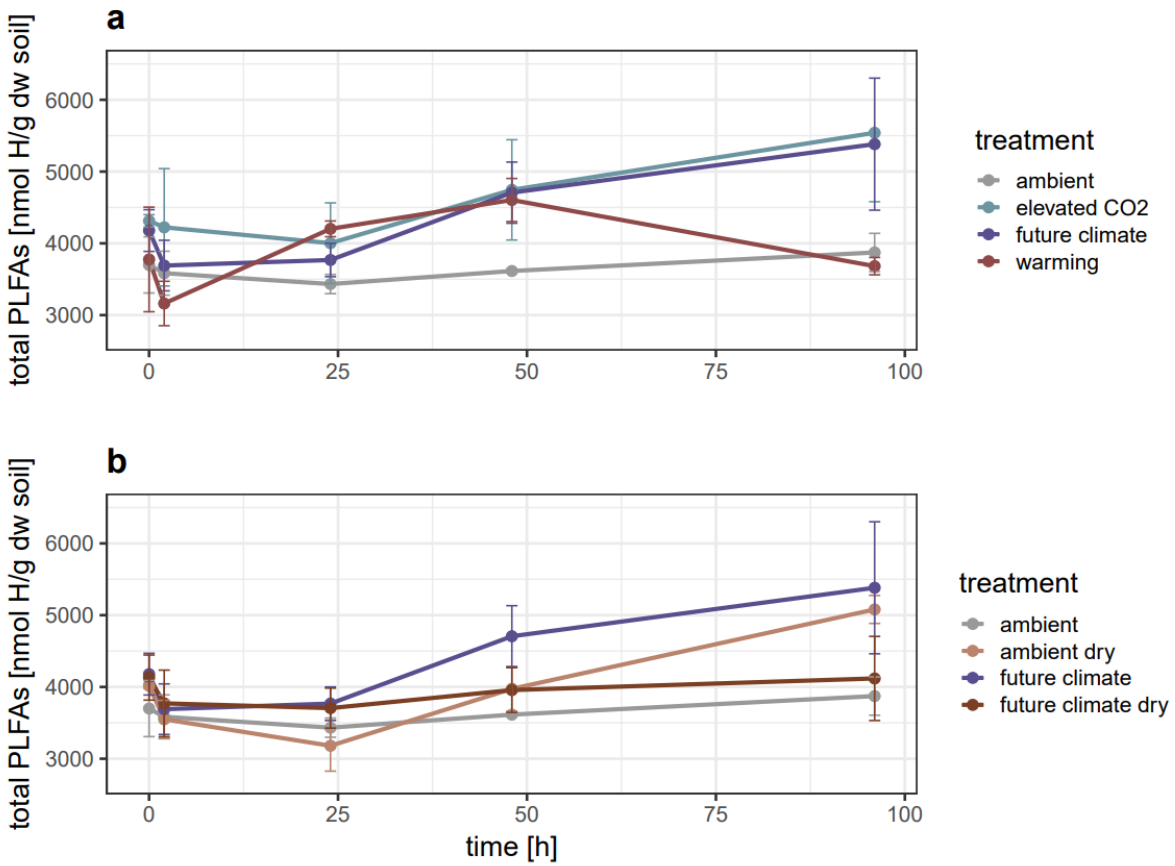

Figure 5: Total PLFAs in nmol H g^{-1} dry weight soil. Data were analyzed using a multifactorial linear mixed-effects model with harvest and Plot-ID as random factors. Colors indicate different treatments. a) The effect of the treatments ambient, eCO_2, future climate and warming on total PLFAs. b) The effect of the treatments ambient, ambient dry (drought), future climate and future climate dry (drought) on total PLFAs. (data: mean ± SE, n = 12 (a), n = 13 (b)).

Climate change treatment effects on PLFA production as a proxy for microbial growth

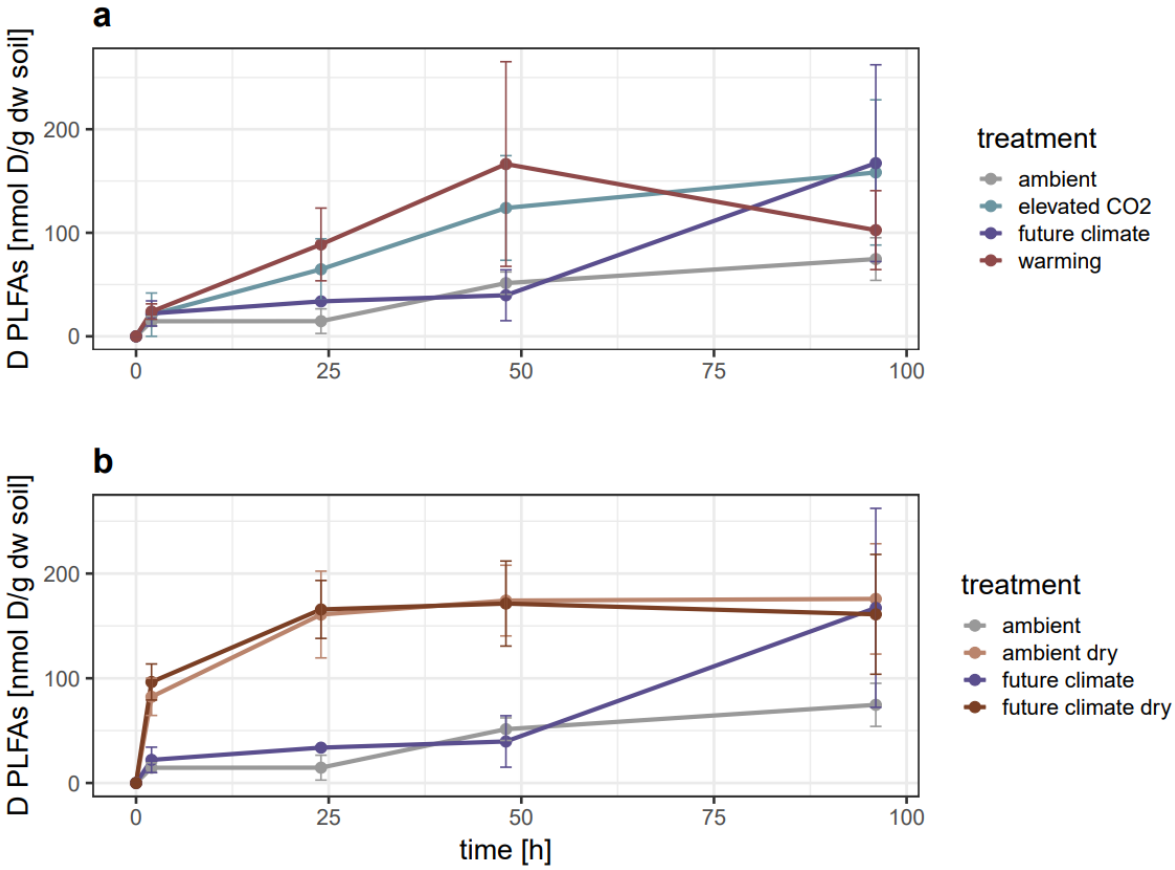

Figure 6: Total D-uptake (corrected for label concentration in the soil water) in nmol D g^{-1} dry weight soil. Data was analyzed using a multifactorial linear mixed-effects model with harvest and Plot-ID as random factors. Colors indicate different treatments. Data was corrected for label concentration in soil water. a) The effect of the treatments ambient, eCO_2, future climate and warming on D-uptake. b) The effect of the treatments ambient, ambient dry (drought), future climate and future climate dry (drought) on D-uptake data: mean ± SE, n = 12 (a), n = 13 (b)).

Climate change treatments significantly influenced D-uptake to PLFAs. It is the amount of PLFAs that have been synthesized in between time point n and time point 0 from the label added. It was corrected for soil water label concentration to account for differences in label concentration between plots (e.g. dry plots had higher D-concentration than ambient rainfall plots, see methods for calculations).

In the factorial comparison of eCO_2 and warming, both factors individually showed a strong, but not significant trend of increased microbial activity, measured as D-uptake (eCO_2: $p = 0.9669$, warming: $p = 0.7767$). The warming effect decreased three days after rewetting. No interactive effects were found (Fig. 6a).

Microbial activity significantly increased after rewetting, in particular in the drought-treated plots indicated by a fast rise in D-uptake within already 2 h after rewetting, peaking after 1

day. D-uptake remained high for 4 days (Fig. 6b). There was no significant effect of future climate drought, but microbial activity increased in future climate treated soils after about 50 h and reached similar activity levels as in previously drought treated soils. (Fig. 6b).

Elevated CO_2, warming and drought influenced different soil microbial groups differently. All groups showed significantly higher total PLFAs in eCO_2 treatments. Warming had no significant effect on total PLFAs of any soil microbial group and it did not cause interactive effects with eCO_2 (Tab. 1).
Future climate significantly increased total PLFAs of bacterial, general and gram+ markers. The interaction of future climate and drought significantly decreased total PLFAs of bacterial and gram+ markers (Tab. 2).
Actinobacterial markers showed significantly higher D-uptake in warmed plots. Significant interaction effects of warming and eCO_2 on D-uptake were found for actinobacterial, bacterial, gram+ and gram- markers. This interactive effect reduced D-uptake of these groups (Tab. 3).
Compared to ambient climate, future climate had no effect on D-uptake of different soil microbial groups (note different datasets used for tables 3 and 4). Drought significantly increased D-uptake of all groups (Tab. 4).

In addition, we analyzed the fatty acid 16:1ω5 in NLFA samples. This marker can be used as marker for arbuscular mycorrhiza. Our results showed no significant influence of any treatment on total PLFAs of, or D-uptake uptake to 16:1ω5. Hence, we can conclude that arbuscular mycorrhiza biomass and activity were unaffected by all treatments.

Table 1: Effects of warming and eCO_2 on total PLFAs of different soil microbial groups. Data was analyzed using a multifactorial linear mixed-effects model with rewetting and Plot-ID as random factors. Values of $p < 0.05$ are indicated in bold. *df* degrees of freedom, *F* F-value.

	actinobacteria			**bacteria**			**fungi**			**general**			**gram (-)**			**gram (+)**	
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
warming	1	0.08	0.778	1	0.1	0.751	1	0	0.966	1	0.05	0.824	1	0.11	0.741	1	0.94	0.337
eCO_2	1	6.19	**0.016**	1	9.98	**0.003**	1	8.34	**0.006**	1	11.38	**0.001**	1	9.72	**0.003**	1	9.71	**0.003**
warming: eCO_2	1	1.24	0.271	1	1.23	0.273	1	0.55	0.463	1	0.65	0.423	1	0.89	0.351	1	1.64	0.205

Table 2: Effects of future climate (future) and drought on total PLFAs of different soil microbial groups. Data was analyzed using a multifactorial linear mixed-effects model with rewetting and Plot-ID as random factors. Values of $p < 0.05$ are indicated in bold. *df* degrees of freedom, *F* F-value.

	actinobacteria			**bacteria**			**fungi**			**general**			**gram (-)**			**gram (+)**	
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
future	1	0.56	0.456	1	4.81	**0.032**	1	1.17	0.285	1	4.42	**0.04**	1	3.1	0.084	1	6.3	**0.015**
drought	1	0.02	0.892	1	0.69	0.41	1	1.73	0.194	1	0.78	0.381	1	2.23	0.141	1	0.02	0.89
future: drought	1	3.39	0.071	1	4.06	**0.049**	1	3.56	0.064	1	3.8	0.056	1	2.54	0.116	1	5.37	**0.024**

Table 3: Effects of warming and eCO_2 on D-uptake of different soil microbial groups. Data was analyzed using a multifactorial linear mixed-effects model with rewetting and Plot-ID as random factors. Values of $p < 0.05$ are indicated in bold. *df* degrees of freedom, *F* F-value.

	actinobacteria			**bacteria**			**fungi**			**general**			**gram (-)**			**gram (+)**	
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
warming	1	5.16	**0.027**	1	0.55	0.462	1	0.1	0.762	1	0.08	0.777	1	0.02	0.88	1	0.38	0.543
eCO_2	1	0.06	0.81	1	0.44	0.51	1	3.3	0.075	1	0	0.967	1	0.46	0.501	1	0.18	0.671
warming: eCO_2	1	4.52	**0.038**	1	0.4	**0.003**	1	3.67	0.061	1	1.5	0.226	1	10.3	**0.002**	1	4.07	**0.049**

Table 4: Effects of future and drought on D-uptake of different soil microbial groups. Data was analyzed using a multifactorial linear mixed-effects model with rewetting and Plot-ID as random factors. Values of $p < 0.05$ are indicated in bold. *df* degrees of freedom, *F* F-value.

	actinobacteria			**bacteria**			**fungi**			**general**			**gram (-)**			**gram (+)**	
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
future	1	3.34	0.073	1	0.05	0.829	1	0.78	0.382	1	0.37	0.546	1	0.03	0.855	1	0.3	0.58
drought	1	31.1	**<.0001**	1	34.1	**<.0001**	1	26.4	**<.0001**	1	33.9	**<.0001**	1	18.6	**0.0001**	1	41	**<.0001**
future: drought	1	1.11	0.296	1	0.811	0.372	1	0.13	0.725	1	0.05	0.82	1	0.48	0.489	1	0.09	0.763

Discussion

The aim of this study was to elucidate the activity of soil microbial groups in response to climate change using D-labeling and PLFA analysis under field conditions. Here, we wanted to take a step further and trace microbial activity in large-scale labeling experiment 'ClimGrass'.

Community composition

In the multifactorial climate change experiment eCO_2 increased the total amount and the amount of soil microbial group PLFAs and moreover changed the microbial community composition significantly (Fig. 3). Our findings are in line with previous studies using PLFA analysis in eCO_2 scenarios. Jin and Evans (2010) used $^{13}CO_2$ tracing and found that actinobacterial and gram+ markers were less abundant in eCO_2 treatments and biomass of general markers was higher at eCO_2. Phillips et al. (2002) found that PLFA biomass of gram- markers increased at eCO_2. In addition, fungal biomass was found to have increased at eCO_2 (Phillips et al. 2002, Jin and Evans 2010).

In contrast, we found no warming effect on total PLFAs. Simon et al. (2020) found that warming induced activity and microbial growth are only visible in autumn. As our sampling period was in August, we can conclude that a different sampling period might have led to different results. Additionally, soil microbes can adapt to long term warming (Bradford et al 2008, Rousk et al 2012). In this case, however, we suggest that, based on findings of Simon et al. (2020) warming has only a visible effect in 'ClimGrass' plots in autumn. We were able to identify a significant decrease in the bacteria:fungi ratio at peak drought in drought samples compared to controls and a decrease in PLFA biomass on drought plots. Even though the promoting effect of drought on fungi has been found in several studies (e.g. Haugwitz et al. 2014, Yuste et al. 2011, Evans and Wallenstein 2012), we could not find significant evidence that supports these findings in our study. Likewise, PLFA biomass of all analyzed soil microbial groups was unaffected by drought. In line with our results, Ren et al. (2018) found that drought has by far stronger effects on total microbial biomass than on community composition. Additionally, other studies also concluded that drought decreases soil microbial biomass (Xu et al. 2020, Hueso et al. 2012). However, Fuchslueger et al. (2016) suggest that drought does not necessarily reduce soil microbial biomass within the 'ClimGrass' experimental setup, supporting our results.

Despite that, as PLFAs are phospholipids, their biomass might not decrease directly under dry conditions as microbes become dormant (Schimel 2018). Canarini et al. (2021) concluded that the recurring summer drought of 'ClimGrass' has produced an ecological memory. This might reduce drought effects on soil microbial communities and drought legacies might have altered the soil microbial communities in the plots in our study, leading to non-detectable drought effects in PLFA data. However, Canarini et al. (2021) additionally found that recurring drought significantly alters community composition compared to plots with no drought whereas our data did not show such evidence.

Rewetting and D-labeling

The response to rewetting was strongly time-dependent. Total PLFAs decreased after rewetting in all plots, presumably because a proportion of soil microbes were killed by the sudden increase in soil moisture. After one day, however, total PLFAs increased again in all plots and even reached levels higher than before. Several research groups found similar results with soil microbial biomass derived from microbial C and N (Cmic and Nmic, Parker and Schimel 2011, Schaeffer et al. 2017, Wu and Brookes 2005) or fatty acid methyl esters (Butterly et al. 2009). All studies found decreasing biomass after rewetting of dry and moist samples. Microbial respiration and growth are being decoupled after rewetting, with respiration peaking directly after rewetting, whereas microbial growth increases after a lag phase (Leizeaga et al. 2022).

Although eCO_2 significantly increased total PLFAs, it had no statistically significant effect on D-uptake. This result is inconsistent with our hypothesis as we expected that eCO_2 would not only increase biomass, but also stimulate soil microbial activity and therefore increase new PLFA synthesis in eCO_2 plots. We suggest that rewetting might have interfered with the effects of eCO_2.

We expected a higher microbial activity due to the elevated temperature and therefore higher D-uptake. Warming increased D-uptake in actinobacteria. Frey et al. (2008) found that actinobacterial FAME-markers increased after 12 years of warming. This does not also fall in line with our results regarding the soil community composition but also supports our findings that actinobacteria on warmed plots synthesized significantly more new PLFAs (higher D-uptake) than on control plots. The interaction of warming and eCO_2 significantly increased D-uptake in bacterial markers compared to ambient controls (actinobacteria, bacteria, gram- and gram+).

We also wanted to test the response of drought plots to rewetting. In line with our hypothesis, soil microbial communities in drought treated plots incorporated more label into

PLFAs (higher D-uptake) than ambient plots (corrected for D available in soil water, see methods section). This leads to the conclusion that microorganisms took up the D to use it for biomass production as rewetting triggered the activity of dormant microbes.

Our results confirm that the increase in microbial activity after rewetting could be studied directly in the field using D-labeled water. So far, D-labeling has mainly been used to study water flow and uptake, for example to roots (e.g. Beyer et al. 2018, Hafner et al. 2017) or groundwater microbes (Taubert et al. 2018). D-labeling of soil samples is being used more and more often in science. Warren (2022) used D-labeling to monitor the synthesis of water-soluble metabolites in soil. No et al. (2022) conducted a drought experiment, using D-labeling to distinguish active and inactive cells. Brückner and Heethoff (2020) used D-labeled glucose in an experiment with mites and extracted PLFAs and NLFAs. Caro et al. (2023) used D-labeling to study soil microbial communities based on D-label uptake to PLFAs. In this study, we were able to show that added D_2O can be used in field experiments. By and large, D-labeling is a robust and well known technique in science that might have an even larger range of application than it is currently known for. D is cheap compared to other stable isotopes and even if it is not stable in DNA, it delivers robust data when it comes to PLFAs.

Summary

Based on D-labeling and PLFA-analysis, we were able to analyze soil microbial communities' reaction to altered climatic conditions (eCO_2, warming and drought) as well as rewetting after drought. Summarizing, we can outline that eCO_2 increased total PLFAs and changed community composition, but did not influence D-uptake into PLFAs. The interaction of warming and eCO_2 increased D-uptake in bacteria after rewetting. Warming did not influence community composition. It enhanced D-uptake in actinobacteria. Drought did not reduce soil microbial biomass but increased D- uptake after rewetting. It did not alter community composition.

The D-method works on a large scale, namely directly in the field, revealing new possibilities for soil science. Using this method in future soil ecological studies could therefore tackle other research questions in soil science.

Supplementary Material

Supplement table 1: Additional data to Fig. 2. Mean values of soil water content (ambient, drought) and soil temperature (ambient + 0 °C, warming + 3 °C) across the drought period (49 days).

soil water content			**soil temperature**		
	cm^3 cm^{-3}	*%*		*°C*	*%*
ambient	0.2780014	100	ambient	17.10405	100
drought	0.1740084	62.6	warming	18.34039	107.2

Supplement table 2: Additional statistical data to Fig. 3. PERMANOVA analysis of community changing effects of different factors based on total PLFAs and group specific markers. *Df* degrees of freedom, *SumOfSqs* Sum of Squares, *F* F-value. (*stress = 0.15*)

	Df	**SumOfSqs**	**R2**	**F**	**p**
harvest	4	0.11	0.16	3.04	**0.019**
warming	1	0	0.00	0.23	0.771
eCO_2	1	0.08	0.12	9.01	**0.001**
harvest: warming	4	0.02	0.04	0.79	0.564
harvest:eCO_2	4	0.04	0.06	1.16	0.334
warming:eCO_2	1	0.01	0.02	1.19	0.27
harvest: warming:eCO_2	4	0.01	0.02	0.43	0.857
Residual	40	0.36	0.556		
Total	59	0.66	1		

Supplement table 3: Additional statistical data to Fig. 4. PERMANOVA analysis of community changing effects of different factors based on total PLFAs and group specific markers. *Df* degrees of freedom, *SumOfSqs* Sum of Squares, *F* F-value. (*stress = 0.18*)

	Df	**SumOfSqs**	**R2**	**F**	**p**
harvest	4	0.14	0.22	4.45	**0.001**
future	1	0.24	0.04	3.21	0.059
drought	1	0.1	0.17	1.38	0.241
harvest: future	4	0	0.01	0.29	0.971
harvest:drought	4	0	0.01	0.28	0.978
future:drought	1	0.3	0.05	3.72	**0.038**
harvest: future:drought	4	0.05	0.08	1.52	0.174
Residual	45	0.34	0.57		
Total	64	0.6	1		

Supplement table 4: Additional statistical data to Fig. 5a. Multivariate linear mixed model of total PLFAs. To account for the repeated sampling over time, harvest was nested in Plot-ID as random factor. *numDf* numerator degrees of freedom, *denDf* denominator degrees of freedom, *F* F-value.

	numDF	**denDF**	**F**	**p**
warming	1	52	0.05	0.824
eCO_2	1	52	11.38	**0.001**
warming:eCO_2	1	52	0.65	0.423

Supplement table 5: Additional statistical data to Fig. 5b. Multivariate linear mixed model of total PLFAs. Harvest nested in Plot-ID was used as random factor. *numDf* numerator degrees of freedom, *denDf* denominator degrees of freedom, *F* F-value.

	numDF	**denDF**	**F**	**p**
future	1	57	4.42	**0.04**
drought	1	57	0.78	0.381
future:drought	1	57	3.8	**0.056**

Supplement table 6: Additional statistical data to Fig. 6a. Multivariate linear mixed model of D-uptake to PLFAs. Harvest nested in Plot-ID was used as random factor. *numDf* numerator degrees of freedom, *denDf* denominator degrees of freedom, *F* F-value.

	numDF	**denDF**	**F**	**p**
warming	1	52	0.08	0.777
eCO_2	1	52	0	0.967
warming:eCO_2	1	52	1.5	0.226

Supplement table 7: Additional statistical data to Fig. 6b. Multivariate linear mixed model of D-uptake to PLFAs. Harvest nested in Plot-ID was used as random factor. *numDf* numerator degrees of freedom, *denDf* denominator degrees of freedom, *F* F-value.

	numDF	**denDF**	**F**	**p**
future	1	57	0.37	0.546
drought	1	57	33.86	**<.0001**
future:drought	1	57	0.05	0.82

Supplement table 8: Bacteria to fungi ratio based on group specific PLFA markers at peak drought. *numDf* numerator degrees of freedom, *denDf* denominator degrees of freedom, *F* F-value.

	numDf	**denDF**	**F**	**p**
warming	1	14	0	0.9879
eCO_2	1	14	0.35	0.5633
drought	1	14	17.22	**0.0010**
warming:eCO_2	1	14	0.13	0.7241

Supplement table 9: Bacteria to fungi ratio based on group specific PLFA markers at peak drought. *b:f ratio* bacteria to fungi ratio.

treatment	**b:f ratio**	**se**
ambient	2.829818	0.094
ambient drought	2.199125	0.093
warming	2.915801	0.089
eCO_2	2.851499	0.118
future climate	2.763280	0.128
future climate dought	2.275517	0.075

Supplement Table 10: All PLFA markers and corresponding microbial groups. x indicates insecurities in the type of fatty acids (amf = arbuscular mycorrhiza).

fatty acid	**group assignment**
10Me16:0	actinobacteria
10Me17:0	actinobacteria
10Me18:0	actinobacteria
18:2ω6,9	fungi
cis18:1ω9	fungi
15:0	general
16:0	general
trans18:1 ω9	general
18:1ωX	general
18:0	general
18:1 ωXX	general
16:1ω7	gram-
16:1ω5	gram- (amf in NLFA)

cy17:0	gram-
cy19:0	gram-
i15:0	gram+
a15:0	gram+
i16:0	gram+
i17:0	gram+
a17:0	gram+
i18:0	gram+

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