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Moisture as a regulator of microbial life in soil

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Moisture as a regulator of microbial life in soil

Moisture as a regulator of microbial life in soil

Ainara Leizeaga Sanchez

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DOCTORAL DISSERTATION

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Abstract

Climate change models predict an increase in the intensity and frequency of drought periods as well as precipitation events. Moisture and its fluctuations have a large impact on soil microorganisms, which are key drivers of the terrestrial carbon (C) cycle. When there is a drought period followed by a rainfall event there is a big CO_2 release from soil to the atmosphere, which can dominate the C budget of some ecosystems. During this period, respiration and microbial growth have been shown to be transiently uncoupled. Earlier studies showed that microbial growth and respiration can respond in two different ways upon rewetting, resulting in differences in microbial carbon use efficiency (i.e., the fraction of used C allocated to growth) and resilience (i.e., the ability of microbial growth to recover to levels before the soils were disturbed). An understanding of how moisture and its fluctuations impact soil microbial communities is thus key to predict terrestrial ecosystem responses to ongoing global change.

The aim of this thesis was to understand how soil microbial communities, and the processes they regulate, are affected by moisture and moisture fluctuations. Specifically, the objectives were to understand (1) what determines the two different microbial response patterns upon rewetting, (2) how the historical conditions microbial communities have been exposed shape their responses to drought and drying and rewetting (DRW) events, and (3) the differences in responses to drought and DRW events between the two major microbial groups, bacteria and fungi.

It was found that (1) the conditions of the DRW disturbance as well as the microbial community's ability to cope with DRW could affect microbial responses to DRW. In addition, individual studies did not show that historical conditions could shape microbial drought tolerance and responses to DRW. However, when taking all the results together with other preliminary results that cover a wider climate range, (2) historical conditions that microbial communities had been exposed to were important. A history of drier condition, as well as a history of higher soil disturbance resulted in more efficient and resilient responses upon rewetting. These results might be due to either (i) microbial adjustment to better cope with disturbances or (ii) differences in resource availability and quality due to differences in climate history or aboveground community. Finally, (3) fungi tolerated drought better than bacteria, and could be equally or more resilient than bacteria after a DRW event.

In summary, to better predict how terrestrial ecosystems will respond to the increase of drought periods and precipitation events, ecosystem models should take into account that bacteria and fungi are differently affected by moisture. In addition, the harshness of the DRW disturbance as well as the previous conditions that microbial communities have been exposed to are important to determine their response to drought and DRW events as well as their carbon use efficiency.

Key words: drying-rewetting, drought, legacy, microbial carbon use efficiency, Birch effect,, microbial growth, respiration, microbial ecology

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Author Contributions

I. AM, AL, JR and EB designed the experiment. AL conducted the lab work. AM and AL analyzed the data. AM wrote the manuscript and all authors provided comments on the manuscript draft.

II. AL, AM, JR and EB designed the experiment. AL conducted the lab work. AL analyzed the data. AL wrote the manuscript and all authors provided comments on the manuscript draft.

III. AL, LCH and JR designed the experiment. CVH conducted the field work and extracted the DNA extractions. LM and AL conducted the bioinformatics analysis. AL analyzed the data. AL wrote the manuscript and all authors provided comments on the manuscript draft.

IV. EV, AT and JR conceived the study. LCH, AL and JR designed the experiment. EN and AT conducted the field work. EN, LCH and AL conducted the lab work. EN, LCH and AL analyzed the data. EN wrote the manuscript and all authors provided comments on the manuscript draft.

V. AL, LCH and JR designed the experiment. AL, LCH, JR, HS and MW conducted the field work. AL, LCH, ACB and DT conducted the lab work. CCP conducted the DNA extractions. CCP and AL conducted the bioinformatics analysis. AL analyzed the data. AL wrote the manuscript and all authors provided comments on the manuscript draft.

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Abstract

Climate change models predict an increase in the intensity and frequency of drought periods as well as precipitation events. Moisture and its fluctuations have a large impact on soil microorganisms, which are key drivers of the terrestrial carbon (C) cycle. When there is a drought period followed by a rainfall event there is a big CO_2 release from soil to the atmosphere, which can dominate the C budget of some ecosystems. During this period, respiration and microbial growth have been shown to be transiently uncoupled. Earlier studies showed that microbial growth and respiration can respond in two different ways upon rewetting, resulting in differences in microbial carbon use efficiency (i.e., the fraction of used C allocated to growth) and resilience (i.e., the ability of microbial growth to recover to levels before the soils were disturbed). An understanding of how moisture and its fluctuations impact soil microbial communities is thus key to predict terrestrial ecosystem responses to ongoing global change.

The aim of this thesis was to understand how soil microbial communities, and the processes they regulate, are affected by moisture and moisture fluctuations. Specifically, the objectives were to understand (1) what determines the two different microbial response patterns upon rewetting, (2) how the historical conditions microbial communities have been exposed shape their responses to drought and drying and rewetting (DRW) events, and (3) the differences in responses to drought and DRW events between the two major microbial groups, bacteria and fungi.

It was found that (1) the conditions of the DRW disturbance as well as the microbial community's ability to cope with DRW could affect microbial responses to DRW. In addition, individual studies did not show that historical conditions could shape microbial drought tolerance and responses to DRW. However, when taking all the results together with other preliminary results that cover a wider climate range, (2) historical conditions that microbial communities had been exposed to were important. A history of drier condition, as well as a history of higher soil disturbance resulted in more efficient and resilient responses upon rewetting. These results might be due to either (i) microbial adjustment to better cope with disturbances or (ii) differences in resource availability and quality due to differences in climate history or aboveground community. Finally, (3) fungi tolerated drought better than bacteria, and could be equally or more resilient than bacteria after a DRW event.

In summary, to better predict how terrestrial ecosystems will respond to the increase of drought periods and precipitation events, ecosystem models should take into account that bacteria and fungi are differently affected by moisture. In addition, the harshness of the DRW disturbance as well as the previous conditions that microbial communities have been exposed to are important to determine their response to drought and DRW events as well as their carbon use efficiency.

Popular science summary

Soils are full of very small inhabitants that are called soil microorganisms. As their name indicates, they are very tiny organisms that are not possible to see without the help of a microscope. There are two major groups of microorganisms: bacteria and fungi. Bacteria are small cells that live in the water that is present in the soil pores. Fungi are slightly bigger organisms that can grow by constructing networks of cells that connect soil pores. A spoon of soil can contain billions of bacterial cells and kilometres of fungal networks. These tiny organisms are crucial for decomposing organic matter that comes from plant leaves or wood. During the decomposition process, they break down the organic matter and use the obtained carbon for different purposes: growth or maintenance. If the carbon is used for growth, meaning that it is used for production of new cells, that carbon can potentially stay in the soil which increases the amount of carbon that can remain buried in the soil. In contrast, if the carbon is used to maintain processes that are happening in the microbial cells, this carbon will be respired and emitted to the atmosphere as carbon dioxide (CO_2). In fact, the CO_2 emissions from soils are six times higher than the anthropogenic emissions. Therefore, soil microbial communities are the gate keepers of carbon between soil and the atmosphere. It is thus important to understand how environmental factors affect soil microorganisms and how they use the carbon derived from organic matter. This will allow a better understanding of how soil carbon stocks will be affected by climate change.

One of the most important factors that affects soil microorganisms is soil moisture. Moisture affects soil microbes directly, where lack of moisture decreases the activity (that is, growth and respiration) of microorganisms. Microorganisms that are less affected by the lack of moisture will be more resistant or drought tolerant. Additionally, the activity of soil microorganisms is strongly affected when there is a drought period followed by a rainfall event (that is, a drying and rewetting event). The drying and rewetting of a soil causes a big release of CO_2 from soil to the atmosphere. However, soil microorganisms grow very slowly and need time to recover to the growth rates they had before being disturbed. Microorganisms have been shown to respond in different ways after drying and rewetting: they can have a resilient response where their growth recovers faster to the growth they had before they were disturbed, or a sensitive response where they will need more time to recover. Depending on the type of response, they will also use carbon differently. If they have a resilient response, more carbon will be used for growth than maintenance resulting in a higher carbon input into the soil. In contrast, if they have a more sensitive response, they will use more carbon for maintenance than for growth, resulting in higher carbon emissions to the atmosphere.

Although the importance of this CO_2 release from soils after drying and rewetting as well as the different responses of soil microorganisms to these disturbances has been recognized, there is no systematic understanding on what factors cause this difference

and how bacteria and fungi are affected by them. The general aim of this thesis was to understand how soil bacteria and fungi are affected by soil moisture. Specifically, I wanted to understand (1) the causes that explain the resilient and the sensitive response to drying and rewetting, (2) if the response of soil microorganisms to drought and drying and rewetting can be affected by the conditions they are exposed to in their natural ecosystems (for example, climate or land-use), and (3) if bacteria and fungi are affected differently by drought and drying and rewetting events.

First, I showed that bacteria had a more resilient or sensitive response depending on how harsh the drying and rewetting disturbance was. For example, if the drying was less harsh (for instance, soils were more wet before being rewetted) bacterial communities responded in a more resilient way. Bacteria also had a more resilient response if they were previously exposed to drying and rewetting cycles, which suggested that soil microorganisms can adjust to cope better with drying and rewetting disturbances and thus perceive them as less harsh.

Second, the separate studies in this thesis showed that the tolerance to drought as well as the resilience after rewetting of soil microbes were not affected by the previous conditions (that is, previous climatic or land-use history) they had been exposed to. However, taking all the studies together with preliminary results of another study that studied soils from a wide range of climates, there were reasons to think that soil microorganisms in drier climates were more drought tolerant as well as more resilient upon rewetting (that is, their growth rates faster) upon rewetting. In addition, soil microbes that had been exposed to drier conditions used carbon more efficiently upon rewetting. This would then result in less carbon emissions from soils to the atmosphere after a drought period followed by a rainfall event.

Finally, in this thesis, I also showed that bacteria and fungi were affected differently by drought and drying and rewetting events. Fungi tolerated better drought, which was probably caused by their thicker cell walls that avoid dehydration as well as their ability to form networks that allow the redistribution of water and the access to more nutrients under dry conditions. In addition, the results of this thesis showed that fungi could recover as fast or sometimes even faster than bacteria after rewetting.

In summary, this thesis provides a deeper understanding about how moisture is regulating bacteria and fungi in soil and provides new insights on how the previous conditions that microbial communities are exposed to can affect their current responses to moisture and moisture fluctuations, which can impact the amount of carbon that is released from soils to the atmosphere. These findings can help us anticipate how soil microorganisms will respond to climate change predicted drought and more intense rainfall events in different parts of the planet. Moreover, the results of this thesis could be incorporated into Earth-system models, which allow the prediction of how soils and the carbon they store will be impacted by climate change.

Resumen

En el suelo habitan unos organismos muy pequeños denominados microorganismos. Como su propio nombre indica, son organismos diminutos que no se pueden ver sin la ayuda de un microscopio y se dividen en dos grupos principales: bacterias y hongos. Las bacterias son pequeñas células que viven en el agua que está presente en los poros del suelo. Los hongos, en cambio, son organismos un poco mas grandes que pueden crecer mediante la construcción de redes de células que conectan los poros del suelo. Si cogemos una cucharada de suelo, ésta puede contener miles de millones de células bacterianas y kilómetros de redes de hongos.

Estos pequeños organismos son cruciales para descomponer la materia orgánica que proviene de las hojas o la madera de las plantas. Durante el proceso de descomposición, los microrganismos pueden utilizar el carbono obtenido de la materia orgánica con diferentes propósitos: crecimiento o mantenimiento de sus funciones celulares. Si el carbono es utilizado para crecer, es decir, para la producción de nuevas células, puede potencialmente permanecer en el suelo, lo que aumenta la cantidad de carbono que puede ser fijado en el suelo. Por el contrario, si el carbono es utilizado para mantener los procesos que están ocurriendo en las células microbianas, será respirado y se emitirá a la atmósfera en forma de dióxido de carbono (CO_2). De hecho, las emisiones de CO_2 de los suelos son seis veces más altas que las emisiones causadas por los seres humanos. Por lo tanto, los microrganismos del suelo tienen un papel muy importante en el intercambio de carbono entre el suelo y la atmósfera. Debido a esto, es importante entender cómo los factores ambientales afectan a los microorganismos del suelo y cómo éstos utilizan el carbono derivado de la materia orgánica. Este conocimiento permitirá una mejor comprensión de cómo las reservas de carbono del suelo se verán afectadas por el cambio climático.

Uno de los factores más importantes que afecta directamente a los microorganismos del suelo es la humedad del suelo. La falta de humedad, disminuye la actividad de los microorganismos, es decir, su crecimiento y respiración. Los microorganismos menos afectados por esta falta de humedad serán más resistentes o tolerantes a la sequía. Además, la actividad de los microorganismos del suelo se ve fuertemente afectada cuando hay un período de sequía seguido de un evento de lluvia (es decir, un evento de secado y humectación). El secado y la humectación de un suelo provoca una gran liberación de CO_2 del suelo a la atmósfera. Sin embargo, los microorganismos del suelo crecen muy lentamente y necesitan tiempo para recuperar la tasa de crecimiento que tenían antes de ser alterados. Se ha demostrado que los microorganismos pueden responder de dos formas diferentes después de que el suelo es secado y humectado. Por un lado, los microrganismos pueden tener una respuesta resiliente, en la que su tasa de crecimiento se recupera rápidamente al nivel de tasa de crecimiento que tenían antes de ser alterados. Por otro lado, pueden presentar una respuesta sensible en la que su tasa

de crecimiento necesitará más tiempo para recuperarse. Dependiendo del tipo de respuesta, también utilizarán el carbono de forma diferente. Si tienen una respuesta resiliente, se utilizará más carbono para el crecimiento que para el mantenimiento, lo que derivará en una mayor cantidad de carbono que puede fijarse en el suelo. Por el contrario, si tienen una respuesta mas sensible, utilizarán mas carbono para el mantenimiento que para el crecimiento, lo que derivará en mayores emisiones de carbono a la atmósfera.

Aunque se ha reconocido la importancia de esta liberación de CO_2 de los suelos después de los eventos de secado y humectación, así como las diferentes respuestas de los microorganismos del suelo a estas alteraciones, no existe un entendimiento sistemático sobre qué factores causan esta diferencia y cómo las bacterias y hongos se ven afectados por estas alteraciones. El objetivo general de esta tesis era comprender cómo las bacterias y los hongos del suelo se ven afectados por la humedad del suelo. Específicamente, se quería entender (1) las causas que explican la respuesta resiliente y sensible al secado y humectación del suelo, (2) si la respuesta de los microorganismos del suelo a la sequía y a las lluvias puede verse afectada por las condiciones a las que están expuestos en sus ecosistemas naturales (por ejemplo, a diferencias en el clima o en el uso del suelo), y (3) si las bacterias y los hongos se ven afectados de manera diferente por la sequía y los eventos de sequía seguidos de humectación.

En primer lugar, se demostró que las bacterias tienen una respuesta más resiliente o sensible dependiendo de la severidad del secado y la humectación del suelo. Por ejemplo, si el secado era menos severo (los suelos estaban algo húmedos antes de volver a humedecerse), las comunidades bacterianas respondían de una manera más resiliente. Las bacterias también presentaron una respuesta más resiliente si habían estado previamente expuestas a ciclos de secado y humectación. Estos resultados sugieren que los microorganismos del suelo pueden adaptarse para hacer frente a las alteraciones de secado y humectación y, por lo tanto, percibir estas alteraciones como menos severas o duras.

En segundo lugar, cada uno de los estudios por separado de esta tesis mostró que la tolerancia a la sequía, así como la capacidad de recuperación después de la humectación de los microorganismos del suelo, no se vieron afectadas por las condiciones previas a las que habían estado expuestos (es decir, antecedentes climáticos o de uso del suelo). Sin embargo, teniendo todo el conjunto de estudios en cuenta junto con resultados preliminares de otro estudio que abarca suelos expuestos a un amplio rango de climas, había razones para pensar que los microorganismos del suelo en climas más secos eran más tolerantes a la sequía y más resilientes a la posterior humectación del suelo. Además, se observó que los microrganismos del suelo que habían estado expuestos a condiciones más secas usaban el carbono de manera más eficiente cuando eran expuestos a un periodo de sequía seguido de la humectación del suelo. Esto, por lo tanto, implicaría que los suelos que están expuestos a condiciones más secas, producirían menores

emisiones de CO_2 a la atmósfera después de un período de sequía seguido de un evento de lluvia, que los suelos que están expuestos a condiciones más húmedas.

Finalmente, en esta tesis también se demostró que las bacterias y los hongos se vieron afectados de manera diferente por la sequía y los eventos de secado y humectación. Los hongos toleraron mejor la sequía, probablemente a causa de sus paredes celulares que son más gruesas y evitan la deshidratación, así como por su capacidad para formar redes que permiten la redistribución del agua y el acceso a más nutrientes en condiciones secas. Además, los resultados de esta tesis mostraron que los hongos pueden recuperarse tan rápido o, a veces, incluso más rápido que las bacterias después de la humectación del suelo.

En resumen, esta tesis proporciona una comprensión más profunda de cómo la humedad regula las bacterias y los hongos en el suelo. Además, proporciona nuevo conocimiento sobre cómo las condiciones previas a las que están expuestas los microorganismos del suelo pueden afectar cómo éstos responden actualmente a la sequía y a las fluctuaciones de la humedad del suelo, lo cual, a su vez, puede afectar la cantidad de carbono que se libera de los suelos a la atmósfera. Se pronostica que el cambio climático causará una mayor cantidad de periodos de sequía y fuertes lluvias y, por lo tanto, estos hallazgos pueden ayudarnos a anticipar cómo los microorganismos del suelo responderán a estos eventos en diferentes partes del planeta. Además, los resultados de esta tesis podrían ser incorporados en los modelos que permiten predecir y entender cómo los suelos y el carbono que almacenan se verán afectados por el cambio climático.

1. Introduction

1.1 Background

Terrestrial ecosystems are the second largest carbon (C) reservoir in the planet after oceans (Le Quéré et al., 2018). Soils are a dominant part of the C pool in terrestrial ecosystems; they contain more C than the atmosphere and vegetation combined (Le Quéré et al., 2018). The processing of this soil C is therefore an important part of the terrestrial C cycle, and the rate and pattern of this soil C processing is regulated by soil microorganisms. The activity of these microorganisms controls the C exchange between land and atmosphere: while their growth regulates the C input into the soil, their respiration regulates the amount of C that is released from the soil (Bardgett et al., 2008; Liang et al., 2017). Soil microorganisms, in turn, are regulated by environmental factors such as pH, moisture and temperature (Conant et al., 2011; Lauber et al., 2009; Rousk and Bååth, 2011; Schimel, 2018).

Moisture is a particularly important factor as it is essential for all living organisms, including microbes. (Schimel, 2018). Moisture determines the rates of soil biological, chemical and physiochemical processes and has a big influence on the type, abundance and growth of microorganisms (Kirchman, 2018). Coping with lack of moisture (i.e., drought) is important for microbial life. Periods of drought are very common globally since one third of the planet is covered by arid, semi-arid, or seasonally arid ecosystems (Gurevitch et al., 2002). Most terrestrial ecosystems are transiently exposed to periods of low moisture availability, and. drought and heavy rainfall events have increased during the last decade (Hartman et al., 2013; Sherwood and Fu, 2014). Moreover, Earth system models predict an intensification of the hydrological cycle, which will result in longer dry periods and a higher frequency of drying and rewetting (DRW) events (Huntington, 2006; Orth et al., 2016). Hence, there is an urgent need to understand how soil microbial communities and the processes they regulate respond to drought and DRW events. An understanding of how microbial processes are influenced by climate change induced drought will enable predictions of how terrestrial ecosystems and the C-cycle will respond to future climate scenarios.

Moisture has direct effects on microbial process rates, such as growth and respiration. As soil dries, conditions become less favourable for soil microorganisms and thus process rates decrease as water grows scarce (Davidson et al., 1998; Howard and Howard, 1993; Manzoni et al., 2012a). While direct effects of moisture are important, drastic moisture fluctuations also considerably affect soil microbes. Rewetting events after drought periods induce enormous pulses of nutrient mineralization and soil respiration known as the "Birch effect" (Birch, 1958). These events can significantly affect the ecosystem C-balance (Schimel et al., 2007) and trigger a cascade of dynamic responses where microbial respiration appears to be highly uncoupled with growth

(Göransson et al., 2013; Iovieno and Bååth, 2008; Meisner et al., 2013). Therefore, due to the multiple important effects that moisture has on microbial communities, it is a key parameter to understand how microbes function in soil. This understanding will allow better predictions of how terrestrial ecosystems and the C-cycle will respond to future climate scenarios (Schimel, 2018).

1.2 Effects of drought on soil microbial communities

When soils dry, soil microorganisms have to deal with a number of challenges. One of them is the lack of water itself as a resource, since all microorganisms require it to perform any type of metabolic activity and grow (Kirchman, 2018). Water acts as a reactant in important biological and chemical reactions, as well as a solvent of nutrients. Thus, moisture is also essential for nutrient acquisition (Schimel, 2018). In addition, lack of moisture exposes soil microorganisms to negative water potentials. The decrease in water availability causes an increase in the concentration of solutes (or even their precipitation), which in turn changes the osmotic pressure and resource availability for microbes (Tecon and Or, 2017). Finally, scarcity of water also disconnects pores impeding nutrient availability for microbes (Carson et al., 2010; Or et al., 2007b).

Soil microorganisms need strategies to deal with these challenges. First, microbes can increase their internal osmolarity by accumulating compatible solutes to equilibrate with environmental conditions (Harris, 1981; Wood, 2015). Both bacteria and fungi have been observed to accumulate such solutes (Csonka, 1989; Witteveen and Visser, 1995). The accumulation of osmolytes in soils has been studied in the last years. Early attempts found no evidence for accumulation of these compounds during drought periods (Boot et al., 2013; Kakumanu et al., 2013). However, later studies report the accumulation of osmolytes such as ectoine, hydroxyectoine and proline in dry soils (Warren, 2020, 2016, 2014). Microbial osmotic regulation has also been proposed to induce a decoupling between growth and respiration upon rewetting, generating a respiration peak immediately after rewetting (Brangarí et al., 2020). Second, it has been proposed that soil microbial communities might deal with desiccation by changing or modifying their environment. For instance, microbes can release a polymeric matrix to produce a biofilm which will reduce the drying stress (Tamaru et al., 2007). Such biofilms consist of a complex mixture of extracellular polymeric substances (EPS), which enhance hydration and transport of solutes (Or et al., 2007a). The EPS production has been suggested to be a useful strategy to retain water at low moisture levels around the microbial cells and thus delay the effects of drying (Brangarí et al., 2018; Schimel, 2018). Third, microbial communities might also shift how they allocate resources during dry periods resulting in a change in microbial carbon use efficiency (CUE) (Schimel et al., 2007). Thus, under dry conditions, one would expect that microbes would allocate less resources to growth and more to survival (e.g., synthetizing osmolytes to cope with osmotic pressure), resulting on a reduced CUE. The few studies

that have looked into the moisture dependence of CUE, have reported that CUE in dry soils can be reduced (Tiemann and Billings, 2011), maintained (Iovieno and Bååth, 2008) or increased (Canarini et al., 2020; Herron et al., 2009). Finally, microorganisms can also become dormant when the environment becomes unfavourable for them. It has been argued that entering this physiological state is an effective way of dealing with physiological and resource stress (Schimel, 2018). When microbes are dormant, their metabolism is reduced and therefore they do not have to cope with the physiological and resource stress that drought can cause. The incorporation of dormant microbes into models has shown that dormancy might be an important mechanism to not only resist desiccation but also to deal with a following wet up (Brangarí et al., 2018; Salazar et al., 2018).

The negative effect of drying and thus the decrease in activity in soil microorganisms has been captured with respiration (Xu et al., 2004a), enzyme activity (Fioretto et al., 2009), nitrogen cycling (Schimel et al., 1989) or microbial growth (Iovieno and Bååth, 2008) measurements. However, the incorporation of the moisture dependence of microbial processes into models has been difficult due to the large variation that has been found in empirical studies (Bauer et al., 2008; Sierra et al., 2015). There are different issues that make the modelling of the microbial moisture dependence difficult: (1) it is not well understood whether historical climate might constrain microbial responses to moisture (see also Section 1.4) and (2) different microbial groups have different moisture dependences (Manzoni et al., 2012a).

Bacteria and fungi are the two main decomposer groups in soils. Even though they share the function of decomposing soil organic matter (SOM), they have fundamental differences. As an example, fungi have filamentous growth (Kirchman, 2018), a wider biomass C/N ratio (De Ruiter et al., 1993), as well as a slower turnover rate than bacteria (Rousk and Bååth, 2007). In addition, fungi respond differently to abiotic factors such as pH (Rousk et al., 2009), temperature (Pietikäinen et al., 2005) and presence of heavy metals than bacteria (Rajapaksha et al., 2004). Fungi and bacteria also seem to be differently affected by lack of moisture. Fungi are generally thought to be more resistant to drought due to their capability of accumulating compatible osmoregulatory solutes, and their filamentous structure that allows redistribution of water (Guhr et al., 2015), and might increase access to spatially separated nutrients in dry soils (Brown, 1990; Magan and Lynch, 1986). However, the variability in drought tolerance that has been found among fungi is high (Manzoni et al., 2012a). The functioning of fungal based food-webs has been suggested to be generally more resistant to changes in moisture (Wardle et al., 2004) and specifically more tolerant to drought than the bacterial based food-webs (De Vries et al., 2012; de Vries and Shade, 2013; Gordon et al., 2008). A dryland survey suggested that drier conditions decrease the diversity of both bacterial and fungal communities (Maestre et al., 2015). The authors also reported that the abundance of the major fungal groups did not change with

aridity, which was consistent with another study that did not observe changes in the abundance of fungi after a summer drought (Barnard et al., 2013). Therefore, even though it is generally suggested that fungi might be favoured over bacteria in dry conditions, it remains unknown if this is a widespread and broadly generalizable pattern.

1.3 Effects of rewetting on soil microbial communities

All droughts end with an input of water into the soil (e.g., rainfall or dew events), which results in a DRW event. When there is a DRW event, a large respiration (i.e. CO_2) pulse is often observed, which can be many times higher than the basal respiration rate in a moist soil (Birch, 1958; Fierer and Schimel, 2003; Kim et al., 2012). When soil microbes, as a result of a period of drought, have been adjusted to high external osmolarity (i.e. high concentration of solutes due to the lack of water) and low matric potentials; and are hit by a sudden rainfall event, they suffer osmotic stress (Morbach and Krämer, 2002), which can kill by cell lysis more than half of the soil microbial community (Kieft et al., 1987; Van Gestel et al., 1993). Microbial cells can also adjust to the water potential shock by getting rid of intracellular osmoregulatory solutes as well as with strong enough cell walls that allow to maintain the solutes inside the cells (Harris, 1981). Any of the effects that the rewetting has on the microbial cells will have consequences for C cycling and the post-rewetting C flush during the "Birch-effect".

The mechanisms underpinning this CO_2 flux after rewetting are not entirely clear, but both abiotic and biotic mechanisms have been proposed (Brangarí et al., 2020; Kim et al., 2012). Some studies suggest that CO_2 might accumulate in dry soils due to a combination of metabolism that does not require high moisture availability and the low connectivity of pores (Liu et al., 2002). After rewetting, this CO_2 would be replaced by water which could explain the initial CO_2 emission after rewetting (abiotic mechanisms) (Marañón-Jiménez et al., 2011). However, it has been suggested that this mechanism does not significantly contribute to the "Birch effect" (Fraser et al., 2016; Inglima et al., 2009). The addition of water into a dry soil can also result in the release of a newly available C pool which is mineralized by microbial communities resulting in a CO_2 release (biotic mechanism)(Schimel, 2018). This newly available C can either be of microbial origin in form of osmolytes or necromass (Fierer and Schimel, 2003; Kieft et al., 1987; Williams and Xia, 2009; Xiang et al., 2008), or newly mobilized C due to the physical disturbance that the rewetting causes in the soil (Denef et al., 2001a, 2001b; Six et al., 2004). The CO_2 response after rewetting will most likely be a combination of both microbially derived C as well as newly presented C by aggregate disruption (Kaiser et al., 2015; Navarro-García et al., 2012). That is, to determine the origin of the C release it is important to consider, how soil microbial communities cope with DRW events, with microbial communities that are not adjusted to DRW events releasing higher amount of C upon rewetting. The physical disturbance that soils have

been previously exposed to also needs to be considered, where more disturbed soils will lead to lower CO_2 releases.

It has been shown that during the "Birch-effect" respiration and microbial growth are uncoupled (see also below)(Göransson et al., 2013; Iovieno and Bååth, 2008; Meisner et al., 2013). This is a surprising phenomenon, since one would expect that microbial growth and respiration are closely related considering that they are both regulated by the availability of C to microbes. However, after a dry soil is rewetted, while initial respiration rates immediately after rewetting are relatively high, microbial growth rates are very low (Fig. 1); which suggests a low microbial survival to the disturbance. One might wonder then: if the size of the surviving microbial population is so small and the "Birch-effect" is mainly biotic, where does the big respiration pulse come from? It seems that even though the CO_2 release is probably microbially derived, these microbes might not be fully functional (Brangarí et al., 2020). It has been suggested that a significant part of the SOM mineralization can be performed by non-cellular machinery (Kéraval et al., 2018; Maire et al., 2013). In fact, enzymatic activity and CO_2 releases have been observed in fumigated soils (Kemmitt et al., 2008; Schimel et al., 2017). When microbial cells do not survive a DRW perturbation, they can release cellular material, such as constituents of enzymatic pathways (e.g. remnant respiratory pathways) with the potential to carry out reactions that result in a CO_2 efflux (Fraser et al., 2016). Therefore, these cells would act as "zombie cells": even though they would not be able to grow and divide, what is left from the original fully functioning cells would nevertheless be able to perform enzymatic activity that could lead to C mineralization. The incorporation of "zombie cells" into models helps explain the uncoupling between growth and respiration during DRW (Brangarí et al., 2020). Taken together, there are lines of evidence that show that C mineralization during the "Birch-effect" is performed not only by intact microbial cells, but also by remnant enzymatic pathways outside cell membranes which can contribute to C mineralization and thus to the CO_2 pulse after rewetting (Fraser et al., 2016).

Rewetting a dry soil not only triggers increased resource availability and mineralization changes, but also causes major shifts in microbial biomass, community structure and growth (Barnard et al., 2020). Since the 1980's, it has been reported that DRW events have a strong influence on microbial biomass and activity (Bottner, 1985; Kieft et al., 1987; Lund and Goksøyr, 1980; Orchard and Cook, 1983). Several studies show that DRW causes a significant reduction of the microbial population biomass size, with a subsequent increase towards a pre-disturbance state level (e.g. Bottner, 1985; Gordon et al., 2008; Van Gestel et al., 1993). It has also been shown that the microbial community structure undergoes significant changes upon rewetting (Barnard et al., 2020). Shifts in the microbial community composition after DRW as indicated by phospholipid fatty acid (PLFA) analysis have been observed in different studies that suggest that the previous conditions that microorganisms have been exposed to might

be important to determine their response (Butterly et al., 2009; Fierer et al., 2003). Community composition shifts after a DRW disturbance have also been shown using molecular tools. Those studies report that different bacterial communities do not respond homogeneously to DRW disturbances. That is, it has been shown that different groups emerge at different moments after rewetting, however a general pattern has not yet be found (Barnard et al., 2015; Placella et al., 2012). The emergence upon rewetting of different microbial taxa has been suggested to explain the C dynamics during DRW.

Several studies have measured growth and respiration rates of microorganisms after DRW of soils, which allows a better understanding of the relevance and impact of soil microorganisms for the C cycle (see Section 3.2). The first attempts that measured both microbial growth and respiration rates during the course of a DRW event surprisingly revealed that microbial growth was decoupled from the respiration response during the first few days after rewetting (Göransson et al., 2013; Iovieno and Bååth, 2008). These two studies identified two types of microbial response patterns to DRW events. In the first pattern ("Type 1"; *sensu* Meisner et al. 2013), bacterial growth rate starts to increase immediately in a linear fashion until a maximum growth level, after which it decreases to growth rates of undisturbed soil. This pattern coincides with initially high respiration rates that then decrease exponentially until they reach pre-DRW respiration rate levels (Fig. 1A) (Iovieno and Bååth, 2008). In the second pattern ("Type 2"; *sensu* Meisner et al. 2013), bacterial growth after rewetting exhibits a clear lag phase with zero or very slow growth (which can last up to 30 h) followed by an exponential increase. The respiration increase is immediate and sustained, sometimes followed by a further increase synchronous with the onset of growth (Fig. 1B) (Göransson et al., 2013). These responses have implications for both the resilience (ability to recover to the pre-disturbance state) and microbial CUE of microbial communities after a DRW event. In the "Type 1" response, bacteria recover faster (i.e., higher resilience) to pre-DRW growth rate levels, with a resulting in a higher CUE (i.e., higher growth per C used). In contrast, in the "Type 2" response, bacteria need a longer time to recover to pre-DRW growth levels (i.e., lower resilience) due to the lag period without growth, which also results in a lower CUE during the disturbance. The high loss of C and the sustained respiration after rewetting in soils with a "Type 2" response has been linked to the presence of "zombie cells" (Brangarí et al., 2020). It has been proposed that microbial communities will have this type of response in environments that have not previously experienced DRW events (i.e. environments that are continuously moist) such as bogs or deep soils (Brangarí et al., 2020). However, it is not well understood what determines the two response patterns. Some studies show that the specific characteristics of the DRW event are important. Prolonged drought can change soil respiration and bacterial growth from a "Type 1" response to a "Type 2" response (Meisner et al., 2015, 2013). In addition, long term storage of soil samples (Meisner et al., 2015) as well as the combination of the drying with salt (Rath et al., 2017) have also been shown to shift

the response from a "Type 1" response to "Type 2". These results suggest that a harsher treatment (i.e., longer dry periods or the combination of drought with inhibitors such as salt) before rewetting results in a "Type 2" response with longer lag periods.

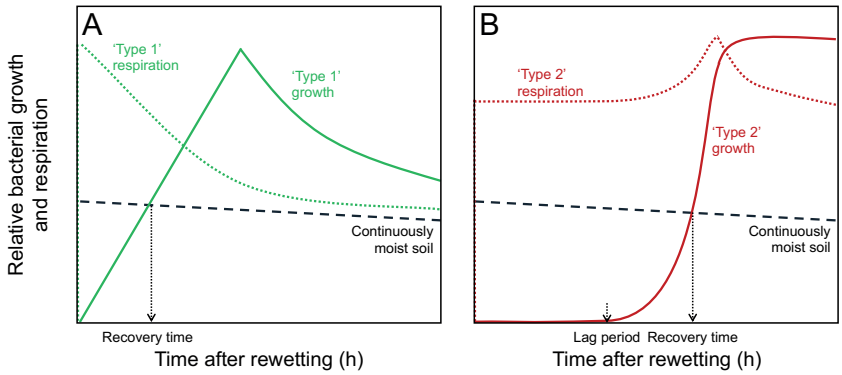

Figure 1. Schematic representation of two bacterial growth and respiration rate response patterns upon drying and rewetting: (A) "Type 1" response and (B) "Type 2" response.

Fungal growth, in the few available studies to date, has been largely unresponsive to DRW, with growth rates typically converging with, but not greatly exceeding, rates in undisturbed soils with constant moisture (Bapiri et al., 2010; Meisner et al., 2013). Besides, fungi responded homogeneously to 4 days and 1 year of air-drying, with a lack of difference in the growth dynamics upon rewetting unlike bacteria (Meisner et al., 2013). These lines of evidence are in line with studies that have explored microbial community structure dynamics and show that fungi generally appear to be more stable during DRW (Barnard et al., 2015, 2013). However, an independent study suggested that fungi can undergo more changes than bacteria (Blazewicz et al., 2014), highlighting the need to further explore fungal responses upon rewetting. So far, microbial functional as well as structural changes upon rewetting have been assessed. However, there are methodological constraints that do not allow the identification and quantification of the taxa that influence the C dynamics after rewetting.

In the last few years, there have been attempts to link soil microbial community structure with function. The use of stable isotope probing, which analyses only the DNA from microorganisms that have incorporated a tracer (usually $H_2{}^{18}O$), is a promising way of identifying the microorganisms that are actively growing (Hungate et al., 2015). This method can track changes in the population that grows during rewetting (Hungate et al., 2015), even resolving growth and mortality rates for individual taxa (Koch et al., 2018). Several studies have tried to resolve the community structure changes upon rewetting using this method. A study by Aanderud and Lennon (2011) showed that the incorporation of $H_2{}^{18}O$ to DNA coincided with CO_2 pulses

after rewetting, with changes in abundance of different microbial groups that initially were rare (Aanderud et al., 2015). Measurements with this method have also revealed high bacterial and fungal mortality and population turnover upon DRW (Blazewicz et al., 2020; Koch et al., 2018), and have reported differences in the responses of fungi and bacteria upon the disturbance (Engelhardt et al., 2018). Engelhardt et al. (2018) reported bigger changes in the abundance and growth rates of bacteria than fungi following rewetting. Thus, this approach can characterize the responding microbial taxa that are actively contributing to the biochemical cycles during DRW events. However, the application of this method has used relatively long incubation times (typically 1-7 days) which can result in missing some of the big changes that happen in microbial growth rates immediately after rewetting.

Taken together, microbial communities can respond quickly and with large changes over a short time to DRW. When a dry soil is rewetted, there are changes in biomass, microbial community composition and function. These changes can be captured by different methods. However, there are still a lot of knowledge gaps that need to be addressed such as (1) what are the causes underpinning the two microbial response patterns upon rewetting, (2) which are the taxa that contribute to the C cycles during DRW disturbances and (3) how the legacy of the environment might shape microbial responses upon rewetting.

1.4 Environmental legacy effects on soil microbial communities

Ecosystem models that include microbial control of biogeochemistry commonly use current environmental factors to predict the response of terrestrial ecosystems to future climate scenarios (Li et al., 2006). However, in the last few years it has been debated whether prior conditions that microbial communities have been exposed to can shape their response to the contemporary environment (Hawkes and Keitt, 2015; Rousk et al., 2013). That is, whether the effects of the past conditions that microbial communities have been exposed to such as moisture and temperature, as well as land-use factors, can persist when the soils are exposed to different factors in the present resulting in a legacy effect. This lasting legacy can be due to persistence of abiotic changes such as resource quality and quantity or presence/absence of aggregates. Alternatively the legacy effect may be driven by biotic changes in microbial communities that might have been shaped by the environment resulting in physiological acclimation, shifts in the community composition or evolutionary changes (Hawkes and Keitt, 2015).

Growing evidence indicates that historical climate can constrain microbial responses to the environment. For instance, differences in historical exposure to drought have been shown to result in differences in microbial respiration (Hawkes et al., 2020, 2017), enzyme activity (Averill et al., 2016), bacterial growth (Hicks et al., 2018) or microbial

community composition (Meisner et al., 2018). In addition, a legacy of drought has been shown to select for more efficient microbial communities (Göransson et al., 2013), higher survival (Veach and Zeglin, 2019) and more resilient and stress-tolerant communities (de Nijs et al., 2019; Evans and Wallenstein, 2014) upon DRW. There are, however, some studies that do not find evidence of a legacy of drought. A study across five European shrublands subjected to long-term summer drought treatments did not find drought legacy effects on microbial process rates (Rousk et al., 2013). Soils across a precipitation gradient that were later manipulated in the laboratory also showed a similar decline in respiratory and enzymatic responses to laboratory DRW disturbances regardless of the site of origin (Tiemann and Billings, 2011). Further, Cregger et al. (2012) investigated soil microbial community responses in semiarid soils from a piñon-juniper woodland with a large-scale precipitation manipulation, and showed that soil microbial composition and abundance varied with seasonal changes and tree species, but not with the exposure to different precipitation regimes (i.e. no legacy effects). These diverging results make it difficult to resolve to what extent legacy of climate can shape soil microbial communities and the functions they regulate. The contrasting observations might be explained by differences in the studied scales, soil types and other factors such as vegetation that can covary with climate.

Land-use might also have lasting legacy effects in soil microbial communities. First, differences in physiochemical factors can shape microbial communities differently in different land-uses (Jangid et al., 2011; Malik et al., 2018). Second, and probably most relevant for this thesis, differences in land-use might also have an influence on the drought history of soils and on how soil microbial communities respond to DRW disturbances (Fierer et al., 2003). In fact, differences in land-use have been suggested to shape the resistance and resilience of soil food-webs to drought (De Vries et al., 2012). To illustrate such differences a comparison between two contrasting land-uses, such as forest and cropland systems can be made (see more in **Paper V**). Forest and croplands have different plant communities which can result in very different conditions that soil microbial communities are exposed to (Osman, 2013). One of the main differences between cropland and forest soils is the quality of soil organic matter (SOM) in terms of its nutrient content or the microbial assimilability the soil C, with cropland soils having a higher quality SOM than forests soils (Woloszczyk et al., 2020). It has been shown that when soil microorganisms have access to higher quality SOM, they can also grow with a higher CUE (Manzoni, 2017; Roller and Schmidt, 2015; Silva-Sánchez et al., 2019). The temperature and moisture that soils are exposed to in forest and cropland systems can also differ within the same climate zone: forest soils are usually cooler and moister than cropland soils due the litter layer that covers the soils as well as the canopy shading. Soil structure also varies between land-uses, with cropland soils having a more compromised structure due to the continuous exposure to disturbance because of harvesting (Sharma and Aggarwal, 1984). Finally, most plants in forest soils, have deeper roots than plants than in agricultural systems (Canadell et

al., 1996), lifting up and redistributing water through upper levels of the soil horizon (Bayala and Prieto, 2020). Consequently, cropland soils have a higher history of disturbances in terms of soil structure and cycles of severe drought and rewetting, which might affect the microbial responses to drought and DRW.

Taken together, several lines of evidence suggest that the conditions that microbial communities are exposed to in their natural ecosystems, can affect their future responses to environmental factors and disturbances. Thus, there is a need to further explore legacy effects of the environment to be able to build more precise models that can better predict how terrestrial ecosystems will respond to future climate scenarios.

2. Aim and objectives of the thesis

The aim of this thesis was to understand how soil microbial communities, and the processes they regulate, are affected by moisture and moisture fluctuations. To accomplish this general aim the following objectives were set.

- Disentangle which are the causes that determine the two distinct microbial response patterns to DRW (**Paper I**, **Paper II, Paper IV**).
- Understand whether the microbial community structural and functional responses to drought and drying and rewetting are affected by the legacy of the environment (**Paper III**, **Paper IV**, **Paper V**).
- Explore the differences in the bacterial and fungal responses to drought and DRW (**Paper III**, **Paper V**).

3. Assessment of moisture effects on microbial life in soil

3.1 Study systems

I have used a combination of different study systems including microcosm experiments, field experiments as well as the assessment of an environmental gradient to understand how moisture regulates microbial life in soil (Fig. 2). This combination is useful to bridge the understanding gained using microcosm studies together with the processes that occur at the ecosystem level, and therefore provide the means to scale up the processes that are studied in the laboratory.

All approaches have shortcomings. Some might argue that microcosm experiments are oversimplified. However, the simplicity of these study systems enables high experimental control and replication (Fig. 2), which are necessary to address many questions that are challenging to answer through field studies and experiments (Stewart et al., 2013). When running a microcosm experiment, the factor of interest can be easily manipulated, in this case moisture, while keeping other factors controlled. This allows an understanding of how moisture regulates soil microorganisms in soils, with its effects being less confounded with other factors than in natural ecosystems. Microcosms are miniaturized ecosystems, where unpredictability and complexity of natural systems are taken away due to simplification of the experimental conditions. Therefore, the goal of these experiments is not to fully reproduce natural ecosystems in a laboratory model system, but rather to simplify complex ecosystems to capture how a single factor of interest modulates soil microorganisms. Besides, as our organisms of interest are soil microorganisms, due to the small size and short generation times of those, it is possible to simulate complex temporal and spatial scales within microcosms. In **Paper I** and **Paper II,** microcosm experiments were carried out where conditions prior to a DRW perturbation were manipulated in the laboratory to understand if the prior conditions of soils can influence microbial responses to DRW. In **Paper I,** the initial water content before soils were rewetted was manipulated, while in **Paper II**, soils were exposed to multiple DRW cycles to elucidate how the responses of soil microorganisms to rewetting developed over the cycles. In **Paper IV**, a similar approach to **Paper II** was used but together with a long-term field rainfall manipulation experiment.

Scaling up the relevance of the processes studied in the laboratory is also important, especially in the context of climate change. In the last decades, the rising interest in the understanding of how soils will respond to ongoing climate change has resulted in an increased number of climate manipulation field experiments. These field experiments have become a very useful tool to assess how soil microbial communities and their processes respond to environmental factors. Although it can be difficult to disentangle cause-effect relationships, field studies are important because they take into account the complexity of natural ecosystems when measuring microbial process rates (Fig. 2). In

Paper IV, a field rainfall manipulation experiment was used, where soils were exposed to summer drought for 18 years. In this case, moisture was the only environmental factor that was manipulated and therefore, whether a history of moisture reduction had a legacy effect on soil microbial communities and the processes they regulate could be assessed. In addition, in **Paper V,** a short-term field experiment where climate change driven temperature increase and drought were simulated, was used. These experiments were placed in two contrasting land-uses, which also allowed the evaluation of the impact of land-use factors in soil microbial communities.

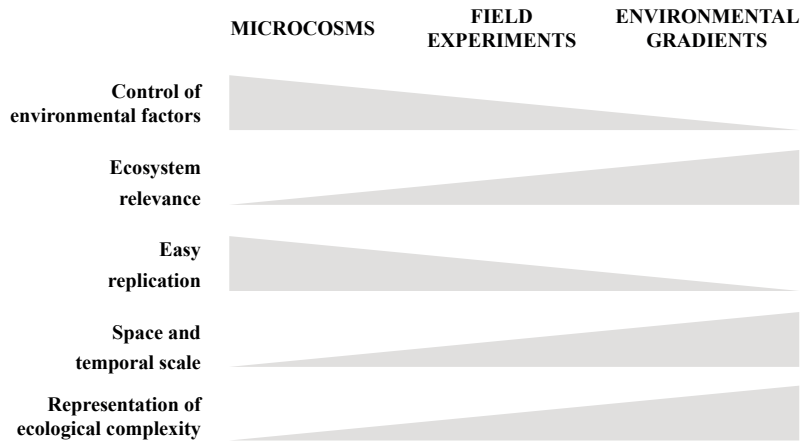

Figure 2. Schematic representation of the relevant aspects of different study systems

Another approach to assess the relationship between environmental factors and soil microbial communities is to measure microbial communities and processes across environmental gradients. When evaluating changes in microbial communities and processes across a gradient, whether historical climate modifies microbial communities in a predictable way can be determined. In **Paper III**, a natural rainfall gradient was used to determine whether exposure to different mean annual precipitation (MAP) (i.e., differences in long-term drought exposure) had a legacy effect on microbial community structure, microbial moisture dependences and responses to DRW. This rainfall gradient was located in Texas and it had a special interest because historical MAP was the most important environmental factor that varied consistently with position across this gradient (see more details in **Paper III**). The use of gradients enables verification of patters that have been observed with laboratory and field experiments, and has been argued to be useful to understand long-term ecological dynamics (Caddy-Retalic et al., 2017; Dunne et al., 2004; Elmendorf et al., 2015). Environmental gradients facilitate the study of sites where the variable of interest already varies (in **Paper III**, MAP). This is useful to look at long-term equilibrated responses to a factor of interest.

3.2 Methods

One important characteristic of a soil microbial community is its species composition. There are some methods that have historically been used to determine microbial community structure such as the analysis and comparison of phospholipid fatty acids (PLFAs) or the community-level physiological profiles (CLPPs). Even though those methods can be used to characterize the type of microorganisms that can be found in soils, this information lacks taxonomic precision. The emergence of molecular methods and their development has led to a better resolved taxonomy of the soil microbial communities. In this thesis (**Paper III** and **Paper V**), amplicon sequencing of fragments of bacterial and fungal DNA (16S for bacteria, and 18S and ITS2 for fungi) has been performed. Amplicon sequence variants (ASVs) were used to determine microbial taxa, since they have been suggested to be more precise, traceable and reproducible than operational taxonomic units (OTUs)(Callahan et al., 2017). The comparison of the obtained bacterial and fungal ASVs with existing databases, allows the identification and classification of taxa with high taxonomic precision and thus a more accurate assessment of the composition, diversity and structure of microbial communities.

Other important characteristics of soil microbial communities are their biomass and activity (Rousk and Bååth, 2011). The most frequently used method to assess how soil microorganisms are affected by environmental factors is to measure biomass (Blagodatskaya and Kuzyakov, 2013; Rath and Rousk, 2015). By measuring the biomass of soil microorganisms, one can quantify the abundance of fungi and bacteria, and thus pools of C and nutrients that can be turned over (Jenkinson et al., 1973). However, is that a relevant way of assessing how soil microorganisms are affected by changes in environmental factors? Do biomass measurements capture how soil microorganisms drive biogeochemistry? To capture how changes in environmental factors influence soil microbiota, sensitive methods that capture responses are needed. Surprisingly, microbial biomass does not respond quickly to changes in environmental conditions, since the biomass level is dependent on different processes, such as growth, death, predation and turnover (Kirchman, 2018). Besides, soil microorganisms can have different metabolic states, ranging from dead, dormant, slowly growing and fast growing, which results in different contributions to the ecosystem functioning (Blazewicz et al., 2013). In fact, it has been suggested that only 0.1-2% of microbial biomass is composed of active microorganisms (Blagodatskaya and Kuzyakov, 2013). Thus, biomass alone is likely to be a poor predictor of how microorganisms are affected by environmental changes and contribute to ecosystem functioning in complex and opaque soil systems (Rousk, 2016).

Instead, microbial process rates can be measured to evaluate the impact of environmental factors on microbial communities and understand how changes in the environment influence ecosystem functioning through soil microorganisms. One

process rate that can be measured is microbial growth, which assesses the rate at which new biomass is produced. Measuring microbial growth enables the detection of the microorganisms" reproduction and/or survival, and the susceptibility or dependence of those on environmental factors (Rousk, 2016). This makes microbial growth a metric with evolutionary and ecological relevance. The growth rates of microorganisms are very sensitive to environmental conditions, and allow the detection of rapid and subtle changes in microbial communities (Rousk and Bååth, 2011). Growth rates can be estimated by measuring the incorporation of a tracer into microbial macromolecules. Different precursors can be used in order to measure bacterial growth and fungal growth separately. In this thesis, bacterial growth has been measured tracking the incorporation of radioactively labelled ^{3}H-leucine into bacterial proteins, following a homogenization-centrifugation technique (Bååth et al., 2001). In **Paper V**, ^{3}H-thymidine incorporation into DNA was also used as a proxy for growth, to establish a conversion factor between the two bacterial growth estimates to later calculate microbial CUE (see below). In contrast, fungal growth has been determined by tracking the incorporation of ^{14}C-acetate into the fungal specific lipid ergosterol (Bååth, 2001; Rousk and Bååth, 2007).

In addition, the respiration of soil microorganisms is a very commonly used measurement to assess microbial activity in soils. Respiration can be estimated by measuring the production of CO_2 from soil. This also appealingly links the activity of soil microorganisms to the impact that they have on ecosystem functioning, since it indicates the amount of C that is released from soil to the atmosphere. If growth and respiration rates are integrated, then microbial CUE can be estimated. CUE determines how much C is used for microbial growth per unit of resource used for microbial metabolism (eq. 1).

$$CUE = \frac{Microbial\ growth}{Microbial\ growth + respiration} \quad \text{(eq.1)}$$

This is an important microbial trait since it integrates their physiology, ecology and evolutionary history (Roller and Schmidt, 2015; Rousk, 2016). Further, microbial CUE also has major implications for ecosystem functioning, since it determines whether soils might serve as sinks or as sources of C. For instance, higher values of CUE indicate higher microbial growth per respiration, which results in a higher potential of C storage in soil via microbial necromass (Liang et al., 2019, 2017). Environmental factors and disturbances caused by changes in those factors are part of the controllers of changes in CUE (Manzoni et al., 2012). Here, in **Paper III** and **Paper IV** CUE could not be determined due to the lack of suitable conversion factors for microbial growth (Rousk and Bååth, 2011). Instead, in **Paper III** the comparison between the relative respiration and growth responses across a precipitation gradient was used to determine the legacy effect of drought in the relative level of CUE. In **Paper IV**, the respiration to

growth ratio was used as a CUE index to compare relative values. In **Paper V**, however, CUE was estimated using conversion factors for growth established by Soares and Rousk (2019).

3.3 Approaches

To understand the effects of moisture and its fluctuations on soil microbial processes, different approaches can be used. The focus during this thesis has been on two different aspects: the direct effect of moisture on microbial processes and the microbial process responses to DRW events.

The direct effects of moisture on microbial process rates have long been regarded as relevant. One way to detect the direct effects of moisture on microbial communities in soils is to assess moisture dependences of soil microbial process rates (bacterial growth, fungal growth and respiration). Based on how microbial processes respond to the dry down of soil, the tolerance of the communities to drought can then be estimated. When microbial communities are exposed to desiccation, communities that can better cope with such stress will be less affected, which will result in a lower decrease of their growth and respiration rates at lower moisture levels. Community drought tolerance can be then quantified as the moisture level that is needed to inhibit activity (growth or respiration) by a certain percentage. Here, the percentages 10% and 50% were used, which are referred as IC_{10} and IC_{50} respectively (Fig. 3A). More tolerant communities to low moisture levels will show lower IC_{10} and IC_{50} values than communities that are more sensitive to drought (Fig. 3B).

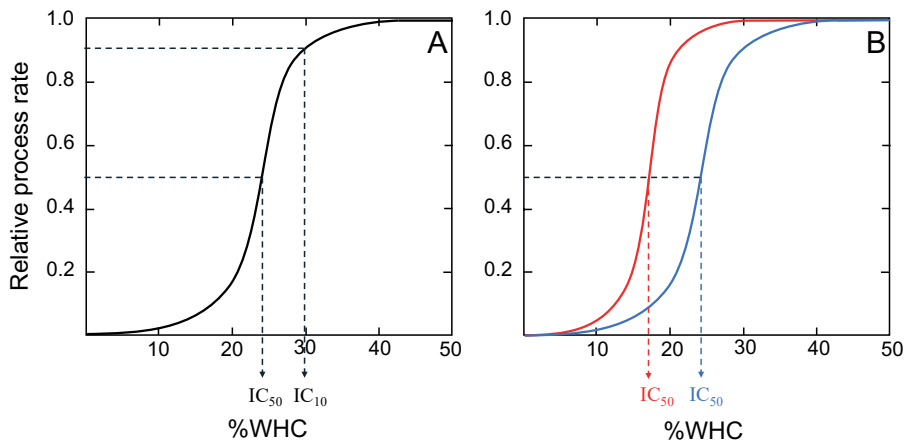

Figure 3. Example of moisture dependences of soil microbial process rates. (A) Estimation of IC_{10} and IC_{50} (moisture level at which the process rate is inhibited by 10% or 50% respectively) from a moisture dependence curve. (B) Examples of estimation of IC_{50} in two different communities, a more drought-tolerant community (red curve) and a less tolerant community (blue curve). WHC = Water holding capacity.

While direct effects of moisture effects are important, fluctuations in moisture can also dramatically affect soil microbial processes, resulting in very large changes in a small time frame of microbial growth and respiration responses. Previous studies have captured these fast dynamics monitoring microbial growth and respiration with high temporal resolution after the DRW event and identified two different type of responses (see Section 1.3). These types of assessments allow accurate determination of lag phase duration (if there is one) and recovery times to pre-disturbance state levels (Fig. 1).

If the microbial moisture dependence and responses to DRW are measured, then microbial functional stability can be assessed. The stability of the microbial community combines (1) how microbial communities and their processes withstand the decrease in water volume in soil, the separated resources and the changes in water potential during desiccation (i.e. "resistance"; *sensu* Griffiths and Philippot 2013), and (2) how microbial communities and their functions recover to a pre-disturbance state after a DRW perturbation (i.e. "resilience"; *sensu* Griffiths and Philippot 2013).

Environmental factors can also affect the composition and structure of microbial communities (Fierer and Jackson, 2006; Tedersoo et al., 2014). It has been argued that microbial community structure might drive its function (Fierer, 2017; Reed and Martiny, 2007). Thus, it is important to understand how it is regulated by environmental factors such as moisture. Here, microbial communities and their structure have been assessed using molecular methods to resolve taxonomic composition (see section 3.2). The use of molecular methods results in a large amount of information, which can be challenging to analyse and interpret in a meaningful way. One way to overcome this challenge is to aggregate estimates of diversity such as alpha and beta diversity, which inform about the variation of taxa in a single sample, and between two different samples respectively (Begon and Townsend, 2021). The use of beta diversity also allows the identification of the causes of the variation between samples. For example, one can identify whether the differences in microbial community structure correlate with environmental variables (Lauber et al., 2008). This approach has been used in **Paper III** to understand how the variation in the environmental and soil physicochemical factors across an environmental gradient might have shaped microbial community structures. However, environmental factors can be autocorrelated, which makes it difficult to disentangle how individual factors affect microbial communities, and whether this effect is direct or indirect (see more in **Paper III**). In addition, beta diversity can also be used to see if microbial communities are structured differently due to the exposure to different treatments. For example, In **Paper V**, beta diversity has been used to assess whether (1) the exposure to different field treatments and land-uses shaped microbial communities differently and (2) microbial community structure changed over the course of a DRW disturbance.

Another way to handle the large amount of information that molecular tools provide is to reduce the complexity of the information by focusing on a subset of interesting and

important taxa. Microbial communities have been described as responding very fast and with big changes upon DRW disturbance. The relative abundances of microbial taxa undergo large changes in a short time, and these changes have been suggested to be linked to their influence in C cycling (see Section 1.3). Therefore, the identification of the most responsive taxa after a DRW event can reveal what happens in terms of microbial community dynamics after a DRW event (**Paper V**). Absolute abundances of taxa are difficult to quantify and therefore when using these type of datasets, relative abundances of the taxa are assessed. This can make it difficult to establish the link between changes in the relative abundance of different taxa with their functional relevance. For instance, a taxon can appear to respond positively to DRW by increasing its relative abundance; however, it might just be that the abundance of this taxa remains stable and the increase of its relative abundance is simply due to the decrease of another taxa. Relative abundances are easier to interpret in situations where there is abundance of resources, where changes in relative increases in relative abundances of taxa will be due to the growth of those. The assumption being that no taxa are likely to decrease in abundance where resources are plenty.

An additional useful analytical approach to understand the information provided by molecular methods and provide a new perspective in microbial community ecology is the use of ecological networks. Network analysis provides a promising starting point to better understand microbial community assembly (Barberán et al., 2012). It allows the identification of potential interactions among soil microorganisms. In **Paper V**, ecological networks of microbial communities are based on co-occurrence analysis, which are based on the correlation between the relative abundances of different taxa. These networks have been suggested to represent interactions between co-existing taxa, which can give an idea about how microbial communities perceive their environment (Qiu et al., 2020; Shi et al., 2016). In addition, the use of network analysis allows the identification of keystone taxa by identifying the taxa that are most connected (i.e., taxa that change in concert) within the network. Keystone taxa have been suggested to be important to maintain the structure and function of a community (Newman, 2006). Taken together, the use of microbial networks opens up a range of possibilities to infer how microbial communities assemble under different conditions, and how these assemblies might change during and after a DRW disturbance (**Paper V**).

4. New insight on how moisture regulates microbial life in soil

4.1 Microbial carbon use efficiency is affected by moisture

The environmental control of microbial CUE is a topic that has gained much attention in the last decade (Manzoni et al., 2012b; Silva-Sánchez et al., 2019; Simon et al., 2020). While it has been widely recognized that the availability of water is a strong driver of microbial physiology (Schimel, 2018), it is not yet fully understood how microbial CUE is affected by moisture. Decreasing moisture availability is a stress for microbial communities, and thus triggers physiological acclimation strategies which have high energetic costs (Schimel et al., 2007). The changes in physiology might lead to shifts in resource allocation. Therefore, one would expect that microbes would allocate less resources to growth and more to a range of metabolic adaptation enhancing survival under lower water availability (e.g. synthetizing osmolytes to cope with osmotic pressure), resulting in a reduced CUE in dry soils (Tiemann and Billings, 2011). However, it can also be that microbial communities are selected to use the resources more efficiently when they are exposed to drought. One of the main constraints in dry conditions is the scarcity of resources (Schimel, 2018). It has been argued that when there is a scarcity of resources, the composition of life history traits of microorganisms can be selected to cope with resource limitation. This selection would then result in a more efficient growth where the uptake and use of resources have been optimized (Roller and Schmidt, 2015).

The number of studies that have addressed the moisture dependence of microbial CUE is quite limited. In this thesis, the comparison between bacterial growth and respiration at different moisture levels provides more insights on how moisture might regulate CUE (**Paper I**; **Paper III**; **Paper IV**). To compare all the studies, a respiration:bacterial growth ratio was used as an index of CUE (Fig. 4).

In these studies, contrasting results were found. **Paper I** reported a constant respiration:growth relationship (Fig. 4) consistent with previous observations (Iovieno and Bååth, 2008), suggesting that CUE was independent of moisture. **Paper III** and **Paper IV**, however, showed that CUE as indicated by respiration:growth ratio was affected by moisture (Fig. 4). The moisture dependence of CUE showed opposite directions in these studies: while CUE decreased at lower moisture levels (i.e. higher respiration:growth) in **Paper IV**, CUE increased (i.e. lower respiration:growth) at drier conditions in **Paper III** which matches other independent laboratory studies (Canarini et al., 2020; Herron et al., 2009). The two study sites represent different ecosystems with contrasting climates. On the one hand, the Dutch soils come from a heathland, with an oceanic climate with a MAP of 1005 mm $year^{-1}$ and mean annual temperature

(MAT) of 8.9°C (**Paper IV**). On the other hand, the Texan soils were sampled from Savannah grassland ecosystems with a semi-arid climate characterised by a higher evapotranspiration. Here, MAP varied from 400 mm $year^{-1}$ to 900 mm $year^{-1}$ and MAT was 19.2°C (**Paper III**). The results suggest that microbial communities in dry climates might have adjusted to use carbon more efficiently at low moisture levels. It has previously been argued that efficient growth is a trait that can be selected for in cultures that mimic complex environments like soil (Bachmann et al., 2013; Hobbie and Hobbie, 2013). Further, in **Paper IV**, the repeated DRW cycles resulted in a shift in the respiration:growth ratio in drier conditions. This indicates that the higher CUE at low moisture levels might be achieved by repeated exposure to drought. Thus, the observed differences in the drought tolerance of microbial processes in these two studies might be explained by the differences in climate. As such, microbial communities in the Texan soils (i.e., with a drier climate, **Paper III**) might have been selected to be more efficient (higher growth per respiration) when soils are dried down.

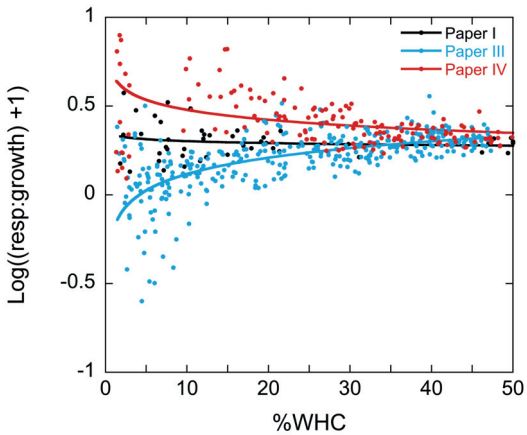

Figure 4. The moisture dependence of CUE in **Paper I**, **Paper III** and **Paper IV**. The ratio between normalised respiration:normalised bacterial growth was used as an index for CUE.

4.2 The legacy of drought might shape microbial moisture tolerance

Historical conditions that soil microbial communities are exposed to have been argued to shape their contemporary responses to environmental factors (Hawkes and Keitt, 2015). Based on this, it was hypothesized in **Paper III** and **Paper IV**, that historical exposure to drought would result in more resistant microbial communities. That is, microbial communities that had been exposed to drier conditions in the field would be less sensitive to the decrease in the moisture availability than the ones that had been exposed to higher incoming rainfall.

Contrary to the expectations, neither **Paper III** nor **Paper IV** showed differences in the drought tolerance in soils that had been exposed to historical differences in incoming

rainfall. In **Paper III**, soils across a precipitation gradient in Texas did not show differences in the IC_{10} and IC_{50} (i.e., drought tolerance) of bacterial growth, fungal growth or respiration. These observations were in line with a previous assessment of the moisture dependence of respiration rates across the gradient, which showed that soils from the historically wettest sites always had higher respiration rates regardless of the moisture they were exposed to (Hawkes et al., 2017). Similarly, in **Paper IV**, the moisture dependence of respiration and bacterial growth showed that there were no differences in the tolerance to drought of microbial processes in soils that had been exposed to an 18-year of consequent summer drought and control soils. Taken together, these results suggest that there might be a lack of adjustment of the microbial tolerance to drought driven by the historical climate, which is also consistent with findings from a meta-analysis that detected no differences in moisture dependences of microbial respiration across biomes and climates (Manzoni et al., 2012).

However, if the results of these two studies are compared, some interesting differences are observed. Dutch soils showed generally higher drought tolerance of respiration than the Texan soils, indicated by lower IC_{50} values (9% WHC vs. 23% WHC). In contrast, IC_{50} values of bacterial growth were higher for the Dutch soils, indicating a lower drought tolerance of bacterial growth than the soils from Texas (19% WHC vs. 13% WHC). As previously discussed (see Section 4.1), difference in exposure to drought in their natural environments might have selected for differences in the use of resources, resulting in respiration and growth curves with different sensitivities to lack of moisture. Thus, microbial communities that have not been adjusted to drier conditions would be more stressed by the lack of moisture resulting in a less efficient growth at low moisture levels. This would then result in growth decreasing more rapidly than respiration during the drying down of the soil in soils form wetter environments (in this case the Dutch soils, **Paper IV**).

An alternative explanation for the differences in the moisture dependence of processes in soils that proceed from different ecosystems, might be differences in physical properties of soils. Soils that proceed from different ecosystems, usually show differences in soil characteristics, which can also result in different water retention curves. For instance, soils with higher clay or SOM content increase the water retention capacity of the soil (Or et al., 2007b). Thus, the matric potential perceived by microbes at moisture contents close to air-dried in different soils might be highly variable due to soil characteristics, resulting in differences in processes even at similar moisture levels that have been measured with our methods.

4.3 Environmental legacies affect microbial community structure

The environmental control of the microbial community composition has been widely studied, and it is well known that environmental factors such as pH, temperature and

moisture are factors that influence community structure (Kirchman, 2018). The results in this thesis support the consensus that the microbial community structure is shaped by the environment they are exposed to (**Paper III**, **Paper V**). Beta diversity proved to be a responsive metric which captured the differences in microbial communities between different soils and resolved the drivers of those differences.

In **Paper III**, soil microbial communities varied across the precipitation gradient and beta diversity best correlated with MAP. However, other variables such as pH or plant productivity also emerged as correlates. The pH range was not considered to be important to constrain microbial community structure and function. Differences in MAP, however, also resulted in differences in plant productivity which probably also shaped fungal community structure due to the close association between plants and fungi (Van Der Heijden et al., 2008; Wardle et al., 2004).

In **Paper V**, in contrast, the strongest driver of microbial community composition was land-use. When the land-use effect was not taken into account, microbial communities in croplands (especially fungal communities) were also constrained by the differences in the field treatments (control, drought and warming). Taken together, these results together with previous literature show that the history of moisture regime of a soil can shape microbial community structure (Hawkes et al., 2017). However, in **Paper V** land-use factors had a stronger effect than the climate manipulation treatments, which could be due to the effect size of the field treatments. Land-use encompasses a wide range of factors that can influence microbial communities such as the aboveground plant community, different soil physicochemical factors, structure and disturbance regimes (Lauber et al., 2008; Postma-Blaauw et al., 2010). Moisture regime of the soil will also be different depending on the land-use (see Section 4.7). Thus, the fact that land-use incorporates many different factors that have previously been shown to affect microbial communities (Jangid et al., 2011; Malik et al., 2018), makes it a strong driver of microbial community structure. However, since many of these factors are autocorrelated and have not been explicitly measured in this study it remains difficult to disentangle which might be the most important factors shaping microbial communities in **Paper V**. An easy way to better understand how each one of the single factors and their interactions might drive microbial community structure is the use of microcosm experiments. Microcosm experiments would allow the manipulation of the factors of interest while controlling for possible confounding factors (see Section 3.1).

4.4 Previous history of moisture and moisture fluctuations can shape the bacterial response upon rewetting

As previously discussed, upon rewetting bacteria can respond with two different patterns (see Section 1.3). The response patterns have been linked to the severity of drying (Meisner et al., 2015; Rath et al., 2017), with a more severe drying before

rewetting resulting in a "Type 2" response (lag period followed by exponential growth, Fig. 1B). **Paper I** and **Paper II** (and **Paper IV**) add further insights about what determines the response patterns when a dry soil is rewetted.

In **Paper I**, when soils that exhibited a "Type 2" response were partially dried, a shift from a "Type 2" to a "Type 1" response was observed (Fig. 5A). This shift happened gradually, where partial drying resulted in shorter lag periods, shorter recovery times and lower maximum bacterial growth rates after rewetting. In addition, lower respiration rates were observed with more remaining moisture before rewetting. These findings demonstrated that partial drying was less severe than air-drying, indicated by (1) a higher level of bacterial growth before rewetting, and (2) a decrease in the respiration rates after rewetting with more remaining moisture in partially dried soils. Previous studies have shown that longer drying periods (Meisner et al., 2015, 2013) and the combination of drying with inhibitors such as salt (Rath et al., 2017), can change the type of response from a "Type 1" to a "Type 2". The results in **Paper I** extend these observations showing that a milder and less severe drying event can shift the response in the opposite direction: from a "Type 2" to a "Type 1". The two response patterns are therefore related the "harshness" of the disturbance and thus the survival of microorganisms after the disturbance.

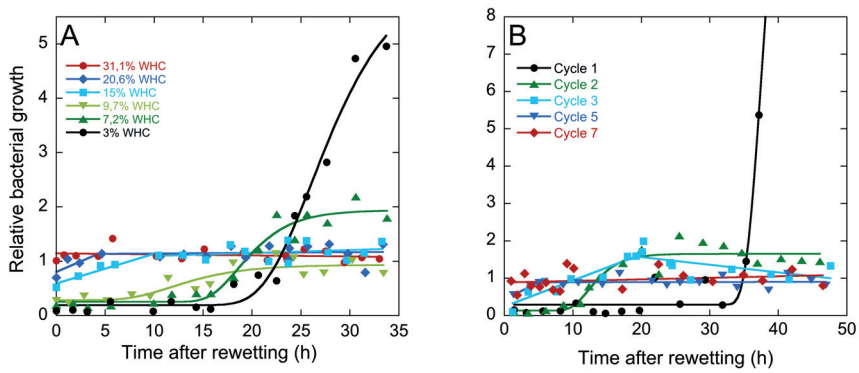

Figure 5. Bacterial growth rate responses upon rewetting with (A) increasing remaining moisture before rewetting and (B) exposure to repeated DRW cycles. See **Paper I** and **Paper II**.

When soils were exposed to repeated DRW cycles: there was a shift from a "Type 2" response to a "Type 1" response (Fig. 5B; **Paper II, Paper IV**). This shift also happened gradually (**Paper II**). However, in this case the disturbance was not changing in each cycle. That is, when soils were exposed to repeated DRW cycles, an identical physical disturbance gradually became less harsh for the bacterial community resulting in a shift towards a "Type 1" response (i.e., immediate growth after rewetting). The changes might be explained by either physiological adjustment of soil microorganisms, trait changes within populations (i.e., evolution) and/or a shift in the microbial community

composition which makes them perceive the subsequent DRW cycles as milder. This is in line with other studies that show that exposure to DRW cycles can select for microbial taxa that can better cope with subsequent such disturbances (Evans and Wallenstein, 2014, 2012).

Taken together, these lines of evidence suggest that bacterial responses to DRW are determined by the harshness of the disturbance as perceived by the microbes. In these studies, the harshness of the disturbance has been experimentally reduced by two mechanisms. First (**Paper I**), the harshness was reduced abiotically by reducing the intensity of the physical perturbation (i.e., drying soils partially before rewetting). Second (**Paper II**; **Paper IV**), the harshness was reduced by biotic mechanisms, where bacterial communities were adjusted to perceive the same physical disturbance less harshly. These results suggest that the conditions that the communities have experienced before rewetting can result in a lasting legacy effect.

Adding these observations to results from earlier studies, a generalized conceptual figure was proposed to describe how the bacterial response to DRW is determined by the harshness of the disturbance perceived by microbes (Fig. 6). This places the response patterns across a continuum rather than in a "Type 1" and "Type 2" dichotomy. In other words, the harsher the disturbance is for the bacteria, the more "Type 2" like response they will show upon rewetting. Bacteria in these soils will be more constrained by the DRW disturbance and will show longer lag periods and recovery times (further to the left in Fig. 6). In contrast, if the DRW disturbance is perceived as mild, bacteria will be less constrained by the disturbance and will be able to start increasing their growth rate faster after rewetting, resulting in shorter lag periods (with even immediate growth after rewetting), as well as shorter recovery times ("Type 1" response, further to the right in Fig. 6). Increasing harshness has been shown to occur when increasing the duration of drought and long-term storage of soil samples before rewetting (Meisner et al., 2015, 2013) as well as combining drought with inhibitors that alter osmotic conditions such as salt (Rath et al., 2017). Decreasing harshness can be due to the decrease of the severity of the drying (i.e., partial drying, **Paper I**) or by exposing soils to repeated DRW cycles (**Paper II**; **Paper IV**). In the latter case, microbial communities shift in order to better cope with the disturbance. At the high end of the "harshness scale", soil bacteria respond to DRW with a "Type 2" response and long lag periods (A in Fig. 6). These soils are predicted to move along the "harshness scale" towards the right-hand side, and shorten the lag period with milder perceived disturbances due to milder disturbances (**Paper I**) or previous exposure to DRW cycles (**Paper II**). They could also increase the lag period with harsher DRW conditions. Soils with an initial "Type 1" response after DRW (see C in Fig. 6), would be predicted to move towards the left-hand side in the harshness scale (i.e. shift towards a "Type 2" response) with prolonged droughts, long-term storage of soil samples and high concentrations of inhibitors combined with the drying (Meisner et al., 2015, 2013; Rath et al., 2017).

With exposure to repeated DRW cycles (**Paper II, Paper IV**) and partial drying (**Paper I**), these soils would exhibit the same response pattern but their recovery time to the pre-disturbance state levels would still decrease. A soil that is in the "middle of the harshness scale" (see B in Fig. 6) would be predicted to shift towards a more "Type 1" or "Type 2" response depending on the conditions prior to DRW. **Paper I** also identified a threshold moisture content above which DRW had no effect on growth (see vertical dashed line in Fig. 6).

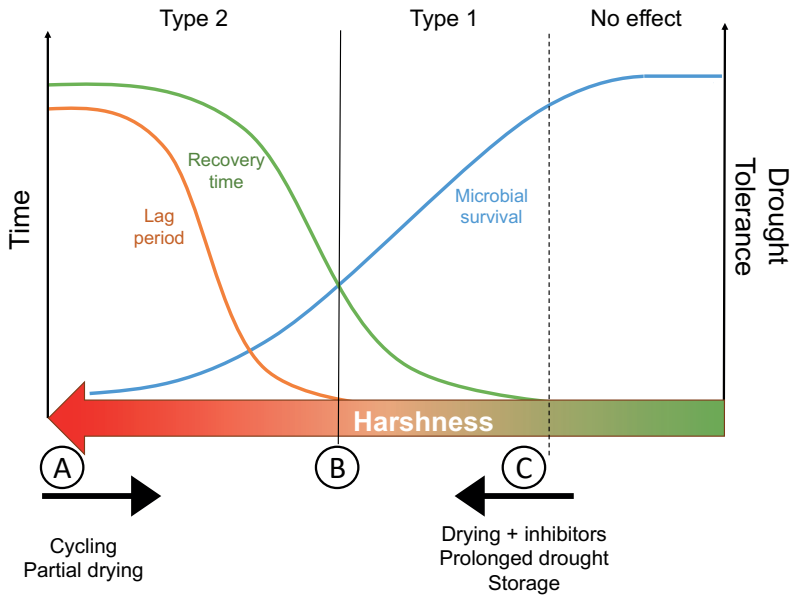

Figure 6. A schematic overview of the response patterns of bacterial growth to drying rewetting and their dependence on the harshness of the disturbance perceived by microbes. An increasing harshness is depicted in the x-axis with increasingly detrimental disturbances for the microbial communities oriented to the left-hand side. Increasing harshness can be achieved by extended duration of drought, combination of drought with inhibitors or drying to lower moisture content before rewetting. Harshness of the disturbance perceived by micorbes can be decreased by partial drying before rewetting or exposure to repeated DRW cycles. The vertical full line indicates the transition from "Type 2" to "Type 1" and the dashed line the harshness of the disturbance below which DRW has no effects in the microbial community (e.g., soils are quite wet before being rewetted, Paper I). Letters indicate microbial communities that perceive the disturbances with different harshness. See **Paper I**.

4.5 Bacteria and fungi respond differently to drought and rewetting

It is generally thought that bacteria and fungi will respond differently to drought and DRW events. Specifically, it is thought that fungi cope better with periods of drought (Evans and Wallenstein, 2012; Manzoni et al., 2012a); while bacteria recover faster to a predisturbance state when the drought period ends after rewetting (de Vries and Shade, 2013). That is, fungi are generally thought to be more resistant and bacteria more resilient.

The results of **Paper III** add to the evidence that fungi are more resistant than bacteria. When soils across the Texan precipitation gradient were dried down, fungi maintained higher growth at lower moisture levels in comparison to bacteria and they were not fully inhibited by lack of moisture (Fig. 7). These results are therefore in line with previous studies that show that fungal-based food-webs are more resistant to drought than bacterial-based food webs (De Vries et al., 2012; de Vries and Shade, 2013; Gordon et al., 2008). The higher resistance of fungi to desiccation is probably due to their thicker cell walls that can resist low matric potentials (Harris, 1981), as well as the ability to redistribute water of mycelia networks which has been proposed to enhance decomposition at low moisture levels (Guhr et al., 2015). Hence, the characteristics of fungi and their mycelia might allow them to maintain their activity and be favoured over bacteria in dry soils.

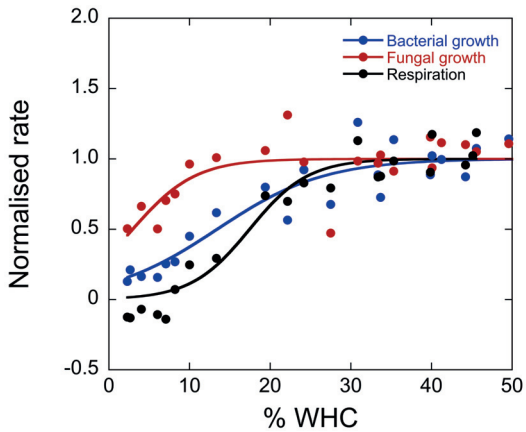

Figure 7. Example of the moisture dependence of bacterial growth, fungal growth and respiration rates in one of the soils from the Texan precipitation gradient. See **Paper III**.

Some of the work that has been done in relation to microbial resistance and resilience to disturbances, suggests that there is a trade-off between resistance and resilience (De Vries et al., 2012; Hedlund et al., 2004; Pimm, 1984). This suggestion leads to the expectation that fungi are less resilient than bacteria to a rewetting event. In line with these expectations, some studies have found that fungi are rather unresponsive in terms of changes in growth rates (Bapiri et al., 2010; Meisner et al., 2013) and co-occurrence network structure (de Vries et al., 2018). However, a recent study shows that fungal growth can be very responsive to DRW events, and that the changes in their growth rates are constrained by bacterial growth after rewetting (Hicks et al., 2019). In that study, bacterial growth was experimentally eliminated using the bactericide bronopol. The inhibition of bacterial growth led to increased fungal growth after rewetting, which indicated a competitive release of fungal growth. In addition, this study also showed that fungi could recover their growth rates faster than bacteria in "Type 2" soils (i.e.,

bacterial growth undergoes a lag period after rewetting), suggesting a higher resilience of fungi at least in these soils.

In **Paper III**, bacteria and fungi showed a similar recovery rate after rewetting. However, fungi did not fully recover to pre-disturbance growth rate levels, probably due to their competition with bacteria which has been shown to occur in stable moisture conditions (Rousk et al., 2010) as well as upon rewetting (Hicks et al., 2019). In **Paper V**, fungi always recovered their growth rates faster than bacteria (Fig.8). This faster recovery after rewetting might be due to their ability to better withstand drought periods due to their thicker cell walls and their ability to redistribute water (Guhr et al., 2015; Harris, 1981). These characteristics might also allow them to better cope with the osmotic shock after rewetting, resulting in a more rapid increase of growth rates than bacteria. It was also observed in **Paper V** that the fungal growth and bacterial growth reached their maximum growth at different time periods (Fig. 8). In fact, when bacterial growth reached its maximum growth fungal growth had decreased, giving further support to the idea of competition between bacteria and fungi upon rewetting (Hicks et al., 2019).

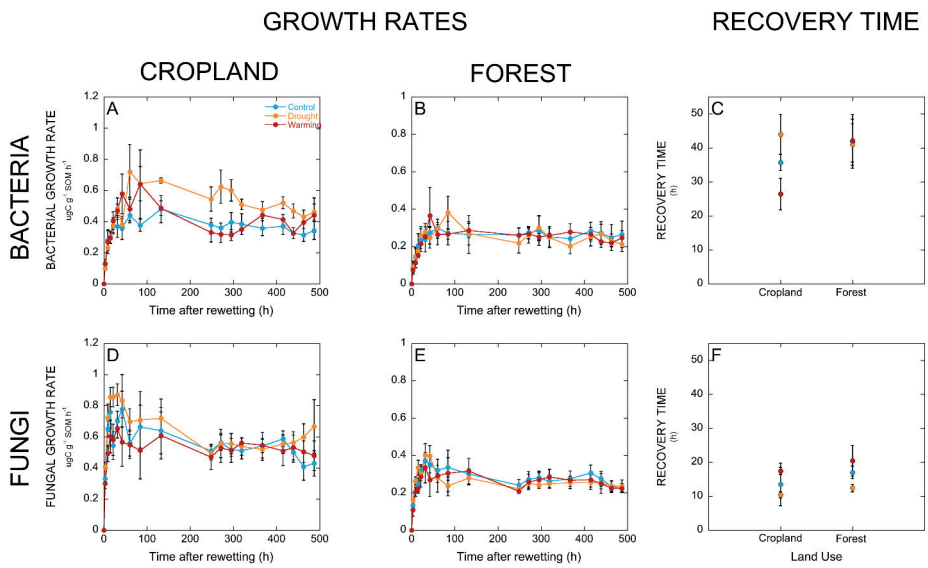

Figure 8. Bacterial and fungal growth dynamics after a DRW disturbance in the laboratory, as well as the recovery times for bacterial and fungal growth to reach levels of growth in continuously moist soils. Bacterial growth rates during 3 weeks after rewetting in soils from (A) cropland and (B) forest, and their (C) recovery times. Fungal growth rates during 3 weeks after rewetting in soils from (D) cropland and (E) forest, and their (F) recovery times. The measurements in the control plots are shown in blue, in the drought plots in orange and in the warming plots in red. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). See **Paper V**.

These results are partly consistent with a study that investigated the growth and death of bacterial and fungal communities in aa annual grassland soil from California. They

found that fungi could increase their growth rates after rewetting similarly to bacteria (Blazewicz et al., 2014). Fungi, therefore, not only are resistant but can also show a resilient response upon rewetting, quickly recovering their growth rates to a pre-disturbance state.

4.6 The legacy of drought can shape microbial resilience upon rewetting

It has been argued that legacy of drought can have an impact on microbial functions (Hawkes and Keitt, 2015). However, when focusing specifically on the effect of drought legacy, it has been shown that microbial response patterns are not affected by the legacy of drought. A legacy of drought did not change the bacterial growth dynamics after rewetting in a set of soil from Belgium and the UK (Göransson et al., 2013; Rahman et al., 2018). Thus, it still remains unresolved whether historical environmental conditions that microbial communities have been exposed to can shape microbial resilience upon rewetting.

In **Paper III**, **IV** and **V**, the legacies of drought on microbial responses to DRW were investigated. The legacies of drought across a rainfall gradient in Texas did not change the microbial responses upon rewetting (**Paper III**). Bacteria and fungi increased their growth rate immediately without delay after rewetting. Respiration rates also increased immediately, which was followed by an exponential decrease to pre-disturbance respiration rate levels, a typical "Type 1" response. This set of dynamics was similar in all studied soils across the gradient. However, the differences in rainfall regime resulted in a tendency for faster recovery time of microbial growth in historically drier sites. In line with these findings, in **Paper V**, a legacy of 1.5 years of drought did not change microbial responses to drought in subtropical soils either. As in the Texan soils, microbial communities from all the studied soils exposed to different field treatments and land-uses responded with a "Type 1" response. Further, in the Ethiopian soils, a legacy of drought induced a faster recovery of fungal growth. Finally, in **Paper IV**, soils from a temperate heathland in the Netherlands were exposed to 18-years of summer drought. Upon rewetting, both control and drought exposed soils exhibited a "Type 2" response. However, soils exposed to 18-years summer drought showed a shorter lag period of bacterial growth after rewetting, which resulted in a shorter recovery time to growth levels before the DRW disturbance. These findings indicate that the exposure to drought might select for microbial communities with higher resilience (i.e., they recover faster) after a DRW disturbance.

It has been argued that previous exposure to a disturbances can shape the resilience of microbial communities to a subsequent disturbance (Griffiths and Philippot, 2013). For example, it has been observed that when soil microbial communities are exposed to a higher frequency of DRW events, a selection for taxa that can better cope with such disturbances (i.e., stress-tolerant taxa) can occur (Evans and Wallenstein, 2014,

2012). A set of laboratory studies also showed that exposure to repeated DRW cycles, select for both bacterial (**Paper II**; **Paper IV**) and fungal (Hick et al. *under review*) communities that recover their growth rates faster to undisturbed soil growth levels. Conversely, when soils were exposed to long periods of drought or constant moisture without any DRW events, bacteria underwent a lag period after rewetting before they increased their growth rate exponentially (a "Type 2" response) (Meisner et al., 2015, 2013). These lines of evidence suggest the previous exposure to DRW events might select for microbial communities that can better cope with such perturbations, perceive the disturbance as a "milder" disturbance and recover more quickly to pre-disturbance growth rate levels (**Paper I**). Therefore, (1) the tendency for a shorter recovery time in historically drier sites in **Paper III**, (2) shorter lag time in **Paper IV**, and (3) the shorter recovery time of fungal growth in drought exposed soils are likely a consequence of microbial communities that have been shaped by the legacy of drought to experience the DRW events as less harsh disturbance.

One of the remarkable results found in these studies is that unlike in **Paper IV**, soils exposed to different precipitation regimes in **Paper III** and **Paper V** generally did not show large differences in the microbial responses to DRW and their resilience. The studied soils in **Paper III** and **Paper V**, are periodically dry environments (Savannah grassland ecosystems in Texas and cropland and forest ecosystems in Ethiopia). Thus, despite the differences in incoming rainfall due to differences in the position across the precipitation gradient (**Paper III**) or to the presence of rain shelters (**Paper V**), the microbial communities did not differ in the response to DRW. The lack of differences in the response to DRW suggests that differences in the environmental gradient or the field treatments might have not been enough to induce a change in the microbial response to DRW. The "Type 1" responses observed in these studies has previously been linked to bacterial communities that show a higher survival after rewetting (Meisner et al., 2015, 2013) and experience DRW as a mild disturbance (**Paper I**, see Section 4.4). Thus, the observed similarity suggests that the historical exposure to frequent DRW events in all soils due to the characteristics of the rather arid ecosystems, have most likely selected for microbial communities that can recover quickly after DRW disturbances (**Paper I**). Taking these results together with previous studies, I suggest that microbial communities exposed to drier climates show a more resilient "Type 1" response (**Paper III**; **Paper V**), than microbial communities exposed to wetter climates that show a "Type 2" response with a lag period (Göransson et al., 2013; Rahman et al., 2018; **Paper IV**). However, it is unknown if this is a generalizable pattern and whether the type of response upon rewetting might be also determined by other soil physiochemical factors.

With the aim of extending these observations, a study across a European climate transect was performed, where 40 soils across a Europe representing a gradient of climates (from N Sweden to S Greece) and a wide range of soil properties were exposed

to a standardized DRW disturbance in the laboratory. Preliminary results suggest that there was a predominance of "Type 1" (i.e., more resilient) responses in wetter climates, whereas a higher number of "Type 2" (i.e., less resilient) responses were observed in the drier climates (Fig. 9A). Thus, the resilience of microbial communities was higher in drier climates indicated by shorter recovery times (Fig. 9A). These findings give further evidence that exposure to different climates might shape the microbial response upon rewetting and therefore their resilience (as previously discussed). While the recovery time of bacterial growth could be explained by climate, it was also strongly correlated with soil pH and %SOM (Figs. 9B, 9C). However, the resilience of the bacterial response could not be explained by the clay content of the soil, as indicated by the lack of correlation between clay content and recovery time (Fig. 9D).

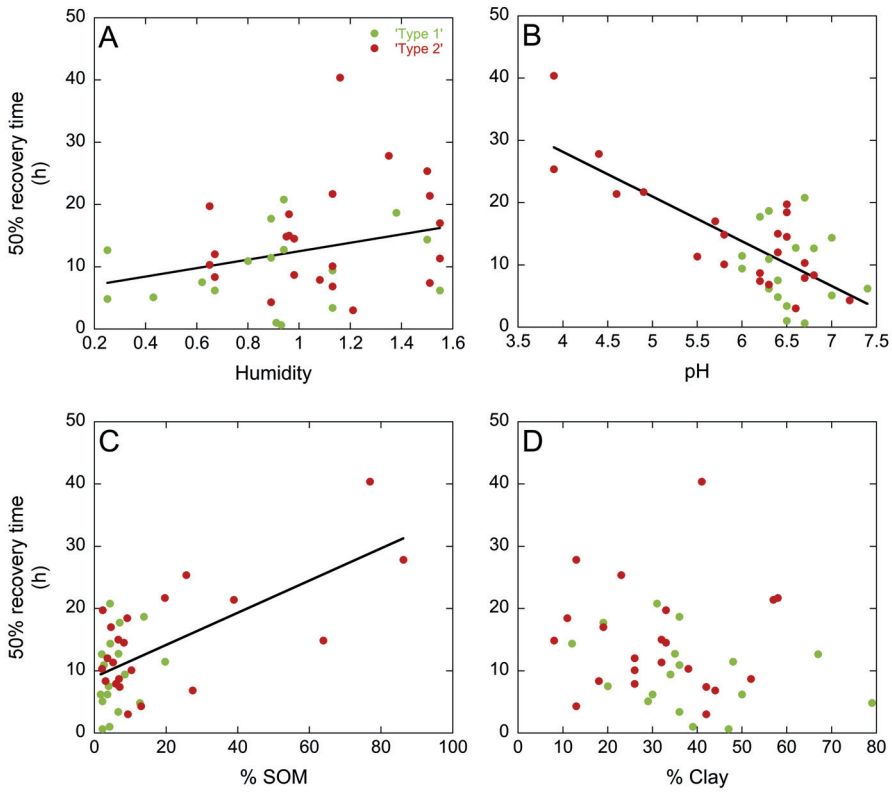

Figure 9. Bacterial growth recovery time (used as an index for resilience) to 50% of the growth rate levels in undisturbed soils in relation to the (A) humidity of the ecosystem where the soils came from (B) pH of the soils, (C) % SOM of soils and (D) % Clay. The humidity index was calculated as the product of mean annual precipitation and mean annual potential evapotranspiration. A lower humidity index indicates a dryer climate and a higher humidity index indicated a wetter climate. (Winterfeldt, 2020).

The recovery time of bacterial growth decreased with higher pH and increased with higher SOM content (Figs. 9B, 9C). In pH ranges lower than 5 only bacterial "Type 2" responses were found, usually linked to a very responsive fungal growth responses upon rewetting previously described by Hicks et al. (2019) (see Section 4.5). It is well known that soil pH strongly affects microbial community composition (Lauber et al., 2009) and function (Rousk et al., 2010). Bacteria are strongly inhibited by low pH (Rousk et al., 2009), which might explain the lag period after rewetting, as well as the more responsive fungal growth. SOM was also strongly correlated with the recovery time (Fig. 9C). It has been argued that soil with higher SOM contents can hold more water (Schimel, 2018), which might result in microbial communities being less exposed to dry periods in these soils. This would then result in bacterial communities that would perceive the DRW disturbance as "harsh" and undergo a lag period after rewetting (see Section 4.4). Soil pH and SOM content are also strongly autocorrelated: low pH soils usually have high SOM contents due to the low decomposition rates. Thus, the strong relationship between pH and SOM with bacterial recovery time after rewetting might be a result of either of the two posed explanations or a combination of them.

4.7 Environmental legacies affect microbial carbon use efficiency upon rewetting

It has been argued that in some ecosystems, the C dynamics that occur during the "Birch-effect" can dominate the C releases from soils to the atmosphere (Schimel, 2018; Xu et al., 2004b). Thus, microbial responses upon rewetting have implications for the microbial C budget. For instance, when microbial communities present a "Type 1" response, a relatively higher amount of resources are used for growth than respiration than when there is a "Type 2" response. The differences in the resource use upon rewetting result in higher C losses upon rewetting in "Type 2" responses that "Type 1" responses. To understand the implications of the observed microbial responses upon rewetting for the C budget of the studied ecosystems, CUE can be determined as the proportion of C used for growth relative to the total amount of C used (see Section 3.2).

In **Paper III**, cumulative microbial growth upon rewetting did not change across the gradient. In contrast, cumulative respiration upon rewetting changed across the gradient, with higher cumulative respiration in historically wetter sites. These results suggested a shift in the microbial CUE upon rewetting across the gradient, where microbial communities in historically drier sites in the precipitation gradient use the C more efficiently (i.e., a higher proportion of C was used for growth) after a DRW perturbation. In line with these results, in **Paper IV**, cumulative respiration to growth ratio 24h after rewetting was lower in drought-treated soil, suggesting again a drought legacy effect in the microbial CUE upon rewetting. However, unlike in **Paper III**, these differences between field treatments were not maintained during the whole study

period, suggesting that the field drought legacy effect on microbial functions was transient. An earlier study also found evidence for an enhanced CUE upon rewetting in soils that had been exposed to drought compared to control soils, which was indicated by a lower respiration per growth ratio (Göransson et al., 2013). The results of these studies thus suggest that soils that have been previously exposed to drought can release less C to the atmosphere upon rewetting.

In **Paper V**, microbial CUE upon rewetting showed a considerable variation between land-uses. Microbial communities in the cropland-used a C more efficiently after rewetting than microbial communities in forest soils. The land-use effect was already obvious 24h after rewetting and it became even larger 3 weeks after rewetting. In this study, the drought and warming treatment did not have an effect on the microbial CUE 24h after rewetting, whereas 3 weeks after rewetting the differences in CUE between treatments became apparent. This suggests that even though the legacy of the field treatments caused variation in the steady-state rates (i.e., stable process rates over time) of microbial CUE, it did not cause variation in the CUE immediately after rewetting.

Thus, **Paper III**, **Paper IV** and **Paper V** indicate that the legacy of the environment can shape how microbial communities use C upon rewetting. There are two main aspects that can be important to explain the observed differences in CUE. On the one hand, the differences can be explained with differences in perturbation history. That is, a DRW disturbance might be more stressful for microbial communities that have not been previously exposed to a high moisture variability (Kieft et al., 1987; Van Gestel et al., 1993; Veach and Zeglin, 2019), which can then result in lower CUE (Schimel et al., 2007). Soils in **Paper III** and **Paper IV** were explicitly chosen to differ in their history of drought. In addition, differences in land-use can also result in differences in the history of moisture variability. Forest soils are usually less exposed to frequent DRW disturbances due to their canopy layer and their root systems that can lift up and redistribute water (see more in **Paper V**). Thus, microbial communities that have not been as exposed to DRW disturbances (historically wetter soils in **Paper III**, control soils in **Paper IV** and forest soils in **Paper V**), probably had a lower physiological readiness to cope with such disturbances, resulting in a higher proportion of C respired upon rewetting.

On the other hand, legacy effects in C mineralization and thus CUE might also be caused by differences in C availability. Drought is also known to affect plants and their productivity (Bréda et al., 2006; Doughty et al., 2015; Zhao and Running, 2010), which can in turn determine the C availability to microbes during DRW events (Barnard et al., 2020). Thus, differences in CUE upon rewetting in **Paper III** and **Paper IV** might be caused by an indirect effect of drought via plants. One fundamental difference between cropland and forests is the variation in their aboveground plant communities which results in differences in C input into the soil. Croplands have been

suggested to have a higher quality of SOM (Woloszczyk et al., 2020), which can then result in higher CUE of microbial communities (Manzoni, 2017; Roller and Schmidt, 2015; Silva-Sánchez et al., 2019). This variation in amount and quality of SOM might then explain the difference in CUE upon rewetting in soils with different histories of drought and in soils with contrasting land-uses (i.e., cropland and forest soils).

4.8 Microbial community structure changes after rewetting

A number of studies have found that the microbial community structure is very responsive to rainfall events after drought periods (e.g. de Vries et al., 2018; Fierer et al., 2003). However, is not well understood (1) which are the taxa that drive these changes over time and (2) how microbial communities assemble after a DRW disturbance. In **Paper V**, these questions were addressed by (1) the identification of the most responsive taxa upon rewetting, and (2) following microbial community networks upon rewetting.

It was found that microbial community structure responded differently to DRW in cropland and forest soils. Microbial community structure changed over the course of a DRW disturbance in the cropland soils, and slowly returned to its original structure in line with previous findings (Jangid et al., 2011). In contrast, microbial community structure did not change in forest soils. These differences were also observed for the number of responsive taxa identified in each land-use: a higher number of responsive taxa upon rewetting could be identified in the cropland soils compared to the forest soils (approximately 20 vs. 3, in each treatment). Differences in microbial community structure changes upon rewetting have previously been linked to a pre-adjustment of communities to DRW due to the moisture fluctuations that they have been exposed to in the natural ecosystems (Fierer et al., 2003). Thus, these results suggest that microbial communities in the forest soils might be preadjusted to better cope with DRW disturbances. However, this contrasts with bacterial and fungal process responses to DRW in the two land-uses (see Section 4.7).

Responsive taxa could only be identified within bacterial communities and mostly in the cropland soils, as already mentioned. The identified responsive taxa supported the idea of a differential resuscitation of bacteria after a DRW disturbance (Blazewicz et al., 2020; Placella et al., 2012). The response patterns of the responsive taxa also suggested that the response strategy might be phylogenetically conserved, that is, the traits that are related to the response upon rewetting might be conversed within bacteria *Phyla* (Placella et al., 2012). The patterns of the changes in the relative abundances of the responsive taxa coincided with a study that suggested that bacteria can be classified in 3 groups based on their response to rewetting: rapid responders (within 1 h after rewetting), intermediate responders (between 1 and 24 h after rewetting) and delayed responders (24-72 h after rewetting)(Placella et al., 2012). The responsive taxa in

control and drought soils summed up to 13% of the total community, whereas the in the warmed soils the abundance of the responsive taxa only represented 5% of the total community. Several studies have suggested that changes in the relative abundance of different taxa upon rewetting might be relevant for ecosystem function (Aanderud et al., 2015; Barnard et al., 2013; Placella et al., 2012). The relative abundances of the responsive taxa are lower than in these studies, which makes difficult to think that only these taxa will drive the community growth or respiration, and thus affect the C-cycle of the ecosystem.

With the network co-occurrence analyses, it was found that the bacterial assemblages formed in the cropland soils were significantly larger than those formed in the forest soils (Fig. 10). In addition, bacterial assemblages developed over time after rewetting and increased complexity peaking at different times after rewetting in cropland and forests soils. Resource and availability have previously been described as drivers of network structure. For example, increase in resource availability in soils due to the addition of glucose (Qiu et al., 2020) or rhizosphere input (Shi et al., 2016) have been suggested as drivers of increased complexity of bacterial networks, which might thus explain changes in complexity upon rewetting. Fungal co-occurrence networks, in contrast, were more complex in forest soils than in cropland soils and their complexity remained relatively stable over the course of the DRW disturbance, which was in line with a previous study that reported that fungal communities were more stable under drought (Fig. 11)(de Vries et al., 2018). Fungal communities performed better in terms of growth than bacterial upon rewetting (see Section 4.5). However, the structural data did not show major changes in fungal communities which could be linked to their functional performance. An explanation for the apparent contrasting functional and structural responses of fungal communities might be the more flexible physiology of fungi. Fungi can grow in a wider range of soil moisture contents (Manzoni et al., 2012a), temperature (Pietikäinen et al., 2005), SOM quality (Strickland and Rousk, 2010) and pH (Rousk et al., 2009). This higher physiological flexibility might thus explain the more stable fungal co-occurrence networks, as well as the lack of responsive taxa in fungal communities.

Taken together, microbial community structure upon rewetting was affected by the legacy of land-use. Additionally, the structure of bacterial and fungal communities was differently shaped by the land-uses and also responded differently upon rewetting. There is a general expectation that microbial community structure is a primary driver of function (Fierer, 2017). Thus, it was expected that there would be a link between the functional and structural data. However, so far, a strong qualitative link could not be found, which calls for a further exploration of the data.

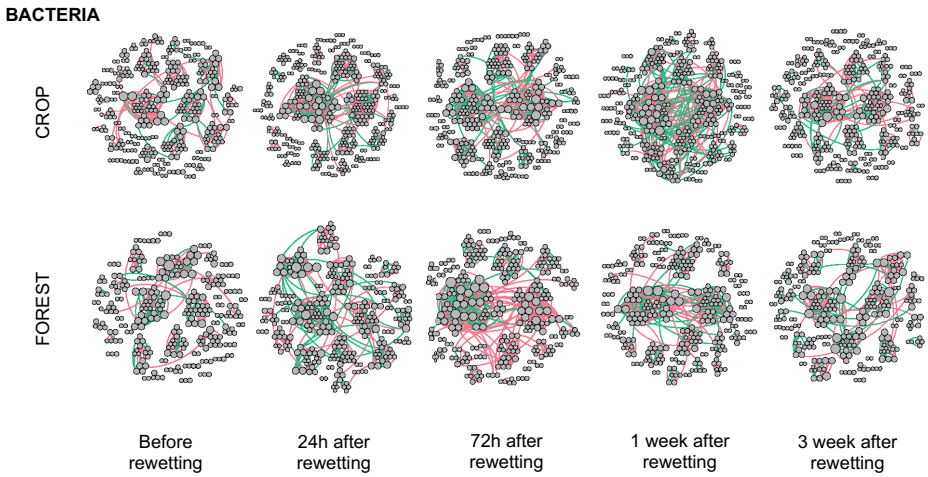

Figure 10. Succession of bacterial community networks over the course of a drying and rewetting (DRW) perturbation (Before rewetting, 24h 72h, 1w and 3w after rewetting) in cropland and forest soils. Networks represent co-occurrence models derived from 11 replicates at each time point. Nodes represent ASVs, and links between nodes represent significant Spearman correlations. The size of the nodes depends on their degree, where bigger nodes indicate nodes with a higher degree. The red and green edges indicate positive and negative correlations respectively. (see **Paper V**)

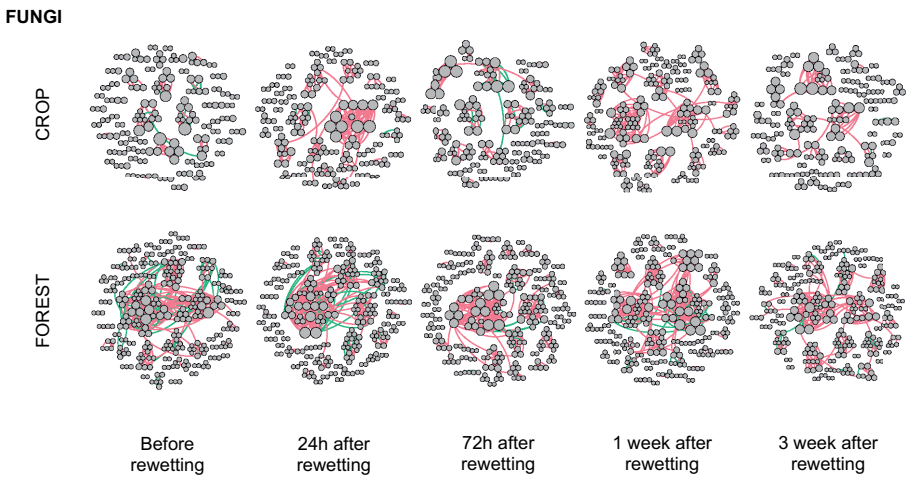

Figure 11. Succession of fungal community networks over the course of a drying and rewetting (DRW) perturbation (Before rewetting, 24h 72h, 1w and 3w after rewetting) in cropland and forest soils. Networks represent co-occurrence models derived from 11 replicates at each time point. Nodes represent ASVs, and links between nodes represent significant Spearman correlations. The size of the nodes depends on their degree, where bigger nodes indicate nodes with a higher degree. The red and green edges indicate positive and negative correlations respectively. (see **Paper V**)

5. Synthesis and future perspectives

To understand how soil microbial communities control biogeochemistry, we need to understand how environmental factors regulate structural and functional aspects of microbial life in soil. Moisture is one of the most well-known factors that regulates soil microorganisms (Kirchman, 2018). Moisture directly affects steady-state rates of microbial processes, which generally decrease with lower moisture (Manzoni et al., 2012a). In addition, moisture fluctuations also dramatically affect microbial process, where a DRW event results in a large CO_2 release to the atmosphere and a transient period where microbial growth and respiration are uncoupled (Göransson et al., 2013; Iovieno and Bååth, 2008). In the last decades, there has been a significant advance in the understanding of how moisture controls microbial life in soils (Barnard et al., 2020; Schimel, 2018). This thesis gives further insights into how moisture regulates soil microbial communities, and how this might impact the C-budget of terrestrial ecosystems.

Paper I and **II** investigated the mechanisms behind the two different microbial response patterns to DRW. **Paper I** showed the harshness of the disturbance could determine the microbial response after rewetting. That is, the harsher the DRW disturbance (i.e., the drier the soils before rewetting) the more "Type 2"-like was the response. **Paper II** showed that the exposure to repeated DRW cycles shifted the microbial response after rewetting from a "Type 2" to a "Type 1". This suggested that microbial communities could be adjusted to perceive the DRW as less harsh, resulting in a more "Type 1" like response. Taken together, these results suggested that the microbial response pattern is controlled by the harshness of the disturbance perceived by microbes (Fig. 6). The results of this thesis together with previous results of laboratory studies also suggested that the history of moisture and moisture fluctuations are important to determine the microbial response upon rewetting.

Paper III, **Paper IV** and **Paper V** showed that in the studied ecosystems history of climate did not result in differences in the resistance and resilience of soil microbial communities to drought and rainfall events. However, these studies did not cover a comprehensive gradient from humid to arid soils. When taking all the results together with preliminary studies across a European climate gradient, the results suggested that microbial resistance and resilience might be shaped by environmental legacies. A drier climate seemed to shape microbial communities to be more drought tolerant and resilient communities (**Paper III**; **Paper IV**). In addition, microbial communities exposed to lower incoming precipitation (**Paper III**; **Paper IV**) as well as a history of soil disturbance (i.e., cropland soils, **Paper V**), resulted in more efficient microbial communities upon rewetting. These results could be explained by microbial adjustment to better cope with perturbations (i.e., drought or DRW events) and/or differences in

resource availability and quality due to differences in climate history or aboveground community.

Finally, bacterial and fungal responses to drought and DRW were explored, as well as their interactions. The results of this thesis suggest that fungi are more resistant to low moisture levels than bacteria (**Paper III**) which was in line with the expectations. However, contrary to the general expectation, fungi appeared to be equally (**Paper III**) or more (**Paper V**) resilient than bacteria after rewetting. It was recently found that bacteria might constrain fungal growth after rewetting (Hicks et al., 2019). The results in this thesis give further support to the idea of competition between bacteria and fungi after rewetting (**Paper III**; **Paper V**).

Much work still needs to be done to fully understand how moisture regulates soil microorganisms and their processes. A way to move forward is the inclusion of plants in the study systems, which has not been done in the studies presented in this thesis but deserves attention. The presence of plants during the DRW experiments will modify (1) the moisture dynamics that microbial communities are exposed to as well as (2) the carbon budget of the system. First, plants modify moisture dynamic of soil since they protect the soil from drying down due to their roots and they can also release EPS that protect microorganisms from drought (see Section1.2). Second, the inclusion of a plant in the study system will also provide a variable carbon input to the system during the DRW disturbance since the plant productivity directly depends on moisture (Keddy, 1992). The carbon input would then be high when the soil is moist and decrease during the drying down of the system. In the experiments that are part of this thesis, the soil moisture levels were well below the wilting point of plants, suggesting that most probably plants would not have survived the disturbances that the soils were exposed to. The death of the plant would in addition change the type of carbon into the system (e.g., dead roots) (Shi et al., 2013), making it difficult to disentangle the direct and indirect effects (through the plant input) of soil moisture on microbial communities. Previous research suggests that the carbon input provided by plants can affect how soil microbial communities respond to DRW. For instance, an study that investigated the microbial responses upon rewetting at different depths, in soils that had been exposed to different precipitation regimes, found that microbial roots and the microbial communities in the rhizosphere were affected by fluctuations in moisture (Engelhardt et al., 2018). In addition, studies that have examined osmolyte accumulation and dynamics during DRW events have shown contrasting results in the presence/absence of plants. When soils were disturbed in the absence of a plant, osmolyte accumulation could not be detected (Boot et al., 2013). In contrast, a recent study that included plants in the study system during the DRW disturbance detected osmolyte accumulation by soil microbes (Warren, 2020). Therefore, these contrasting findings suggest that the presence of plants can shape how microbes allocate carbon, which could then affect their response to DRW disturbances. In addition, it was also

suggested in **Paper III** and **Paper V** that differences in aboveground communities and productivities could be a possible explanation for differences in the microbial carbon budget during drought and DRW events. Thus, the effects of plants in the microbial response to drought and DRW is a research gap that needs to be further explored with the incorporation of plants into the study systems as well as the use of intact plant-soil systems.

In Section 4.6, it was suggested that the history of climate might shape microbial responses and their recovery upon rewetting. Besides, it was also shown that the recovery after a DRW disturbance was strongly correlated with soil physicochemical factors such as pH or %SOM. The use of laboratory experiments where one or more of these soil physiochemical factors are factorially modified would allow to disentangle whether these factors are important to determine the response of soil microbes to DRW, as well as, how important they are and if they interact with other factors. The use of environmental gradients where one of the target factors varies would be another interesting tool to answer this question. As an example, soil pH would be an interesting factor to investigate since it has been shown to strongly influence microbial community structure (Lauber et al., 2009; Rousk and Bååth, 2011) and function (Rousk et al., 2009). Soil pH could be manipulated in the laboratory by liming (addition of calcium- or magnesium-rich materials to neutralize soil acidity), which is a procedure that has been used before to investigate pH effects in soil microorganisms (Silva-Sánchez et al., 2019). Then, the knowledge obtained in the lab could be scaled up using soils along a pH gradient (e.g., the Hoosfield acid strip at Rothamsted Research, UK). The study of the effect of pH on microbial responses upon rewetting would also allow a better understanding of the bacterial and fungal interactions upon rewetting, since pH has been shown to inhibit bacterial growth, which in turn has been suggested to constrain fungal growth upon rewetting (Hicks et al., 2019).

Another aspect that has been explored in this thesis is the effect of moisture in the microbial community structure. However, the used methods could not directly link the microbial community structure with its function, as it was unknown which was the physiological state (active, inactive, dormant or dead) of the characterised microorganisms. A way to overcome this problem is the use of stable isotope probing (SIP) which analyses exclusively the DNA from the microorganisms that have incorporated a tracer (usually $H_2{}^{18}O$), which are the ones that are growing and thus contributing to ecosystem function (see Section 1.3). The use of this method would for example allow to unveil whether the change in the response pattern (from "Type 2" o "Type 1") observed in **Paper II** is due to a microbial community shift. In addition, it would also be useful to understand microbial assembly upon rewetting (**Paper V**), which would provide a better understanding about whether changes in microbial assembly explain changes in function. In addition, the construction of microbial networks with only the taxa that are actively contributing to ecosystem functions,

would allow the identification of keystone taxa that are important for the maintenance of structure and function of microbial communities.

The verification of the results found in **Paper III** and **Paper V** would be another interesting way of continuing the work presented in this thesis. The results of these studies suggested that both previous exposure to drought as well as land-use can shape soil microbial community responses to DRW. To verify these results, in November 2017, an environmental climate gradient was chosen in a subtropical location (Ethiopia, **Paper V**), where rain exclusion experiments were established in two contrasting land-uses in each location of the precipitation gradient (Fig. 12A). In the gradient, MAP spanned a range of 1235 mm range, with 2200 mm $year^{-1}$ in the wettest end of the gradient to 965 mm $year^{-1}$ in the dry end of the gradient. The 5 locations varied in altitude, therefore differences in MAT were also found from 15 °C in the most elevated and wet sites to 27°C in the lower and drier sites. The established field experiments across the gradient aimed to compare the variation across the precipitation gradient (long-term drought effects) to that created within sites where rain exclusion experiments (short-term effects) were placed. Therefore, the whole field experiment consisted of 5 locations, two contrasting land-uses (natural forest vs. degraded crop soils, Figs. 12D, 12E) at each location, and 4 control plots and 4 drought plots (Fig. 12B) at each land-use, which were constructed as in **Paper V** (Fig. 12C). Three and a half years after the establishment of the experiments, soils will be sampled and microbial responses to drought and DRW will be assessed. This study will provide a better understanding of (1) climate and land-use legacy effects on microbial responses to drought and DRW, (2) whether there is an interaction between climate and land-use (i.e., if climate legacy strengthens or weakens the effects by land-use and vice versa), and (3) how long it takes to the legacy effects to be apparent and relevant.

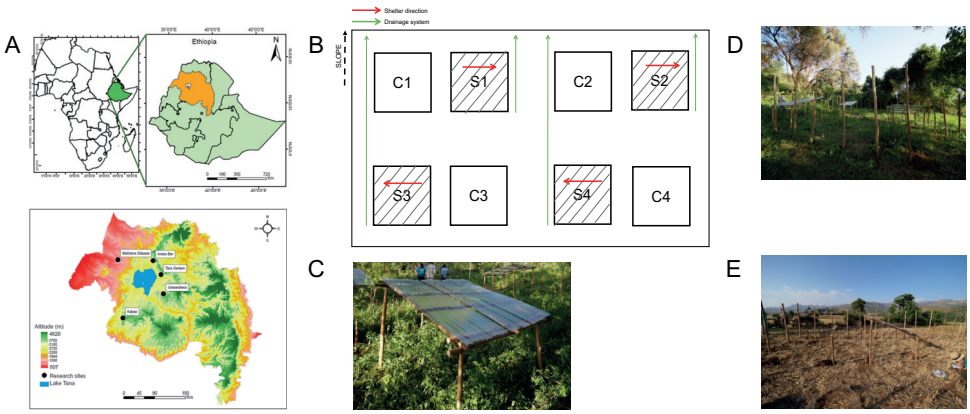

Figure 12. (A) Location of the precipitation gradient and study sites. (B) Schematic representation of the field experiment in each one of the sites. (C) Example of the constructed rain shelters. (D) Example of a forest site. (E) Example of a cropland site.

So far, all the studies that have investigated microbial growth responses upon rewetting a dry soil have been done in the laboratory. A logical continuation of this work would then be to test whether the patterns observed in the lab really reproduce the patterns that occur in natural ecosystems. Therefore, it would be interesting to dry soils in the field and rewet them *in situ* and follow microbial growth and respiration responses with high spatiotemporal resolution. This assessment will allow us the scale up the knowledge gained in the laboratory during the last years and explore the contribution of soil microorganisms to C fluxes during drought and rainfall events in natural ecosystems.

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Paper I

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Partial drying accelerates bacterial growth recovery to rewetting

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ABSTRACT

Fluctuations in soil moisture create drying-rewetting events affecting the activity of microorganisms. Microbial responses to drying-rewetting are mostly studied in soils that are air-dried before rewetting. Upon rewetting, two patterns of bacterial growth have been observed. In the Type 1 pattern, bacterial growth rates increase immediately in a linear fashion. In the Type 2 pattern, bacterial growth rates increase exponentially after a lag period. However, soils are often only partially dried. Partial drying (higher remaining moisture content before rewetting) may be considered a less harsh treatment compared with air-drying. We hypothesized that a soil with a Type 2 response upon rewetting air-dried soil would transform into a Type 1 response if dried partially before rewetting. Two soils were dried to a gradient of different moisture content. Respiration and bacterial growth rates were then measured before and during 48 h after rewetting to 50% of water holding capacity (WHC). Initial moisture content determined growth and respiration in a sigmoidal fashion, with lowest activity in air-dried soil and maximum above ca. 30% WHC. Partial drying resulted in shorter lag periods, shorter recovery times and lower maximum bacterial growth rates after rewetting. The respiration after rewetting was lower when soil was partially dried and higher when soils were air-dried. The threshold moisture content where transition from a Type 2 to a Type 1 response occurred was about 14% WHC, while >30% WHC resulted in no rewetting effect. We combine our result with other recent reports to propose a framework of response patterns after drying-rewetting, where the harshness of drying determines the response pattern of bacteria upon rewetting dried soils.

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1. Introduction

Moisture is an important determinant of microbial activity in soil (Manzoni et al., 2012a). Fluctuations in moisture conditions create drying and rewetting events, which affect microbial growth rates and soil respiration rates (Kieft et al., 1987; Blazewicz et al., 2014), and it is well known that a pulse of carbon dioxide (CO_2) often is observed after rewetting a dry soil (Jarvis et al., 2007; Sponseller, 2007; Kim et al., 2012). Most studies of drying-rewetting events assess completely air-dried soils that are rewetted to optimal moisture (Chowdhury et al., 2011; Barnard et al., 2015; Meisner et al., 2015), but soil moisture content will vary spatially (Rey et al., 2017) and temporally (Cregger et al., 2012). Thus, the moisture content before rewetting will vary and is frequently much higher than in air-dried soils (Lado-Monserrat et al., 2014). The increase in respiration rate induced by rewetting has been shown to be less evident when soil is partially dried before rewetting (Kim et al., 2010; Yan and Marschner, 2014) and is only detectable when soil is dried to a moisture content below a threshold level (Fischer, 2009). Thus, rewetting completely air-dried soils could be considered a harsher perturbation than rewetting partially dried soils. It is generally assumed that the size of the respiration pulse will correlate with the amount of microorganisms killed by the drying-rewetting event (Kieft et al., 1987; Blazewicz et al., 2014; Fraser et al., 2016), although mobilization of carbon (C) released from soil organic matter (Xiang et al., 2008; Schimel et al., 2011) or the accumulation of osmolytes in microbial biomass (Warren, 2014; but see Boot et al., 2013) will also contribute to the respiration pulse.

Two patterns of bacterial growth have been observed upon rewetting a dry soil (Fig. 1). In the first pattern ("Type 1 response"; Fig. 1), bacterial growth rates increase linearly from low values

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upon rewetting without a lag period (Iovieno and Bååth, 2008). In the second pattern ("Type 2 response"; Fig. 1), bacterial growth rates start to increase exponentially after a clear lag period of up to 20 h of very low levels of bacterial growth (Göransson et al., 2013). These differences in growth patterns also result in a shorter recovery time for the Type 1 response, and higher rates of maximal growth in the Type 2 response (Meisner et al., 2015). Previous work showed that a prolonged drying period can shift the response pattern from a Type 1 to a Type 2 within the same soil (Meisner et al., 2013, 2015). It was hypothesized that a lower survival of microbes due to prolonged drying was the reason for this shift in response pattern (Meisner et al., 2015), suggesting that a harsher treatment would result in a Type 2 response with increasingly longer lag periods.

Since partial drying could be considered a less harsh treatment than air-drying, we hypothesized that a soil with a Type 2 response to rewetting air-dried soil would transform into a Type 1 response if dried only partially before rewetting (Fig. 1). As such, the aims of the current study were: (1) to test how partial drying affect the bacterial growth response upon rewetting a soil with a Type 2 response; (2) to determine at what moisture level the transition from a Type 2 into a Type 1 occurs. We expected that partial drying before rewetting would result in shorter lag periods before the increase of bacterial growth, lower maximum growth rates after rewetting and a shorter recovery time to values matching those in a constantly moist soil compared to air drying. In addition, we expected that partial drying before rewetting would result in a lower CO_2 release upon rewetting. A prerequisite for our study was that respiration and bacterial growth rates are reduced at lower water contents before rewetting (Iovieno and Bååth, 2008; Manzoni et al., 2012a).

2. Material and methods

2.1. Soil

Selected soils exhibited a Type 2 response after rewetting following 4 days' air drying, with an increase in bacterial growth after lag periods of around 15–20 h at 17 °C. Soil from Greenland was collected in August 2014 at Østerlien, which is located close to the Arctic Station, Qeqertarsuaq, Disko Island in Central West Greenland. The soil at this site was formed by quaternary deposits on pre-quaternary formations of crystalline, breccia and plateau-basalt lavas, of the order Gelisols (USDA, 1992) or Cryosols (FAO, 1989). The soil was sampled from the A-horizon (pH_{water} = 6.7; SOM = 5.7%). Soil from the U.K. was collected in August 2014 at the Henfaes Experimental Research Station, which is located 12 km east of Bangor, U.K. The soil was a fine loamy brown earth over gravel (pH_{water} = 5.3; SOM = 8.4%) classified as a Dystric Cambisol (FAO, 1989) or a Fluventic Dystrochrept (USDA, 1992) and was collected under ca. 12 year old Alder (*Alnus glutinosa*) or Beech (*Fagus sylvatica*) monocultures describes previously (Göransson et al., 2013). All soils were sieved (<2.8 mm) fresh, and stones and roots were picked out by hand. Soils were stored at 4 °C until use.

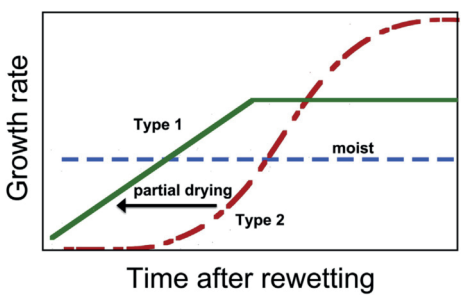

Fig. 1. Schematic overview of the two response patterns of bacterial growth found after drying-rewetting. In a Type 2 response (red stippled line), bacteria increase their growth rates after a clear lag period, whereas in a Type 1 response (green line), bacteria increase their growth rate linearly immediately after rewetting. The blue line indicates the bacterial growth rate in the constantly moist control soil. The arrow indicates the hypothesis that partial drying before rewetting changes the Type 2 into a Type 1 response. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Experiments

Four experiments were made. For the U.K. soils, the alder and beech forest soils were treated as replicate experiments. The Greenland soil was only sampled at one place, but two separate experiments with this soil were made in order to replicate the experiment. We combined non-independent experimental assessments of the same location for curve fitting.

2.2.1. Drying of soils

The soils were dried at room temperature (22–23 °C) under a fan until they reached the intended range of water contents. Before drying, 50 g field moist soil was placed into 500 ml microcosms and adjusted to 50% of its maximum water holding capacity (WHC). The time to reach the desired water content varied from 0 h (for 50% WHC) to 2 days (for air-dried soils). Once the approximate moisture content was reached, the microcosms were lidded and the water content was determined gravimetrically.

All the microcosms were placed at 17 °C and kept with lids closed for 1–4 days. Then bacterial growth and respiration were measured in the moisture gradient of soils one day before rewetting to estimate the direct effect of moisture content on growth and activity. The growth rate assessments used 1 h incubations and the respiration rate assessments used 24 h.

2.2.2. Rewetting of soils

Dried soils were rewetted to 50% WHC and incubated at 17 °C together with a moist control always kept at 50% WHC. Upon rewetting, bacterial growth was measured every 2–3 h during 48 h. To allow this sampling scheme, two sets of soils were prepared from each microcosm on the day of rewetting by placing 15 g subsamples of soil into 150 ml plastic vials. One set was rewetted in the evening and one set the following morning to allow for response curves with a high temporal resolution as has been performed previously (Meisner et al., 2013, 2015).

2.3. Microbial analyses

2.3.1. Bacterial growth

Bacterial growth was measured by the incorporation of ^{3}H-Leucine (Leu) into extracted bacteria (Bååth et al., 2001). Briefly, at each time point, one gram of soil was mixed with 20 ml demineralized water by vortexing for 3 min. The supernatant with a bacterial suspension was sampled after low speed centrifugation (1000 *g* for 8 min) and the incorporation of Leu was measured in 1.5 ml aliquots of the bacterial suspension. A combination of non-radioactive and tritiated Leu ([^{3}H]Leu, 37 MBq ml^{-1}, 5.74 TBq mmol^{-1}, Perkin Elmer, USA) was added to yield a final concentration of 275 nM. The extracted bacteria were incubated for 1 h at 17 °C. The samples were washed (Bååth et al., 2001) and the radioactivity of the incorporated Leu was measured on a liquid

scintillator. Bacterial growth was expressed as the amount of Leu that was incorporated in the extracted bacteria per g dry soil and per h.

2.3.2. Soil respiration

Soil respiration was measured using a GC equipped with a methanizer and a FID detector. One gram of soil was put in a 20 ml glass vial, purged with pressurized air, sealed and incubated at 17 °C for 24 h. Three time periods were measured: 24 h before rewetting, 0–24 h after rewetting and 24–48 h after rewetting. The respiration rates were expressed as μg CO_2 per g dry soil and per day.

2.4. Modeling

2.4.1. Modeling bacterial growth

Bacterial growth and respiration values were standardized for the response by the maximum value for bacterial growth and respiration before rewetting. After rewetting, the standardization was done by dividing with the activity in the 50% WHC moist control soil. This standardization was done to be able to compare bacterial growth and respiration rates against soil moisture for the four experiments.

Since two response patterns of bacterial growth are found after rewetting, two types of models were used to calculate the lag period (time before bacteria start growing exponentially), maximum growth rate and recovery time (the time point where bacterial growth reached the value in the constantly moist control soil (50% WHC)) (Meisner et al., 2015). Curves were fitted using Kaleidagraph version 4.5.2 for Mac.

When bacterial growth started to increase exponentially after a lag period (Type 2), the response was modeled with the modified Gompertz equation (Zwietering et al., 1990) for the period before the maximum growth rate of the bacteria was reached:

$$G_t = \mathrm{B} + \left\{ A^{*} e^{\left(-e^{\left(\frac{\mu max^{*} e}{A} (\lambda - t) + 1 \right)} \right)} \right\} \quad (1)$$

G_t is the standardized growth rate at time t. B is the asymptotic growth rate at t_0, that is initial growth rate before rewetting. A is the difference between the lower and higher curve asymptotes. μmax is the specific bacterial growth rate. λ is the lag time, the time point after which growth starts to increase exponentially. The recovery time point is the time point where G_t equals growth in the moist control, and maximum growth rate was calculated as the sum of A and B.

When bacterial growth started to increase immediately upon rewetting (Type 1) the response was modeled with a linear function until the growth rate was stable. The linear model was also used when it was not possible to fit a Gompertz equation or the model fit for the Gompertz equation was below $R^2 = 0.75$. The lag period for the linear model was per definition 0 h for this response type. The recovery time point was the time point where growth calculated with the linear function equaled growth in the moist control, and maximum growth was calculated as the average growth rate for all the measurements after stable growth was reached.

2.4.2. Modeling the relationships between moisture and characteristics of activity

The relationships between soil moisture and bacterial growth as well as soil moisture and soil respiration were modeled with a logistic equation. The relationships between soil moisture before rewetting and lag time or recovery time were also modeled with a logistic equation. The relationships between soil moisture content before rewetting and maximum growth rates or respiration rates after rewetting were modeled with a negative exponential equation. We checked if the data from the two sites could be combined into one curve fit by calculating the F-ratio that was based on the sum of squares and degree of freedoms of both fits separate and the sum of squares when the model was fit with all data (Motulsky and Ransnas, 1987). A large F value indicated that two separate curve fits for each site was better (Table S1).

Before rewetting, we considered a lower threshold moisture level when there was no further decrease in growth or respiration with decreasing % WHC and a higher threshold value (saturation) when there was no further increase in response variables with increasing % WHC. After rewetting, we considered the presence of a threshold when there was no further increase with decreasing % WHC and a saturation point when there was no further decrease with increasing % WHC. The threshold and saturation values for the logistic equations were calculated according to McDowall and Dampney (2006). In brief, the y-value at 0.05 and 0.95 of the curve is calculated and we considered the corresponding x-values as the threshold and saturation value, respectively. For the negative exponential equations, we considered the x-value when modeled y-values exceeded 0.05 of the difference between maximum and minimum values of the curve.

3. Results

3.1. Respiration and bacterial growth before rewetting

Both bacterial growth and respiration rates increased with soil moisture content according to a logistic model (Fig. 2). The respiration and growth rates did not increase further above around 30% WHC, with half of maximum rates around 15% WHC. A lower threshold was found around 3% WHC, which was similar to moisture content in air-dried soil. The similar effect of moisture content before rewetting on bacterial growth and respiration rate made them positively correlated at an almost 1:1 relationship (Fig. 3; $R^2 = 0.90$; $P < 0.001$).

3.2. Bacterial growth after rewetting

Moisture content before rewetting affected the bacterial growth pattern after rewetting (Fig. 4). A Type 2 response with a clear lag period followed by an exponential increase in growth rate was observed when soils were air-dried or dried to low soil moisture contents before rewetting in both soils. For example, rewetting air-dried soil (~3% WHC) from Greenland resulted in a lag period of around 20 h (Fig. 4a). The lag period then became shorter when soil was dried less severely prior to rewetting in both the Greenland (Fig. 4a) and the U.K. soils (Fig. 4b). An exponential growth increase was still found after the lag period when there was a Type 2 response. At even higher remaining initial moisture content before rewetting, bacterial growth started at higher levels and also started to increase immediately in a linear fashion, showing a Type 1 response.

The relationship between soil moisture content before rewetting and the length of the lag period was modeled with a logistic relationship ($R^2 = 0.91$ for both Greenland and U.K. soils (Fig. 5a)). The lag period had a maximum duration of around 20 h in both air-dried soil from Greenland and the U.K. No lag period was observed when soils were air-dried to about 14% WHC or higher before rewetting for both soils, suggesting a threshold where the response changes from a Type 2 into a Type 1 (Fig. 5a, vertical solid line).

The effect of moisture content before rewetting on the recovery

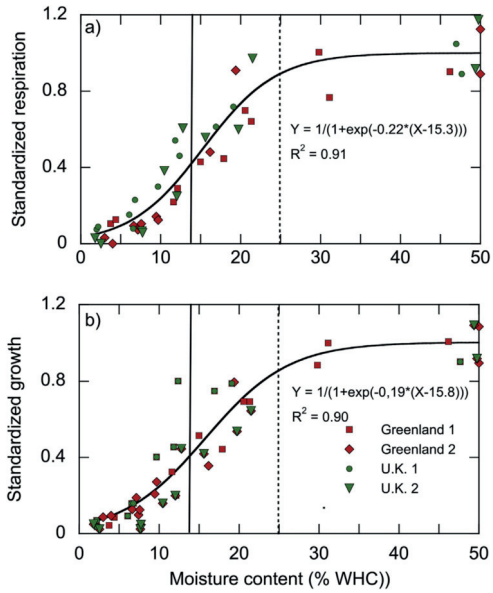

Fig. 2. Soil moisture content versus standardized respiration (a) and growth rates (b) before rewetting. Values were standardized by the maximum respiration and growth rates. Respiration and growth rate were fitted with a logistic equation. The vertical solid line indicates the transition from a Type 2 into a Type 1 response, and the vertical stippled line the transition between a Type 1 response and no effect of rewetting (see Fig. 5).

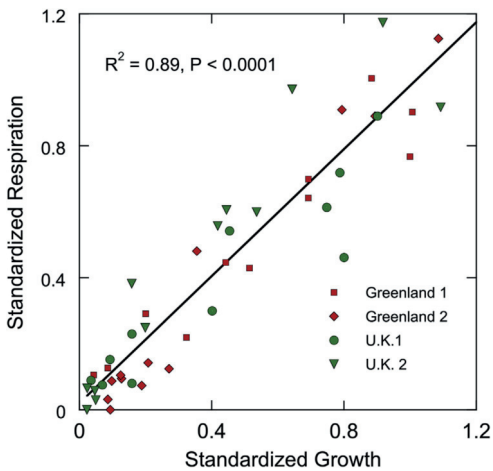

Fig. 3. The relationship between standardized bacterial growth and respiration at different moisture contents before rewetting the soil. Values were standardized by the maximum respiration and growth rates.

time could be modeled with a logistic equation, with no differences between the soils (Table S1; $R^2 = 0.86$, Fig. 5b). The maximum time

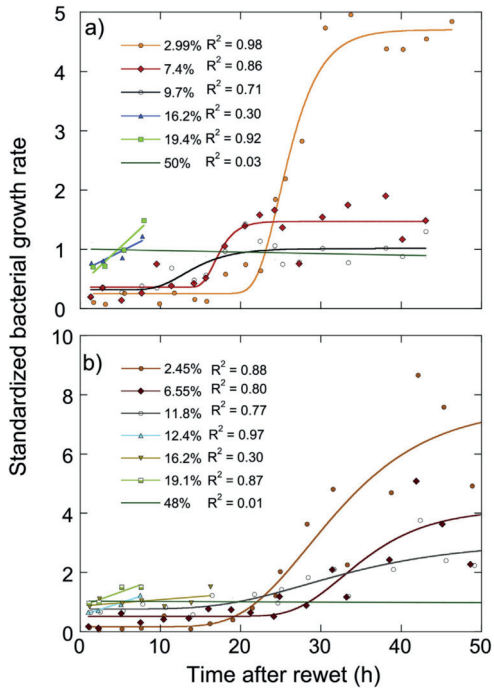

Fig. 4. Time after rewet versus standardized bacterial growth rate for soil from Greenland (a) and soil from U.K. (b). Values for the control soil with 50% WHC were set to 1. Six moisture contents were used for soil from Greenland and seven for soil from U.K. to illustrate when bacteria grew with a Type 1 pattern upon rewetting (diamond, circle), or a Type 2 pattern (triangle, square). The moist control is indicated with a green line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

period for the bacterial growth to recover was around 23 h and occurred when soil was dried $\leq$5% WHC before rewetting. When soil was dried to around 25% WHC or higher, the modeled growth rate was not different from the moist control at 50% WHC, resulting in a recovery time of 0 h (Fig. 5b, vertical stippled line). Thus, above this threshold moisture content before rewetting there were no effect of rewetting the soil on the bacterial growth response.

The maximum bacterial growth rates found after rewetting were higher when soils were dried to a lower moisture content (i.e. more severe drying) before rewetting (Fig. 5c). For example, air-dried soil (3% WHC) had almost 5 times higher maximum growth rates after rewetting than the moist control for the Greenland soil, whereas soil with initial moisture content of 7.4% WHC had only ca. 1.5 times higher growth rates (Fig. 4a). The effect of partial drying on the maximum growth could be modeled with an exponential equation ($R^2 = 0.66$ for the U.K and 0.83 for Greenland soils, Fig. 5c). The maximum growth rate was around 5 times higher in air-dried soil than the constantly moist soils from Greenland and around 7 times higher in soils from the U.K. This maximum growth rate reached values matching those in the moist control at lower moisture contents for soil from Greenland compared to soils from the U.K. (Fig. 5c).

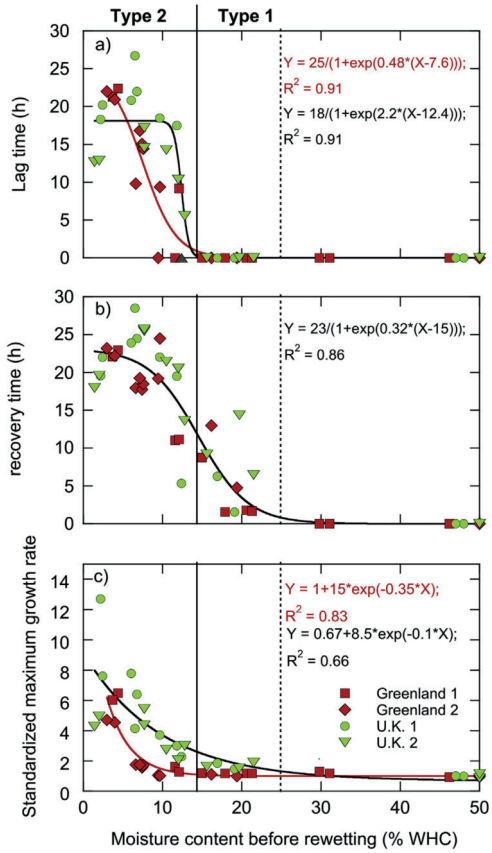

Fig. 5. Soil moisture content before rewetting versus bacterial growth characteristics: Lag time (a), recovery time (b) and standardized maximum growth rate after rewetting (c). Values for the control soil with 50% WHC were set to 1. Red lines and symbols indicate soil from Greenland (squares, diamonds) and green lines and symbols indicate soil from the U.K. (circles, triangles). Moisture content before rewetting and lag time was fitted with a logistic equation. The curve fit for recovery time was combined for both soils and could be fitted with a logistic equation. Standardized maximum growth rates were fitted with negative exponential equations. The vertical solid line indicates the transition from a Type 2 into a Type 1 response, and the vertical stippled line the transition between a Type 1 response and no effect of rewetting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

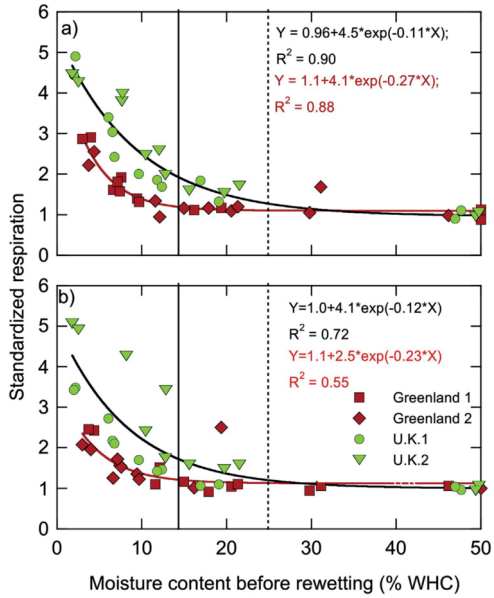

Fig. 6. Soil moisture content before rewetting versus standardized respiration rates during 0–24 h (a) and 24–48 h after rewetting (b). Values for the control soil with 50% WHC were set to 1. Curves were all fit with a negative exponential equation. The vertical solid line indicates the transition from a Type 2 into a Type 1 response, and the vertical stippled line the transition between a Type 1 response and no effect of rewetting (see Fig. 5).

3.3. Respiration after rewetting

The drier the soil was before rewetting, the higher was the amount of respiration produced during 0–24 h (Fig. 6a) or during 24–48 h after rewetting (Fig. 6b). For air-dried soil, the respiration released during 0–24 h was 3 and 5 times higher than in the constantly moist soils from Greenland and U.K., respectively (Fig. 6a). The corresponding values for the time period 24–48 h were similar (Fig. 6b). Partial drying before rewetting decreased the amount of respiration released 0–24 h and 24–48 h after rewetting compared with air-dried soils ($R^2 \geq 0.81$ in all cases). An increased release of CO_2 after rewetting could only be detected when soil was dried below 12% WHC before rewetting for soil from Greenland and below around 20–30% WHC before rewetting for soil from the U.K. The respiration after rewetting from moisture contents above these values remained similar to the 50% WHC moist control soil during the entire experiment.

4. Discussion

4.1. Partial drying is less severe than air-drying

We predicted that partial drying would be a less harsh treatment to the bacterial community compared to air-drying. Several lines of evidence support that the severity of drought increased with more complete drying, and could be reduced by incomplete, partial, drying. First, the level of bacterial growth immediately after rewetting has earlier been used as an index for the status of soil bacterial activity (Meisner et al., 2013, 2015), where higher growth rates show that bacteria have been less inhibited by drought and rewetting. The rate of growth directly after perturbation has also been similarly used to determine the status of the bacterial activity in soils following freezing-thawing (Koponen and Bååth, 2016). In the present study, bacterial growth was very low in air-dried soils, but the level increased with higher moisture in the partially dried soils (Fig. 2), thus suggesting that the latter treatments were less detrimental. Similar results have previously been observed for a Swedish grassland soil (Iovieno and Bååth, 2008) and for growth of cultivable bacteria (Seifert, 1961). Second, the respiration during the first 24 h after rewetting was highest in air-dried soils and

decreased with more remaining water in partially dried soils (Fig. 6). This result is consistent with empirical and modeling studies of partial drying before rewetting (Fischer, 2009; Lado-Monserrat et al., 2014; Manzoni et al., 2016). Increased respiration rates due to harsher drying may capture substrate that became available from a more extensive killing of the microbial biomass induced by the drying and rewetting perturbation (Kieft et al., 1987; Fraser et al., 2016), by higher osmolyte concentration in microbial biomass induced by the drying (Warren, 2016), or by a higher C-release from SOM upon rewetting (Schimel et al., 2011). Still, lower respiration rates in partially dried soils suggested that partial drying before rewetting reduced the harshness of the perturbation. Increasing substrate release in more dried soils was also reflected by the maximum bacterial growth rate reached after rewetting, which was highest in air-dried soil and decreased with more remaining water in partially dried soils (Fig. 5c).

4.2. Reduced severity of drying changed the bacterial response from Type 2 to Type 1

We demonstrate that partial drying, with higher soil moisture remaining before rewetting, changed the response pattern of bacterial growth to rewetting from a Type 2 response (increase in growth after a lag period) into a Type 1 response (immediate increase in growth rates) (Figs. 1 and 4). As such, our results support our main hypothesis. It has previously been observed that a soil with a Type 1 response could be changed into a Type 2 response when air-dried for longer periods (Meisner et al., 2013, 2015), or when air-drying was combined with salt (Rath et al., 2017). The underlying reason for these changes were interpreted to be related to an increased harshness of drying to the bacterial community. We can now extend these results and also show that a milder and less severe drying event, partial drying, affects the bacterial growth response to rewetting in the opposite way, changing a Type 2 to a Type 1 response.

We also found evidence for a gradual transition in the extent of the Type 2 response in partially dried soils. The lag period grew shorter with higher moisture content before rewetting, to eventually reach zero, and thus transition into a Type 1 response (Fig. 5a). As predicted, this is the opposite effect of increasing the severity of drying, where extended periods of air-drying initially resulted in a transition from a Type 1 to a Type 2 response, with even longer lag periods after rewetting resulting from longer periods of air-drying (Meisner et al., 2015).

Our second objective was to determine at what moisture level a switch from a Type 2 to a Type 1 bacterial growth response would occur. This moisture threshold was around 14% WHC, in both soils (Fig. 5a). It is likely that soils with less pronounced Type 2 responses to drying-rewetting, *i.e.* with shorter lag-periods after rewetting from air-dried conditions, have threshold values for this transition at lower moisture contents.

There was also a gradual transition within the range of moisture contents in the partially dried soils that resulted in a Type 1 response after rewetting (>14% of WHC). The recovery time to levels of bacterial growth matching the moist reference soil became shorter with higher moisture content before rewetting up to ca. 24% WHC (Fig. 5b). Thus, for soils originally having a Type 1 response when rewetting air-dried soil (e.g. Iovieno and Bååth, 2008), partial drying is expected to still result in a Type 1 response but with shorter recovery times (also see section 4.5.).

4.3. Threshold moisture for no rewetting effect

A threshold moisture content of ca. 30% of WHC could be determined, above which drying and rewetting had no effect on growth (Fig. 5) or respiration (Fig. 6). Similar results have previously been reported for soil respiration upon partial drying, suggesting a moisture threshold for no effect (Fischer, 2009; Lado-Monserrat et al., 2014). The similarity of the threshold for cumulative respiration and maximum growth rate is consistent with increased substrate availability after rewetting driving both microbial variables. Furthermore, this suggests that respiration and bacterial growth are not affected by moisture changes within a relatively broad range centered around the expected optimal moisture (between ca. 30%–50% WHC in the studied soils).

4.4. The dependence of carbon-use efficiency on soil moisture and rewetting

The effects of environmental factors and perturbations on microbial carbon-use efficiency (CUE) have recently become an intense line of study, both empirically (Geyer et al., 2016; Öquist et al., 2016; Spohn et al., 2016a, 2016b) and theoretically (Wetterstedt and Ågren, 2011; Manzoni et al., 2012b; Roller and Schmidt, 2015). Although not explicitly studied here, comparing bacterial growth to respiration can provide an index for the microbial CUE as affected by moisture and rewetting events. CUE appeared to be stable under a wide range of stable moisture conditions, but low during the first 48 h after rewetting dry soils. Prior to rewetting when moisture levels were stable, respiration and bacterial growth were well correlated, with a near 1:1 relationship (Fig. 3), which is consistent with earlier laboratory experimental work (Iovieno and Bååth, 2008). This close correlation between growth and respiration suggests that different soil moisture levels will not affect CUE of soil bacteria during stable moisture conditions. However, after rewetting dry soil the link between growth and respiration was strongly uncoupled as initial growth rates were low and respiration rates were high. The underlying reasons for this disconnect have been previously discussed (Göransson et al., 2013; Meisner et al., 2013, 2015). Briefly, the initial respiration pulse is likely determined by substrate available for respiration without subsequent microbial growth. This interpretation is consistent with previous work on the source of the respiration pulse, which observed that both biochemical (extracellular) and organismal sources contribute to soil respiration when rewetting dry soil (Fraser et al., 2016). However, it remains to be resolved how the well-linked respiration and microbial growth during stable moisture balances against the dynamics triggered by variable moisture at ecosystem levels over longer time-periods.

4.5. Concluding remarks and outlook

We show that a soil with a Type 2 pattern in the bacterial growth response after drying-rewetting can be changed into a Type 1 pattern by partial rather than complete drying. We also suggest that the two response patterns of bacterial growth after rewetting dry soils are related to the amount of surviving microorganisms and thus to the harshness of drying.

Adding these observations to results from earlier studies, we propose a generalized conceptual figure to describe how the bacterial response to rewetting dry soil is determined by the harshness of drying (Fig. 7). Increasing harshness has been shown to occur with increasing duration of drought, or drought combined with altered osmotic conditions due to salt, and decreasing harshness due to partial drying. In addition, different soils can respond to rewetting from different positions along the 'harshness scale' when they are air-dried for 4 days (see A, B and C in Fig. 7 and description in legend). We choose soils in the present study at the high end of the harshness scale with a Type 2 response and long lag periods (A in Fig. 7). Soils with a Type 2 response, but with only a short lag

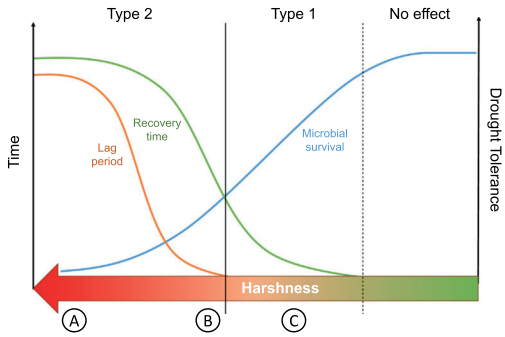

Fig. 7. A schematic overview of the response patterns of bacterial growth to drying-rewetting and their dependence on the harshness of drying. An increasing harshness of the drying event is depicted on the x-axis with increasingly detrimental perturbations for the microbial community oriented to the left-hand side. Increasing harshness can be achieved by extended duration of drought (Meisner et al., 2013, 2015) or drying to lower moisture content before rewetting (present study). For the latter case, starting to the right, at optimal moisture conditions, going left along the x-axis, at a threshold value of moisture (vertical stippled line), bacterial growth will be impaired, but will rapidly recovery after rewetting, resulting in Type 1 response. A longer recovery time is found in harsher treatments (further to the left). The transition from a Type 1 to a Type 2 response (solid vertical line) is indicated by the presence of a lag period before exponential growth. This lag period will be short near this threshold, but lag period and recovery period will increase with harsher treatments (e.g., more extensive drying or longer periods of drought). Soils from different locations that are air-dried for 4 days can have different response patterns after rewetting. Soils studied here are at the high-end of the "harshness scale" (A), soils having a Type 2 response but with only a short lag period further to the right (B), and soils with a Type 1 response even further to the right (C).

period after 4 days drying (B in Fig. 7), are predicted to change to a Type 1 response when partially dried to a moisture content only marginally wetter than air-dried conditions before rewetting. Prolonged droughts, on the other hand, will always result in Type 2 responses for these soils for all points on the harshness scale, but with longer lag periods correlating to length of drought. Soils with initially a Type 1 response after air-drying-rewetting (C in Fig. 7), would be predicted to only decrease the recovery time with partial drying, whereas prolonged droughts would result in a transition to a Type 2 response with longer lag periods with increasingly longer drought periods (Meisner et al., 2015).

One question that remains unanswered is how identical experimental drying-rewetting treatments (air-drying during 4 days) can result in two response patterns in different soils? Different physio-chemical environmental factors affecting microbial survival and respiration may be one reason (Balogh et al., 2011; Kaiser et al., 2015). Another explanation may be that microorganisms from different climates are adapted to different moisture regimes with differences in microbial drought tolerance affecting the response pattern (Allison and Goulden, 2017). In order to identify the mechanisms underpinning different response patterns, we thus need to study the microbial responses to drying-rewetting in soils from different regions, including wide ranges of edaphic factors and with different legacies of drought and rewetting episodes.

An additional aspect to consider is the moisture content the soil is rewetted to after drying (Rey et al., 2017). This was not studied here, since soils were always rewetted to 50% WHC. However, moisture levels post-rewetting may be important for the bacterial response, since more CO_2 is produced when dry soil is rewetted to higher water content after rewetting (Evans et al., 2014; Lado-Monserrat et al., 2014). We expect, however, that the response type of bacterial growth would be mainly determined by amount of remaining water prior to rewetting due to the importance of the harshness of drying in determining the growth pattern after rewetting shown here.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.05.016.

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Paper II

Repeated drying and rewetting cycles accelerate bacterial growth recovery after rewetting

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Abstract

Drastic fluctuations in soil moisture, such as drying and rewetting (DRW) cycles, affect soil biogeochemical processes. A pulse of CO_2 is released upon rewetting a dry soil, which coincides with changes in microbial growth, biomass, and nutrient mineralization. Soils are commonly exposed to repeated DRW cycles, where the number of cycles affects the microbial communities and the processes they regulate. However, it is not well understood how the exposure of soils to repeated DRW cycles affects bacterial growth after rewetting. Two patterns of bacterial growth response upon rewetting have been identified by previous studies. Bacterial growth can either start increasing immediately in a linear fashion ("Type 1") or start increasing exponentially after a lag period ("Type 2"). Three soils, with different response patterns upon DRW (one with "Type 1" response and two with a "Type 2" response with different lag periods), were exposed to 7 DRW cycles. Respiration and bacterial growth were measured for 48 h after 1, 2, 3, 5 and 7 cycles. In addition, the time that bacterial growth needed to recover to predisturbance growth levels was estimated (recovery time). Exposure to repeated DRW cycles accelerated growth recovery after rewetting, as bacteria shifted their growth responses from a "Type 2" pattern to a "Type 1" pattern. This change could be detected in both bacterial growth and respiration dynamics after rewetting. Soils that initially had a "Type 1" response did not change the response pattern after repeated DRW cycles, but bacterial growth recovery after rewetting tended to be faster. Exposure to repeated DRW cycles thus resulted in the reduction and eventual loss of the lag periods and shorter recovery times. Our results show that exposure to repeated DRW cycles will affect the outcome of future DRW cycles, which might be mediated by either a shift in the species composition or in the physiological conditions of bacteria. The previous exposure to DRW events might thus have a legacy effect on the future microbial dynamics when there is a drought period followed by a rainfall event.

Key words: drying-rewetting cycles, birch effect, bacterial growth, respiration

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1. Introduction

Soil moisture is one of the canonical factors regulating soil microbial communities and their activity (Waksman and Gerretsen, 1931; Kirchman, 2018). Soil moisture not only affects steady state rates of microbial processes (Manzoni et al., 2012), but fluctuations in moisture will also cause dynamics of microbial activity. Especially drastic changes in soil moisture, like drying and rewetting (DRW) cycles, lead to one of the most dynamic events in soil microbial ecology (Schimel, 2018). When a dry soil is rewetted, initially a large release of CO_2 to the atmosphere is observed (Birch, 1958; Kim et al., 2012), which can account for a significant C loss of ecosystems (Schimel et al., 2007; Manzoni et al., 2020).

Most terrestrial ecosystems are exposed to fluctuations in moisture availability. Consequently, soil microbial communities are to some extent globally exposed to DRW events. During those events, changes in microbial growth and biomass (Bottner, 1985; Kieft et al., 1987; Iovieno and Bååth, 2008), as well as in nutrient mineralization and availability take place (Birch, 1958; Fierer and Schimel, 2002). DRW events can be single events, but it is common that soils are exposed to repeated DRW cycles during the year (Jarvis et al., 2007; Inglima et al., 2009). The effects of DRW cycles on soil C mineralization have frequently been studied in soils from different ecosystems, both in the field (Xu et al., 2004; Tang et al., 2005; Jarvis et al., 2007) and in the laboratory (Orchard and Cook, 1983; Mikha et al., 2005; Xiang et al., 2008; Shi and Marschner, 2014). In laboratory studies, C mineralization has generally been found to decrease with increasing number of DRW cycles, which has been linked to the depletion of available C (Fierer and Schimel, 2002; Mikha et al., 2005). In addition, it has been suggested that soil microbial communities that have been exposed to repeated DRW cycles may become more resistant to an additional DRW cycle (Fierer et al., 2003; Evans and Wallenstein, 2014;). This is in line with other studies suggesting that exposure to repeated DRW cycles can select for faster growing microorganisms (Fierer and Schimel, 2002; Evans and Wallenstein, 2012), that can recover to a pre-disturbance state more rapidly (de Nijs et al., 2019). This changes in the communities can persist for several weeks after the disturbances (Meisner et al., 2018).

When dry soils are rewetted, bacterial growth and respiration have been shown to be transiently uncoupled (Iovieno and Bååth, 2008; Göransson et al., 2013). While respiration rates show high rates immediately after rewetting, bacterial growth rates are very low immediately after the disturbance. Two different bacterial growth response patterns upon DRW have been identified (Meisner et al., 2013). On the one hand, bacterial growth rates start increasing immediately after rewetting in a linear fashion reaching a maximum level, coinciding with an immediate increase in respiration rate after rewetting followed by an exponential decline to moist control levels (henceforth, “Type 1” response) (Iovieno and Bååth, 2008). On the other hand, bacterial growth rates start to increase exponentially after a clear lag period with no increase in growth. In this case, respiration also increases immediately after rewetting but is then sustained over some time, sometimes with a secondary increase in sync with the bacterial growth rate increase. Eventually, respiration decreases to pre-disturbance state levels (henceforth, “Type 2” response) (Göransson et al., 2013).

It has been proposed that the bacterial response to DRW will be shaped by the harshness of the disturbance as perceived by the community (Meisner et al., 2017). If a DRW event is “harsh” for the bacterial community, soil microorganisms will be more compromised by the disturbance. Thus, a longer time to increase their growth rate will

be needed, resulting in a lag period ("Type 2" response). In contrast, if the DRW event is less "harsh" for the bacterial community, they will not be as compromised by the drought, resulting in an immediate increase of their growth rate ("Type 1" response). Even if harshness is not an absolute quality, it may still be useful as a relative attribute. Thus, as an example, longer drying periods, and drying soils to very low moisture levels both are considered as "harsher" conditions (Meisner et al., 2017, 2015). In addition, a recent study, where soils from a temperate heathland were exposed to repeated DRW cycles, found a shift in the type of response (from a "Type 2" to a "Type 1" response) upon rewetting after two DRW events (de Nijs et al., 2019). This suggests that with subsequent DRW cycles, the bacterial community experiences the DRW disturbance as less "harsh". However, it remains unknown whether this pattern can be generalized.

In the present study, we exposed three different soils to repeated DRW cycles. We selected soils to cover a range of bacterial growth patterns upon DRW. The soils were subjected to seven DRW cycles, and bacterial growth and respiration were followed to characterize the response to rewetting. Based on previous studies, we chose two soils with a "Type 2" response but with different lag periods, as well as one soil that exhibited a "Type 1" response with no lag period (Meisner et al., 2017, 2013). We hypothesized that repeated DRW cycles would change the microbial response after rewetting. This would be seen as a (i) reduction or eventual loss of the lag period for bacterial growth (i.e., a transition from a "Type 2" response to a "Type 1" response), a (ii) reduction in the recovery time to pre-DRW growth rates, and (iii) a shift from a sustained respiration response ("Type 2") to an immediate maximum followed by continually decreasing rates ("Type 1").

2. Materials and methods

2.1 Soils

Based on previous studies, three soils were selected for the experiment with different response patterns upon rewetting. One was a managed grassland soil from south Sweden, classified as a sandy loamy brown earth soil (soil S; pH_{water} = 6.5; SOM by loss on ignition (600 °C) = 8.4%). The soil exhibited a "Type 1" bacterial growth response after 4-days air-drying and rewetting (Fig. 1A). This soil was previously studied by Meisner et al. (2013). The other two soils exhibited a "Type 2" bacterial growth response after 4-days air-drying and rewetting (Fig. 1A). One of them was collected in Greenland (soil G), and was a soil formed by quaternary deposits on pre-quaternary formations of crystalline, breccia and plateau basalt lavas (pH_{water} = 6.8; SOM = 2.6%), which was previously described by Meisner et al. (2017). The other soil was mixture of soils collected under Alder (*Alnus glutinosa*) or Beech (*Fagus sylvatica*) monocultures in Wales (soil W) previously described by Göransson et al. (2013). Both soils were fine loamy brown soils and were mixed since they both exhibited the same bacterial response upon rewetting (pH_{water} = 5.5; SOM = 7.9%). Soils were sampled in autumn 2014 and the experiments were run within 3 moths after sampling. Meanwhile, soils were stored field moist at 5 °C. All soils were then wet-sieved in the laboratory using a 2 mm mesh-size before starting the experiment.

2.2. Experimental drying and rewetting cycles

All soils were exposed to 7 repeated DRW cycles (Fig. 1B). For each soil 6 microcosms (500 mL plastic beakers with lids) were prepared with 60 g of fresh soil. To dry the soils, microcosms were left without lids under a ventilator at room temperature (~22 °C) for 3 days until they were air-dried (i.e., they reached a constant weight). Then, soils were rewetted to optimum moisture, that is 50% of their maximum water holding capacity

(WHC). Distilled water was added to the dry soil using a pipette and soil was then mixed with a spatula for approximately 10 s. After rewetting, soils were sampled, measured and kept in a temperature-controlled room at 17 °C.

Bacterial growth and respiration during 48 h after rewetting were measured. This time-frame after rewetting has previously been shown to capture the growth and respiration dynamics after rewetting (Meisner et al., 2013). To allow high temporal resolution measurements, soils were rewetted in 2 sets, one rewetted in the evening and the other rewetted in the morning and measured in parallel as previously described (Meisner et al., 2013, 2015, 2017). For bacterial growth, samples were taken approximately every 2 h, whereas for respiration 3 samples were taken for the following time periods: 0 to 6 h, 6 to 24 h, and 24 to 48 h. Bacterial growth and respiration were measured after cycles 1, 2, 3, 5, and 7 by destructive sampling of one of the microcosms. The whole experiment was repeated twice with the three different soils.

Controls for each cycle consisted of soils from the previous cycle that was maintained at 50% WHC. One microcosm was moistened to 50% WHC a week before the experiment started and kept moist as a control soil for the first DRW cycle. For the following cycles, soil from the previous cycle was kept moist to use as a control. Control soils were also mixed in parallel with cycled soils although no water was added.

2.3 Measurements

2.3.1 Bacterial growth

Leucine incorporation into bacterial proteins was measured as a proxy for bacterial growth. Measurements of leucine incorporation were done essentially according to the homogenization/centrifugation technique (Bååth, 1994), using modifications by Bååth et al., (2001). Shortly, 1g of soil was weighed into a 50mL centrifuge tube and mixed with 20mL of distilled H_2O for 3 min using a multivortex shaker. The soil-water mixture was then centrifuged at low speed (10 min at 1000 g), which resulted in a supernatant with bacterial suspension. 1.5 mL of the suspension were placed into a 2 mL microcentrifugation vials and incubated with radioactively labelled leucine (Leu) for 1 h at 17 °C. The added mixture consisted of 2 µl of 1-[4,5-^{3}H]-Leucine (5.7 TBq mmol^{-1},

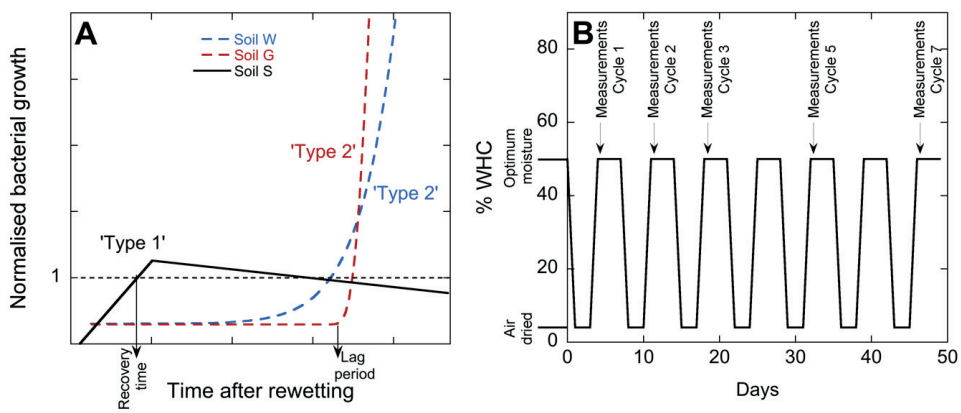

Figure 1. (A) Schematic representation of the initial bacterial growth response to 4-days drying and rewetting of the three soils that were used for the experiment. The full black line (soil S) indicates a "Type 1" bacterial growth response and the blue (soil W) and red (soil G) dashed lines indicate the "Type 2" responses with different lag periods. The dashed horizontal line indicates the bacterial growth level of undisturbed soils, which was used to standardize growth data. (B) Experimental design indicating the changes in the soil moisture during the repeated drying and rewetting cycles. The arrows indicate when the measurements were taken.

Perkin Elmer, USA) and unlabeled Leu which resulted in a final concentration of 275 nM. After incubation, growth was terminated by adding 75 µL of 100% trichloroacetic acid (TCA) resulting in a final concentration of 5% TCA. Non-incorporated Leu was washed away following the washing steps described by Bååth et al. (2001). Finally, 1mL of scintillation cocktail (Ultima Gold; PerkinElmer, USA) was added to the sample and radioactivity was measure using a liquid scintillation counter (PerkinElmer Liquid Scintillation Analyzer, Tri-Carb 2910 TR). Obtained values are presented as pmol Leu incorporated g^{-1} dry soil h^{-1}.

2.3.2 Respiration

Average respiration rates were estimated using gas chromatography for the following time periods: 0-6h, 6-24h and 24-48h. 1g of soil was weighed into 20 mL glasses vials. The vials were then purged with pressurized air to have a constant initial background level of CO_2 in all samples and sealed. Samples were incubated for the appropriate amount of time and the CO_2 production was quantified using a gas chromatograph equipped with a methanizer and an FID detector. Due to technical errors with the gas chromatograph, respiration rates in cycle 5 could not be measured.

2.4 Data analysis

2.4.1 Bacterial growth

Bacterial growth rates after rewetting were normalized to the control soil growth rates. For the 5th cycle, due to technical issues we did not have a control soil; instead, the average value of the 3rd and 7th cycle was used to normalize the data.

Since two different patterns after rewetting dry soil were found in the experiment, two types of models were used to model bacterial growth after rewetting (Meisner et al., 2017). When bacterial growth started to grow immediately ("Type 1" response), two linear curves were used to model it; one for the immediate increase and a second to describe the stable/decreasing phase of the growth. These two curves were separated by an inflection point were bacterial growth reached maximum growth. The inflection point was estimated using a "broken stick model" (Toms and Lesperance, 2003) as described in Leizeaga et al. (2020) using JMP Pro 15 (SAS institute).

When bacterial growth after rewetting showed a lag period before it started increasing exponentially ("Type 2" response), the response was modelled using a Gompertz equation described by (Zwietering et al., 1990):

$$Bacterial\ growth = D + A * e^{-e^{B-Cx}} \quad (1)$$

where A is the difference between the initial and the maximum growth, B and C are mathematical parameters modelling the slope and the curvature when bacterial growth is exponential, and finally D is the initial growth.

After modelling bacterial growth, additional parameters that describe the bacterial growth pattern characteristics were estimated. (i) The lag period, which indicates time-point at which the bacterial growth rates start increasing exponentially. The lag period is 0h in the "Type 1" responses and is calculated using eq. 2 in the "Type 2" responses (Meisner et al., 2017).

$$Lag\ phase = \frac{B-1}{C} \quad (2)$$

(ii) The recovery time was also estimated, which indicates the time that the bacterial growth needs to reach the bacterial growth rates at the moist control soil (50% WHC).

Spearman's rank correlations were used to test the effect of increasing number of drying and rewetting cycles in the lag periods and recovery times. All the statistical analyses were performed using using JMP Pro 15 (SAS institute).

2.4.2. Respiration

Soil respiration was measured 0-6h, 6-24h and 24-48h after rewetting. To be able to compare the respiration response type of all three soils and after each DRW cycle, a respiration index (RI) of early respiration divided with later respiration was calculated similar to Slessarev et al. (2020), however we used the 0-6 h and 24-48 h time-periods. This ratio was then log-transformed. A "Type 1" respiration pattern, where respiration is highest early after rewetting, is characterized by a higher RI; a "Type 2" respiration pattern, with similar or even higher respiration in the latter compared to the early phase, will have lower values (Rath et al., 2017; Slessarev et al., 2020).

Differences in the RI in each DRW cycle and soil were tested with a two-way ANOVA. The factors considered were "soil" (with 3 levels: soil S, soil, W and soil G) and "DRW cycle" (with 5 levels: 1, 2, 3, 5 and 7) and their interaction. Tukey's HSD comparisons were used to compare treatments with an $\alpha = 0.05$.

3. Results

3.1. Bacterial growth

Bacterial growth after rewetting responded differently to an initial cycle of DRW in the three soils (Fig. 1A), as anticipated. Soil S exhibited a "Type 1" response where the bacterial growth rate started increasing in a linear fashion immediately after rewetting. In contrast, bacterial growth in the W and G soils exhibited a "Type 2" response, where a lag period with almost no growth was followed by exponential growth. The "Type 1" soil exhibited a faster recovery to bacterial growth levels in an undisturbed soil

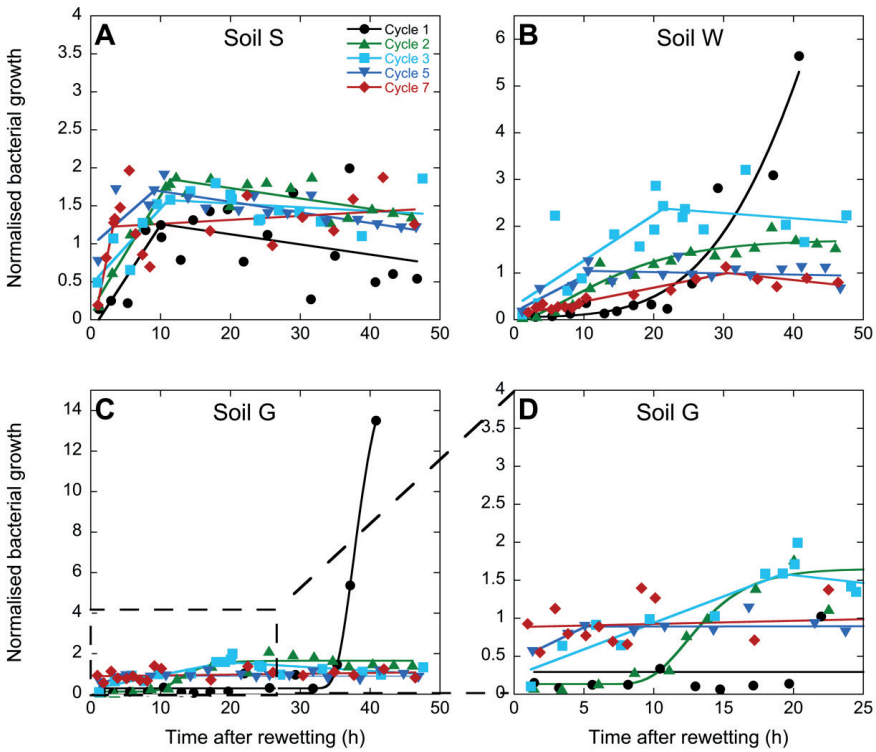

Figure 2. Bacterial growth responses to repeated 4-days drying and rewetting in soils from (A) Soil S, (B) Soil W and (C, D) Soil G. The bacterial growth values are normalized to the control (y=1), that is to the level of bacterial growth of a soil that has undergone one cycle less of drying and rewetting. The data points represent the average values of the 2 experiments.

compared to the "Type 2" soils, with recovery times of 8.5 h, 25.0 h, and 32.7 h, in soils S, W and G. The "Type 2" soils exhibited higher maximum growth rates relative to the control, with maximal levels 5.6 and 13.5-fold higher than in the undisturbed soils for soils W and G, respectively. Soil G had a longer lag period, 20.6 h, than did soil W, 8.1 h.

The exposure to repeated DRW cycles in a "Type 1" response soil (soil S) resulted in a consistent "Type 1" response pattern after each subsequent DRW cycle (Fig. 2A). In contrast, exposing soils G and W, with initial "Type 2" responses, to repeated DRW cycles resulted in a gradual transition from a "Type 2" to a "Type 1" response (Figs. 2B, 2C, 2D). After the second DRW cycle, these soils still exhibited a "Type 2" response, but with a shorter lag period. From the third DRW cycle and on, bacterial growth in these soils always exhibited a "Type 1" response after rewetting without a lag period.

The characteristics of the bacterial growth response after rewetting changed with the exposure to additional DRW cycles, even when the type of response was the same (Fig. 3). The lag period decreased with the number of cycles in the soils that exhibited a "Type 2" response (Fig. 3A). The lag period in soil W decreased from 8.1 h to 1.4 h from the first to the second cycle, whereas in soil G it decreased from 20.6 h to 4.9 h. After three DRW cycles the lag period had disappeared for both soils, having transitioned into a "Type 1" response. Increasing the number of cycles gradually decreased the recovery time (Fig. 3B). A decrease was observed during the first 3 cycles in the soils G and W ("Type 2" soils), linked to the reduction and loss of the lag period. In soil W, no further decrease in the recovery time was observed after the third cycle, resulting in a non-significant tendency for a shorter recovery time with more DRW cycles (ρ=-0.80, p=0.20). Soil G decreased its recovery time with each subsequent DRW

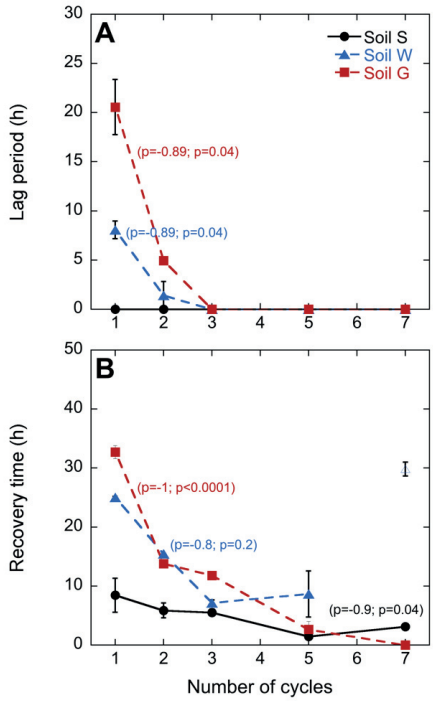

Figure 3. Bacterial growth characteristics after repeated 4-days drying and rewetting cycles: (A) lag period and (B) recovery time. Black circles and full line indicate soil S, blue triangles and stippled line soil W and red squares with stippled line soil G. Data points are mean (±SE, n=2). The blue triangle without filling indicates an outlier. In brackets next to each curve the Spearman correlation coefficient and p-value (ρ and p respectively).

cycle (Fig. 3B). There was also a decrease in the recovery time with increasing number of DRW cycles in soil S, which had an initial "Type 1" response (Fig. 3B). The recovery time decreased from 8.5 h in after the first DRW cycle to 3.1 h in the last cycle. Thus, even though there was not a change in the response pattern for soil S, bacterial growth after rewetting needed less time to recover to the control level with subsequent DRW cycles.

3.2. Respiration

Exposure to repeated DRW cycles generally decreased respiration rates (Fig. 4), but different respiration response patterns upon rewetting were observed. Respiration in soil

S peaked immediately upon rewetting and then decreased exponentially after each DRW cycle (a "Type 1" respiration pattern), starting from a lower level with each DRW cycle (Fig. 4A). However, soil W and G had different respiration responses depending on the number of DRW cycles that they had been exposed to (Fig. 4B-C). After the first cycle, the respiration rate increased immediately upon rewetting for soil W, and was then sustained during the remaining measurement period, suggesting a largely maintained respiration rate during at least 48 h (a "Type 2" respiration pattern). After the second cycle, the respiration rate peaked immediately and then decreased. This decrease was even more pronounced after DRW cycles 3 and 7. Soil G behaved similarly to soil W, although after the first DRW cycle, the respiration rate even increased during the 24-48 h period after rewetting (Fig. 4C). After the second cycle, the respiration rate was sustained during the first 12 h after rewetting and then decreased. After cycles 3 and 7, the respiration rate showed an exponential decrease after the immediate peak after rewetting.

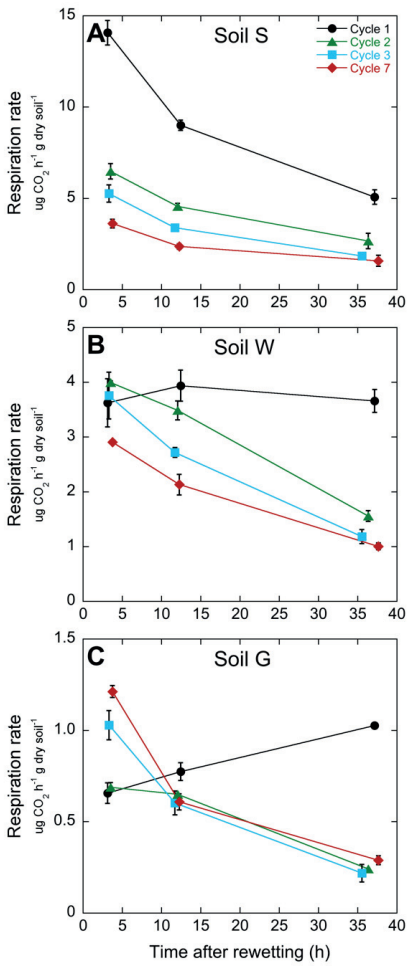

Figure 4. Respiration rates after repeated 4-days drying and rewetting cycle in (A) soil S, (B) soil W and (C) soil G. Data points are mean respiration rates (±SE, n=2).

A respiration index (RI) was calculated to better capture the 2 types of respiration responses. The RI differed between the "Type 1" and "Type 2" soil in the first cycle. Soils W and G had low values of RI after one cycle, typical for a "Type 2" response (Fig. 5); while soil S had high values characteristic of a "Type 1" pattern. Soil S showed a stable RI during subsequent DRW cycles, indicating that there was no shift in the response pattern. Soils W and G both had a transition from low values of RI to higher values during the first 3 DRW cycles. Then, RI was stable over the subsequent cycles for these soils.

4. Discussion

Previous studies have described two types of microbial response patterns to DRW (Iovieno and Bååth, 2008; Göransson et al., 2013; Meisner et al., 2013, 2015, 2017; Rath et al., 2017; de Nijs et al., 2019). Those studies cover a wide range of responses: from an immediate increase of the growth rate after rewetting ("Type 1") to a 20 h lag period before the exponential increase of the growth rate ("Type 2"). Here, we use three different soils that cover the whole range: soil S as a "Type 1" soil, soil W as an intermediate "Type 2" soil with an 8.1 h lag period, and soil G as an extreme "Type 2" soil with a 20.6 h lag period. Bacterial growth measured at a high temporal resolution after rewetting has

mostly been used to differentiate between the "Type 1" and "Type 2" response patterns (e.g. Meisner et al., 2013; Rath et al., 2017), since it is a sensitive metric to differentiate between a long lag period or an immediate increase in growth after rewetting. In contrast, soil respiration is often measured at a lower temporal resolution after rewetting a dry soil and therefore do not capture these two types of response patterns (Miller et al., 2005; Xiang et al., 2008; Butterly et al., 2009). However, we have identified a few studies with high temporal resolution where these two response patters could be distinguished (Göransson et al., 2013; Fraser et al., 2016; Sawada et al., 2016; Slessarev et al., 2020). In a recent study by Slessarev et al. (2020) the authors suggested the use of a respiration index between early and late respiration after rewetting in order to differentiate these patterns. Here, we used a similar respiration index (RI; Fig. 5), showing that we can distinguish between "Type 1" and "Type 2" responses based on early and late respiration rates.

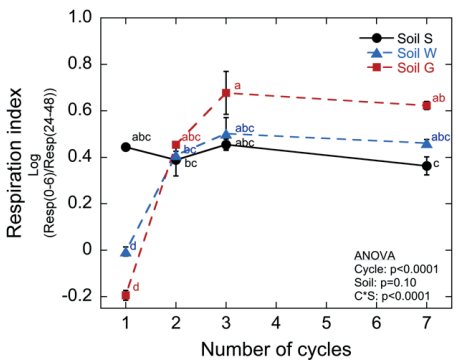

Figure 5. Respiration index (RI) for "Type 1" and "Type 2" responses calculated as Log (respiration (0-6h)/respiration(24-48h)). Lower values of the RI indicate a "Type 2" pattern while higher values indicate a "Type 1" pattern. Black circles and full line indicate soil S, blue triangles and stippled line soil W and red squares with stippled line soil G. Data points are mean values (±SE, n=2). DF = 11, F ratio = 32.95 for the used model.

Exposing a "Type 2" soil to repeated DRW cycles shifted the response pattern upon rewetting to a "Type 1" pattern: the lag period was first shortened and eventually disappeared with increasing number of DRW cycles (Fig. 2B-D). This was in line with our predictions and verifies observations by de Nijs et al. (2019), who showed that exposing one soil to two repeated DRW shifted the response pattern from a "Type 2" to a "Type 1". However, our study not only shows the transition in the response pattern, but also shows that this is gradual indicated by the recovery times of bacterial growth rates. When the "Type 1" response soil (soil S) was subjected to repeated DRW cycles, the response pattern upon rewetting did not change. However, a more rapid recovery time to a pre-disturbance growth level with each subsequent DRW cycles was observed (Fig. 3B), indicating that the microbial community in this soil also had been affected by repeated disturbances. This decrease in the recovery time with increasing number of DRW cycles, was thus a general pattern observed in the three studied soils, which was in line with our expectation (Fig. 3B).

The shift in the type of response was also captured with respiration: in soils W and G there was a shift from a sustained response of respiration after rewetting to response where the respiration was decreasing rapidly after the immediate increase after rewetting, which resulted in an increase of the RI (Figs. 4B-C). Similar results can be deduced from respiration data on tropical soils from Thailand and Japan by Sawada et al. (2016), where five DWR cycles resulted in those soils shifting from a "Type 2" to a "Type 1" response pattern. Fraser et al. (2016) subjected a grassland soil from Wales to four DRW cycles, also resulting in a shift in the response pattern, detectable by respiration data. In the study by Slessarev et al. (2020), the DRW response of several Californian soils was studied. However, the soils were subjected to a DRW pretreatment before the

main study. Thus, the soils were actually exposed to two DRW cycles. The authors found a large proportion of "Type 1" responses, which also matches our observations: some soils might actually have had a "Type 2" response during the first DRW cycle, which might have been shifted to a "Type 1" response after the second cycle. Taken together, the transition of soils having a "Type 2" to a "Type 1" response after repeated DRW cycles appears to be a general phenomenon.

The exposure to repeated DRW cycles resulted in bacterial communities that were less constrained by the disturbance, and therefore could recover their growth rates faster, even if the DRW treatment was the same every cycle. This could be interpreted as the bacterial community perceiving as the same disturbance as less "harsh" after repeated DRW cycles. The response pattern upon rewetting has been suggested to be determined by the "harshness" of the disturbance as perceived by microbes, with a less "harsh" disturbance resulting in a "Type 1" response and "harsher" one in a "Type 2" response (Meisner et al., 2017). This results in a continuum of responses rather than in a "Type 1" and "Type 2" dichotomy. A partial drying is a less "harsh" disturbance for the microbes, which results in a "Type 1" response while air-drying results in a "Type 2" response (Meisner et al., 2017). Prolonged drying (a "harsh" treatment) results in a change from a "Type 1" to a "Type 2" response with increasing lag times (Meisner et al., 2013; 2015). The combination of drought with inhibitors (e.g., salt, which alters osmotic conditions) results in even "harsher" drying conditions, resulting in a shift from a "Type 1" to a "Type 2" response (Rath et al., 2017). In all these studies, differences in the disturbance was the mechanism explaining differences in the "harshness". However, in the present study we subjected the soils to a standardized disturbance in each cycle: four days air-drying followed by rewetting to 50% WHC. Thus, microbial communities perceived the same DRW disturbance as less "harsh". The decrease in the perceived "harshness" with the higher exposure to DRW cycles thus changed the response upon rewetting from a "Type 2" to a "Type 1". Thus, we show that soil microbial communities not only respond differently to DRW due to variation in the disturbance conditions shown by previous studies (Meisner et al., 2017, 2015, 2013; Rath et al., 2017), but also that a community adjustment via changes in community composition or microbial physiology can result in communities that perceive the DRW as less "harsh", resulting in a shift in the response pattern.

The change in the response pattern might be explained by a shift in the microbial community composition with the repeated DRW cycles or a change/adjustment in the microbial physiology. The exposure to a DRW cycle can select for microbial taxa that can better cope with subsequent such disturbances (Evans & Wallenstein, 2012; 2014). The decrease in recovery time, including the shift from a "Type 2" to a "Type 1" response, has been suggested to be due to either a physiological adjustment of the community or a microbial community shift (de Nijs et al., 2019). On the one hand, soil microbes can survive the drying period through physiological adjustments, including osmotic acclimation, production of extracellular polymers, dormancy and spore formation (Schimel, 2018). Those strategies would increase the survival of the microbial communities during the drying period allowing them to become more common after rewetting. Bacteria that for example have been observed to become more abundant after DRW cycles are *Firmicutes*, which includes the spore forming *Bacillus* (Clark and Hirsch, 2008; Martí et al., 2012). On the other hand, DRW events result in newly released substrate (Denef et al., 2001; Williams and Xia, 2009; Slessarev et al.,

2020) which can increase the abundance of copiotrophs that rapidly will increase their abundance after rewetting (Placella et al., 2012; Barnard et al., 2013, 2015). During the next DRW cycles, the larger abundance of these bacteria will result in even a shorter recovery time of overall bacterial growth. A DRW event could also result in a larger part of the remaining bacteria being in a growing or in a less starving condition during the following DRW episode. The length of starvation will affect the lag time when starvation is broken and growth starts again (Mochizuki and Tsutomu, 1994; Lin and Crowley, 2001; Jacobsen and Koch, 2006). Thus, an additional DRW cycle could "activate" part of the community and even if only a minor part of the community becomes "active" that will determine the community growth response after rewetting (Moreno-Gámez et al., 2020). Exposing microbial communities to repeated DRW cycles will thus function as enrichment culture, favoring microbes that can better cope with DRW, including microbes that become more abundant after the dry period, microbes with characteristic of rapid growth and those with an "activated" physiological state phase.

Several studies have shown that different soils can have different response patterns after one DRW cycle, with a large variation in lag phase length and recovery times (e.g., Göransson et al., 2013; Meisner et al., 2015; Rath et al. 2017). Our study brings new insights into what is determining the microbial response to DRW events. We show that the number of previous DRW cycles determines the microbial response to a subsequent DRW cycle, resulting in a legacy effect. While predicting the type of microbial response upon rewetting based on the physical "harshness" of the disturbance is feasible after monitoring environmental factors (e.g., how long the soil was dry, how much the soil was dried), understanding and knowing the history of DRW that a soil has been exposed to in the field is more difficult. In addition, the history of DRW of a soil also includes the extent of drying of each one of the DRW cycles, which will also shape the type of response that microbial communities will have when a dry soil is rewetted again. Last, we do not know for how long the legacy of earlier DRW events will still be detectable, that is how rapid and to what extent the transition from a "Type 2" to a "Type 1" response will reverse under conditions of adequate moisture. Taken together, the history of DRW events will thus obscure attempts to use only other abiotic factors to elucidate if a soil has a "Type 1" or a "Type 2" response after DRW.

5. Acknowledgements

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RECONCILING RESILIENCE ACROSS ECOLOGICAL SYSTEMS, SPECIES AND SUBDISCIPLINES

Research Article

Drought legacy affects microbial community trait distributions related to moisture along a savannah grassland precipitation gradient

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Abstract

1. Ecosystem models commonly use stable-state assumptions to predict responses of soil microbial functions to environmental change. However, past climatic conditions can shape microbial functional responses resulting in a 'legacy effect'. For instance, exposure to drier conditions in the field may shape how soil microbial communities respond to subsequent drought and drying and rewetting (DRW) events.
2. We investigated microbial tolerance to low moisture levels ('resistance') and ability to recover after a DRW perturbation ('resilience') across a steep precipitation gradient in Texas, USA.
3. Although differences in precipitation regime did not result in differences in resistance and resilience of soil microbes, microbial communities appeared to be generally resilient and resistant across the gradient, suggesting that frequent exposure to drought had characterised the trait distributions of microbial communities. Moreover, microbial communities from historically drier sites used carbon more efficiently during a DRW perturbation suggesting that long-term drought history leaves a legacy effect on microbial functions. This may have been due to an indirect effect of drought caused via precipitation-induced differences in primary productivity, influencing the availability of soil organic matter to microbes. Alternatively, different exposures to drought might have shaped the microbial 'readiness' to cope with the DRW disturbance. Microbial community composition was also linked to drought history, but was unrelated to variation in function.
4. *Synthesis.* Exposure to drought can have both direct and indirect effects on soil microbial communities, which can result in lasting legacy effects on the functions they control.

KEYWORDS

carbon use efficiency, drought legacy, drying-rewetting, microbial growth, resilience, resistance

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1 | INTRODUCTION

Soil moisture determines rates of soil biological, chemical and physicochemical processes and is a primary controller of the abundance, community composition and growth rate of microorganisms (Fierer & Schimel, 2002; Kirchman, 2018). As soil dries, conditions become more unfavourable for microorganisms and thus microbial process rates decrease as water grows scarce (Davidson et al., 1998; Howard & Howard, 1993; Manzoni et al., 2012). Moisture fluctuations also radically affect soil microbes, whereby rewetting events after drought periods induce enormous pulses of nutrient mineralisation and soil respiration known as the 'Birch effect' (Birch, 1958), which can characterise the carbon (C) cycle of whole ecosystems (Schimel, 2018; Schimel et al., 2007). These events trigger a cascade of dynamic responses where microbial growth appears to be transiently uncoupled from the respiration pulse (Göransson et al., 2013; Iovieno & Bååth, 2008). Due to the effect that soil moisture and its variation have on microorganisms, moisture is a key parameter for predicting how microbial communities function in soils, but remains poorly represented in models.

Ecosystem models that include microbial processes commonly use steady-state assumptions to predict responses of soil microbial functions to environmental change. They assume that contemporary chemical and climatic factors are the main controllers of microbial activity (Sierra et al., 2015). However, past climatic conditions (e.g. drought) can have a lasting legacy on soil microbial community composition and function (Hawkes & Keitt, 2015). That is, exposure to different conditions in the field might have an impact on how microbial communities will respond to future environmental conditions. Growing evidence supports that historical contingencies can modulate the responses of microbial processes to abiotic factors. For example, a history of drought can have strong and persistent effects on microbial respiration (Hawkes et al., 2017, 2020), potential enzyme activities (Averill et al., 2016), soil microbial community composition (Meisner et al., 2018) and the microbial carbon use efficiency (CUE) during a drying and rewetting (DRW) perturbation (de Nijs et al., 2019; Göransson et al., 2013). A legacy of summer drought and a DRW history have also been shown to select for more resilient and stress-tolerant communities (de Nijs et al., 2019; Evans & Wallenstein, 2014). These studies indicate that the composition, function and response to perturbations of soil microbial communities can be shaped by rainfall history. However, some studies have not shown legacy effects of warming or drought (Cregger et al., 2012; Rousk et al., 2013), making it difficult to resolve to what extent the legacy of past climate shapes microbial functions across different ecosystems where other factors like soil type and vegetation covary.

Microorganisms have two different strategies to cope with cycles of drought. Microbial communities can either withstand the drying down and thus maintain activity at lower moisture levels ('resistance', sensu Griffiths & Philippot, 2013) or they can become faster at recovering towards the pre-disturbance state after a DRW event ('resilience', sensu Griffiths & Philippot, 2013). It is possible to assess the resistance of microbial communities to drought by monitoring the process rates during soil dry down. Resilience can be quantified by following the microbial response to a rewetting event. Previous studies have shown that bacteria can respond in two different manners upon rewetting a dry soil (Meisner et al., 2013) that may depend on historical drought regimes: (a) a more resilient type of response where bacteria start recovering immediately after rewetting, coinciding with respiration rates that peak immediately and then decrease exponentially, or (b) a less resilient response in which bacteria undergo a lag period with no growth before they start growing exponentially, coinciding with a sustained period of elevated respiration, sometimes followed by a further increase in sync with the onset of growth. Previous studies have related the response upon rewetting to the harshness of the disturbance perceived by the microbes, with more severe drying resulting in a less resilient type of response (Meisner et al., 2015, 2017). For instance, extending the drying period (Meisner et al., 2015) or combining the drying with inhibitors such as salt (Rath et al., 2017) results in a less resilient response upon DRW (i.e. slower recovery of growth to levels before rewetting). Differences in growth and respiration responses upon rewetting also lead to differences in microbial CUE during the perturbation, which can be indexed as the ratio of growth relative to the total amount of C consumed. A more resilient response to DRW would then result in a higher CUE during the perturbation (de Nijs et al., 2019). It has been argued that the resilience of soil microorganisms can be influenced by previous exposure to disturbances, where in each disturbance cycle, communities are structured for improved resilience (Griffiths & Philippot, 2013). Furthermore, some studies have shown an increased CUE upon rewetting in soils with a history of drought treatments (de Nijs et al., 2019; Göransson et al., 2013). These observations suggest that exposure to different precipitation regimes could filter for communities that cope differently with DRW disturbances, and thus a change in the type of response to DRW would be expected, as well as a change in the CUE during perturbation.

Here, we investigated the legacy of drought on the moisture dependence ('resistance') of microbial processes and their responses to DRW ('resilience'). Specifically, we investigated whether exposure to different historical precipitation regimes can induce differences in tolerance to low contemporary moisture levels and microbial responses to DRW, as well as alter the balance of microbial C-use between growth and respiration. To this end, we used soils from a ~500-mm precipitation gradient that spanned ~400 km in central Texas across the Edwards Plateau, where savanna grasslands grow in rocky clay soils on a single geological formation (Hawkes et al., 2017) with minimal variation in soil type, pH and soil organic matter (SOM). Previous work in this system has shown persistent legacies of historical precipitation on soil respiration and enzyme activities (Averill et al., 2016; Hawkes et al., 2017, 2020; Waring & Hawkes, 2018). However, the moisture dependence of microbial growth and the microbial responses to DRW have never been resolved across this gradient. We determined microbial process dynamics after rewetting dry soils and measured the moisture dependence of those microbial processes across the gradient. We hypothesised that historical

differences in precipitation regime would affect the microbial functional stability (i.e. resistance and resilience). Specifically, we anticipated that microbial communities would be (1) more drought resistant and (2) more resilient during DRW events in the historically drier sites than in the historically wetter sites. In addition, we hypothesised that these differences in functional stability would also result in differences in CUE, including both more efficient microbes (3a) at low moisture levels and (3b) during DRW in the historically drier sites. It has been proposed that the two main decomposer groups in soils, bacteria and fungi, respond differently to low moisture and DRW disturbances. Therefore, we hypothesised that (4) fungi would be more resistant to drought (Manzoni et al., 2012) and less resilient (De Vries et al., 2012; de Vries & Shade, 2013) to DRW perturbations than bacteria. Finally, based on prior observations on this gradient (Hawkes et al., 2017; Waring & Hawkes, 2018), we hypothesised that (5) microbial community structure would track differences in precipitation regime that correlate with the above-mentioned functional differences across the gradient (Fierer, 2017; Reed & Martiny, 2007).

2 | MATERIALS AND METHODS

2.1 | Rainfall gradient and sampling

The 400-km rainfall gradient was located in central Texas across the Edwards Plateau where mean annual precipitation (MAP) declined from ~900 mm/year in the west to ~400 mm/year in the east, while mean annual temperature was more consistent across the gradient, averaging 19.2°C. Soils were shallow, rocky and calcareous (primarily Mollisols). Sampling locations within sites were selected to minimise differences in topography (<2% slope) and the plant community (native grassland with <50% woody plant cover; Averill et al., 2016; Hawkes et al., 2017). Normalised difference vegetation index (NDVI) was used as a proxy for plant productivity. NDVI data were extracted from Didan (2015) using the Appears software (AppEEARS Team, 2020). NDVI is a widely used reflectance vegetation index (Kerr & Ostrovsky, 2003), which has previously been used to accurately estimate plant productivity (Awaya et al., 2004; Wu, 2012). NDVI data were averaged from 10/06/2015 to 26/06/2015. Previous comparisons of satellite and ground based NDVI measurements have shown that even though there is variation in the measurements there is a high agreement between the two estimations (Tittebrand et al., 2009), and that remote sensing NDVI measurements are especially suitable for grasslands (Wilson & Meyers, 2007).

The 17 sites across the rainfall gradient were sampled in June 2015. The entire rainfall gradient experienced drought from 2011 to 2014, followed by normal rainfall in 2015 due to an El Niño-Southern Oscillation (ENSO) event (https://waterdatafortexas.org/drought). At each site, 20 × 20 m plots were identified and a composite sample was formed by sampling at least 10 separate points to a depth of 10–15 cm. Soils were then subsampled and frozen at −80°C for microbial analysis, or sieved to 2 mm and air dried in the laboratory until they reached constant weight at room temperature before further experiments and measurements.

2.2 | Soil and microbial community characterisation

Total C and nitrogen (N) were analysed by Dumas dry combustion using a C/N elemental analyzer (VarioMAX CN, Elementar). Soil pH and electrical conductivity (EC) were measured in a 1:5 (w:V) water extraction using an electrode. SOM and soil inorganic carbon (SIC) were measured using a loss on ignition (LOI) procedure according to Wang et al. (2011). The maximum amount of water that soils could hold after gravity loss was measured to determine the water holding capacity (WHC) of soils as described in Hicks et al. (2018).

For analysis of microbial community composition, DNA was extracted from two ~0.25 g aliquots of frozen soil from 16 of the sites (Table 1) using MoBio Power Soil Kits (MoBio). DNA was quantified fluorimetrically (Qubit, Invitrogen) and standardised to 10 ng/μl. For PCR, Illumina TruSeq V3 indices were used (Illumina) linked to 16 rRNA bacteria-specific primers (S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 primers; Klindworth et al., 2013) and 28S rRNA fungal-specific primers (NL1-NL4; O'Donnell, 1993). For PCR reactions, we used platinum PCR Supermix (Invitrogen), 1.25 μl of each primer (10 μM), 0.5 μl of BSA (20 mg/ml) and 2 μl (~20 ng) of DNA. Bacterial PCR ran with a hot start at 95°C for 5 min, 25 cycles of 95°C for 40 s, 55°C for 2 min, 72°C for 60 s and a final extension step of 72°C for 7 min. The fungal reactions ran with a hot start at 93°C for 5 min, 35 cycles of 93°C for 60 s, 58°C for 60 s, 72°C for 60 s and a final extension step of 72°C for 10 min. For each sample, PCR reactions were run in duplicate, combined, cleaned with Agencourt AMPure XP magnetic beads (Beckman Coulter) and again quantitated with a Qubit fluorometer. Samples were then pooled in equal amounts and sequenced on Illumina MiSeq v3 (2 × 250 bp for bacteria, 2 × 300 bp for fungi) at the University of Texas Genome Sequencing and Analysis Facility.

2.3 | Moisture dependence ('resistance') experiment

Soils were weighed into microcosms and adjusted to 50% WHC (optimum moisture) before being left to stabilise for 2 weeks at 17°C, which corresponds to a typical spring soil temperature. Microcosms were then placed under a ventilator at room temperature until they reached constant weight (i.e. air dry). Subsamples were taken every 2–3 hr during the drying down for measuring water content, respiration, bacterial growth and fungal growth. Subsamples were briefly kept at 5°C and subsequently processed together. This procedure was repeated twice and the two datasets were combined, fitting a single sigmoidal curve to all data points for further data analysis. A total of 24 measurements were conducted for each parameter to determine the moisture dependence of microbial processes (Figure 1).

2.4 | Drying and rewetting ('resilience') experiment

Soils were air dried until they reached constant moisture under a ventilator at room temperature and rewetted to 50% WHC. Responses of bacterial growth, fungal growth and respiration upon

TABLE 1 Physiochemical characteristics of soils across the rainfall gradient

MAP (mm/year)	C/N (mass fraction)	SOM (%)	SIC (%)	WHC (%)	pH	EC	Sand (%)	Clay (%)	Silt (%)	Total PLFA	Bacterial PLFA	Fungal PLFA	NDVI
407	40	6.1	9.3	50	8.5	132	39	28	33	205.1	106.2	5.0	0.37
442	30	6.2	8.2	49	8.4	118	18	32	50	145.7	71.2	4.5	0.36
533	15	11.2	6.4	71	7.9	135	26	42	32	172.8	85.5	3.9	0.38
604	27	8.7	3.8	84	7.9	160	19	48	33	259.0	125.3	5.4	0.54
631	27	11.3	5.7	76	8.0	155	40	35	25	171.8	87.4	4.3	0.68
662	13	11.4	2.8	97	8.1	149	22	50	28	156.2	68.7	4.4	0.54
685	16	14.8	2.9	107	7.9	195	35	40	25	262.8	144.1	4.6	0.64
695	35	10.6	13.8	72	8.1	142	41	36	23	137.2	70.3	3.7	0.53
726	23	13.8	4.4	75	8.0	147	33	24	43	259.8	130.8	6.0	0.61
749	14	14.0	3.0	90	7.8	155	33	40	26	202.0	100.9	4.5	0.64
769	16	18.3	5.8	72	7.8	209	18	40	43	244.2	138.5	5.7	0.67
814	11	8.9	1.5	71	8.0	160	49	21	31	283.0	143.7	6.0	0.52
850	51	8.7	11.7	72	8.0	131	42	26	32	243.9	109.9	16.8	0.53
852	7	3.8	0.3	61	7.8	69	60	15	25	357.0	175.8	9.3	0.63
859	39	11.7	11.5	66	8.1	143	34	32	34	159.5	72.3	6.1	0.47
886	8	7.3	0.5	74	7.7	84	25	27	48	390.0	200.9	14.9	0.66
889[a]	16	12.2	5.8	89	8.0	154	37	35	28	256.6	115.1	9.5	NA

Note: PLFAs were stored and measured dry and values are reported as nmol of PLFA per SOM.

Abbreviations: EC, electrical conductivity; MAP, mean annual precipitation; NDVI, normalised difference vegetation index; PLFA, phospholipid fatty acids; SIC, soil inorganic carbon; SOM, soil organic matter; WHC, water holding capacity.

[a]This site was not included in the microbial community analysis.

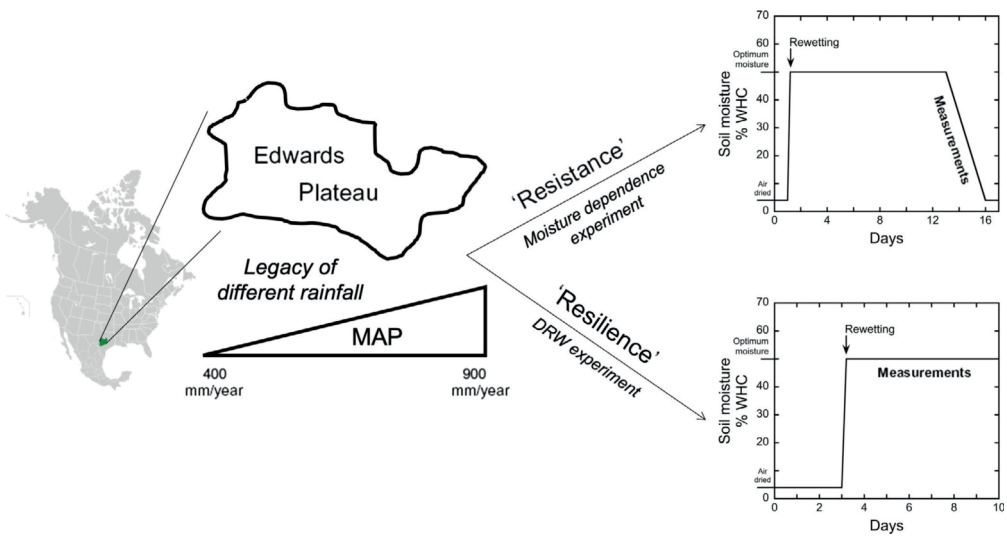

FIGURE 1 Experimental design. Soils with a legacy of different rainfall were sampled across a precipitation gradient in the Edwards Plateau, Texas, US. The soils were exposed to two different experiments. In the first experiment (upper panel), the resistance of the processes regulated by microbes was tested. To do so, air dried soils was rewetted to 50% WHC followed by a 2 weeks incubation to stabilise rates. Then, soil was dried down during 3 days until it was air dried, and measurements were taken during the dry down process. In the second experiment (lower panel), the resilience of soil microbial communities was tested: soils were air dried and subsequently rewetted to 50% WHC (optimum moisture); then the responses of microbial processes were measured during 1 week

rewetting were followed during 1 week at a constant temperature of 17°C. To allow high temporal resolution of measurements, two sets of samples were used, one rewetted in the evening and the other rewetted in the morning and measured in parallel (Meisner et al., 2013, 2015, 2017). Phospholipid fatty acids (PLFAs) were extracted in each soil before rewetting and 1 day after rewetting to assess the level of destruction exerted on the microbial community by DRW (Figure 1).

Continuously moist soils were used as a reference point ('control') for the DRW experiment. To have process rate measurements for these soils, subsamples of each air dried soil were adjusted to 50% WHC and kept for 2 weeks to let microbial process rate stabilise at 17°C. Once the rates were stable, bacterial growth, fungal growth and respiration were measured once a week for 1 month and the average of these values were used as microbial process rates in continuously moist soils. These values were later used to normalise the DRW data.

2.5 | Measurements

2.5.1 | Bacterial growth

We measured protein production as a proxy for bacterial growth (Rousk & Bååth, 2011). To do so, a radioactively labelled precursor of proteins was used: ^{3}H-leucine. Tracking the incorporation of the precursor into proteins allowed the estimation of protein production. The ^{3}H-leucine incorporation into bacteria was estimated according to the homogenisation/centrifugation technique (Bååth, 1992, 1994; Bååth et al., 2001), using the modifications in Meisner et al. (2013). The amount of leucine incorporated into extracted bacteria (pmol Leu incorporated g^{-1} SOM hr^{-1}) was used as a proxy for bacterial growth.

2.5.2 | Fungal growth

We measured the production of the fungal-specific lipid ergosterol as a proxy for fungal growth (Rousk & Bååth, 2011). To do so, radioactively labelled ^{14}C-acetate was used to track its incorporation to newly synthesised ergosterol (Bååth, 2001; Meisner et al., 2013; Rousk et al., 2009). The amount of incorporated acetate into extracted ergosterol (pmol acetate incorporated g^{-1} SOM hr^{-1}) was used as a proxy for fungal growth.

2.5.3 | Respiration

For moisture dependence measurements, 1.0 g of soil was weighed into 20-ml glass vials, which were purged with pressurised air, sealed with crimp caps and incubated for 24 hr in the dark at 17°C. CO_2 production was measured using a gas chromatograph equipped with a methaniser and an FID detector. For the DRW experiment the same procedure was followed, however, the incubation time varied between 6 hr (at the beginning of the experiment) and 72 hr (at the end of the experiment).

2.5.4 | Determination of biomass using phospholipid fatty acid analysis

Phospholipid fatty acids were extracted from soil subsamples using the method described by Frostegård et al. (1993) with modifications by Cruz-Paredes et al. (2017). The sum of the following PLFAs was used as a measure of the bacterial biomass: i14:0, i15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0, while 18:2ω6,9 was used as a measure of fungal biomass (Frostegård & Bååth, 1996).

2.6 | Data analysis

Microbial community sequence data were processed using DADA2 (Callahan et al., 2016) to determine the amplicon sequence variants (ASVs) with default settings. All raw sequences have been deposited in the National Center for Biotechnology Information Short Read Archive (BioProject PRJNA62748). For the bacterial community analysis, only the forward reads were used which were 230 bp long after trimming for quality. In the case of fungi, the forward (200 bp) and reverse reads (160 bp) were trimmed and concatenated with 'Ns' between them. The ASVs were then curated using LULU (Frøslev et al., 2017) and further filtered if they were present in only one sample and if the total counts of those were below 10. This resulted in a total of 552 and 357 ASVs for bacterial and fungal communities respectively. QIIME2 (Bolyen et al., 2019) was used to rarefy the samples with a sampling depth of ~5,000 and ~4,000 sequence reads for bacterial and fungal communities, respectively, to analyse the alpha and beta diversity of the ASVs. To understand how the conditions of the soil influence the soil microbial communities, soil environmental and physiochemical variables (see Table 1) were correlated with the diversity metrics using the *envfit*() function from the VEGAN package in R (Oksanen et al., 2019). Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities was used to visualise these data. When bacterial communities were too dissimilar the visualisation resulted in a 'horseshoe effect' resulting in a loss of information about community dissimilarities. Therefore, an alternative method to transform the data was performed which used a nonsaturating distance metric, Earth Mover Band Aware Distance (EMBAD; Morton et al., 2017; Rath et al., 2019). Mantel tests with the whole environmental matrix and the single environmental variables were performed, as well as partial Mantel tests to address drivers of community structure.

To assess microbial resistance to desiccation, the moisture dependence of microbial growth and respiration were modelled using a logistic model according to Rath et al. (2017). Then,

microbial drought tolerance was estimated with the IC_{10} and IC_{50} (moisture level at which the process rates are inhibited by 10% and 50% respectively) values for bacterial growth, fungal growth and respiration at each site of the rainfall gradient. Fungal growth inhibition across the studied moisture range was insufficient to calculate IC_{50} values.

Microbial resilience was assessed by modelling growth and respiration responses to a DRW perturbation. Two linear curves were used to model bacterial growth upon DRW; one for the immediate increase in growth after rewetting (Meisner et al., 2013) and a second to describe the decrease of bacterial growth after reaching a maximum growth rate ('inflection point'). In order to find the inflection point, we used a 'broken stick model' which is a regression model where the relationships between the response and the explanatory variable are piecewise linear. The slope changes in different parts along the *X*-axis and thus, this is represented by two straight lines connected by the inflection point. The model is based in the following equation:

$$y = \beta_0 + \beta_1(X) + \beta_2(X - C)^+ + \varepsilon, \quad (1)$$

where β_0 is the intercept, β_1 is the slope before the inflection point *C* and β_2 is the difference in slope after the inflection point. Thus, the slope after the inflection point is $\beta_1 + \beta_2$. The variable $(X - C)^+$ is a derived variable that takes the value of 0 for values of $X < C$, and the values $(X - C)^+$ for values of $X > C$. To estimate the inflection point *C* we used a piecewise linear model (Toms & Lesperance, 2003) in JMP Pro 14 (SAS institute). Once the inflection point was estimated, bacterial growth at the inflection point was calculated, plotted and used to fit both linear curves. A double-first-order exponential-decay function was used to describe respiration (Rousk et al., 2011) whereas a logarithmic function was used for fungal growth. The time that bacterial growth and fungal growth needed to recover to the 50% of the moist control levels were calculated as an index of resilience.

Cumulative bacterial growth, fungal growth and respiration during 1 week following the DRW perturbation were calculated, as well as cumulative growth during 1 week in the continuously moist control soils. To assess only the effect of the perturbation on microbial growth and respiration rates, and to compensate for the effect of any other factor that might cause variation in microbial process rates, DRW data were normalised to the continuously moist control data. This allowed us to focus on the relative responses of microbes to DRW across the gradient.

Linear regressions were used to assess relationships between microbial resilience (50% of recovery of growth rates and inflection point) and resistance (IC_{50} and IC_{10} values) with MAP. In addition, regressions were also used to explore relationships with cumulative processes during 1 week at constant moisture and during 1 week perturbation (both absolute and normalised) with MAP. This was also tested with plant productivity proxied with NDVI. Differences in the recovery time between fungi and bacteria were tested with a one-way ANOVA, using MAP as a blocking variable to isolate the variance related to the difference of the microbial groups.

The amount of total, bacterial and fungal biomass per SOM was estimated before rewetting and 24 hr after rewetting. A regression analysis was used to assess whether cumulative respiration during 24 hr could be linked to destruction of biomass. In addition, a one-way ANOVA was used to compare the amount of biomass before and 24 hr after rewetting, using MAP as a blocking variable to isolate the variance related to the difference of the biomass.

The link between bacterial and fungal community structure and functional traits across the gradient were evaluated using Pearson correlations. The correlation between the principal coordinates of bacterial and fungal communities with cumulative processes (bacterial growth, fungal growth and respiration) for 1 week after rewetting was tested, as well as with the microbial tolerance to desiccation (using IC_{10} and IC_{50} estimates of the processes).

Kaleidagraph 4.5 (Synergy Software) and JMP Pro 14 (SAS Institute) were used for curve fitting, visualising and statistical analyses.

3 | RESULTS

3.1 | Soil and microbial community characterisation

Soil characteristics varied among sites across the gradient (Table 1). Plant productivity increased with higher MAP, shown by the significant relationship between NDVI and MAP ($R^2 = 0.40$ $p = 0.01$). However, soil physiochemistry did not change consistently across the rainfall gradient, as shown by the lack of relationship with MAP ($p > 0.05$). pH was alkaline across the whole gradient and varied within a narrow range from 7.7 to 8.5, showing a small decrease with higher MAP ($R^2 = 0.41$, $p = 0.005$). Total ($p = 0.02$), bacterial ($p = 0.05$) and fungal ($p = 0.005$) biomass measured as PLFAs all showed a significant increase with MAP across the gradient.

The alpha diversity of bacterial communities increased slightly but significantly with MAP ($p = 0.01$, Figure 2a), whereas fungal alpha diversity did not change across the gradient (Figure 2b). Both bacterial and fungal beta diversities were constrained by MAP ($R^2 = 0.86$, $p = 0.001$ and $R^2 = 0.51$, $p = 0.01$ respectively) and pH ($R^2 = 0.44$, $p = 0.03$ and $R^2 = 0.40$ $p = 0.05$ respectively; Figure 2c,d). MAP was significantly linked to the PCo1 of the bacterial community variance ($R^2 = 0.50$, $p = 0.002$; Figure 2e) as well as to the PCo2 of the fungal community variance ($R^2 = 0.35$, $p = 0.02$; Figure 2f). Mantel tests showed that only MAP had a significant effect on both bacterial ($p = 0.008$) and fungal ($p = 0.02$) beta diversity. This was confirmed through partial Mantel tests, which showed a non-significant relationship between the bacterial and fungal beta diversity with the environmental matrix when MAP was excluded.

Microbial process rates determined at stable and optimal moisture (50% WHC) also varied across the gradient (Figure 5d–f). Both

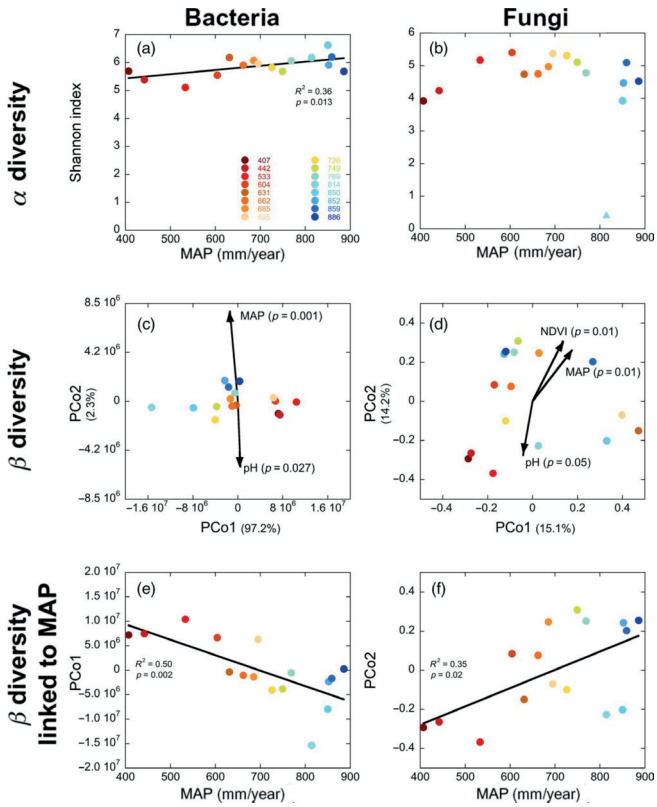

FIGURE 2 Soil microbial communities across the precipitation gradient where each colour represents a single site from the gradient. The amount of precipitation regime of each site is reported in mm/year. Alpha diversity for (a) bacterial and (b) fungal communities shown as Shannon diversity index (an outlier is shown as a cyan triangle for fungal communities). Beta diversity with vectors indicating significant drivers of differences for (c) bacterial (with EMBAD transformation) and (d) fungal communities. The arrows are scaled by their R^2, that is 'weak' predictors have shorter arrows than 'strong' predictors. Extracted principal coordinate values regressed with MAP for (e) bacterial and (f) fungal communities

bacterial growth ($R^2 = 0.49$, $p = 0.001$; Figure 5d) and fungal growth ($R^2 = 0.25$, $p = 0.04$; Figure 5e) significantly increased in soils from sites with higher MAP. By contrast, respiration was stable across the precipitation gradient (Figure 5f).

3.2 | Microbial resistance to drying

The moisture dependence assessments showed that growth and respiration rates decreased with reductions in contemporary moisture (Figure 3). Responses of all three measured microbial processes were modelled well by sigmoidal curves, in all soils across the gradient ($R^2 = 0.64 \pm 0.05$ for bacterial growth, $R^2 = 0.61 \pm 0.04$ for fungal growth and $R^2 = 0.88 \pm 0.01$ for respiration; Figure S1). Generally, respiration rates started declining at higher moisture levels than bacterial or fungal growth, suggesting a higher sensitivity of respiration to moisture. Thus, drier conditions were required to inhibit growth than respiration, especially fungal growth which was sustained at high rates across a broad moisture content range and was never completely inhibited.

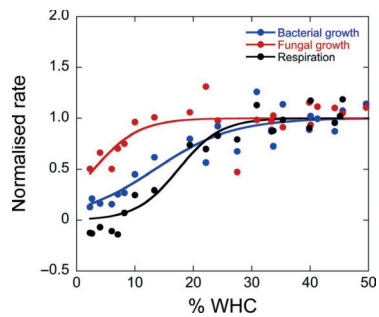

FIGURE 3 The moisture dependence of bacterial growth, fungal growth and respiration in one of the soils from the precipitation gradient (859 mm/year). Sigmoidal curves were fitted to model moisture dependences for the three measured process rates in all soils across the gradient (Figure S5)

Microbial resistance to drought did not change across the gradient based on the moisture content at which the growth and respiration rates were inhibited by 50% and 10% (IC_{50} and IC_{10}; Table 2),

TABLE 2 IC_{10} and IC_{50} (moisture level at which rates are inhibited by 10% and 50% respectively) values for bacterial growth, fungal growth and respiration across the rainfall gradient

	Bacterial growth		Fungal growth	Respiration	
MAP	IC_{10}	IC_{50}	IC_{10}	IC_{10}	IC_{50}
407	55.3	17.3	19.1	41.7	26.3
442	51.0	15.8	30.9	55.1	31.7
533	NA	NA	23.0	27.7	19.5
604	28.4	8.7	16.7	21.7	13.6
631	20.5	8.4	29.9	42.8	25.4
662	14.2	7.7	27.0	48.7	29.5
685	26.5	NA	NA	40.3	25.9
695	26.5	7.2	37.8	33.9	16.5
726	32.1	11.2	9.9	42.2	24.6
749	15.1	8.8	NA	39.6	24.8
769	21.9	12.2	NA	44.4	30.1
814	45.3	17.7	34.7	34.4	20.2
850	23.6	9.4	21.3	48.6	25.2
852	30.6	11.5	21.9	21.7	15.3
859	28.1	13.4	11.5	25.4	17.5
886	29.0	14.0	21.2	32.4	17.4
889	38.1	11.6	27.4	42.4	23.9

Note: IC_{50} and IC_{10} values are reported as %WHC. Since fungal growth was not completely inhibited it was not possible to estimate IC_{50} values for fungi. Note that due to the sigmoidal curve not being a good curve fit in some cases, some estimates for IC_{10} and IC_{50} are not available (NA).

that is changes in MAP or NDVI across the gradient were not linked to changes in IC_{50} and IC_{10} of microbial processes. IC_{50} was always higher for respiration than the growth measurements suggesting a lower tolerance of respiration than growth to reduced moisture. This resulted in higher growth per respiration at low moisture levels across the whole precipitation gradient.

3.3 | Microbial resilience to DRW

The response of microbial processes to a DRW event was similar in all sites across the gradient (Figures S2–S4). Bacteria started growing immediately until they reached a maximum, before decreasing to converge with the level of the continuously moist control (Figure 5a; Figure S2). Thus, bacterial growth responses were modelled by two linear curve fits, an increasing curve ($R^2 = 0.75 \pm 0.05$) and decreasing curve ($R^2 = 0.21 \pm 0.05$) which were separated by an inflection point of maximum growth at 22.2 ± 2.2 hr (Figure 4a; Figure S2). Fungi started growing immediately, starting from levels lower than in the moist control soil and gradually stabilising after about 20 hr in all sites. However, fungi never recovered to the levels of the continuously moist soils (i.e. values never reached 1; Figure 4b; Figure S3). Fungal growth dynamics were modelled well in all instances by a logarithmic curve

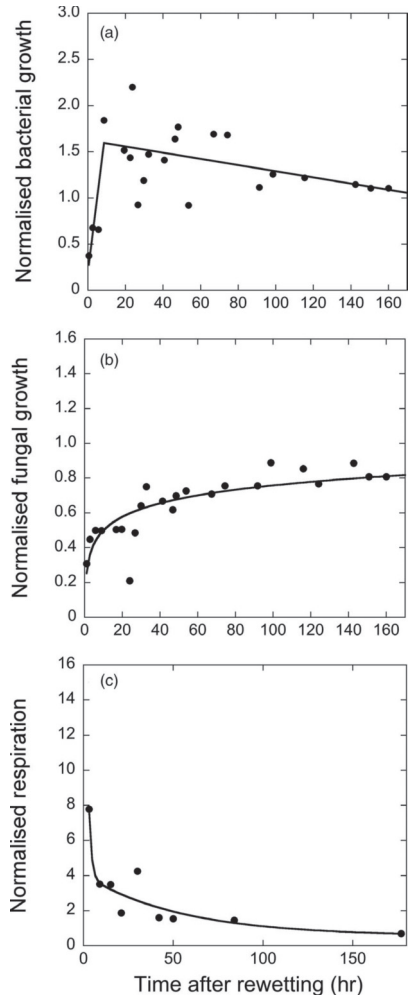

FIGURE 4 Microbial growth and respiration after a drying and rewetting (DRW) perturbation. Example of responses to DRW for (a) bacterial growth, (b) fungal growth and (c) respiration (852 mm/year). Bacterial growth was modelled with two linear curve fits which were separated by an inflection point (see Section 2). Fungal growth and respiration were modelled with a logarithmic and a double exponential curve, respectively (see Section 2). DRW responses for all 17 individual sites are provided in the Supporting Information (Figures S1–S3)

($R^2 = 0.43 \pm 0.06$; Figure 4b; Figure S3). The 50% of recovery of bacterial growth and fungal growth rates to levels of continuously moist soil was not significantly linked to MAP ($p > 0.05$); however, a tendency of increase with higher MAP was observed. In addition, there were no significant differences ($p > 0.05$) between the recovery time of bacteria

and fungi. In addition, the inflection point for bacterial growth did not show a significant link with MAP.

Respiration rates peaked immediately after rewetting in all soils. The peak was between 2 and 14 times higher than in the continuously moist control soils, after which the rates decreased and gradually converged with levels of the controls (Figure 4c; Figure S4). These dynamics were modelled well by a double exponential curve in all cases ($R^2 = 0.76 \pm 0.05$). To determine if observed CO_2 releases were due to microbial biomass destruction, we calculated the proportion of destroyed PLFAs 24 hr after rewetting (Table S2). PLFA destruction did not vary consistently across the gradient ($p > 0.05$) and could not explain the respiration pulse after rewetting. Microbial biomass measured as PLFAs even increased significantly 24 hr after rewetting ($p < 0.0001$), suggesting a net growth since rewetting.

To assess how the microbial C-budget during the DRW perturbation varied across the gradient, cumulative values were estimated for the microbial processes. Cumulative bacterial growth and respiration following the DRW disturbance changed systematically across the precipitation gradient, having the highest rates for both parameters in the historically wettest sites (Figure 5a,c). In contrast, fungal growth in response to DRW did not show a significant change across the gradient (Figure 5b). These processes did not change with variation in plant productivity which was shown by the lack of correlation with NDVI ($p > 0.05$ in all cases).

To specifically resolve the effect of the DRW perturbation on microbial processes, the variation caused by other factors was removed by normalising data using cumulative growth and respiration of continually moist soils from each site, over an equivalent 1-week period (Figure 5d–f). Normalised data showed that both bacterial and fungal growth were unaffected by the precipitation regime (Figure 5g,h). Cumulative bacterial growth during the perturbation matched that in the continuously moist soils (i.e. values equaled 1, Figure 5g). In contrast, cumulative fungal growth never reached 1; that is, values never recovered to moist control levels in the week following the DRW

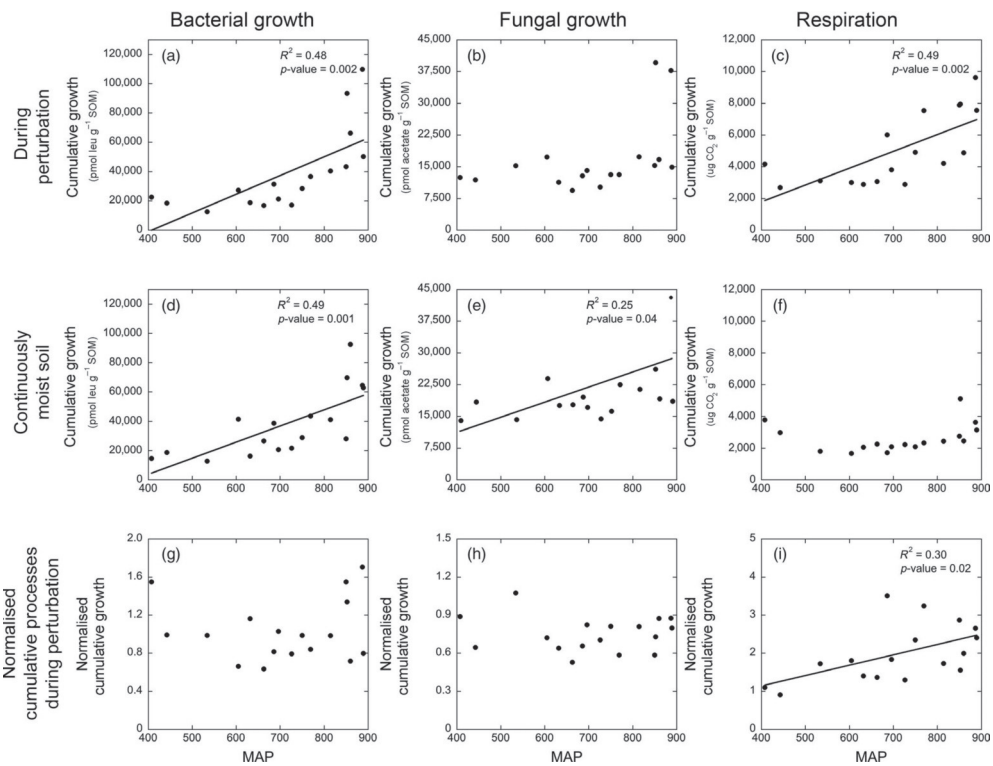

FIGURE 5 Cumulative (a) bacterial growth (b) fungal growth and (c) respiration during 1 week after a drying and rewetting (DRW) perturbation across the precipitation gradient. In addition, cumulative (d) bacterial growth (e) fungal growth and (f) respiration during 1 week in continuously moist soil from across the precipitation gradient. Finally, cumulative (g) bacterial growth (h) fungal growth and (i) respiration during 1 week of DRW perturbation normalised to moist control values across the precipitation gradient

perturbation (Figure 5h). However, differences in respiration during DRW could be linked to differences in MAP, with lower cumulative respiration in the historically drier sites (where respiration over 1 week after rewetting matched that in the moist control soils) and an almost 3-fold higher cumulative respiration in the sites with a higher MAP (Figure 3i; $R^2 = 0.30$, $p = 0.02$). These differences across the gradient resulted in a higher respiration per growth rate in the historically wetter sites than in the historically drier sites. The same patterns were observed when the normalised data were regressed against plant productivity proxied with NDVI: while there was a lack of correlation of plant productivity with cumulative bacterial growth and fungal growth ($R^2 < 0.01$, $p = 0.97$; $R^2 = 0.17$, $p = 0.1$), the cumulative respiration increased significantly with higher NDVI measurements ($R^2 = 0.28$, $p = 0.04$).

3.4 | Linking community structure to function

Pearson correlations did not show any significant relationships between the structure of bacterial and fungal communities and cumulative processes 1 week after rewetting, or with the IC_{10} and IC_{50} values of microbial processes (Table S3).

4 | DISCUSSION

4.1 | Validation of the precipitation gradient

We here investigated the legacy effect of drought on microbial responses to DRW perturbations and drought tolerance using soils from a precipitation gradient in Texas. As such, our assessment relied on MAP being the main factor varying consistently across the gradient. Most physiochemical parameters showed no significant relationship with MAP (Table 1), nor did plant cover (Hawkes et al., 2017). Soil pH, which showed a significant relationship with MAP, was alkaline across the entire precipitation gradient, varying only within a narrow range between 7.7 and 8.5. The decrease of pH with higher MAP is common due to higher leaching of basic cations in soils exposed to higher precipitation (Voroney & Heck, 2015). NDVI measurements also showed a significant relationship with MAP, suggesting an increase of plant productivity with MAP. Therefore, historical MAP was the single most important environmental factor that varied consistently across this gradient, which also had an effect on plant productivity since moisture is one of the main constraints for plants in ecosystems subjected to drought (Reichstein et al., 2013). This was also confirmed with the changes in the microbial community structure across the gradient. Soil microbial communities were mainly constrained by MAP (Figure 2). The bacterial and fungal beta diversity was also affected by pH (Figure 2c,d). Soil pH is known to have a great influence on fungi and bacteria (Rousk et al., 2009). However, the pH range that these soils have has been shown to not strongly influence microbial functions (Rousk et al., 2009), nor community composition and structure (Lauber et al., 2009; Rousk, Bååth, et al., 2010). Therefore, the observed effect of pH on microbial structure differences is probably due to the correlation between MAP and pH. Plant productivity also had an effect on the fungal beta diversity (Figure 2d). Fungi are strongly coupled with plants (Van Der Heijden et al., 2008; Wardle et al., 2004) which suggests that this link is probably an indirect effect of drought.

4.2 | Microbial resistance and resilience to drought were independent of historical precipitation

We hypothesised that (1) exposure to different MAP would structure microbial communities with a higher drought tolerance in historically drier sites. Contrary to our expectations, we did not observe differences in drought tolerance across the gradient for any of the microbial processes that we measured. This is in line with results from a field experiment that showed the resistance of both respiration and bacterial growth was similar in control soils and those exposed to 18 years of experimental drought (de Nijs et al., 2019). Moreover, a meta-analysis showed similar moisture dependences of microbial communities across biomes and climates (Manzoni et al., 2012). These results were consistent with prior observations that drier sites on the gradient showed always lower respiration regardless of moisture (Hawkes et al., 2017), which was also found in assessments of moisture sensitivity of respiration in experimental treatments (Hawkes et al., 2020).

We further hypothesised that (2) the responses of soil microbial communities to DRW would depend on MAP, expecting more resilient communities in the historically drier sites. Contrary to our predictions, when soils were exposed to a DRW event in the laboratory, bacterial growth, fungal growth and respiration exhibited a similar general set of dynamics after rewetting along the whole gradient (Figures S2–S4). After rewetting, respiration and growth responses started to increase without delay in all instances. Respiration rates increased immediately after rewetting reaching several-fold (2–14) higher levels than in the continuously moist soils, which then was followed by an exponential decrease (Figure 3c; Figure S4). Bacterial growth started from low levels and increased rapidly in a linear fashion to typically reach maximum growth around 22 hr before decreasing to align with moist control levels (Figure 4b; Figure S2). These type of responses have been reported before (Iovieno & Bååth, 2008; Meisner et al., 2013), and they have been linked to highly resilient communities (de Nijs et al., 2019), which perceive DRW disturbances as mild perturbations and recover quickly to a pre-disturbance state (Meisner et al., 2017). Therefore, we find no evidence for a legacy effect of drought on microbial responses to DRW. This finding is in line with another study that did not find drought legacy effects on microbial response to DRW (Rahman et al., 2018). Although we did not find a legacy effect of drought on the resilience of bacterial and fungal growth, we observed a tendency for a faster recovery time in the historically drier sites. This is consistent with other studies that showed that a legacy of drought can induce a shift towards a more resilient bacterial community after rewetting (de Nijs et al., 2019; Göransson et al., 2013), shortening the lag period before bacterial

growth starts to increase and recovers to the pre-disturbance state (Meisner et al., 2017). This might indicate that differences in drought history across the gradient were insufficient to induce a detectable difference. Instead, the general aridity of the ecosystem exceeded the signal of historical differences across the gradient.

There is no consensus in the literature regarding the resilience of microbial communities to DRW at the field scale. A set of studies reported that exposure to a higher frequency of DRW events selected for more stress-tolerant taxa (Evans & Wallenstein, 2012, 2014). Two field studies also showed contrasting legacy effects of drought on bacterial resilience to DRW, where a legacy of drought induced a higher resilience in a well-drained organic temperate heathland soil in the Netherlands (de Nijs et al., 2019), while in a forest soil from the UK a legacy of summer drought resulted in a tendency for a lower bacterial resilience (Göransson et al., 2013). It has previously been argued that prior exposure to disturbances can affect the resistance and resilience of microbial communities to new disturbances (Griffiths & Philippot, 2013). For instance, previous laboratory studies showed that if bacteria were exposed to a series of DRW cycles they were selected to better cope with such perturbations, resulting in a more resilient response (de Nijs et al., 2019; Leizeaga Sanchez, 2015). Moreover, a less resilient response has been observed when soils in the laboratory undergo a long period of either drought or constant moisture without DRW events (Meisner et al., 2015). These lines of evidence suggest that the previous exposure to DRW events might be the factor determining the resilience of soil microbes to DRW. The savannah grassland soils we studied here are all periodically dry environments: despite average differences in rainfall across the gradient, the entire Edwards Plateau experienced severe drought from 2011 to 2014, followed by above normal rainfall in 2015. Therefore, the observed similarity in both resistance and resilience across the gradient is likely due to selection on soil microbes from repeated historical exposure to drought and DRW events rather than the average amount of rainfall.

4.3 | Microbial carbon use efficiency was affected by historical precipitation

Microbial communities had a generally high CUE at low moisture levels across the precipitation gradient, but used C more efficiently upon DRW in sites at the driest end of the gradient. This is largely consistent with our expectations (3a, 3b), and suggests high drought tolerance across the gradient but with shifts in the microbial C-use as drought history becomes more severe. Decreasing moisture availability is a stressor for microbial communities, which can induce the need for physiological acclimation strategies that might lead to shifts in resource allocations (Schimel et al., 2007). Consequently, there would be a need for microbes to allocate less resources to growth and more to survival, resulting in a decrease in CUE as soil dries (Schimel, 2018). In our study, we compared the moisture dependences of growth and respiration in the different soils across the gradient (Figure S1). These comparisons showed a higher growth per respiration ratio at dry conditions in all sites. That is, at low moisture levels microbial communities allocated relatively more C for growth, suggesting a high CUE; consistent with recent assessments of steady-state rates in an Austrian set of soils (Canarini et al., 2020), but contrasting with results from a temperate heath (de Nijs et al., 2019). In a laboratory experiment where soils were dried to different moisture contents, a higher CUE was found at low moisture levels and attributed to accumulation of osmolytes based on ^{13}C tracing (Herron et al., 2009). This in an unlikely explanation in our case since the proxies used for growth were determined by tracking changes in rates of protein and membrane production and therefore were unlikely to capture C accumulation inside the cells. As noted earlier, these savanna grassland soils commonly undergo drought periods, which might select for microbial taxa with more efficient growth in dry conditions across the entire gradient.

Shifts in CUE may be a common response to drought. A previous field experiment that assessed respiratory responses upon DRW in control and drought-treated soil showed higher C releases through respiration in control soils (i.e. historically wetter sites) than drought-treated soils (Evans & Wallenstein, 2012). Göransson et al. (2013) also investigated microbial dynamics upon DRW in soils with a history of summer drought versus control soils. In line with our results, they observed differences in CO_2 releases during the perturbation, while cumulative growth was similar; which also resulted in a higher CUE in the drought-treated soils compared to the control. It has been postulated that the C made available to microorganisms by a DRW event may be due to the destruction of microbial biomass induced by the perturbation (Fierer et al., 2003; Kieft et al., 1987). To evaluate the contribution of this putative C source in our study, we determined the change on PLFA concentrations induced by the DRW. Surprisingly, we found that DRW did not decrease PLFA concentration in any of the sites, and there was also no link between PLFA concentration changes and MAP or plant productivity (NDVI). All this suggests that the destruction of microbial biomass was not an important source of C in any of the studied soils, and that microbial cells in these drought-prone soils were relatively resistant to the DRW disturbance. Alternative sources of C released could be intracellular solutes that microorganisms accumulate during drought (Fierer & Schimel, 2003; Warren, 2014, 2016; Xiang et al., 2008) or newly mobilised C (Denef, Six, Bossuyt, et al., 2001; Denef, Six, Paustian, et al., 2001; Six et al., 2004).

Drought legacies of C mineralisation could also be caused by differences in C availability, which can be primarily linked to differences in plant input. Evapotranspiration and therefore primary productivity are linked to differences in precipitation (Allen et al., 1984), which we also find evidence for across the gradient, with more vegetation productivity at higher MAP (Table 1). In addition, a history of drought has also previously been reported to decrease plant productivity (Bréda et al., 2006), which can have an impact on soil microbial communities through plant–soil feedbacks (Fuchslueger et al., 2014; Williams & de Vries, 2020). Some studies have shown drought legacy effects on mineralisation rates (Evans & Wallenstein, 2012; Hawkes et al., 2017; Martiny et al., 2017),

which may be due to differences in the lability and availability of C to microbes (Hicks et al., 2018). However, there is no evidence that soil C varies with precipitation history across the Texas gradient sites: there was no consistent variation of SOM, SIC or C/N across the gradient (Table 1). In this study, when we used respiration per SOM as an index of C quality available in soil (Fierer et al., 2005, 2006; Robertson & Paul, 2000) it also did not show significant changes across the gradient at optimum moisture conditions (Figure 5f). Nevertheless, these high-level C pools may not capture the accessibility of SOM to microbes, which is partly driven by the molecular-scale physiochemical interactions with mineral surfaces and physical protection of organic matter (Cotrufo et al., 2013; Lehmann & Kleber, 2015). Plants therefore can determine the C availability to microbes during DRW events (Barnard et al., 2020). This opens up new research questions where intact plant–soils systems should be considered.

Differences in moisture history can affect plant input and C rhizodeposition (Canarini & Dijkstra, 2015); while it might simultaneously affect the accessibility of C to microbes via mineral-associations that stabilise SOM and increase the residence time of C pools (Das et al., 2019). DRW disturbances are well known to mobilise organic material (Schimel, 2018), and therefore could mobilise minerally stabilised C, making it available for soil microbial communities. This could explain the higher proportion of respired C in historically wetter sites in a study where drought-prone soils were compared with irrigated soils (Williams & Xia, 2009), as well as in the historically wetter sites in our study system. However, there is also a decoupling between growth and respiration, as while the relative respiration upon rewetting increased with MAP, both bacterial and fungal growth remained stable, generating the above-mentioned differences in CUE. This could be explained by differences in the quality of SOM in terms of amount of nutrients and energy content that would result in differences in CUE (Manzoni, 2017; Roller & Schmidt, 2015; Silva-Sánchez et al., 2019; Soares & Rousk, 2019). Alternatively, the higher respiration upon rewetting in the historically wetter sites could also be explained by stress. Microbial communities in the wetter end of the gradient may have had a lower physiological readiness to cope with a DRW disturbance due to a reduced sensitivity to moisture fluctuations caused by historical drought (Veach & Zeglin, 2019), which would result in a lower CUE (Schimel et al., 2007).

4.4 | Fungi are more resistant but similarly resilient to drought compared to bacteria

We found support for our hypothesis that (4) fungi are more resistant to drought and less resilient to DRW perturbations than bacteria. We showed that in this ecosystem fungi were more resistant than bacteria since they were able to maintain their activity at lower moisture levels. Fungi are generally thought to be more resistant to drought due to their filamentous structure that gives them exploratory surface. In fact, fungal-based food webs have been suggested to be generally more resistant to environmental changes (Wardle et al., 2004) and specifically more tolerant to drought (De Vries et al., 2012; Gordon et al., 2008) due to their ability to redistribute water (Guhr et al., 2015) and their thick chitinous cell walls (Harris, 1981). Yet fungi were less resilient in terms of time needed to recover to pre-disturbance levels. Even though fungi started growing immediately, they mostly failed to fully recover to pre-disturbance growth rates (Figure S3). Bacteria typically recovered and exceeded pre-disturbance growth rates within 22 hr after water was added to the dry soil. However, using 100% recovery as our baseline did not allow for robust evaluation of differences between fungi and bacteria, due to the observed partial recovery of fungi. Instead, when we used 50% of recovery as a proxy for resilience, we found that bacteria and fungi needed a similar time to recover; suggesting that bacteria and fungi were similarly resilient in these soils up to a point. It has previously been suggested that when the conditions are optimal for bacteria, their growth can inhibit fungal growth at steady-state conditions (Rousk, Brookes, et al., 2010). Therefore, it is possible that fungal recovery beyond 50% was inhibited by competition with bacteria (Hicks et al., 2019).

4.5 | Precipitation regime shaped microbial communities

Finally, we confirmed that (5) bacterial and fungal communities differed across the rainfall gradient, but this did not explain observed differences in function. Variation in bacterial and fungal communities was mostly linked to MAP (Figure 2), which is consistent with previous studies that have shown that precipitation regime can structure community composition (Allison & Martiny, 2009; Evans et al., 2014; Toberman et al., 2008). There is a general expectation that microbial community structure is a primary driver of function (Fierer, 2017), which has also been previously reported in this particular system (Waring & Hawkes, 2018). While it has been found that microbial community structures can be shaped by a DRW perturbation (Barnard et al., 2013; Blazewicz et al., 2020; Placella et al., 2012), this will not obscure dissimilarities in community structures due to other environmental differences, including the precipitation regime, between the soils (Engelhardt et al., 2018; Evans & Wallenstein, 2012; Veach & Zeglin, 2019). In our study, the differences in microbial communities were not linked to differences in functional responses upon rewetting nor to differences in the resistance of bacterial growth, fungal growth and respiration (Table S3). Previous studies have shown that microbial functions assessed at controlled, steady-state conditions are less sensitive to differences in precipitation regime (Rousk et al., 2013), while here the dynamic functional responses resolved over time have picked up signals from the drought legacy. Irrespective, the observed functional differences across the gradient upon DRW were better linked directly to precipitation regime, suggesting a direct link to environmental factors rather than an indirect link, via the community structure.

5 | CONCLUSIONS

Precipitation history did not result in differences in microbial resistance to drought and resilience to moisture fluctuations, indicating that there was no drought legacy effect on microbial functional stability in this ecosystem. This suggested that long-term differences in precipitation regime did not shape microbial community responses to DRW. Instead, previous exposure to DRW events in this drought-prone ecosystem likely shaped the communities similarly across the gradient, resulting in generally resilient bacterial and fungal communities to DRW. Different histories of drought did, however, result in differences in the C-use of microbial communities during DRW, with lower C losses through respiration in historically drier sites. In addition, there was a generally high CUE at low moisture levels across the entire gradient. This may be due to the high frequency of historical exposure to drought in this particular ecosystem. Taken together, these results suggest that previous exposure to drought and DRW events can shape the stability of both microbial communities and the ecosystem processes they control. This needs representation in soil C models to improve our ability to predict how ecosystems will respond to future climate scenarios.

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COMPETING INTERESTS

The authors do not have any conflict of interest to report.

AUTHORS' CONTRIBUTIONS

J.R. conceived the idea; C.V.H. conducted the field work; A.L., L.C.H. and J.R. designed the experiment; C.V.H. conducted the DNA extractions; A.L. and L.C.H. conducted the laboratory experiments; L.M. and A.L. conducted the bioinformatic analysis; A.L. analysed the data; A.L. lead the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA AVAILABILITY STATEMENT

Raw sequence data can be accessed via the National Center for Biotechnology Information Short Read Archive under BioProject PRJNA627480. All the data provided in this manuscript as well as the calculation done with can be found in https://doi.org/10.5061/dryad.r2280gbbn (Leizeaga et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary information

Materials and methods

Site information

Samples were collected from the Edwards Plateau in central Texas at the sites and locations indicated in Table S1. MAP serves as a key to the sites throughout.

Table S1. Site name, latitude and longitude (decimal), and mean annual precipitation (MAP).

Site	Latitude	Longitude	MAP (mm year^{-1})
FLASHS	30.67 N	101.70 W	407
SCATSP	29.69 N	101.32 W	442
DRITSP	29.94 N	100.93 W	534
KCATSP	29.61 N	100.45 W	605
MORECO	29.74 N	100.10 W	632
CASECO	30.15 N	99.99 W	663
HENECO	29.75 N	100.13 W	686
KOOECO	29.77 N	100.08 W	696
KERWMA	30.07 N	99.51 W	726
KENECO	30.04 N	99.39 W	750
MOCECO	29.77 N	99.81 W	769
COLECO	30.33 N	98.44 W	814
BREECO	30.29 N	98.09 W	850
INGECO	30.33 N	98.45 W	852
KERECO	30.13 N	98.53 W	860
LBJWFC	30.18 N	97.87 W	887

Results

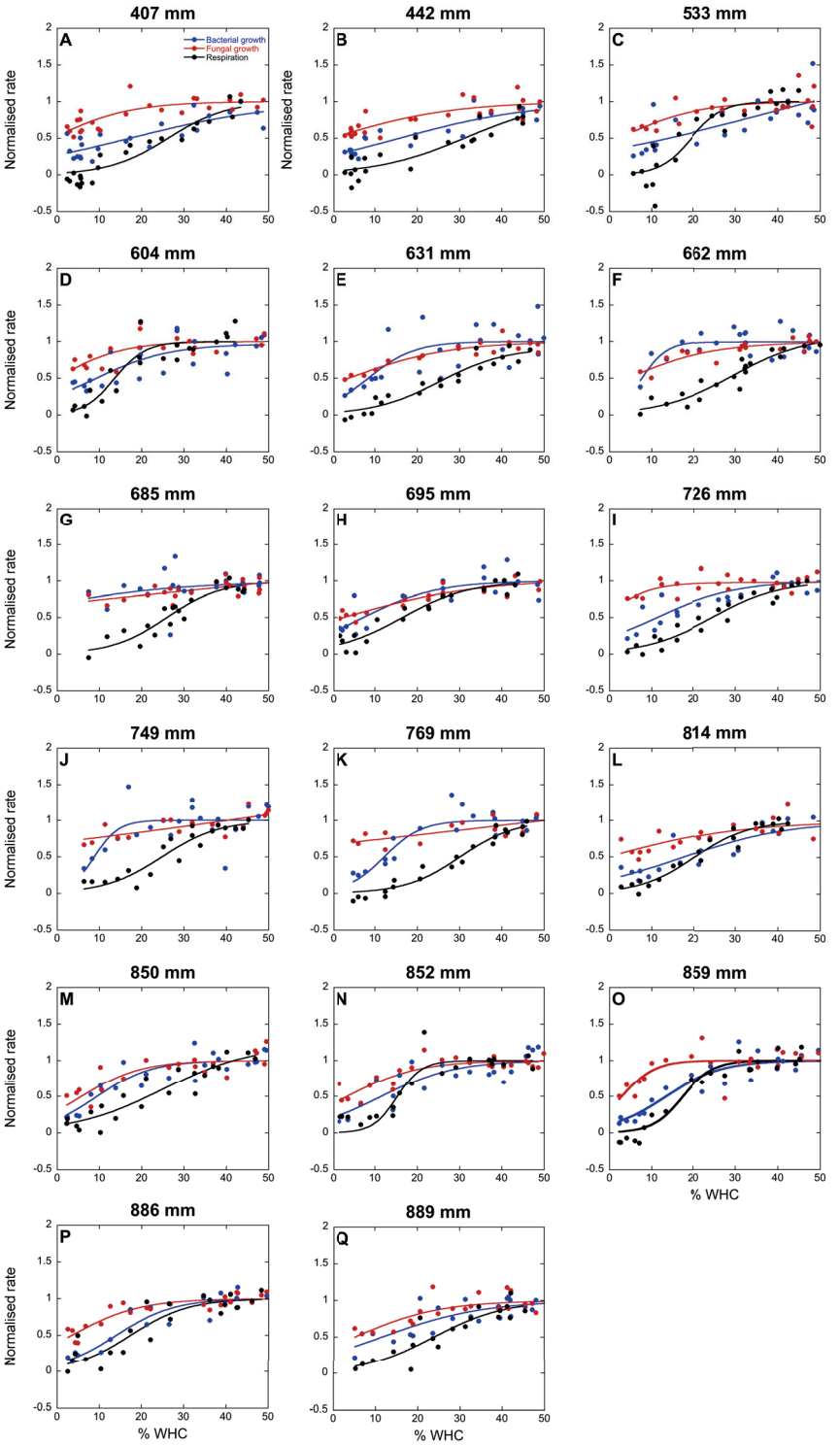
Table S2. Total PLFAs before rewetting and 1 day after rewetting.

MAP (mm year^{-1})	PLFA before rewetting	PLFA 1 day after rewetting	Destroyed total biomass*
407	205.1	237.9	-16.0
442	145.7	172.0	-18.0
533	172.8	189.4	-9.6
604	259.0	370.8	-43.2
631	171.8	201.0	-17.0
662	156.2	223.6	-43.2
685	262.8	296.9	-13.0
695	137.2	184.5	-34.5
726	259.8	299.2	-15.2
749	202.0	253.4	-25.4
769	244.2	304.8	-24.8
814	283.0	335.9	-18.7
850	243.9	199.5	18.2
852	357.0	443.4	-24.2
859	159.5	218.8	-37.1
886	390.0	283.3	27.4
889	256.6	305.8	-19.2

*Total destroyed biomass is showed as the difference between the PLFAs before rewetting and 1 day after rewetting. The % of destroyed biomass in relation to the biomass before rewetting is showed.

Table S3. Pearson correlation table for principal coordinates of soil microbial communities and microbial functions

	Drying and rewetting						**Moisture dependence**									
	Cumulative bacterial growth		Cumulative fungal growth		Cumulative respiration		Bacterial IC_{10}		Bacterial IC_{50}		Fungal IC_{10}		Respiration IC_{10}		Respiration IC_{50}	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
PCo1 Bacteria	-0.2	0.48	--	--	-0.15	0.6	0.3	0.26	0.3	0.2	--	--	0.1	0.72	0.18	0.51
PCo1 Fungi	--	--	0.28	0.3	-0.22	0.42	--	--	--	--	-0.2	0.55	-0.35	0.19	-0.37	0.16
PCo2 Fungi	--	--	-0.1	0.83	-0.1	0.72	--	--	--	--	-0.4	0.16	-0.08	0.77	0.12	0.65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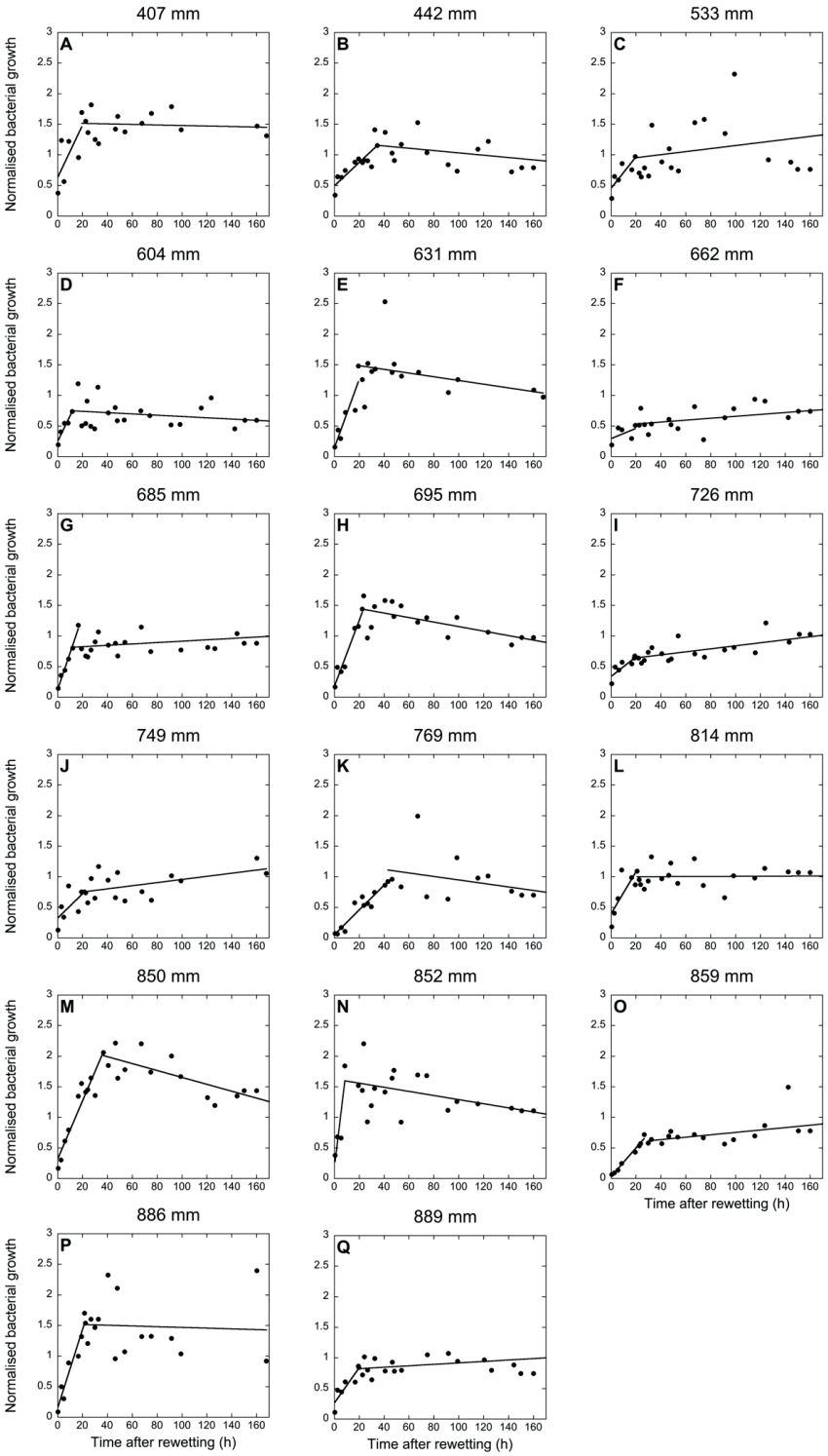
Bacterial growth
Fungal growth
Respiration
Normalised rate
% WHC

Figure S1. Moisture dependences of bacterial growth, fungal growth and respiration across the precipitation gradient (A) 407 mm $year^{-1}$(B) 442 mm $year^{-1}$ (C) 533 mm $year^{-1}$ (D) 604 mm $year^{-1}$ (E) 631 mm $year^{-1}$ (F) 662 mm $year^{-1}$ (G) 685 mm $year^{-1}$ (H) 695 mm $year^{-1}$ (I) 726 mm $year^{-1}$ (J) 749 mm $year^{-1}$ (K) 769 mm $year^{-1}$ (L) 814 mm $year^{-1}$ (M) 850 mm $year^{-1}$ (N) 852 mm $year^{-1}$ (O) 859 mm $year^{-1}$ (P) 886 mm $year^{-1}$ (Q) 889 mm $year^{-1}$. Moisture dependences in all instances we modelled using sigmoidal curves; $R^2 = 0.64 \pm 0.05$ for bacterial growth, $R^2 = 0.61 \pm 0.04$ for fungal growth and $R^2 = 0.88 \pm 0.01$ for respiration.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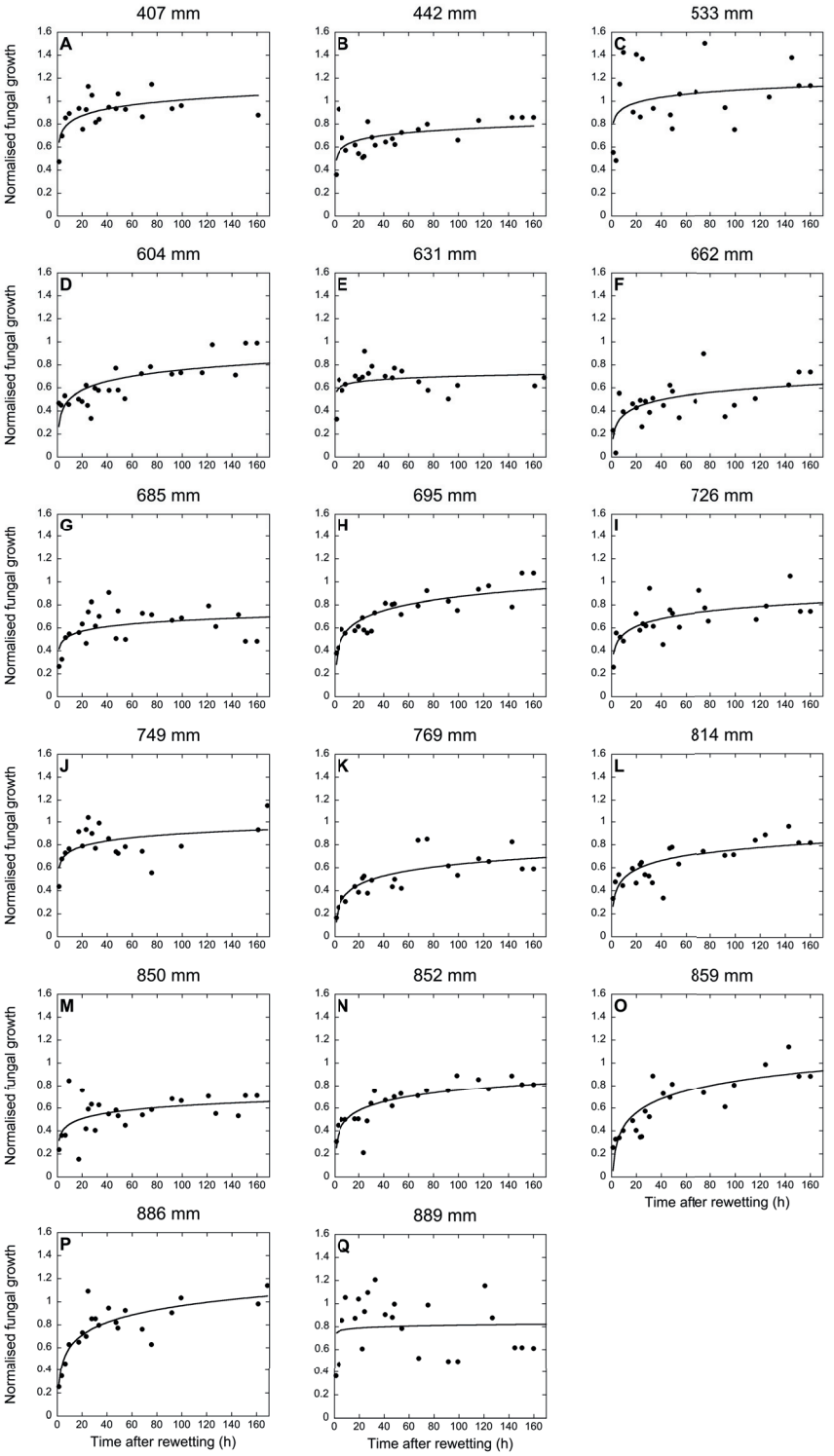
Normalised bacterial growth
Time after rewetting (h)
0 20 40 60 80 100 120 140 160
0 0.5 1 1.5 2 2.5 3

Figure S2. Bacterial growth responses after rewetting dry soils across all the precipitation gradient (A) 407 mm $year^{-1}$ (B) 442 mm $year^{-1}$ (C) 533 mm $year^{-1}$ (D) 604 mm $year^{-1}$ (E) 631 mm $year^{-1}$ (F) 662 mm $year^{-1}$ (G) 685 mm $year^{-1}$ (H) 695 mm $year^{-1}$ (I) 726 mm $year^{-1}$ (J) 749 mm $year^{-1}$ (K) 769 mm $year^{-1}$ (L) 814 mm $year^{-1}$ (M) 850 mm $year^{-1}$ (N) 852 mm $year^{-1}$ (O) 859 mm $year^{-1}$ (P) 886 mm $year^{-1}$ (Q) 889 mm $year^{-1}$. All soils had a similar response patter where bacterial started growing immediately after rewetting reaching a maximum growth level before growth stabilized or started decreasing to moist control levels. These dynamics were modelled using 2 linear models (R^2 = 0.75 ± 0.05; R^2=0.21 ± 0.05) with an intersection of maximum growth at 22.2 h ± 2.2.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Normalised fungal growth
Time after rewetting (h)
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0.2
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0.8
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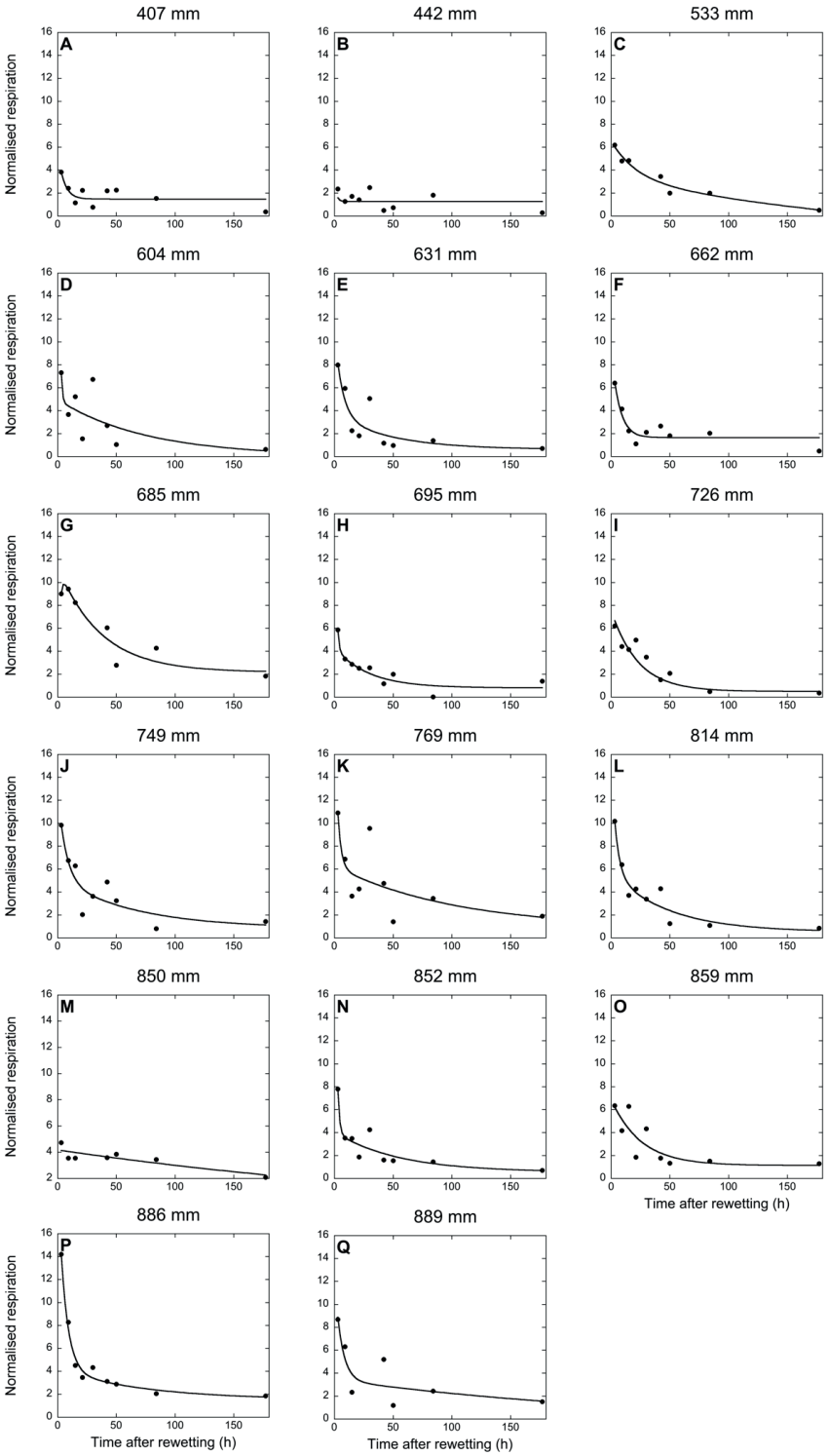
80
100
120
140
160

Figure S3. Fungal growth responses after rewetting dry soils across the precipitation gradient (A) 407 mm $year^{-1}$ (B) 442 mm $year^{-1}$ (C) 533 mm $year^{-1}$ (D) 604 mm $year^{-1}$ (E) 631 mm $year^{-1}$ (F) 662 mm $year^{-1}$ (G) 685 mm $year^{-1}$ (H) 695 mm $year^{-1}$ (I) 726 mm $year^{-1}$ (J) 749 mm $year^{-1}$ (K) 769 mm $year^{-1}$ (L) 814 mm $year^{-1}$ (M) 850 mm $year^{-1}$ (N) 852 mm $year^{-1}$ (O) 859 mm $year^{-1}$ (P) 886 mm $year^{-1}$ (Q) 889 mm $year^{-1}$. All soils had the same response pattern where fungi started growing immediately and stabilized c. 20h after rewetting, never reaching the continuously moist soil growth levels. Fungal dynamics were modelled using a logarithmic fit in all instances (R^2=0.43 ± 0.06).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Normalised respiration
Time after rewetting (h)

Figure S4. Respiration responses after rewetting dry soils across the precipitation gradient (A) 407 mm $year^{-1}$ (B) 442 mm $year^{-1}$ (C) 533 mm $year^{-1}$ (D) 604 mm $year^{-1}$ (E) 631 mm $year^{-1}$ (F) 662 mm $year^{-1}$ (G) 685 mm $year^{-1}$ (H) 695 mm $year^{-1}$ (I) 726 mm $year^{-1}$ (J) 749 mm $year^{-1}$ (K) 769 mm $year^{-1}$ (L) 814 mm $year^{-1}$ (M) 850 mm $year^{-1}$ (N) 852 mm $year^{-1}$ (O) 859 mm $year^{-1}$ (P) 886 mm $year^{-1}$ (Q) 889 mm $year^{-1}$. All soils exhibited the same respiration dynamics after rewetting. Respiration peaked immediately and decreased exponentially until moist control levels within 1 week. Respiration responses to DRW were modelled in all soils with a double exponential fit (R^2=0.76± 0.05).

Paper IV

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PRIMARY RESEARCH ARTICLE

WILEY Global Change Biology

Soil microbial moisture dependences and responses to drying–rewetting: The legacy of 18 years drought

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Abstract

Climate change will alter precipitation patterns with consequences for soil C cycling. An understanding of how fluctuating soil moisture affects microbial processes is therefore critical to predict responses to future global change. We investigated how long-term experimental field drought influences microbial tolerance to lower moisture levels ("resistance") and ability to recover when rewetted after drought ("resilience"), using soils from a heathland which had been subjected to experimental precipitation reduction during the summer for 18 years. We tested whether drought could induce increased resistance, resilience, and changes in the balance between respiration and bacterial growth during perturbation events, by following a two-tiered approach. We first evaluated the effects of the long-term summer drought on microbial community functioning to drought and drying–rewetting (D/RW), and second tested the ability to alter resistance and resilience through additional perturbation cycles. A history of summer drought in the field selected for increased resilience but not resistance, suggesting that rewetting after drought, rather than low moisture levels during drought, was the selective pressure shaping the microbial community functions. Laboratory D/RW cycles also selected for communities with a higher resilience rather than increased resistance. The ratio of respiration to bacterial growth during D/RW perturbation was lower for the field drought-exposed communities and decreased for both field treatments during the D/RW cycles. This suggests that cycles of D/RW also structure microbial communities to respond quickly and efficiently to rewetting after drought. Our findings imply that microbial communities can adapt to changing climatic conditions and that this might slow the rate of soil C loss predicted to be induced by future cyclic drought.

KEYWORDS
bacterial growth, climate change, drought adaptation, drying–rewetting, long-term field experiment, resistance and resilience, respiration

1 | INTRODUCTION

The climate is changing, driven by increased concentrations of greenhouse gases in the atmosphere (IPCC, 2013). A major contributor to the carbon dioxide (CO_2) flow from the terrestrial to the atmospheric system is soil respiration, mainly coming from heterotrophic respiration by microorganisms (Schimel, 1995; Yuste et al., 2011). Temperature and moisture are the two most powerful environmental factors to control soil microbial processes and thus

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biogeochemistry (Le Quéré et al., 2009; Waksman & Gerretsen, 1931). The IPCC predicts that climate change driven alterations in the global water cycle will result in bigger spatial and temporal contrasts in precipitation (Ciais et al., 2005; IPCC, 2013; Orth, Zscheischler, & Seneviratne, 2016). Consequently, more extensive periods of drought followed by heavy precipitation events can be expected in Europe (Dai, 2013). An understanding of how drying–rewetting (D/RW) events influence microbial growth and respiration is therefore critical to enable predictions of how climate change induced drought might affect microbial communities, and thus regulate the carbon (C) cycle of the terrestrial biosphere (Reichstein et al., 2013).

Microbes are the most abundant soil organisms and depend strongly on moisture since it is the medium through which they interact with their environment, obtain resources, and disperse (Hueso, García, & Hernández, 2012; Pulleman & Tietema, 1999; Tecon & Or, 2017). The relation between microbial processes and soil moisture is complex, and fluctuating moisture levels can affect growth rates, size, and composition of the microbial community (Barnard, Osborne, & Firestone, 2015; Kim, Vargas, Bond-Lamberty, & Turetsky, 2012). The microbial dependence on moisture is a twofold phenomenon. First, stable state microbial process rates depend on water availability and normally decrease with lower levels of moisture (Manzoni, Schimel, & Porporato, 2012). In addition, one of the most dramatic events that occur in soil is rewetting after a period of drought. A rewetting event triggers a transient period of very high rates of respiration, known as the Birch effect (Birch, 1958). During the Birch effect, microbial biomass, growth, and biogeochemistry are disconnected during explosively fast dynamics (Göransson, Godbold, Jones, & Rousk, 2013; Kim et al., 2012). Microbial communities have two main strategies to adapt to cycles of drought. Microbial communities can either grow more tolerant to reduced moisture during the drying ("resistance," sensu Griffiths et al., 2000; Griffiths & Philippot, 2013), or they can become faster at recovering after the drought has ended and the soil is moist again ("resilience"; sensu Griffiths et al., 2000; Griffiths & Philippot, 2013).

Growing evidence suggests that historical conditions play a major role in microbial responses to changing environments (Averill, Waring, & Hawkes, 2016; Evans & Wallenstein, 2012; Fierer, Schimel, & Holden, 2003; Hawkes & Keitt, 2015; Hawkes, Waring, Rocca, & Kivlin, 2017). Even a single D/RW event can result in a community shift toward more stress tolerant taxa (Evans & Wallenstein, 2012), thus influencing the response to additional environmental change. Furthermore, it has also been shown that the history of soil moisture can affect microbial responses to D/RW cycles in soil. For instance, Fierer et al. (2003) showed that communities in soils with a history of variable moisture were more resistant to additional cycles of this disturbance. This could indicate that microbial communities which have been exposed to severe drought had been structured to better cope with the exposure to an additional D/RW cycle.

Meisner, Bååth, and Rousk (2013) showed that microbial communities can respond in two different manners when exposed to D/RW perturbations. Using experimental microcosm systems, they distinguished: (a) a more resilient type of response where microbes start growing immediately with a quick recovery after rewetting and (b) a less resilient response where the community starts growing exponentially only after an extended lag-period of no growth (Meisner et al., 2013; Meisner, Leizeaga, Rousk, & Bååth, 2017; Meisner, Rousk, & Bååth, 2015). While the mechanisms driving these differences remain elusive, it has been shown that the intensity of the D/RW perturbation—through variation of the duration of drought (Meisner et al., 2015), the moisture level prior to rewetting (Meisner et al., 2017), combining the drying with inhibitors (Rath, Maheshwari, & Rousk, 2017), or preselecting for D/RW-competent communities (Leizeaga Sanchez, 2015)—can determine the type of bacterial growth response induced. As such, a shift in growth responses has been linked to the harshness of drying as perceived by the microbes (Meisner et al., 2015, 2017; Rath et al., 2017).

Microbial resilience to D/RW cycles also has implications for the microbial C budget, with the microbial carbon use efficiency (CUE) determining the proportion of C used for growth relative to the total amount of C consumed. Microbial CUE will be affected by the resilience to D/RW, since a faster recovery of growth rates to values matching those before the rewetting during a period of elevated respiration will result in a higher CUE compared to a slow recovery. An earlier study found support for an enhanced CUE due to changes in resilience in soils with a history of drought when exposed to a D/RW cycle, compared to a soil exposed to ambient conditions, as indicated by a lowered respiration per growth ratio (Göransson et al., 2013). Hence, if microbial communities exposed to D/RW experience each subsequent D/RW event as a less harsh perturbation, they should become better at coping with the perturbation via increased resistance to drought or resilience to rewetting after drought (Griffiths & Philippot, 2013; Schimel, Balser, & Wallenstein, 2007), thus resulting in an enhanced CUE during the perturbation. Yet, while laboratory experiments to date suggest that exposing a community to repeated cycles of D/RW, where the community can grow adapted to the perturbation, should induce a more resilient growth response of the community with an enhanced CUE, the verification of these suggestions at the ecosystem level is thus far lacking. This calls for assessments in field experiments.

At a heathland site in the Netherlands, a drought treatment has been active for the last 18 years, with a >30% precipitation reduction during the growing season simulating an increased frequency of summer droughts, projected due to climate change (Beier et al., 2004; IPCC, 2013). While there has been no significant change to standing plant biomass and plant species richness (Peñuelas et al., 2007), the drought treatment has resulted in reduced aboveground net primary productivity and soil respiration (Reinsch et al., 2017). There is also evidence that drought-treated soils have been more exposed to D/RW events, highlighted by the observed pulse of CO_2 release from drought-exposed soils following rewetting at the end of the summer drought period (Kopittke, Tietema, Loon, & Asscheman, 2014). Thus, this field-experiment offers a valuable opportunity to investigate how a history of drought influences microbial resistance to low moisture and ability to recover when rewetted after drought. Specifically, here we test whether a history of drought conditions

can induce tolerance to lower moisture levels, can induce faster recovery to rewetting of bacterial growth rates, and can alter the balance between respiration and bacterial growth during perturbation events. Our experimental assessment followed a two-tiered approach. First, the effect of the long-term summer drought on microbial resistance to drought and resilience to D/RW was characterized. Second, we challenged the microbial communities with repeated D/RW cycles in the laboratory, to determine if a history of drought had altered microbial resistance and resilience to additional perturbation cycles. We hypothesized that field experimental exposure to long-term summer drought in an intact ecosystem would change microbial community function to (1a) become more resistant to drought and (1b) respond with higher resilience to rewetting after drought. We also hypothesized that exposure to experimental D/RW in the laboratory would induce a higher (2a) resistance to drought and (2b) resilience to D/RW, thus demonstrating a possible mechanism underlying the microbial responses to drought in the field experiment. In addition, we hypothesized that (3) both field drought treatments and microcosm D/RW cycles would select for communities with a lowered ratio of respiration to growth (i.e., higher CUE) during subsequent D/RW perturbations, thus identifying the target trait that microbial communities had been selected for.

2 | MATERIALS AND METHODS

2.1 | Site description

The soil samples came from a heathland site, mainly covered with *Calluna vulgaris*, located in Oldebroek, the Netherlands (52°24′N, 05°55′E), with a mean annual temperature of 8.9°C and mean annual precipitation of 1,005 mm. The site is located on a well-drained moderately acidic soil, classified as a Haplic Arenosol (FAO, 2006). At this site, three plots of 20 m^2 received a summer drought treatment since 1998 and three plots served as a control. During 2–3 months in the summer, a retractable transparent and waterproof cover prevented precipitation from entering the plot of the drought treatment (Beier et al., 2004), leading to a decadal average reduction of precipitation by 30% during the growing season (Rousk, Smith, & Jones, 2013). Soil volumetric water content and temperature have been measured using Decagon soil moisture and temperature sensors (ECH_2O 5TM), with five sensors located in two of the three control and drought-treated plots. Measurements show a clear reduction in soil moisture (Figure 1a) reaching approximately 60% during summer (Figure 1c), with no effect on soil temperature (Figure 1b,d) in the period of April 2014 to the time of sampling.

2.2 | Soil sampling

Soil was collected at the end of April 2017, just before the onset of the drought treatment period of that year. Three composite samples from the organic horizon (2–8 cm thick) of each of the experimental field treatments "drought" and "control" were taken, resulting in three independent replicates for each field treatment. Immediately after sampling, the soils were homogenized by hand and wet-sieved (<6 mm). Samples were stored in the dark at 5°C until the start of the experiments (ca. 5 days). The following soil characteristics were assessed after sampling: water content (gravimetric, 24 hr at 105°C), soil organic matter (loss on ignition, 12 hr at 550°C), soil pH and

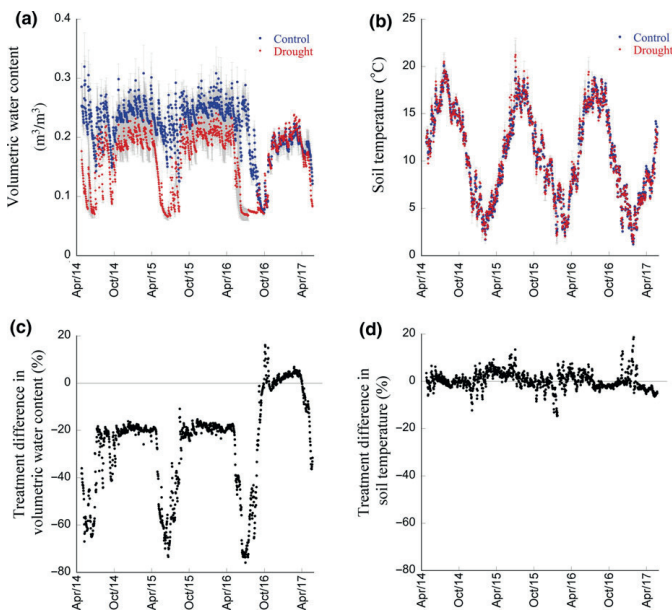

FIGURE 1 (a) Volumetric soil water content (m^3 m^{-3}) and (b) soil temperature (°C) in control and drought-treated plots (5 cm depth; mean ± SE, n = 2) and the relative % difference in (c) volumetric water content and (d) soil temperature between control and drought-treated plots April 2014–April 2017

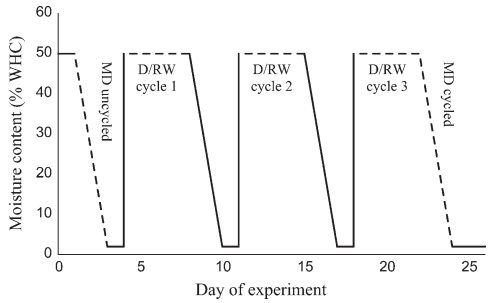

FIGURE 2 A schematic representation of the experimental approach (dashed lines denote measurement of bacterial growth and respiration). The moisture dependence (MD) was determined for uncycled and cycled soils and responses to rewetting were determined for three drying–rewetting (D/RW) cycles

conductivity (1:5, soil:distilled water extraction), and water holding capacity (WHC; maximum water held by soil after draining for 6 hr). Finally, basal bacterial growth and respiration were assessed (see below), for field-moist soils ("basal rates"). Hereafter, all soils were adjusted to 50% WHC, reflecting an optimal moisture content, and left for 48 hr at room temperature to stabilize (a sufficient duration for these small adjustments; Meisner et al., 2017) before the moisture dependence was determined.

2.3 | Microbial moisture dependence

The moisture dependences of drought-treated and control soils were established after sampling ("uncycled soils") and after 3 D/RW cycles ("cycled soils"; see Figure 2 and following section). In each case, measurements were conducted during two independent assessments, with the moisture dependence of the uncycled soils determined both at the start of the experimental period and again at the same time as the cycled soils (after storage at 5°C in the dark for 3 weeks; see Figure 2) to control for any storage effects. As there was no significant difference between the two assessments (Supporting information Table S1), data from the two repetitions were combined and used for subsequent data analysis.

Subsamples of ca. 50 g soil at 50% WHC per replicate were put into 500 ml microcosms and placed under a ventilator at room temperature to dry. Every 1–2 hr, subsamples were taken to measure respiration, bacterial growth, and water content. This was continued for ca. 24 hr until moisture content stabilized at air dry (ca. 2.15% WHC), resulting in 12 subsamples at different moisture levels for each soil during each assessment. Subsamples were briefly kept at 5°C until sampling was completed and were subsequently processed together. The methods used for measuring respiration and bacterial growth are described below.

2.4 | Experimental drying–rewetting cycles

Soils from both drought and control treatments were exposed to three D/RW cycles (Figure 2). To dry samples, the microcosms were placed for 65 hr under a ventilator at room temperature without lids. The gravimetric moisture content (24 hr at 105°C) of the air-dried soil samples was ca. 2.15% WHC. To avoid disturbance caused by subsampling from microcosms over time, air-dried soil subsamples of 0.5 g were weighed into 18 plastic tubes and seven glass vials for each soil, sufficient for subsequent bacterial growth and respiration measurements, respectively, by destructive sampling. These air-dried soil samples were then rewetted to 50% WHC (optimum moisture) using demineralized water and gently mixed using a spatula. After rewetting, all samples were incubated at 16°C, matching a summer soil temperature for the site. Respiration and bacterial growth rates were measured at intervals until 70 hr after rewetting, since previous research has shown that this covers the increase in activity upon rewetting a dried soil (Meisner et al., 2013, 2015, 2017). To ensure

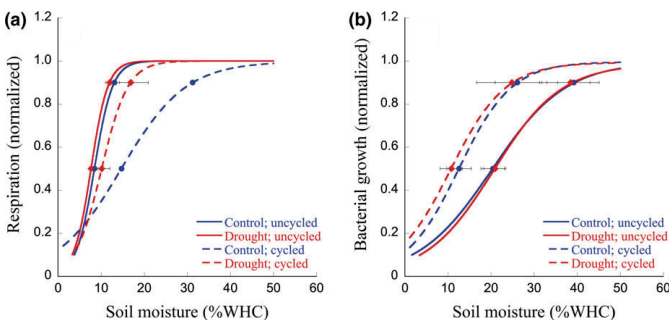

FIGURE 3 The moisture dependence of (a) microbial respiration and (b) bacterial growth showing the associated IC_{10} and IC_{50} values (mean ± SE, n = 3; where error bar cannot be seen, the bar is smaller than the symbol). Sigmoidal curves were fitted for the moisture dependence of the drought and control field treatment replicates for both the uncycled and cycled soils individually (n = 24 for each curve). The continuous lines represent the uncycled soils and characterize the field experiment effects. The dashed lines represent the soils after 3 D/RW cycles. Fitted curves for each individual replicate can be found in Supporting information (Fig. S1)

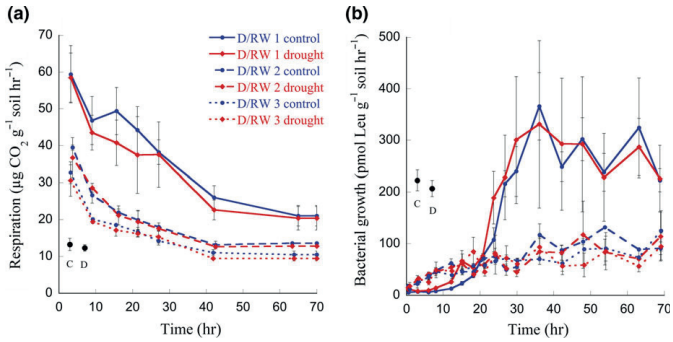

FIGURE 4 (a) Microbial respiration and (b) bacterial growth during 3 D/RW cycles (see legend for treatment). Data represent mean ± SE (n = 3). Mean respiration and bacterial growth rates in continuously moist soils (set to 50% WHC; C = control, D = drought) during all three cycles, measured three times per soil per cycle

a high time resolution of measurements, two sets of samples were prepared for each soil, which were rewetted in either the morning or the evening and monitored in parallel, as previously described (Meisner et al., 2015).

2.5 | Respiration and bacterial growth measurements

For respiration, seven measurements were made following each rewetting event, starting with an interval of 6 hr until 24 hr after rewetting followed by a measurement interval of 12–24 hr (Figures 2, 4a). Respiration was measured by the accumulation of CO_2 in 20 ml headspace vials containing 0.5 g soil. Vials were first purged with pressurized air, before being sealed and incubated in the dark at 16°C. After the predetermined incubation time, the CO_2 concentration was analyzed on a gas chromatograph equipped with flame ionization detector and methanizer. Respiration was expressed as µg CO_2 per gram dry weight soil per hour.

For bacterial growth, a total of 18 measurements were made following each rewetting event, with an interval of 3 hr for the first 36 hr after rewetting, followed by an interval of 6 hr, and increasing to 12 hr at the end of the measurement period (Figures 2, 4b). Bacterial growth was determined by measuring the rate of ^{3}H-leucine (Leu) incorporation into extracted bacteria (Bååth, Pettersson, & Söderberg, 2001), which captures the rate of protein synthesis and reflects the bacterial growth rate occurring in the soil at the time of sampling (Bloem, Hopkins, & Benedetti, 2005; Rousk & Bååth, 2011). 0.5 g soil (f.w.) was mixed with 20 ml demineralized water, vortexed for 3 min and centrifuged (10 min at 1,000 g). The resulting bacterial suspension was incubated at 16°C for 1 hr, with 2 µL 1–[4,5–^{3}H]-Leucine (5.7 TBq mmol^{-1}, Perkin Elmer, USA) and unlabeled Leu with a final concentration of 275 nM Leu in the bacterial suspension. Bacterial growth was terminated after 1 hr by adding 75 µL of 100% trichloroacetic acid. Centrifugation and washing were performed as described by Bååth et al. (2001). Scintillation cocktail (Ultima Gold; PerkinElmer, USA) was added, and radioactivity was measured using a liquid scintillation counter. The amount of leucine incorporated into extracted bacteria (pmol Leu incorporated g^{-1} soil h^{-1}) was used as a measure of bacterial growth.

2.6 | Data analysis

In order to evaluate the resistance of bacterial growth and respiration to drying, moisture-response relationships were determined. To do so, moisture dependence data were normalized to the average of the values at optimal rates (where no inhibition of process was observed, which occurred between 35% and 50% WHC), in a two-step procedure (Rath, Maheshwari, Bengtson, & Rousk, 2016). This resulted in normalized values between 1 (no inhibition of the process) and 0 (complete inhibition of the process), with data fitted using a sigmoidal curve (Rath et al., 2016).

A logistic model with the following equation was applied:

$$y = \frac{c}{1 + e^{-b(x-a)}} \quad (1)$$

Y [unit-less] represents respiration or bacterial growth rates normalized by the average of the rates between 35% and 50% WHC. It describes the activity at a certain x-value (i.e., moisture level), where a is the 50% inhibition value, b is a parameter which indicates the inhibition rate, and c represents the process rate for the optimum moisture (Rath et al., 2016). The IC_{10} and IC_{50} values were determined for individual soils, whereby the IC_{10} and IC_{50} represent the moisture level at which respiration or bacterial growth were inhibited by 10% or 50%, respectively.

After the first D/RW event, the bacterial growth responses started with a lag-period of zero growth for several hours, which is a response previously observed for some soils (Meisner et al., 2015). To estimate the duration of these lag-periods, bacterial growth for cycle 1 was fitted with the Gompertz model (Meisner et al., 2015). This model uses the log-transformed data to describe bacterial growth:

$$G_t = G_{t0} + A \times e^{-e^{b-ct}} \quad (2)$$

$$\text{Lag time} = \frac{b-1}{c} \quad (3)$$

The G_t is the logarithm of the log-transformed bacterial growth data [pmol leu g^{-1} hr^{-1}], A represents the difference between the extreme values, b and c are fitted parameters. The lag-time for bacterial growth in hours was calculated according to Equation (3). Cumulative values of bacterial growth and respiration, and the ratio

of respiration to bacterial growth were calculated for the first 24 hr period and the full 75 hr period. The first period was chosen to investigate the initial effect of rewetting, while the latter covered the total measured period.

Kaleidagraph 4.5.3 (Synergy Software) and JMP 13.2.1 (SAS Institute) were used for curve fitting, visualizing, and statistical analyses. The effect of drought treatment on the lag-time was tested using one-way ANOVA. Main and interactive effects of drought field treatment and cycling on the moisture dependence IC_{10} and IC_{50} values were tested using two-way ANOVA. Cumulative values of respiration and bacterial growth and their ratios were tested using repeated-measures ANOVA for the factors field treatment, cycling, and their interaction. Significant differences were identified where $p < 0.05$.

3 | RESULTS

3.1 | Soil characteristics

Soil characteristics and basal microbial process rates were assessed immediately after sampling (Table 1). There was no significant difference between control and drought-exposed soils.

3.2 | Field treatment effects on microbial resistance

Respiration and bacterial growth rates decreased with lower soil moisture, with these responses modeled well in all instances by a sigmoidal curve (respiration: R^2 = 0.88 ± 0.01, Figure 3a and bacterial growth: R^2 = 0.80 ± 0.03, Figure 3b). The field treatments did not show a significant difference in resistance to drying before exposure to the experimental D/RW cycles (Figure 3). The inhibition of bacterial growth started at a higher moisture level and showed a slower rate of decrease with lower moisture levels compared to the moisture dependence of respiration, with no discernible differences between the field treatments.

3.3 | Effect of drying–rewetting cycles on microbial resistance

For all treatments, the level of respiration decreased with lower moisture, as modeled well by sigmoidal curves (R^2 = 0.86 ± 0.02, Figure 3a), and from the fitted curves two indices for resistance were estimated: the moisture level that inhibited respiration by 10% ("IC_{10}") and the moisture level that inhibited the respiration by 50% ("IC_{50}"). Exposure to D/RW cycles resulted in a shift toward lower resistance for respiration (a right-shifted curve; Figure 3a). Cycling resulted in more moisture-sensitive respiration, which started declining at higher moisture levels, and influenced the control soils more strongly than drought-treated soils, as reflected by a significant cycling × field treatment interaction (p = 0.04) for the IC_{10} values (cycling p = 0.002, treatment p = 0.02). Cycling had a strong effect on the IC_{50} of respiration (p = 0.004). Field drought also had a small but significant effect on the IC_{50} of respiration (p = 0.04), but the uncycled drought and control subsets could not be distinguished (Tukey HSD on ANOVA; Figure 3a).

As for respiration, bacterial growth was modeled well by a sigmoidal function (R^2 = 0.68 ± 0.05) and rates decreased with lower moisture for all treatments. In the case of bacterial growth, cycling significantly increased the resistance of bacterial growth to low moisture (a left-shifted curve; Figure 3b), as reflected by lower IC_{50} values for growth after cycling (p = 0.01). There was no significant effect of field treatment, or field treatment x cycling interaction on the IC_{50} for bacterial growth (p = 0.82 and p = 0.67, respectively).

TABLE 1 Soil characteristics and basal microbial process rates in control and drought-exposed soils

	Control Mean (SE)	Drought Mean (SE)
Water content (%)	187.0 (28.7)	153.9 (14.9)
SOM (%)	72.2 (8.9)	74.8 (1.7)
pH 1:5 extraction	4.3 (0.01)	4.3 (0.10)
Conductivity (mS m^{-1}) 1:5 extraction	5.2 (1.3)	4.6 (1.0)
WHC (%)	486 (63.0)	522 (34.6)
Basal respiration (μg CO_2 g^{-1} soil h^{-1})	16 (1.7)	15.1 (2.3)
Basal bacterial growth (pmol Leu g^{-1} soil h^{-1})	194 (41.1)	155 (19.6)

3.4 | Field treatment effects on microbial resilience to drying–rewetting

Respiration peaked immediately after rewetting, with no clear difference between drought-treated and control soils (Figure 4a). Bacterial growth showed a pronounced lag-period with no growth upon rewetting, after which growth increased exponentially until rates stabilized (Figure 4b). Bacterial growth of the drought-treated soils started increasing earlier compared to the controls, resulting in a shortened lag-time before growth (i.e., increased resilience). The lag-time for the drought-treated soils was significantly shorter with 6.6 ± 1.0 hr compared to 9.9 ± 0.5 hr for the control soils (p = 0.04).

There was no difference in cumulative respiration during the first 24 hr following rewetting between the control and drought-treated soils (Figure 5a). However, the shorter lag-period before the onset of growth in the drought-treated soils lead to higher cumulative bacterial growth during the first 24 hr (Figure 5b). Consequently, the ratio of respiration to bacterial growth for the first 24 hr after rewetting was significantly lower for the drought-treated soils (Figure 5c; p = 0.001), indicating a higher CUE. When considering the 70 hr after rewetting, however, there was no significant effect of field treatment on cumulative respiration and growth, nor the ratio of respiration: growth (Figure 5d–f).

3.5 | Effect of drying–rewetting cycles on microbial resilience

Respiration rates increased immediately upon rewetting and decreased over time for all cycles (Figure 4a). Respiration after the first D/RW cycle was highest, with rates maintained for a longer

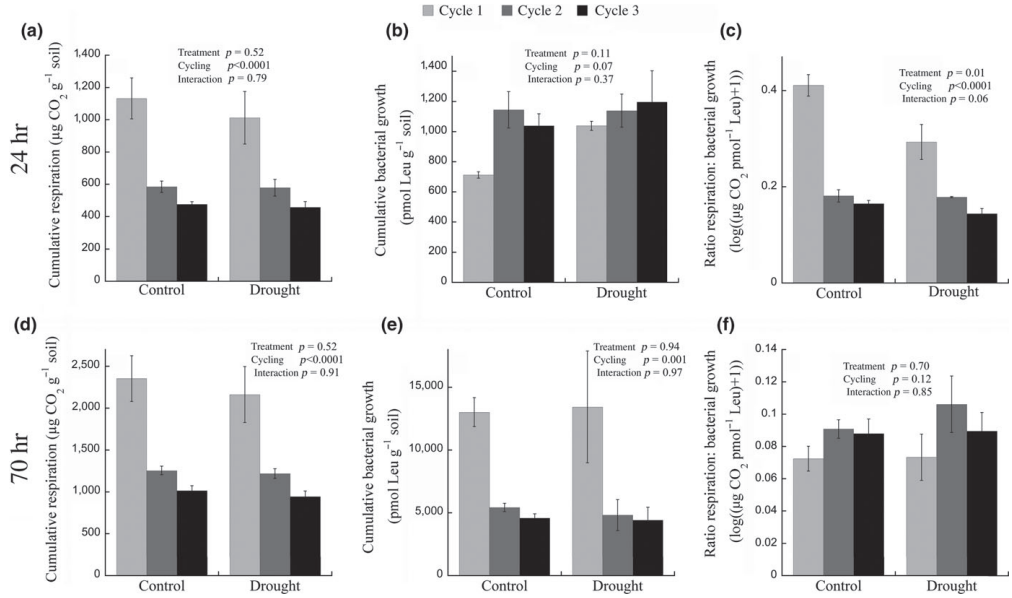

FIGURE 5 Cumulative values for 24 hr after D/RW for (a) respiration, (b) bacterial growth and (c) respiration: bacterial growth ratio, and 70 hr after D/RW for (d) respiration, (e) bacterial growth and (f) respiration: bacterial growth ratio

period of time, whereas after subsequent D/RW cycles respiration started at a lower rate and exhibited a faster decrease after rewetting (Figure 4a). Subsequent cycles of D/RW resulted in decreased cumulative respiration after both 24 and 70 hr periods (Figure 5a,d; $p < 0.0001$). Most of this effect for both time frames occurred between the first and second cycle, accounting for 46% of the observed reduction, while the decrease from cycle two to three was only a further 10%. There was no significant effect of field treatment on cumulative respiration.

Bacterial growth following the first D/RW event exhibited a clear lag-period without growth, after which growth increased exponentially (Figure 4b). In the subsequent cycles, growth started immediately upon rewetting for both field treatments, and growth rates stabilized at lower rates compared to the first cycle. This resulted in a significant decrease in cumulative bacterial growth over the 70 hr following rewetting, driven by the treatment-factor cycling ($p = 0.001$). Most of this effect occurred between the first and second cycle, accounting for a decrease of 61%, with a further reduction in growth by 5% between the second and third D/RW cycles (Figure 5e).

The ratio of respiration to bacterial growth was used as an index to compare CUEs among soils from different field treatments and D/RW cycles (Figure 5c,f). Field treatment and D/RW cycle both had a significant effect on the ratio of respiration: growth during the first 24 hr after rewetting ($p = 0.01$ and $p < 0.001$, respectively), with a marginal interactive effect of field treatment and cycle also evident ($p = 0.06$). Over the course of the three D/RW cycles, the ratio of respiration: bacterial growth decreased, indicating an increased CUE. The effect of the field treatment depended on the cycle, with the most marked difference between control and drought-treated soils occurring after the first D/RW event. When considering the total 70-hr study period, the ratio of respiration: bacterial growth showed a small increase after the first cycle, however, this effect was not significant (Figure 5f).

4 | DISCUSSION

4.1 | Soil microbial responses to experimental field drought

We hypothesized that exposure to long-term summer drought in an intact ecosystem would change the microbial community function to (1a) become more resistant to drought and (1b) respond with higher resilience to rewetting after drought. The moisture dependence of the uncycled soils showed that the resistance for both respiration and bacterial growth to drought was similar for control and drought-exposed soils (Figure 3), in contrast to our hypothesis. However, this finding is consistent with results from a meta-analysis where there was no difference in the drought tolerance of respiration for communities extracted from dry compared to moister environments (Manzoni et al., 2012). Although microbial resistance to drought was unaffected by the field drought treatments, we did find that

exposure to 18 years of summer drought had an effect on microbial resilience to drought. In line with hypothesis 1b, the onset of bacterial growth after rewetting started significantly earlier in the soils exposed to 18 years of summer drought compared to the control treatments (Figure 4b). This result is consistent with a study conducted in a more arid environment (Tall grass prairie, Kansas, USA) that showed that a decade of increased D/RW-stress resulted in a community with a faster microbial recovery following rewetting (Evans & Wallenstein, 2014). However, in a shorter-term study investigating the legacy of 2 years of summer drought in a forest plantation in a moist ecosystem in Wales, UK, no effects on the microbial recovery after a D/RW cycle could be observed (Göransson et al., 2013).

Microbial resistance and resilience will depend on the history of the studied community and its interaction with any putative disturbance factor (Griffiths & Philippot, 2013). For example, exposure to a particular disturbance may lead to the adaptation of the microbial community to subsequent disturbances of the same type, thus resulting in an enhanced functional stability. The field experiment investigated here did not lead to differences in resistance to drought (Figure 3), but did result in a clear difference in the resilience of microorganisms following the exposure to D/RW (Figure 4b). This suggested that the drought treatment had selected for communities that recover more quickly following rain after a drought, but not communities which could sustain activity during drying. This interpretation would imply that the recovery of growth rates following rewetting events, rather than resistance to low water potentials during periods of drought, is the selective force that has shaped the communities formed following 18 years of summer drought treatment. It is likely that the chance of a D/RW event occurring in the field is increased with rain exclusion treatments, as was shown by marked increases in soil moisture at the end of each summer drought treatment (Figure 1a,c). Indeed, at the studied Oldebroek site, a pulse of CO_2 release from soil was induced by rewetting after the summer drought treatment but not in the control treatments, consistent with a drought treatment induced Birch effect (Kopittke et al., 2014). The frequency of D/RW events will naturally also increase with the duration of the study, and thus differences between field treatments will compound with the age of the experiment. This interpretation is consistent with the apparent increased effect with longer-term drought treatments (no effect after 2-year treatments; Göransson et al., 2013; but clear effects after >10 years; Evans & Wallenstein, 2014; and this study) and also with the results on the exposure to laboratory D/RW cycles (see Section 4.2 below). The type of bacterial growth response following D/RW has also been linked to the harshness of the event as perceived by the microorganisms (Meisner et al., 2017). Thus, the shortened lag-time and hence more resilient response to D/RW in the drought-treated soil is likely a consequence of a microbial community structured by its historical environment to experience the rewetting event as a less harsh perturbation, consistent with the suggestions of Griffiths and Philippot (2013).

4.2 | Soil microbial responses to experimental drying–rewetting cycles

We hypothesized that (2a) D/RW cycles in the laboratory would induce a higher resistance to drought. The microbial resistance to drought changed after being subjected to repeated D/RW cycles. However, the moisture dependences of bacterial growth and respiration responded along different trajectories. The resistance of microbial respiration to drought decreased after three D/RW cycles for both field treatments, and the response in control soils was larger than that for field drought-treated soils (Figure 3a). The more pronounced change in response to D/RW of microbes derived from moist compared to dry environments is consistent with earlier studies (Engelhardt et al., 2018; Fierer & Schimel, 2002). In contrast with respiration, the exposure to repeated D/RW cycles increased the resistance of bacterial growth to drought in both field treatments (Figure 3b), and this asymmetry between growth rate and respiration has implications for microbial CUE during drying perturbations (see Section 4.3 below).

It was also hypothesized that (2b) laboratory cycles of D/RW would induce an increased resilience to D/RW perturbations. After each D/RW event, respiration rates immediately peaked followed by an exponential decrease, characteristic of the Birch effect (Kim et al., 2012). However, higher rates of respiration were maintained for longer after the first D/RW event compared to subsequent cycles (Figure 4a). The gradual reduction of cumulative respiration induced by each D/RW cycle could be due to a reduced C availability in the soils after each D/RW event, as has been previously reported (Borken & Matzner, 2009; Fierer & Schimel, 2002; Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2014; Fuchslueger et al., 2016). However, despite the likely depletion of C resources after each D/RW event, this did not constrain microbial recovery to subsequent D/RW. A reduction in resources has been previously linked to the formation or extension of the lag-phase before bacterial growth (Meisner et al., 2015). Yet here, after the second D/RW cycle, the soils from both field treatments no longer exhibited a lag-period before bacterial growth (Figure 4b), resulting in a faster recovery following the perturbation. These findings are consistent with our hypothesis of increased resilience after a series of D/RW perturbations. The increased resilience to D/RW cycles could be explained by a change in the microbial physiology, trait changes within populations (i.e., evolution), or a shift in relative abundance of different microbial taxa within the community. Although we do not explicitly resolve between these possibilities, the high functional and taxonomic diversity of microbial communities (Delmont et al., 2011; Raes & Bork, 2008), along with recent experimental work (Fiegna, Moreno-Letelier, Bell, & Barraclough, 2015; Gravel et al., 2012), suggest that functional shifts due to species sorting swamp those due to physiological or evolutionary changes in soil systems. Consistent with this interpretation, in one of the few studies with the power to resolve between growth responses of different microbial taxa to rewetting of dry soil, pronounced community changes were induced by a D/RW cycle (Koch et al., 2018). Hence, the observed change in

functional response to D/RW most likely results from a microbial community shift, rather than evolutionary or physiological change.

4.3 | Responses of soil microbial carbon use during drying–rewetting

We hypothesized that (3) both field drought and laboratory D/RW cycles would select for communities with a lower respiration to growth ratio during the D/RW perturbation. The ratio of respiration to growth in the first 24 hr after the first rewetting event was significantly lower for the drought-treated communities, which can partly be explained by the shortened lag-time of no bacterial growth in these soils (Figure 5c). In addition, there was a pronounced decrease in the ratio after the second D/RW event for both field treatments (Figure 5c). This response coincided with the loss of the lag-period after rewetting, and both these changes indicate a shift toward a community functioning with higher resilience and greater stability (Griffiths & Philippot, 2013; Orwin, Dickie, Wood, Bonner, & Holdaway, 2015). The cumulative values of growth and respiration for the full study period were similar for both field treatments, however, suggesting that the field drought legacy effect on microbial functioning was transient (Figure 5f).

It was not possible to determine absolute levels of microbial CUE here, partly due to a lack of suitable conversion factors to estimate bacterial C production from leucine incorporation (Rousk & Bååth, 2011). However, we can use the relative shifts in the ratio of respiration to growth, yielding an index of CUE. An added benefit of such a ratio is that the influence of total C resource availability is self-standardizing. The asymmetrical responses in the resistance of respiration and bacterial growth to drought resulted in a shift toward lower rates of respiration to growth during dry conditions (i.e. at low %moisture; Figure 3). This effect was made stronger following a history of experimental D/RW treatments, suggesting the microbial communities in those treatments had become shaped to grow with higher CUE when exposed to low soil moisture.

In pure culture studies in laboratory systems, microorganisms that grow fast are normally favoured, leading to a selection for high growth rates (Roller & Schmidt, 2015). However, when a spatial structure has been introduced to mimic aspects of a complex matrix where the movement of both microorganisms and substrates are limited, such as in a soil environment (Hobbie & Hobbie, 2013), it has been demonstrated that high CUE is a trait that can be selected for (Bachmann et al., 2013). However, to date it remains unknown if this finding can be extended to also hold true for complex microbial communities occurring in soil systems (Roller & Schmidt, 2015). Our findings provide the first circumstantial evidence that selection for high CUE is a trait that can be favoured on a community level in a real soil system during exposure to drought, and during the perturbations induced by D/RW cycles.

5 | CONCLUSION

A history of summer drought in the field selected for increased microbial resilience but not resistance to drought, suggesting that it was the rewetting rather than exposure to drought that was the selective pressure on the microbial community. Our results suggest that a history of drought will increase microbial resilience following D/RW by reducing the lag-period before the start of bacterial growth by about 30%. This resulted in approximately 40% more bacterial growth in the 24 hr following rewetting compared to soils which did not have a history of summer drought. Cycles of laboratory D/RW treatments also selected for communities with a faster growth-recovery after rewetting rather than a higher resistance to drought. The ratio of respiration to growth during a D/RW event was lower in communities derived from field drought compared to control soils and also decreased when exposed to cycles of D/RW. These responses suggest that both field experimental rain reduction and the exposure to experimental D/RW cycles in the lab restructure microbial communities to be quick responders to rewetting after drought, and to use C with a high efficiency during the perturbation. Taken together, these results imply that microbial communities can adapt to changing climatic conditions and that the shift from catabolic to anabolic microbial C use (Liang, Schimel, & Jastrow, 2017) might mitigate some of the soil C loss predicted to be induced by future cyclic drought.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary Information

Results

Table S1 IC_{10} and IC_{50} values for respiration (resp.) and bacterial growth (bac.) from two independent assessments (A1 and A2), where data represent mean (SE; n=3). No significant difference between the first and second assessment (ANOVA of assessment effect for both respiration and bacterial growth on IC_{10} values $p > 0.17$ and IC_{50} values $p > 0.10$).

		Control; uncycled	Drought; uncycled	Control; cycled	Drought; cycled
IC_{10} resp.	**A1**	15.8 (3.0)	11.0 (0.4)	19.4 (4.1)	22.5 (1.3)
	A2	12.1 (1.9)	18.8 (5.2)	13.4 (3.6)	17.9 (1.0)
IC_{50} resp.	**A1**	9.6 (1.6)	7.4 (0.1)	16.2 (1.3)	13.2 (0.9)
	A2	8.5 (1.0)	10.2 (1.3)	12.1 (0.6)	5.6 (5.8)
IC_{10} bac.	**A1**	41.3 (4.3)	33.4 (5.1)	26.4 (3.3)	33.5 (2.2)
	A2	34.0 (5.3)	38.6 (7.8)	22.8 (9.6)	16.7 (3.8)
IC_{50} bac.	**A1**	23.9 (2.1)	18.4 (1.0)	14.0 (2.2)	17.9 (3.7)
	A2	16.6 (2.9)	22.2 (4.4)	11.8 (4.2)	8.9 (1.4)

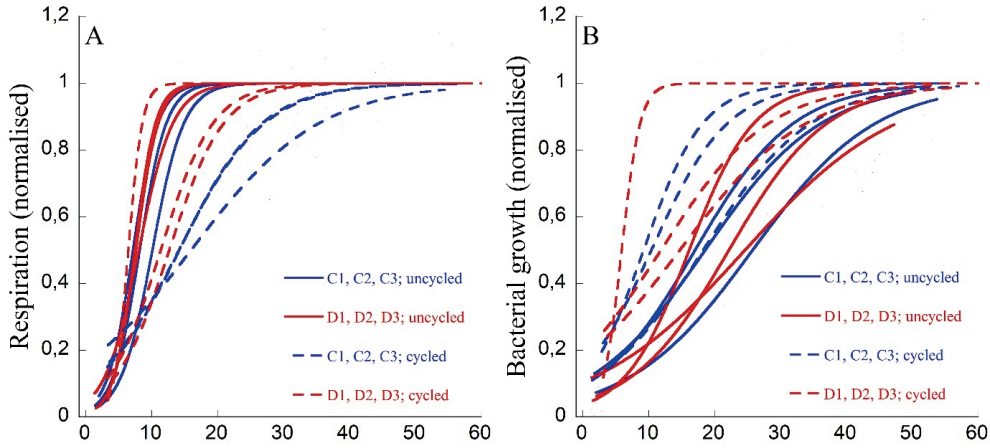

Figure S1 The individual curve fits for moisture dependence of (A) microbial respiration and (B) bacterial growth. Sigmoidal curves were fitted for the moisture dependence of the drought and control field-treatment replicates for both the uncycled and cycled soils individually (respiration: R^2=0.86±0.02 (A); and bacterial growth: R^2=0.68±0.05 (B)). The continuous lines represent the uncycled soils and characterize the field experiment effects. The dashed lines represent the soils after 3 D/RW cycles.

Paper V

Soil microbial communities are more structurally responsive and functionally efficient during rewetting after drought in subtropical cropland than forest soils

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Abstract

Climate change will increase temperatures and the frequency and intensity of extreme drought and rainfall events. When a drought period is followed by a rainfall event, there is a big CO_2 pulse from soil to the atmosphere which is regulated by soil microorganisms. In the present study, we set out to investigate how simulated drought and warming affect soil microbial responses to drying and rewetting (DRW), and how these responses interact with the level of land degradation. To do so, rain shelters and open top chambers (OTC) were installed in Ethiopia in contrasting land-uses; a degraded cropland and a pristine forest. Rain shelters reduced soil moisture by up to 33%, with a bigger effect in the forest than in the cropland soils. OTCs increased the soil temperature by up to 5°C, especially in the crop soils. Warming also decreased soil moisture, with a bigger effect in the cropland than forest soils. Soils were sampled (1.5 years after field treatment establishment) and exposed to a DRW cycle in the laboratory. Microbial growth and respiration responses were followed with high temporal resolution over 3 weeks. In addition, bacterial and fungal DNA was extracted and sequenced over the course of the experiment. Microbial communities in both land-uses and treatments universally showed a highly resilient type of functional responses with both bacterial and fungal growth increasing immediately upon rewetting, linked with the expected respiration pulse. The field treatments simulating drought and warming did not affect the already high resilience of soil microbial communities to DRW. However, differences in the resilience (i.e., the time that growth needed to recover to growth levels in undisturbed soil) between fungi and bacteria were observed: fungi were more resilient than bacteria. Microbial CUE upon rewetting was always higher in cropland soils than in forest soils. Simulated drought reduced the microbial CUE during rewetting in cropland and slightly increased it in forest soils. The CUE was also elevated in the warming treatments in both land-uses. Taken together, the responses in microbial CUE during the rewetting of dry soils were likely linked to either (i) differences in resource availability and quality, or (ii) selection of more efficient microbial communities due to a higher previous exposure to DRW events. The structure of microbial communities was more responsive upon rewetting in cropland than forest soils. In addition, community structure of bacteria and fungi responded differently upon rewetting. While the complexity of bacterial networks changed over the course of a DRW disturbance; fungal networks remained stable after the DRW disturbance. Our results suggest that environmental legacies need to be taken into account to understand how microbial function and structure will respond to climate change induced more frequent and intense drought and rainfall events.

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1. Introduction

The intensification of land-use, together with climate change and its associated temperature increase and hydrological intensification, are anthropogenic threats to terrestrial ecosystem functioning. Soil microbial communities govern key processes in the terrestrial carbon (C) cycle (Bardgett et al., 2008), controlling the main C releases to the atmosphere through respiration (Adachi et al., 2017) and C sequestration through their growth (Liang et al., 2017). Soil microorganisms, in turn, are strongly regulated by their environment (Kirchman, 2018), with moisture and temperature being the two main factors that regulate microbial communities and processes (Sierra et al., 2015; Waksman and Gerretsen, 1931). Land-use is also an important controller since it encompasses both aboveground (e.g. plant communities and productivity) and belowground (e.g. soil disturbance level and structure) processes that have an important influence on soil microorganisms (Malik et al., 2018). Therefore, our ability to determine how soils are responding to ongoing climate change rely on the understanding of how soil microorganisms and the processes they regulate are affected by the environment they live in.

Tropical ecosystems remain understudied. In fact, the urgent need to study climate change effects in these ecosystems has been recognized (Cavaleri et al., 2015; Zhou et al., 2013), resulting in increasing recent attention. These studies usually use reciprocal transplant experiments to assess climate change effects (e.g. Looby and Treseder, 2018; Nottingham et al., 2019) , with very few field-scale manipulation experiments to date. There are only a handful of exceptions that have simulated soil warming (Kennard et al., 2020; Nottingham et al., 2020) or drought (Bouskill et al., 2016; Cleveland et al., 2010; Davidson et al., 2008) in the tropics. To date, studies that investigate climate change effects in the tropics have focused on how soil respiration (Cleveland et al., 2010), enzyme activities (Looby and Treseder, 2018), as well as microbial community structure (Bouskill et al., 2016) are affected by altered temperature or precipitation. However, there is a shortage of assessments of how temperature and precipitation alterations affect the dynamic process rates of the soil microbial communities to drying and rewetting (DRW) in different land-uses.

Dry soils are known to quickly release CO_2 upon rewetting (Birch, 1958; Kim et al., 2012). These C releases from soils to the atmosphere have been suggested to have a strong influence on the C budget of many ecosystems including mesic grasslands (Hoover and Rogers, 2016), Mediterranean ecosystems (Inglima et al., 2009; Jarvis et al., 2007), and tropical forests (Waring and Powers, 2016). Rewetting dry soil is one of the most dynamic events that is known in microbial ecology since big changes in both microbial communities (Barnard et al., 2015, 2013; Placella et al., 2012) and processes (Fierer and Schimel, 2002; Iovieno and Bååth, 2008) occur during a short period of time. During a DRW event, microbial growth and respiration have been shown to be uncoupled: while respiration rates increase immediately to high rates relative to an undisturbed soil, microbial growth rates are initially very low (Hicks et al., 2019). Microbial growth rates then gradually recover to rates matching those in undisturbed samples (Meisner et al., 2013). The time that microbial growth rates need to recover to undisturbed levels can vary depending on how "harsh" the microorganisms perceive the disturbance, resulting in differences in resilience and carbon use efficiency (CUE) (Leizeaga et al., 2020; Meisner et al., 2017). When microbial communities perceive the disturbance as "harsh", they have a less resilient response resulting in higher CO_2 releases to the atmosphere per C input into the soil through biomass synthesis. In

contrast, when the DRW disturbance is perceived as less "harsh", microbial communities recover their growth rates faster, resulting in a higher CUE during the perturbation. This can have implications for the ecosystem C balance (Brangarí et al., 2020; de Nijs et al., 2019).

DRW disturbances are also known to increase resource availability (Fierer and Schimel, 2003; Slessarev et al., 2020). The induced resource pulses will trigger a succession of community assembly dynamics, starting with the colonization of free resources and eventually an intensification of interactions when large microbial populations again compete for limiting resources (Placella et al., 2012; Shi et al., 2016). Yet, understanding these successional dynamics is often a challenge since microbial communities consist of a high number of taxa whose distribution of traits remain mostly unexplored. Growing literature suggests that co-occurrence networks can be a useful tool to understand how taxa interact within communities and how these communities respond to changes in the environment (Barberán et al., 2012; Deng et al., 2012). Positive interactions in these networks have been suggested to indicate cooperative relationships that could arise from synergetic of facilitative interactions, while negative links would indicate competitive or predatory interactions (Faust and Raes, 2012). Besides, other network properties such as connectivity or modularity have been suggested to be linked with the stability of the community (Coyte et al., 2015). In fact, studies that have used co-occurrence networks to assess community structure responses to changes in their environment have reported an increase in positive interactions and complexity with an increase of resource availability (Qiu et al., 2020; Shi et al., 2016), as well as differences in the bacterial and fungal community responses to drought (de Vries et al., 2018). Although the use of co-occurrence networks to understand microbial community structure has increased during the last few years, to our knowledge there are no previous assessments of how microbial assembly changes over the course of a DRW disturbance and how the interactions within microbial communities are affected by other factors such as land-use.

It has been argued that the historical conditions that microbial communities have been exposed to can shape microbial communities and their functional traits, resulting in a lasting legacy effect (Hawkes and Keitt, 2015). Examples of legacies of drought have been shown for C mineralization rates (Fierer and Schimel, 2002; Magid et al., 1999), and soil microbial community composition and the associated representation of ability to cope with disturbances (Evans and Wallenstein, 2014, 2012). In addition, drought history has been shown to select for more resilient and/or efficient microbial communities in soils from ecosystems in Northern Europe and semi-arid grasslands in Texas (de Nijs et al., 2019; Göransson et al., 2013; Leizeaga et al., 2020). Warming can also shape the structure of microbial communities (DeAngelis et al., 2015; Oliverio et al., 2017) as well as their functions. It has been shown that microbial communities can align their trait distributions to changes in environmental temperatures to become more warm- or cold-tolerant (Bárcenas-Moreno et al., 2009; Birgander et al., 2013). A decrease in moisture is often an indirect effect of the exposure to experimental soil warming, resulting in soils that not only have a history of warming but also a history of drought. Warming and drought can also indirectly affect microbial communities via plant communities, as moisture and temperature are two important regulators of aboveground productivity, which in turn can alter microbial community structure and function (Fuchslueger et al., 2014; Williams and de Vries, 2020). The exposure to drought and warming might therefore both directly and

indirectly shape microbial communities and their processes, all of which may characterize their responses to a DRW event.

Differences in land-use might also have an influence on the drought history of soils and on how soil microbial communities respond to DRW disturbances (Fierer et al., 2003). Forest and croplands have different plant communities which can result in very different microbial resource environments (Osman, 2013). One of the main differences between cropland and forest soils is the quality of soil organic matter (SOM) in terms of its nutrient content or the microbial assimilability the soil C, with cropland soils having a higher quality SOM than forests soils (Woloszczyk et al., 2020). It has been shown that when soil microorganisms have access to higher quality SOM, they can also grow with a higher CUE (Manzoni, 2017; Roller and Schmidt, 2015; Silva-Sánchez et al., 2019). The availability and quality of SOM due to differences in plant input have also been suggested to shape the response of soil microorganisms to DRW (Leizeaga et al., 2020). The temperature and moisture that soils are exposed to in forest and cropland systems can also differ within the same climate zone: forest soils are usually cooler and moister than cropland soils due the litter layer that covers the soils as well as the canopy shading. Soil structure also varies between land-uses, with cropland soils having a more compromised structure due to the continuous exposure to disturbance because of harvesting (Sharma and Aggarwal, 1984). Finally, most plants in forest soils, often perennials, have deeper roots than plants, often annuals, in agricultural systems (Canadell et al., 1996), lifting up and redistributing water through upper levels of the soil horizon (Bayala and Prieto, 2020). Consequently, during dry periods, cropland soils might dry out faster than forest soils that experience less severe moisture limitation. Taken together, these lines of evidence suggest that microbial communities in forest and cropland soils might experience drought and rainfall events differently due to different exposures to drought and differences in the quality of OM available in the system.

Here, we investigated how climate change-induced drought and warming and its interactions with land-use affect microbial communities and their structural and functional responses to DRW, and consequently the terrestrial C budget in tropical ecosystems. To this end, we used climate manipulation experiments in Northern Ethiopia. Rain exclusion shelters and open top chambers (OTC) were installed in two contrasting land-uses: a degraded cropland and a pristine forest. One and a half years after installation of the manipulation treatments, soils were sampled and exposed to a DRW event in the laboratory. Then, soil microbial functions and community composition were followed for three weeks after the perturbation. We hypothesized that (1) microbial communities would be shaped by the field treatments and land-uses, resulting in differences in the responding taxa over the course of a DRW experiment. In addition, we hypothesized that over the course of a DRW experiment we would find a (2) shift in the interactions between taxa during the initial period of resource colonization dominated by positive interactions followed by a period dominated by negative interactions due to the gradual increase in the competition for limiting resources with time after rewetting. We also expected that (3) microbial functions would be shaped by the field treatments and land-uses. Specifically, we hypothesized that (3a) soils exposed to drought would select for altered bacterial and fungal communities with more resilient and efficient responses to a DRW disturbance, while (3b) community changes induced by warming would not affect their traits related to moisture. In addition, we hypothesized that (3c) cropland soil microbial communities would have a more resilient and efficient response to DRW than

forest microbial communities due to their history of higher exposure to DRW events or structural disruption and access to higher quality SOM deposited by plant crops. Finally, we hypothesized based on previous studies that (4) the fungal communities would be less responsive to a DRW disturbance than bacteria (de Vries et al., 2018).

2. Materials and Methods

2.1 Site description

The studied cropland and forest sites were located in the north-western Ethiopian Amhara region at 2110 m elevation (12°9'42''N, 37° 44'21'E; 12° 8'42''N, 37° 44' 35'' E respectively). The study sites were selected from previously studied sites in the Amhara National Regional State (Assefa et al., 2017). The site is characterized by a mean annual temperature (MAT) of 19°C and mean annual precipitation (MAP) of 1100 mm $year^{-1}$. Precipitation is distinctly seasonal. Based on soil moisture fluctuations we determined a "wet period" and a "dry period". The "wet period" started in 27th May 2018 and ended 20th January 2019. Therefore, the "dry period" was between 8th February 2018-26th May 2018 and between 21st January 2019-10th February 2019. Two contrasting land-uses were selected where the field experiments were established. The forest site, is a mixed forest constituted by indigenous tree species (Aerts et al., 2016). In the cropland site crop rotation is a common practice between years, as well as tillage (to a 10-20cm depth). The most common crops that are grown in this site during the wet season are *Eragrostis tef*, *Eleusine coracana*, *Sorghum bicolor*, *Zea mays*, *Triticum aestivum*, *Guizotiaabyssinica* and *Vicia faba*.

2.2 Field experiment description and monitoring

In November 2017, at each selected site a 12 m x 6 m area was fenced where 4 drought and 3 warming plots were established as well as corresponding control plots in a blocked design. Drought was simulated by the use of rain shelters. A 1.8 m x 1.8 m area was selected above which a rain shelter was built. 4 eucalyptus wooden beams were used to support the structure, with two of them being 1.5m high and the other two 1m to form an inclination in the shelter so that the water would flow in the same direction as the slope in the site. The beams were buried to 0.5 m depth to stabilize the structure, topped with a square wooden frame to which a transparent plastic cover was secured covering 80 % of the area. This was done with the aim of reducing 80% of the incoming rainfall. Four 1.8 m x 1.8 m plots served as control plots. Finally, three additional plots were chosen to establish warming treatments using 3 mm thick and 35cm tall open top chambers (OTCs), with 85 cm of diameter in the top and 150 cm diameter in the base. These were designed to increase the air temperature, and were based on the International Tundra Experiment (ITEX) design (Marion et al., 1997). The fenced areas where the field experiments were established were maintained and monitored weekly. Vegetation was continuously removed by weekly mowing in both the cropland and the forest to compensate the lack of grazing, thus ensuring a similar vegetation before and after the fencing. Soil volumetric water content and temperature were monitored twice weekly during the course of the field experiment (1.5 years). The moisture and temperature probes (SM150T, Delta-T Devices and a digital thermometer with a stainless-steel sensor marked at 5 cm depth) were inserted carefully in the soil five times within each plot and the average of this values was reported as the measurement. All measurements were taken between 2pm and 4pm.

2.3 Soil sampling

Soils were collected in February 2019, at the end of the dry season. Within each plot, 5 soil samples (0-5 cm depth) were collected resulting in a composite sample from the top 5 cm. This resulted in 22 samples: 2 land-uses

which had 3 treatments replicated 4 times (control and drought) or 3 times (warming). After sampling the soils were stored in double Ziploc bags and were shipped express to Lund University in Sweden for analysis, within 1 week of sampling.

2.4 Soil characterization

On arrival, all soil samples were sieved (<4 mm) and stones and roots were manually removed. A 1:5 (w:v) water extraction was used to measure soil pH and electrical conductivity (EC) using an electrode. Soil organic matter (SOM) was estimated using a loss on ignition (LOI) procedure (600°C during 12h). Water holding capacity (WHC), the maximum amount of water that soils could hold after gravity loss, was measured according to the procedure described in Hicks et al. (2018).

2.5 Experimental drying and rewetting

Soil samples were air dried until their water content reached equilibrium with air moisture under a ventilator at room temperature. Then, soils were rewetted to 50% WHC (optimum moisture) and bacterial growth, fungal growth and respiration rates were measured with high temporal resolution during 3 weeks at a constant temperature of 17°C. To allow a high temporal resolution of the measurements, two sets of samples were used. One was rewetted in the morning and the other in the afternoon and measured in parallel (Meisner et al., 2017, 2015, 2013). In addition, samples were collected for microbial community characterization before drying and rewetting and 24h, 72h, 1 week and 3 weeks after drying and rewetting.

2.6 Microbial community characterization

DNA was extracted from 250 mg of freeze-dried soil using MoBio Power Soil Kits (MoBio, Carlsbad, CA, USA) following the manufacturer instructions. DNA was quantified fluorometrically (Qubit, Invitrogen, Carlsbad, CA, USA) and DNA extracts were sent to BGI for amplicon sequencing. For bacterial communities, the V3-V4 region of the 16S was amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'). For fungal communities the ITS1-ITS2 region was amplified using the primers ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3').

2.7 Microbial growth, respiration and carbon use efficiency

Bacterial growth was determined in 1 g soil using the ^{3}H-leucine (Leu) incorporation into protein method (Bååth et al., 2001; Rousk et al., 2009), with modifications described in Meisner et al. (2015). The amount of incorporated ^{3}H-leucine into extracted bacteria (pmol Leu incorporated g SOM^{-1} h^{-1}) was used as a proxy for bacterial growth rate. To be able to determine bacterial C-production, first a conversion factor between Leucine incorporation and Thymidine incorporation was established for these specific soils. To do so, a bacterial suspension was made and both Leucine incorporation into protein and Thymidine (Thy) incorporation into DNA (Bååth, 1992) were measured in these samples. Then, the Leu incorporation rates were converted to Thy incorporation rates, which could then be converted to bacterial C-production using a conversion factor reported by Soares and Rousk (2019) which resulted in bacterial growth rates as μgC g^{-1}SOM h^{-1}.

Fungal growth was determined by estimating the ergosterol synthesis in 1 g of soil. In order to do so, the ^{14}C-acetate incorporation into ergosterol method was used (Bååth, 2001; Rousk et al., 2009) with modifications described in Meisner et al. (2013). The amount of incorporated acetate into extracted ergosterol (pmol acetate incorporated g^{-1}SOM soil h^{-1}) was used as a proxy for fungal growth. The fungal growth rate was

converted to fungal C-production using the conversion factor by Soares and Rousk (2019), resulting in fungal growth rates as $\mu gC\ g^{-1}SOM\ h^{-1}$. The ratio between bacterial growth and fungal growth (both in units of C) was then calculated.

For respiration, 1 g of soil was weighed into 20mL glass vials, purged with pressurized air, sealed with crimp caps and incubated between 6h and 72 h (with a shorter incubation time at the start of the experiment) in the dark at 17°C. CO_2 production was measured using a gas chromatograph equipped with a methanizer and a flame ionization detector. The µg of C respired per g SOM and h was calculated.

Microbial carbon use efficiency (CUE) was estimated as the ratio of the total microbial C production (bacterial C production + fungal C production) to the total microbial carbon use (total microbial C production + respired C).

2.8 Data analysis

2.8.1 Soil microbial communities

All sequence data was processed using DADA2 (Callahan et al. 2016) to determine the amplicon sequence variants (ASVs). For the bacterial community analysis, the forward (200 bp) and reverse reads (160 bp) were trimmed and merged. For fungi the DADA2 ITS Pipeline Workflow (1.8) was used with default parameters. To understand how microbial communities had been affected by the two land-uses and treatment, bacterial and fungal alpha and beta diversity from the dry soils (i.e., before rewetting) were estimated. Shannon diversity index was calculated to estimate alpha diversity. Differences between land-uses and treatments in alpha diversity were tested with a two-way ANOVA. For beta diversity analysis ASVs were filtered by only keeping ASVs with at least 5 counts and samples were then transformed to even sampling depth with the function *transform_sample_counts* from the *phyloseq* package (McMurdie and Holmes, 2013). Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities were used to visualize the beta diversity data. PERMANOVA was performed to understand if there were differences in the beta diversity between land-uses and treatments. In addition, to better understand whether the communities had been shaped by the treatments, each land-use was also tested separately for bacterial and fungal communities. Finally, changes in bacterial and fungal community structure over the course of a DRW disturbance were also tested for each of the land-uses separately. The original dataset was separated by the two land-uses and filtered by removing ASVs that did not appear more than 5 times in more than half the samples for bacteria and in more than one fifth of the samples for fungi. These datasets were then used to calculate beta diversity and for further analyses. To find the significant taxa in determining the community differences across time points (henceforth, "responsive taxa") we performed a LEfSe analysis which combines the non-parametric test and linear discriminant analysis. These was done using the function *trans_diff* from the *microeco* package (Liu et al., 2021) with default parameters.

Co-occurrence networks were analyzed for fungal and bacterial communities for each land- use and each time point after rewetting separately. Thus, each network was based on 11 samples. All network analyses were done using the function *trans_network* from the *microeco* package in R. Interactions consisted of Spearman's rank correlations and co-occurrence networks were constructed using only highly significant correlations ($p < 0.001$) of $\rho > 0.8$. Global network properties were characterized according to Deng et al. (2012). We conducted a one-way analysis of covariance (ANCOVA) to test whether the network properties significantly differed over time between cropland and forest soils. Networks were visualized in gephi

(Bastian et al., 2009). The modularity (*M*) of networks was characterized in the study and $M > 0.4$ was used as the threshold to define the modular structure of a network (Newman, 2006). A modular structure of a network indicates that the network is constructed by groups of nodes that are highly connected within the group and have a small number of connections outside the group (Newman, 2006). The connectivity of each node was determined based on the within-module connectivity (*Zi*) and among-module connectivity (*Pi*) (Guimerà and Amaral, 2005), which were calculated using the function *cal_node_type* from the *microeco* package. Keystone taxa were identified as nodes that had a high connectivity following the criteria in Shi et al. (2016): $Zi > 2.5$ or/and $Pi > 0.62$. Random networks were constructed using the function *erdos.renyi.game* from the *igraph* package (Csárdi & Nepusz, 2006).

2.8.2 Microbial processes: microbial growth, respiration and CUE

Cumulative bacterial growth, fungal growth, respiration, CUE and the fungal to bacterial growth ratio during 1 day and 3 weeks following the DRW disturbance were calculated. To do so, the area under the fitted curve from the dynamics of each parameter was estimated. Functional differences between land-uses and treatments were tested on the cumulative values of microbial growth, respiration, CUE and fungal to bacterial growth ratio 1 day and 3 weeks after rewetting. This was done with a mixed effect model, considering the factors 'land-use' (forest, cropland) and 'treatment' (control, drought and warming) as fixed effects. 'Block' (8 blocks, 4 per land-use) was considered as a random effect, with 'treatment' being nested within 'block'. For factors with more than 2 levels, Tukey's HSD pair-wise comparisons were used to compare treatments with an $\alpha = 0.05$.

The recovery time of bacterial growth and fungal growth to a steady-state growth rate level were calculated. First, the steady-state bacterial growth and fungal growth rate values were estimated by averaging the growth rates values between 250h and 500h after rewetting (i.e., when growth rates had reached a steady-state). Second, a linear curve was used to model the increasing part of bacterial growth and fungal growth. Finally, the time that the bacteria and fungi needed to reach the steady-state level growth was calculated using the increasing linear model. Differences in recovery times between fungi and bacteria were also tested using a mixed effect model, considering the factors 'organism' (bacteria, fungi), 'land-use' (forest, cropland) and 'treatment' (control, drought and warming) as fixed effects. 'Block' was considered as a random effect, with 'treatment' being nested within the 'block'. As there was an interaction between 'organism' and 'treatment' the effect of the treatment in the microorganisms was tested for each microorganism separately, only using 'land-use' and 'treatment' as fixed effect and 'block' as the random effect. Statistics were performed with JMP Pro 15 (SAS institute).

2.8.3 Soil moisture and temperature

Differences in soil moisture and temperature between treatments were tested for each one of the land-uses separately. A mixed effect model was used, considering the factors 'treatment' and 'date' as fixed effect and using the 'block' as a random effect, with the sampling 'plots' being nested within the block. For pairwise comparisons between treatments the general linear hypotheses (*glht*) function was used in R (R Core Team, 2020).

3. Results

3.1 Field treatment effects

Soil moisture differed between treatments in both land-uses during the wet period ($p < 0.001$; Figs. 1A, 1B). In the cropland, soils in the drought treatment were drier, especially during the second part of the wet period with soil under the shelters being up to 33% drier than the control (Figs. 1A, S1A). Soils in the warming treatments were also drier than the control in the cropland (up to 25% drier) and this effect was maintained during the whole wet period (Figs. 1B, S1B). In the forest, the warming treatment had a slight drought effect, where the soils got up to 10% drier than in the control treatments. The drought treatment had a bigger effect, where the soils were up to 33% drier than the control soils during the whole wet period. During the dry period, in the cropland, moisture in the warmed and drought treated soils were significantly reduced compared to the control treatments: soils were approximately 5% drier in both drought and warming treatments ($p=0.007$; Figs. 1A, S1A). In contrast, during the dry period there were not differences in the soil moisture between treatments in the forest soils ($p=0.52$; Figs. 1B, S1B).

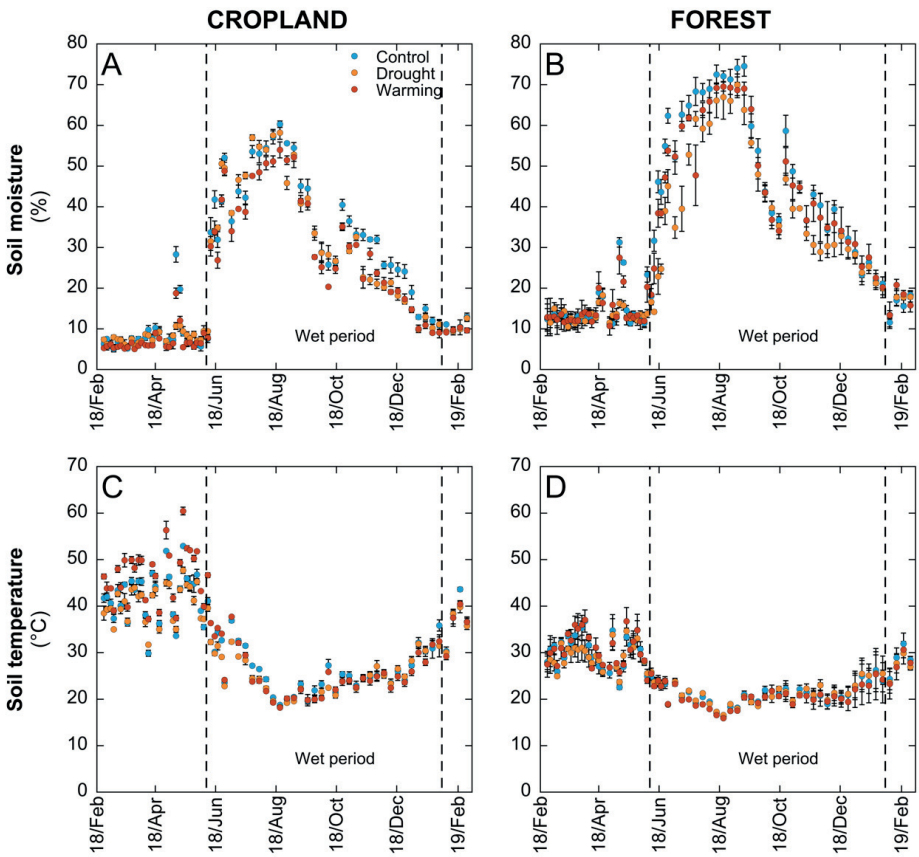

Figure 1. Soil moisture (%) and temperature data (°C) for the (A, C) cropland and (B, D) forest sites. The measurements in the control plots are shown in blue, in the drought plots in orange and in the warming plots in red. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). The vertical dashed lines indicate the starting and ending point of the wet period.

Table 1. Soil characteristics

Land use	Treatment	pH	EC	% SOM	100% WHC
Cropland	Control	6.8 (0.03)	46 (1.2)	8.8 (0.15)	71 (1.1)
Cropland	Drought	6.4 (0.03)	52 (4.2)	9.3 (0.39)	72 (3.8)
Cropland	Warming	6.7 (0.21)	46 (4.7)	8.6 (0.13)	70 (1.6)
Forest	Control	6.7 (0.03)	66 (3.1)	18.9 (0.80)	85 (1.0)
Forest	Drought	6.7 (0.04)	71 (3.7)	19.5 (1.53)	84 (3.3)
Forest	Warming	6.7 (0.06)	61 (6.3)	18.5 (0.67)	80 (1.7)

Values denote means ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). Soil pH and EC were measured on a water extraction with a weight to volume ratio (w:V) of 5.

Soil temperature in the cropland differed between treatments during both the wet and the dry period ($p<0.001$; Figs. 1C, S1C). The warming treatment showed an increase of 5°C compared to the control; whereas the drought treatment showed a decrease in the temperature. The forest soils only showed differences in the soil temperature during the dry period ($p=0.031$; Figs. 1D, S1D): the warming treatment showed an increase in the temperature (up to 5°C) in comparison to the control, while the drought treatment showed a slight decrease during the beginning of the dry period (by up to 5°C).

3.2 Soil and microbial community characterization

Soil EC, SOM and WHC were unaffected by the field treatments; while the soil pH was significantly lower in the drought treatment (pH = 6.5) than in the control (pH = 6.8) ($p = 0.027$). Land-use, however, had an effect on EC, SOM and WHC ($p < 0.0001$) but did not have a significant effect on soil pH (pH = 7 in all instances).

The alpha diversity of both bacteria and fungi did not differ between land-uses and treatments, bacterial and fungal Shannon diversity were approximately 6.5 and 4.5 respectively (Figs. 2A, 2B). Beta diversity appeared to be strongly constrained by land-use for both bacterial and fungal communities ($p = 0.001$); but treatment did not have an effect on the bacterial and fungal community structure ($p = 0.39$ and $p = 0.33$, respectively) (Figs. 2C, 2D). Community structure differences were also tested separately for bacterial and fungal communities in soils from cropland and forest to understand whether the field treatment had resulted in differences in beta diversity (Fig. S2). Bacterial community structure had a marginal effect of the field treatments in crop soils ($p=0.06$); whereas bacterial communities from the forest soil did not show an effect of the field treatments ($p=0.95$). Fungal community structure was affected by the field treatments in the crop soils ($p=0.02$) but to not in the forest soils ($p=0.96$).

3.3 Microbial processes after DRW

The response of all measured microbial processes to a laboratory DRW disturbance were similar in both land-uses and all treatments (Figs. 3, S3). Overall microbial growth rate started increasing immediately after rewetting (Figs. 3A, 3B), to reach maximum growth rate levels at 20h in the cropland soils and 30h in the forest soils. Microbial growth was then maintained at a relatively stable rate during the first week, after which it showed a slight decrease and then stayed stable for the duration of the time that measurements were made. Generally, microbial growth rates were higher in soils from the cropland than in the soils from the forest. The cumulative microbial growth differed between land-uses (Figs. 3C, 3D; $p < 0.001$), being higher in the cropland than in the forest. There were also differences between treatments in the cumulative growth

1 day after rewetting (Fig. 3C; p = 0.032), although these differences did not remain when taking into account the whole time period (Fig. 3D; p = 0.42). During the first day after rewetting, the soils from the drought and warming treatments had significantly different responses, which were in opposite directions compared to the control: while microbial growth was higher in the drought in comparison to the control, microbial growth had a tendency to be lower in the warming treatment (Fig. 2C).

By looking at the fungal to bacterial growth ratios, differences in the growth dynamics of the two decomposer groups could be observed (Figs. 3M, 3N). After rewetting, fungi responded faster, as shown by a higher fungal to bacterial growth ratio during the first 50h after rewetting, which then decreased. Fungal growth reached a maximum peak at around 31h after rewetting (Figs. S3E, S3F) which was before the peak in bacterial growth which occurred at around 60 h after rewetting (Figs. S3A, S3B). Around 50h after rewetting, the fungal to bacterial growth ratio reached approximately 1, that is the bacteria and fungi were growing at similar rates. The dynamics were the same in all treatments and both land-uses; however, the fungal to bacterial growth ratios reached higher values in the crop soils. These

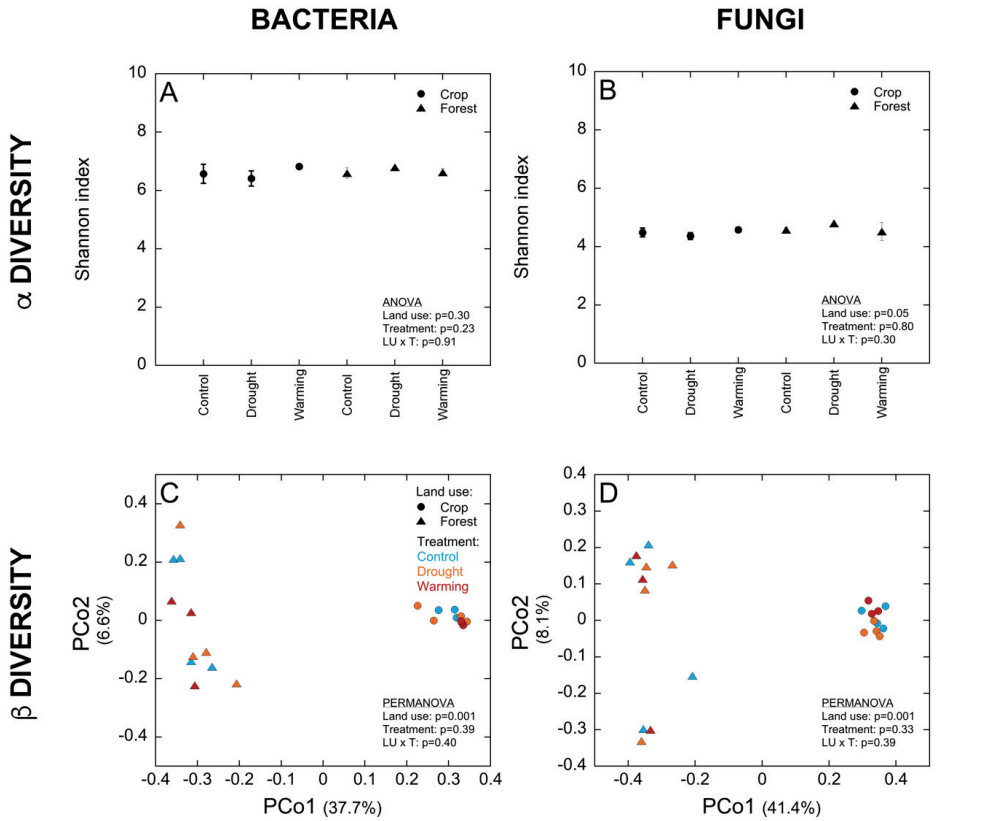

Figure 2. Soil microbial communities in dry soils (i.e. before rewetting). Alpha diversity for (A) bacterial and (B) fungal communities reported as Shannon diversity index. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). Beta diversity for (C) bacterial and (D) fungal communities. The symbols denote each one of the sampled plots. Estimations of beta diversity in the crop soils are shown as circles, whereas the forest soils are shown as triangles. Control plots are shown in blue, in the drought plots in orange and in the warming plots in red.

responses and differences resulted in significant effects on the cumulative bacterial growth (Figs. S3C, S3D), fungal growth (Figs. S3G, S3H) and cumulative fungal to bacterial growth ratio (Figs. 3O, 3P). The cumulative fungal to bacterial growth ratio was higher in the cropland than in the forest throughout the experiment, with a more pronounced difference 1 day (Fig. 3O) than 3 weeks after rewetting (Fig. 3P). This was caused by a large difference in the cumulative fungal growth during the first day after DRW (Fig. S3C) compared to more subtle differences in cumulative bacterial growth (Fig. S3G). 3 weeks after rewetting, growth rates of bacteria and fungi were similar in

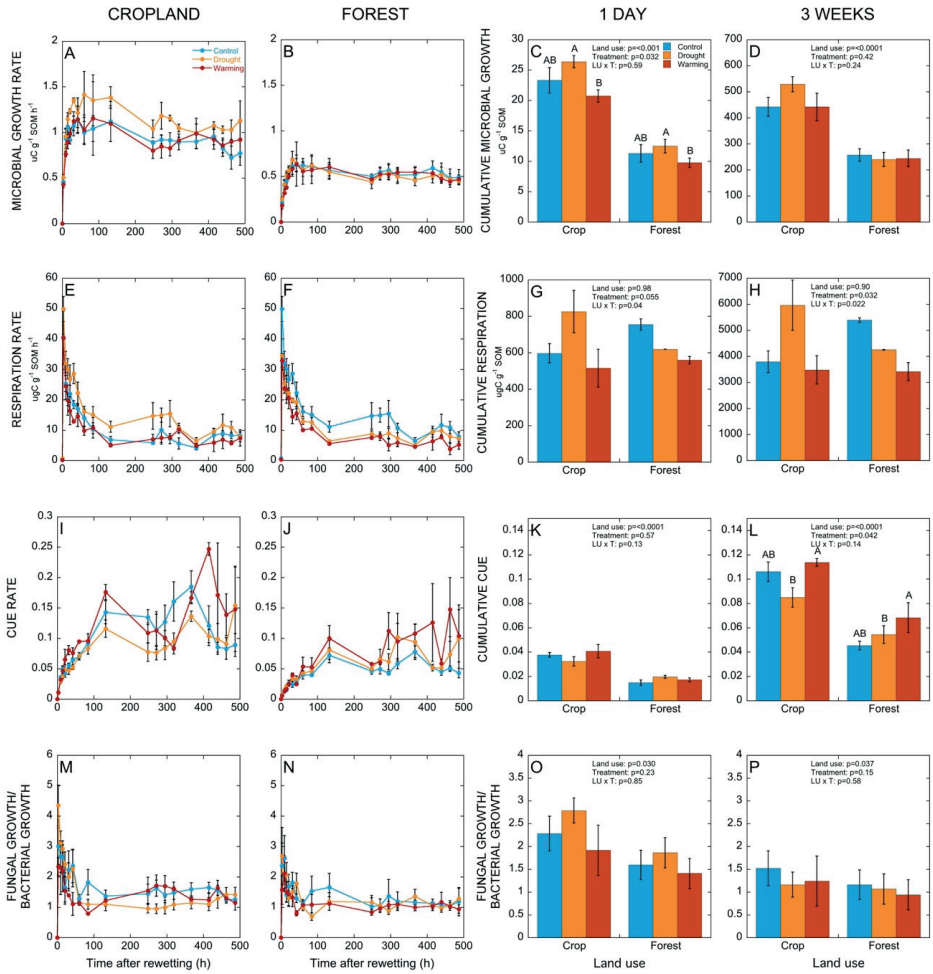

Figure 3. Cumulative microbial processes and their dynamics after a DRW disturbance in the laboratory. Microbial growth rates during a week after rewetting in soils from (A) cropland and (B) forest, and their resulting cumulative microbial growth (C) 1 day and (D) 3 weeks after rewetting. Respiration rates during a week after rewetting in soils from (E) cropland and (F) forest, and their resulting cumulative respiration (G) 1 day and (H) 3 weeks after rewetting. The rate of CUE during a week after rewetting in (I) cropland and (J) forest, and their resulting cumulative CUE (K) 1 day and (L) 1 week after rewetting. The ratio of fungal growth to bacterial growth rates during a week after rewetting in (M) cropland and in and (N) forest, and their resulting ratio of cumulative fungal growth to bacterial growth (O) 1 day and (P) 1 week after rewetting. The measurements in the control plots are shown in blue, in the drought plots in orange and in the warming plots in red. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). Letters indicate significant difference between treatments after a Tukey's pair-wise comparison.

both crop and forest soils (Figs. S3B, S3F), resulting in a cumulative fungal to bacterial growth ratio of approximately 1 (Fig. 3P).

Bacterial growth and fungal growth had significantly different recovery times (Fig. 3, $p < 0.001$). Fungi showed a 15 ± 1 h recovery time, which was shorter than the bacterial recovery time after rewetting (39±3 h). The recovery time was not affected by treatment or land-use ($p=0.63$ and $p=0.11$ respectively). However, an interaction between treatment and organisms was observed ($p = 0.0023$). To better understand such interaction, the model was run for each one of the organisms separately. While bacterial recovery time was unchanged by land-use and treatment (Fig. 5, $p = 0.21$ and $p = 0.44$ respectively), the recovery time of fungal growth was affected by treatment and not land-use (Fig. 5, $p = 0.01$ and $p = 0.12$ respectively). Warming and drought had the opposite effect in the fungal recovery time in both land-uses: while warming increased the recovery time in relation to the control, drought decreased it.

The respiration rate in all instances peaked immediately after rewetting followed by an exponential decrease to a steady respiration rate, which was reached approximately 1 week after rewetting (Figs. 3E, 3F). Unlike microbial growth rates, the levels of the respiration rates were similar in the cropland and forest soils. In the cropland soils, drought treated soil showed the highest respiration rates during the course of the experiment; while in the forest, the control soils had the highest respiration rates. These dynamics resulted in differences in the cumulative respiration after 1 day and 3 weeks of DRW (Figs. 3G, 3H). Cumulative respiration did not differ between cropland and forest soils in any of the reported time-points. However, there was an interaction between land-use and treatment both 1 day after rewetting (Fig. 3G; $p = 0.04$) and 3 weeks after rewetting (Fig. 3H; $p = 0.02$). In the cropland soils, cumulative respiration responded in opposite directions in the drought and warmed treated soils, with drought soils being higher and warmed soils lower than the control. In forest soil, however, cumulative respiration in both drought and warming treatments was lower than the control, with cumulative respiration in the warmed soils being lowest.

The microbial CUE was dynamic over time and followed a similar pattern in both land-uses and all treatments during the course of the experiment (Figs. 3I, 3J). CUE was very

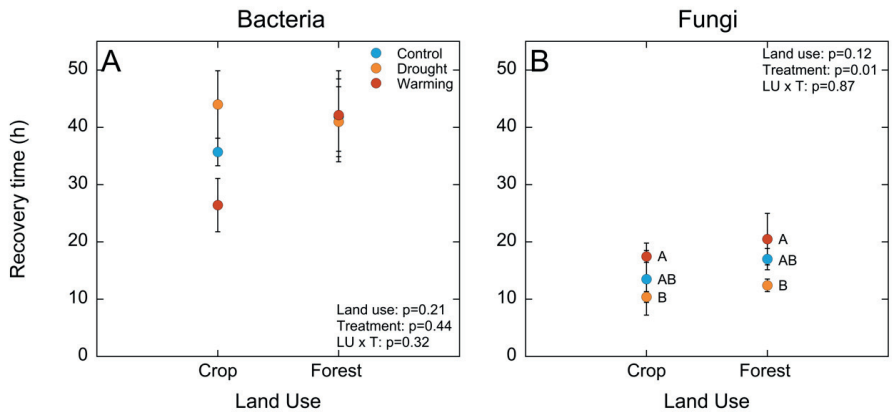

Figure 4. The recovery times of (A) bacterial growth rates and (B) fungal growth rates after rewetting to the rates of soils that have not been disturbed (i.e., their moisture was kept constant). The recovery times in the control plots are shown in blue, in the drought plots in orange and in the warming plots in red. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment). Letters indicate differences between treatments after a Tukey's pair-wise comparison.

low (close to 0) immediately after rewetting and increased during the first week after rewetting. After the first week, the CUE reached relatively stable levels, ranging between 0.10 and 0.15 in the cropland soils and between 0.05 and 0.10 in the forest soils. In both land-uses, the CUE in the warmed soils was consistently higher throughout the experiment, and the rates were always higher in the cropland soils than in the forest soils. These responses and the differences between them resulted in significant effects of land-use on the resulting CUE 1 day after rewetting (Fig. 3K; $p < 0.0001$) and of land-use and treatment on the resulting CUE 3 weeks after rewetting (Fig. 3L; $p < 0.0001$ and $p = 0.04$ respectively).

3.4 Responses of microbial community structure to DRW

Microbial community structure significantly changed over the course of a DRW disturbance in the cropland soils for bacterial and fungal communities (Fig. S4). To understand how microbial communities were changing over the course of a DRW

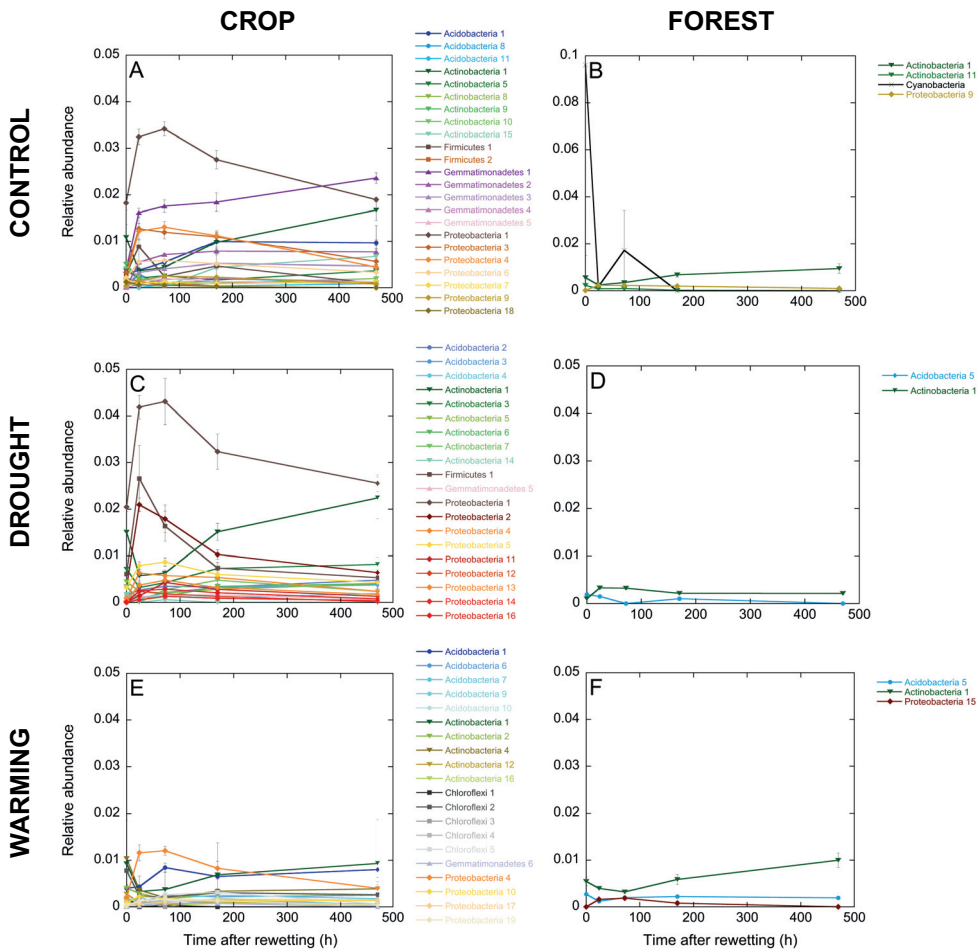

Figure 5. Responsive taxa of bacterial communities during 3 weeks after a DRW disturbance. Responsive taxa of the (A) control (C) drought treated and (E) warmed soils in the cropland soils, as well in the (B) control, (D) drought treated and (F) warmed soils from the forest. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment).

disturbance, responsive taxa were identified. That it, taxa that differentially changed in their relative abundances over the course of the experiment. Responsive taxa could only be identified for bacterial communities, and the number of responsive taxa greatly differed between land-uses (Fig. 5).

In the cropland soils, the 3 treatments had a similar number of responsive taxa (23 in control, 20 in drought treated and 20 in warmed soils); however, the levels of abundance of the responsive taxa reached in the control and drought treated soils were higher than in the warmed soils (Fig. 5). The identified responsive taxa in the 3 treatments belonged to 6 different Phyla. All responsive taxa within *Acidobacteria* slowly increased throughout the studied period after rewetting; while taxa within *Actinobacteria* decreased 1 day after rewetting to later increase and stabilize. Responsive taxa within *Firmicutes*, which were only present in control and drought treated soils, increased in abundance during the first 24h after rewetting and then decreased to match the relative abundance levels they had before rewetting. Responsive taxa within *Gemmatimonadetes*, which were mainly present in control soils, increased their abundance within the first 24h after rewetting and their abundance then remained stable during the course of the experiment. The relative abundance of responsive taxa within *Proteobacteria* increased 24h after rewetting and then decreased to levels matching those before rewetting during the following 2 weeks. Finally, responsive taxa belonging to *Chloroflexi*, which were exclusively present in warmed soils, did not follow a common pattern.

In contrast, in forest soils, bacterial communities only showed a tendency to change over the course of a DRW disturbance. This resulted in the identification of very few responsive taxa (4 in control, 2 in drought treated and 3 in warmed soils). Most of them remained stable during the course of the DRW experiment. The most responsive taxon to DRW responsive was a *Cyanobacteria*, that started with a very high relative abundance before rewetting and then decreased (Fig. 5B).

All bacterial and fungal networks were significantly more clustered than random networks. Bacterial networks properties did not significantly change over the course of the

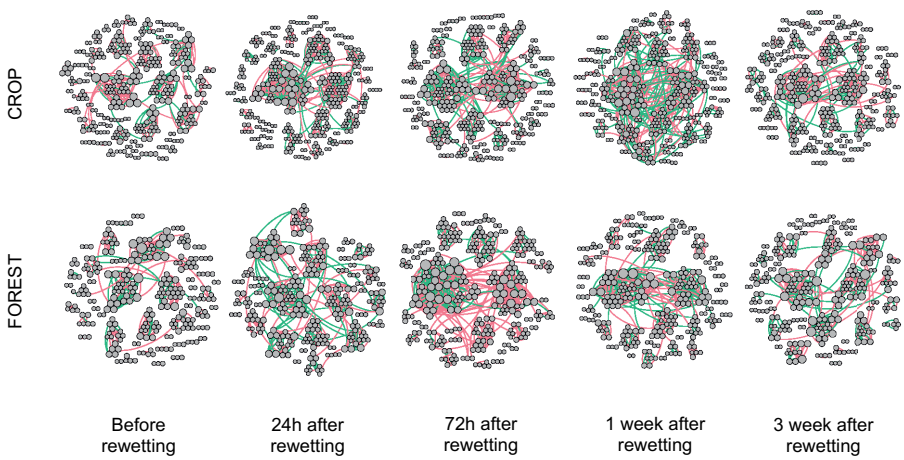

Figure 6. Succession of bacterial community networks over the course of a drying and rewetting (DRW) perturbation (Before rewetting, 24h 72h, 1w and 3w after rewetting) in cropland and forest soils. Networks represent random co-occurrence models derived from 11 replicates at each time point. Nodes represent ASVs, and edges between nodes indicate significant Spearman correlations. The size of the nodes depends on their degree, where bigger nodes indicate nodes with a higher degree. The red and green links indicate positive and negative correlations respectively.

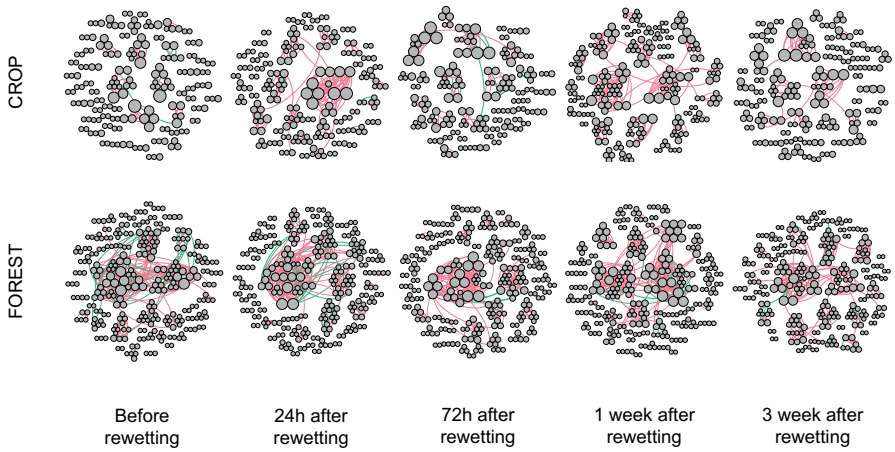

Figure 7. Succession of fungal community networks over the course of a drying and rewetting (DRW) perturbation (Before rewetting, 24h 72h, 1w and 3w after rewetting) in cropland and forest soils. Networks represent random co-occurrence models derived from 11 replicates at each time point. Nodes represent ASVs, and edges between nodes indicate significant Spearman correlations. The size of the nodes depends on their degree, where bigger nodes indicate nodes with a higher degree. The red and green links indicate positive and negative correlations respectively.

DRW experiment in the two different land-uses (Fig. 6, Table S1). However, the complexity of bacterial networks in cropland soils peaked 170h after rewetting, indicated by an increase in the number of edges, degree (connection strength between nodes) and a decrease in the average path length (Table S1). Keystone taxa were identified in the networks to distinguish which taxa were well connected within the network. More keystone taxa were identified in the cropland soils. The number of keystone taxa also peaked at 170h after rewetting. In contrast, in the forest soils the complexity peak of the bacterial networks occurred at 72h after rewetting and the number of keystone taxa remained relatively stable over the course of the DRW experiment. Bacterial networks were always significantly bigger in the cropland soils than in the forest soils, as indicated by a larger number of nodes.

Fungal networks were smaller than the bacterial networks, and did not significantly change over the course of a DRW disturbance (Fig. 7, Table S1). In addition, no major peaks in any of the network parameters were observed, indicating no changes in the network complexity over the course of the DRW experiment. However, the fungal networks differed between land-uses: they were more complex in the forest than cropland soils, as indicated by a higher average degree (connection strength between nodes) and a smaller average harmonic geodesic distance (distance between 2 nodes). The number of keystone taxa was lower in the fungal networks. In the cropland soils only a keystone taxon was identified 3 weeks after rewetting; whereas in the forest soils the community started with 4 keystone taxa and decreased to 1 taxon over the course of the DRW experiment (Table S1).

4. Discussion

4.1 The field experiments

In the present study, we investigated the effects of induced drought and warming on the microbial responses to DRW in two contrasting land-uses. As such, our assessment relied on the effect of the field treatments on soil temperature and moisture. We found that soil moisture was significantly reduced in both drought (by up to 33%) and

warming (by up to 25%) treatments in both land-uses. These seemed a reasonable size effect in comparison to other studies that manipulated incoming rainfall in the tropics and reduced moisture between 22 and 55 % (Bouskill et al., 2016; Cleveland et al., 2010; Davidson et al., 2008). Thus, the warming treatment also resulted in an indirect drought effect which has previously been observed in other temperature manipulation experiments (Kennard et al., 2020; Nottingham et al., 2020). The effect size in drought treatments was higher than in the warming treatments, and the warming treatment only slightly reduced soil moisture in the forest soils. Soil temperature was also significantly affected by field treatments. While warming treatments increased soil temperature by up to 5 °C, which was similar to previous warming studies in the tropics (Kennard et al., 2020; Nottingham et al., 2020) drought treatments slightly decreased soil temperature, probably caused by a small shading effect by the rain shelters.

4.2 The environmental legacy effect on the microbial functional responses to DRW

Contrary to our expectations, differences in land-use did not affect microbial resilience (i.e., the recovery of microbial growth to microbial growth rates in undisturbed soil) after DRW. In both land-uses, microbial growth rates increased immediately after rewetting. The respiration rates also increased immediately after rewetting which was followed by an exponential decrease. This response pattern has been previously observed in semi-arid grasslands (Leizeaga et al., 2020), and interpreted as a resilient response, where microbial communities perceive DRW as a mild disturbance (de Nijs et al., 2019; Meisner et al., 2017). Thus, the dry climate of the studied ecosystems, where microbial communities are prone to be exposed to DRW cycles might explain the generally resilient response of microbial communities upon rewetting.

Despite the lack of difference in the recovery of microbial growth after DRW, there were differences in the microbial CUE, which were especially large between land-uses. Microbial communities in the cropland used C more efficiently upon rewetting. These differences were already obvious 24h after rewetting and became larger 3 weeks after rewetting, indicating the legacy of the land-use was not transient. There are two possibilities that might explain the differences in CUE upon rewetting. One possibility could be that land-uses differed in the quality and availability of resources, which are factors that have been suggested to shape microbial growth efficiency (Roller and Schmidt, 2015). Thus, the observed higher CUE in the studied cropland soils might be explained by the higher quality of C that cropland systems are usually characterized by (Woloszczyk et al., 2020). If we use respiration per SOM as an index of C quality in these soils (Fierer et al., 2005; Robertson and Paul, 2000), there were no differences between land-uses, suggesting that C quality was probably not the reason for the observed differences in CUE. A second possibility could be that a history of DRW exposure had selected for a community well-disposed to cope with such disturbances (Veach and Zeglin, 2019). Microbial communities have been suggested to allocate more C to survival strategies than to growth when they are under stress, resulting in a decrease in CUE (Schimel et al., 2007). Thus, if microbial communities can better cope with DRW disturbances, less stress would be induced, leading to an increased allocation of C to growth which would increase their CUE upon rewetting, and potentially lower C losses upon rewetting (de Nijs et al., 2019). Cropland soils, similarly to grassland soils, are more exposed to disturbances due to the management of the soils, as well as alternating periods of drought due to the lack of canopy and deep roots (James et al., 2003). Hence, the higher frequency of disturbances that cropland soils are exposed to might explain

the more efficient use of C upon rewetting of microbial communities in the cropland.

The field treatments also affected the cumulative processes upon rewetting. Field drought resulted in higher microbial growth and respiration upon rewetting, which was especially noticeable 24 h after rewetting. Since the relative increase in respiration was bigger, this resulted in lower CUE. Based on previous assessments of drought legacy effects on the microbial response to DRW, we anticipated that experimental drought would result in higher CUE upon rewetting (de Nijs et al., 2019; Leizeaga et al., 2020). In contrast to our expectation, in the cropland both microbial growth and respiration in the drought treatments increased upon rewetting. Microbial growth and respiration are both processes that are regulated by the availability of resources (Kemmitt et al., 2008), which suggests that soil microorganisms had access to resources that increased both their respiration and growth. Probably, those resources were the result of accumulation of organic matter that was not decomposed during the dry period due to lack of moisture, which then became available after rewetting (Schimel, 2018). The drought treatment in the forest, however, matched our expectations, as microbial communities had a similar growth rate to the control but a lower respiration rate, resulting in a tendency for a higher CUE. The differences between the cropland and forest soils might be explained by the bigger and longer effect of the drought treatment in the forest soils. In line with previous findings that have monitored respiration rates in warming experiments, soil warming resulted in a decrease in cumulative respiration which might be explained by the depletion of SOM (Melillo et al., 2017). However, surprisingly, this pattern was not observed for microbial growth. Microbial growth in the warmer soils was similar to the control soils, which resulted in a higher CUE of the warmed soils.

The field experiment did not result in large changes in microbial processes after rewetting, which might be driven by the relatively small size effects of the field treatments or the short time that soil microorganisms have been exposed to environmental change. Previous studies that have investigated microbial process responses to DRW have reported contrasting results. While some have found legacy effects of drought microbial processes upon rewetting, with more efficient responses historically drier sites (de Nijs et al., 2019; Leizeaga et al., 2020), others have only found marginal drought legacy effects (Göransson et al., 2013) or even lack of effects (Rahman et al., 2018). These studies varied in the time that soil microorganisms have been exposed to environmental change, with longer times resulting in more noticeable differences. Our field experiment had a smaller effect size than the studies that presented clear differences between treatments. Additionally, the field experiments were only running for 1.5 years. Thus, the observed subtle differences between field treatments might be explained by the short duration of the field experiment and/or the effect size of the treatments. This experiment is, however, part of a larger scale study which will allow the comparison of these results with effects that might occur when incoming rainfall has been manipulated for a longer time period.

4.3 Environmental legacy effects on microbial structural responses to DRW

The structure of bacterial and fungal communities was shaped by the legacy of the environment, as we anticipated. While land-use was a strong driver of the microbial community structure, field treatments did not drive community structure differences (Figs. 2C, 2D). However, if land-use was disregarded, the drought and warming treatments resulted in differences in microbial community structure in the cropland soils but not in the forest (Fig. S2). These differences could be explained by the

different aboveground communities in these contrasting land-uses (Wardle et al., 2004). Forests have a more diverse aboveground community, that might support a higher heterogeneity of microhabitats in these systems, resulting in a higher variability of microbial communities which could obscure possible effects by the field treatments.

After a disturbance, microbial community structure changes but usually returns gradually to their original structure (Jangid et al., 2011). Our results are only partly in line with these findings. While microbial community structure in the cropland soils significantly changed over the course of a DRW disturbance; microbial communities in the forest soils did not (Fig. S4). These contrasting results in community structure were also found in the number of responsive bacterial taxa upon rewetting that were identified: cropland soils showed a higher number of responsive taxa than forest soils. The preadaptation of microbial communities to moisture fluctuations changes was suggested to explain differential responses of microbial community structure in contrasting land-uses by a previous laboratory study (Fierer et al., 2003). A higher frequency of stress-tolerant taxa have also been observed after previous exposure to DRW disturbances (Evans and Wallenstein, 2014, 2012). Thus, these results suggest that microbial communities in the forest soils might be preadjusted to better cope with DRW disturbances. However, this contrasts with the bacterial and fungal process responses to DRW that we observed in the two land-uses.

As already mentioned, the responsive taxa could only be identified within bacterial communities. Bacterial communities have been suggested to have a differential resuscitation after a DRW disturbance (Blazewicz et al., 2020; Placella et al., 2012). In addition, in has been suggested that the response strategy is phylogenetically conserved, that is, the traits that are related to the response upon rewetting might be conversed within bacteria *Phyla* (Placella et al., 2012). The authors of this study classify bacteria in 3 different groups: rapid responders (within 1 h after rewetting), intermediate responders (between 1 and 24 h after rewetting) and delayed responders (24-72 h after rewetting). In line with this, our results show that all the responsive taxa belonging to the same phylum had a similar response pattern. Even though we could not identify the same groups as in the previously mentioned study, the responsive taxa in our study also showed a differential resuscitation. *Actinobacteria* were the first responsive group, followed by *Gemmatiomonadetes*, *Acidobacteria* and *Proteobacteria*. The responsive taxa in control and drought soils summed up to 13% of the total community, whereas the in the warmed soils the abundance of the responsive taxa only represented 5% of the total community. Several studies have suggested that changes in the relative abundance of different taxa upon rewetting might be relevant for ecosystem function (Aanderud et al., 2015; Barnard et al., 2013; Placella et al., 2012). The relative abundances of the responsive taxa are lower than in these studies, which makes it difficult to think that only these taxa will drive the community growth or respiration, calling for further exploration of the dataset.

We anticipated that co-occurrence networks would show a shift in the type of interactions between taxa over the course of a DRW disturbance, driven by resource availability. Specifically, we expected to find a shift in the from positive interactions to negative interactions. We did not find support for that. Instead, we found that the bacterial assemblages formed in the cropland soils were significantly larger than those formed in the forest soils. In addition, we found that these assemblages developed over time after rewetting and increased in complexity, peaking at different times after rewetting in cropland and forest soils. Resource

availability has previously been described as drivers of network structure. For example, increase in resource availability in soils due to the addition of glucose (Qiu et al., 2020) or rhizosphere input (Shi et al., 2016) have been suggested as drivers of increased complexity of bacterial networks. Hence, the observed responses of bacterial assemblages might be explained by changes in resource availability after DRW (Göransson et al., 2013). If that were the case, one would expect that the peak of community complexity would coincide with the greatest rate of resource availability provisioning. The total microbial use of C can be used as a proxy for the availability of microbial resources (Fierer et al., 2005; Rousk and Frey, 2015). The peak in microbial resource use occurred consistently between 80-120 h after rewetting in both land-uses (data not shown). In contrast, network complexity peaked after about 172 h in the crop while it peaked at about 72 h in the forest, disqualifying that complexity was linked to the provisioning of microbial resourced by DRW. It is possible that the link between resource availability and community complexity should only manifest under the condition that availability of resources matched the stoichiometric need of the microbial users, and that otherwise nutrient limitations would ensue. If that were the case, then a lower nutrient limitation induced in the forests could explain the earlier peak in complexity than in cropland soils. Fungal networks, in contrast, were more complex in forest soils than in cropland soils and their complexity remained relatively stable over the course of the DRW disturbance, which was in line with a previous study that reported that fungal communities were more stable under drought (de Vries et al., 2018). There are two possibilities that can explain the higher complexity of fungal networks in forest soils. On the one hand, the ability of fungi to process SOM with a wider nutrient content (Strickland and Rousk, 2010) and their association with plants (Wardle et al., 2004) might allow them to build more complex networks. On the other hand, a lower nutrient limitation upon rewetting might also support a higher complexity of fungal networks.

4.4 Bacterial and fungal responses to drying and rewetting

It has long been argued that there is a trade-off between resistance and resilience (De Vries et al., 2012a; Hedlund et al., 2004; Pimm, 1984). Fungal communities have been shown to be more tolerant to periods of drought in a number of different studies (De Vries et al., 2012b; Gordon et al., 2008; Leizeaga et al., 2020; Manzoni et al., 2012), which leads to the expectation that fungal communities are more resistant but less resilient than bacterial communities (de Vries and Shade, 2013). The results of this study contrast with this expectation. Regarding microbial functions, studies that have specifically investigated fungal growth responses to DRW disturbances have shown that fungi are rather unresponsive (Bapiri et al., 2010; Meisner et al., 2013) or can be similarly resilient to bacterial communities (Leizeaga et al., 2020). Our results, however, show that in the studied soils fungal communities were more resilient than bacterial communities, indicated by shorter recovery times to continuously moist soil growth levels, which were shortest in the drought treated soils. In addition, fungi also seemed to be favored in the cropland soils, as indicated by a higher fungal to bacterial growth ratio upon rewetting 1 day and 3 weeks after rewetting. These observations suggest that fungal communities were adjusted to better cope with DRW disturbances in those soils, which might be mediated by their ability to better withstand drought periods due to their thicker cell walls and their ability to redistribute water (Guhr et al., 2015; Harris, 1981). The increase in bacterial growth resulted in a decrease of fungal growth rate, which suggests that even though fungi might be more resilient than bacteria, their growth is constrained by

bacterial growth (Hicks et al., 2019). These findings highlight the need to further explore bacterial and fungal interactions during DRW disturbances. One way to test the interaction between fungi and bacteria could be the construction of the co-occurrence networks. If there is competition between bacteria and fungi, we would expect to observe a high number of negative edges between bacteria and fungi, which would change over time. However, the taxonomic resolution that we have for each one of the groups is different, which might be an issue to interpret the results obtained with such networks.

In terms of community structure, fungal communities have been suggested to be less responsive and more stable during the recovery of the system after a drought period (Barnard et al., 2013; de Vries et al., 2018). Our results are in line with these findings: we observed a lack of responsive fungal taxa upon rewetting, as well as no major changes in the fungal community assembly. Thus, fungal communities perform better upon rewetting than bacterial communities; however, we could not identify major changes in the fungal community structure that were linked their functional performance. Fungi are known to have a more flexible physiology, which allows them to grow in a wider range of soil moisture contents (Manzoni et al., 2012), temperature (Pietikäinen et al., 2005), SOM quality (Strickland and Rousk, 2010) and pH (Rousk et al., 2009). This higher physiological flexibility might thus explain the lack of responsive taxa in fungal communities, as well as, the stable fungal networks upon rewetting.

4.5 Conclusions

We show that land-use legacies can shape both microbial community structural and functional responses to DRW. Additionally, we show that bacterial and fungal communities have different responses upon rewetting in subtropical soils: fungi are functionally more resilient than bacteria, and structurally more stable. Thus, if we want to predict how soil microbial communities in subtropical environments will respond to climate change induced more frequent and intense drought and rainfall events, we need to consider (1) the legacy effect of the environment in the function and structure of soil microbes, as well as (2) the different functional and structural responses of bacteria and fungi.

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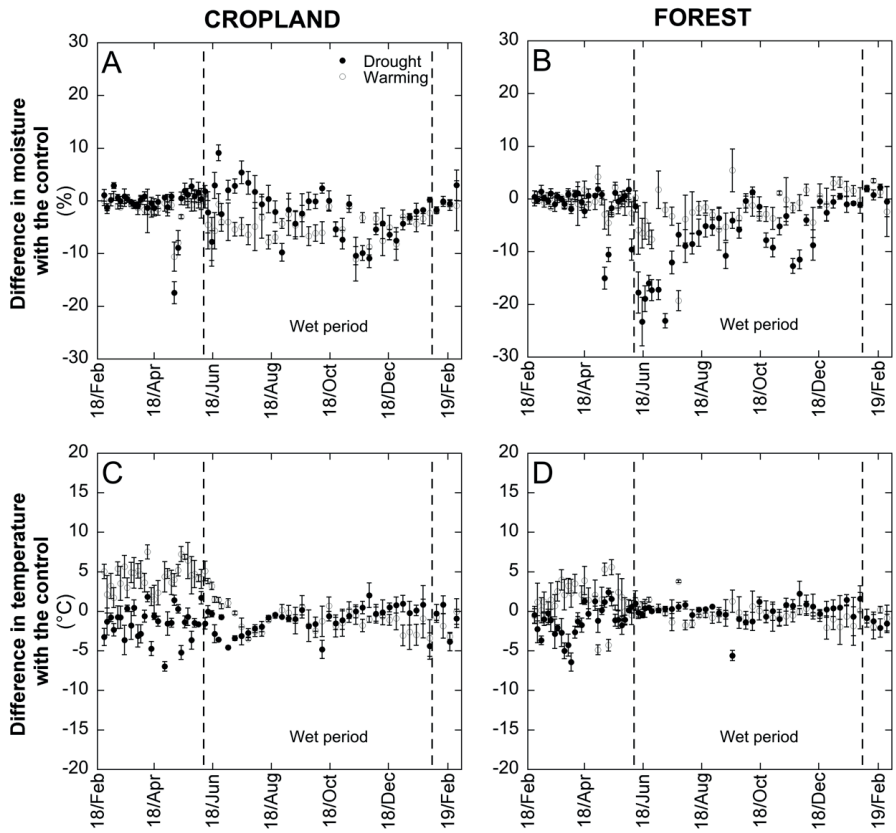
Supplementary material

Results



Figures S1. Difference in moisture (%) between the drought treated soils and the control and warmed treated soils and the control in the (A) cropland and (B) forest. Difference in temperature (°C) between the drought treated soils and the control and warmed treated soils and the control in the (C) cropland and (D) forest. The black and white circles indicate the drought and warming treatments respectively. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment).

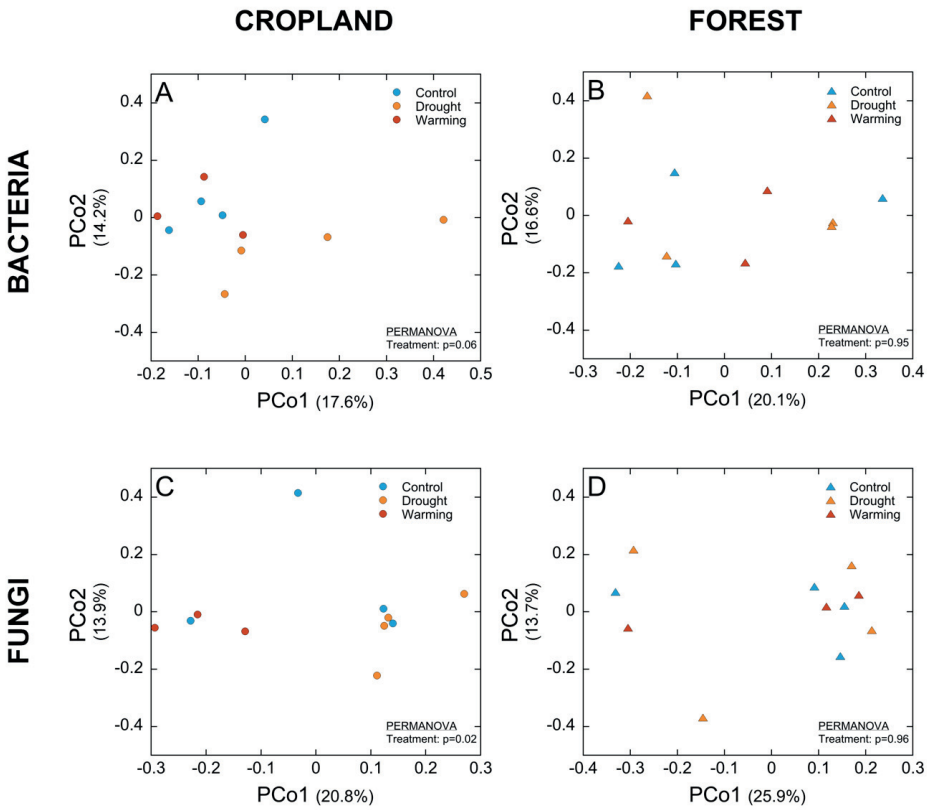


Figure S2. Beta diversity of soil microbial before rewetting for bacteria in (A) cropland and (B) forest soils, and fungi in (C) cropland and (D) forest soils. The symbols denote each one of the sampled plots. Estimations of beta diversity in the crop soils are shown as circles, whereas the forest soils are shown as triangles. Control plots are shown in blue, in the drought plots in orange and in the warming plots in red.

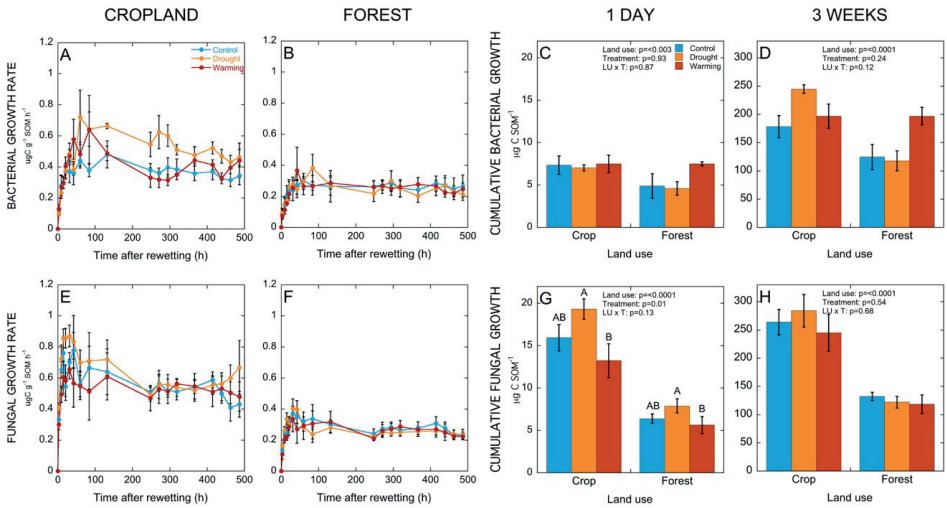


Figure S3. Cumulative bacterial and fungal growth and their dynamics after a DRW disturbance in the laboratory. Bacterial growth rates during a week after rewetting in soils from (A) cropland and (B) forest, and their resulting cumulative bacterial growth (C) 1 day and (D) 3 weeks after rewetting. Fungal growth rates during a week after rewetting in soils from (E) cropland and (F) forest, and their resulting cumulative fungal growth (G) 1 day and (H) 3 weeks after rewetting. The measurements in the control plots are shown in blue, in the drought plots in orange and in the warming plots in red. The symbols denote mean values ±SE (n=4 in control and drought treatments and n=3 in the warming treatment).

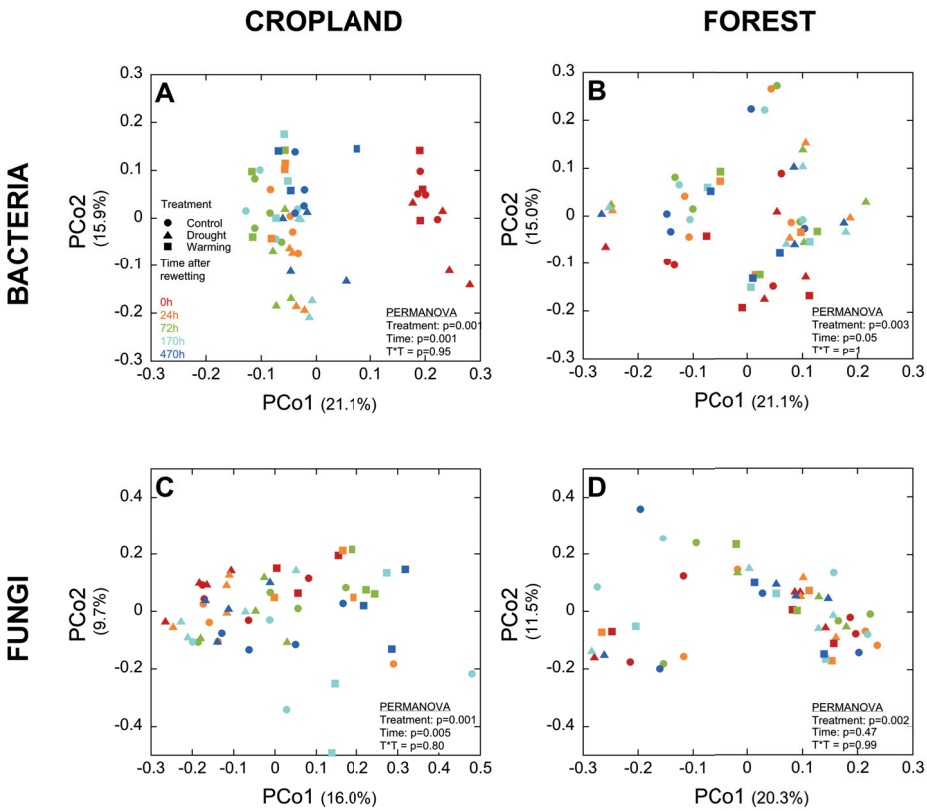


Figure S4. Beta diversity of soil microbial communities over the course of a drying and rewetting (DRW) disturbance. The beta diversity was estimated for both bacterial communities in (A) cropland and (B) forest soils, as well as fungal communities in (C) cropland and (D) forest soils. The symbols denote each one of the sampled plots. Estimations of beta diversity in the control soils are shown as circles, drought treated soils are indicated with triangles and warmed soils with squares. The different colors indicate the different sampling times during the DRW experiment.

Table S1. Topological properties of the empirical networks of bacterial and fungal communities over the course of a drying and rewetting disturbance in cropland and forest soils.

Organism	Land-use	Time after rewetting (h)	Number of nodes	Number of edges	Positive edges	Negative edges	Average degree	Network diameter	Harmonic geodesic distance	Modularity	Number of keystone species
Bacteria	Crop	0	364	389	64.0	36.0	2.14	21	0.46	0.88	2
Bacteria	Crop	24	402	567	53.1	46.9	2.82	21	0.45	0.82	4
Bacteria	Crop	72	375	517	53.4	46.6	2.76	21	0.44	0.81	7
Bacteria	Crop	170	383	626	51.8	48.2	3.27	16	0.46	0.72	16
Bacteria	Crop	470	387	448	52.7	47.3	2.32	21	0.46	0.86	5
Bacteria	Forest	0	284	318	65.7	34.3	2.24	22	0.46	0.89	3
Bacteria	Forest	24	307	428	54.7	45.3	2.79	23	0.34	0.84	6
Bacteria	Forest	72	306	527	64.3	35.7	3.44	23	0.37	0.77	2
Bacteria	Forest	170	314	411	55.5	44.5	2.62	22	0.44	0.82	5
Bacteria	Forest	470	300	365	58.1	41.9	2.43	24	0.42	0.85	4
Fungi	Crop	0	194	156	75.0	25.0	1.61	7	0.61	0.96	0
Fungi	Crop	24	207	234	95.7	4.3	2.26	10	0.66	0.87	0
Fungi	Crop	72	185	176	82.4	17.6	1.90	13	0.66	0.93	0
Fungi	Crop	170	239	305	99.0	1.0	2.55	15	0.57	0.92	0
Fungi	Crop	470	205	187	88.8	11.2	1.82	11	0.74	0.93	1
Fungi	Forest	0	296	396	72.7	27.3	2.68	14	0.56	0.75	4
Fungi	Forest	24	285	374	75.1	24.9	2.63	18	0.60	0.77	2
Fungi	Forest	72	290	360	92.8	7.2	2.48	16	0.63	0.86	0
Fungi	Forest	170	294	432	85.4	14.6	2.94	14	0.61	0.84	1
Fungi	Forest	470	353	291	84.7	15.3	2.43	15	0.54	0.88	1

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Moisture as a regulator of microbial life in soil

Moisture and its fluctuations have long been regarded as important regulators of soil microorganisms, which act as gate-keepers for the carbon exchange between soil and the atmosphere. Hence, there is an urgent need to understand how soil microbial communities will respond to the more frequent and intense drought and rainfall events expected due to climate change. This thesis provides a deeper understanding of how microbial communities respond to moisture fluctuations, and explores possible mechanisms that affect the response. In addition, it examines how these responses might be shaped by the history of climate or land-use of the ecosystems, and the implications for the ecosystem carbon budget.

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