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Legacy effects of temperature alterations on microbial resistance and resilience to drying and rewetting

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Legacy effects of temperature alterations on microbial resistance and resilience to drying and rewetting

Effekter av temperaturförändringar på mikrobiell resistens och återhämtningsförmåga vid torkning och återfuktning

Master degree thesis, 30 credits in Environmental Changes in High Latitudes (EnCHIL)

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Master thesis, 30 credits, in Environmental Changes in High Latitudes (EnCHIL)

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1. Research aims

1.1 General introduction and Research aims

Soil is one of the main pools of accessible terrestrial carbon; much of that carbon is in the arctic (Crowther et al., 2019). Since the scientific consensus on climate change was established, it has been a consideration of what becomes of this carbon when cold can no longer keep it from being decomposed (Schuur et al., 2015). The answer to this likely lies in the microbial community of these ecosystems, as decomposition is generally a microbially driven process (Lehmann and Kleber 2015). These communities are complex and foster a multifaceted interaction with many of the variables within the environment they are hosted in. Thus, in order to understand what becomes of the stored carbon in these systems, I need to understand the basics of these interactions.

Moisture is a key factor in the regulation of microbial activity (Evans et al., 2022, Sierra et al., 2015). Both its quantity and periodicity can have massive effects on how microbes grow and what functionality they are capable of (Evans et al., 2022). Soils that are dried often adapt to have either resistance or resilience to such events. Subsequent rewetting can cause massive pulses of greenhouse gas emissions (Xu et al., 2004). These drying rewetting events strongly impact the future responses of soil to being rewetted after drying (Leizeaga et al., 2022). With the precipitation and temperature alterations that will be seen in the arctic and subarctic, more knowledge of how these soil microbial communities will be altered with them in terms of both their growth and respiration (Iovieno and Bååth 2008). As most literature in high latitudes has focused on temperature regulation of microbes and most studies on moisture dependance have taken place in arid or mid-latitude systems, this thesis aims to address the gap of; what occurs in microbial communities' moisture response in high latitude systems when they are warmed.

In this thesis, I examined how a history of warming has altered the microbial legacy and thus changed the response to future disturbances. I particularly interrogated a microbial community's resistance to drought and resilience when rewetting. I investigated this using growth rate measurements and respiration to quantify how microbes used C by either binding it into biomass versus respirating it. Additionally, I looked at field data for moisture and temperature via on-site

sensors and field measurements to contextualize and explain the microbial response with previous history of moisture to specific sites. I expected that as soil was warmed in the summer soil microbial communities would be dried and thus microbial resistance and resilience would increase. I also expected soil microbial communities warmed in the winter to be wetter and less resistant and resilient than controls.

2. Introduction and background

2.1 Background and context of temperature

Due to the large quantity of soil carbon (C) in the arctic and the fact that decomposition is often thermally limited in high latitude systems, understanding climate change's effect on these systems will be paramount (Curtin et al. 2014, Crowther et al. 2019). With increasing temperature, the rate of decomposition by fungi, bacteria and other microbes will accelerate (Kätterer et al. 1998). To account for these anticipated changes in models and subsequent C budgets, a precise quantification of the decomposition processes in arctic and subarctic soils is required (Poppeliers et al. 2022). To do this, the microbial ecology underpinning decomposition must be understood both in terms of responses to temperature and the various secondary effects of temperature.

The most obvious and direct effect of climate change is rising temperatures; which are expected to increase 2-5° C before the end of the century (IPCC 2021). The rate of warming since 1979 is nearly four times higher in the arctic compared to mid-latitudes (Rantanen et al. 2022). Temperature limitation has allowed C to accumulate in the arctic over time as the cold temperature causes decomposition to be slower than the production from plants. However, as climate change affects the arctic, the microbial response within soil will determine the fate of this vast store of C (Crowther et al. 2019). With the arctic and subarctic being adapted to cold temperatures, microbial research in this arena is a critical piece of the C budget puzzle. With an increase in temperature, there is evidence that microbial activity will also accelerate; in fact temperature is one of the strongest predictors of microbial activity (Panikov 1999, Yuste et al. 2007). Temperature is a major control for microbial activity because of the underlying mechanisms in biochemistry that govern life at a fundamental level. At lower temperature

ranges, the reactions that allow life to occur are halted but slowly increase again with warmth, because temperatures also increase the rate at which these biochemical reactions occur (Ritchie 2018). Heterotrophic respiration generally increases along with microbial growth rates as metabolic processes increase (Curtain et al. 2012, Rousk et al. 2012, Ritchie 2018). When function is greatly reduced at 40 C, it is because this is the temperature at which proteins denature, enzymes no longer undergo their normal reactions and thus without special modifications, these cease to function (Daniel and Danson 2013, Ritchie 2018).

Beyond the direct effects on microbes, altered temperatures can also cause many indirect effects in ecosystems. Warmer temperatures can dry out soils by increasing evapotranspiration (Brown 2014). In addition, in cold conditions, warming can release liquid water by thawing ice, resulting in wetter soils (Schwingshakl et al. 2017). Given the complexity of temperature effects on soil moisture dynamics in subarctic systems and the subsequent effects on microbes, there is a need to study the effects of altered moisture on soil microbial communities.

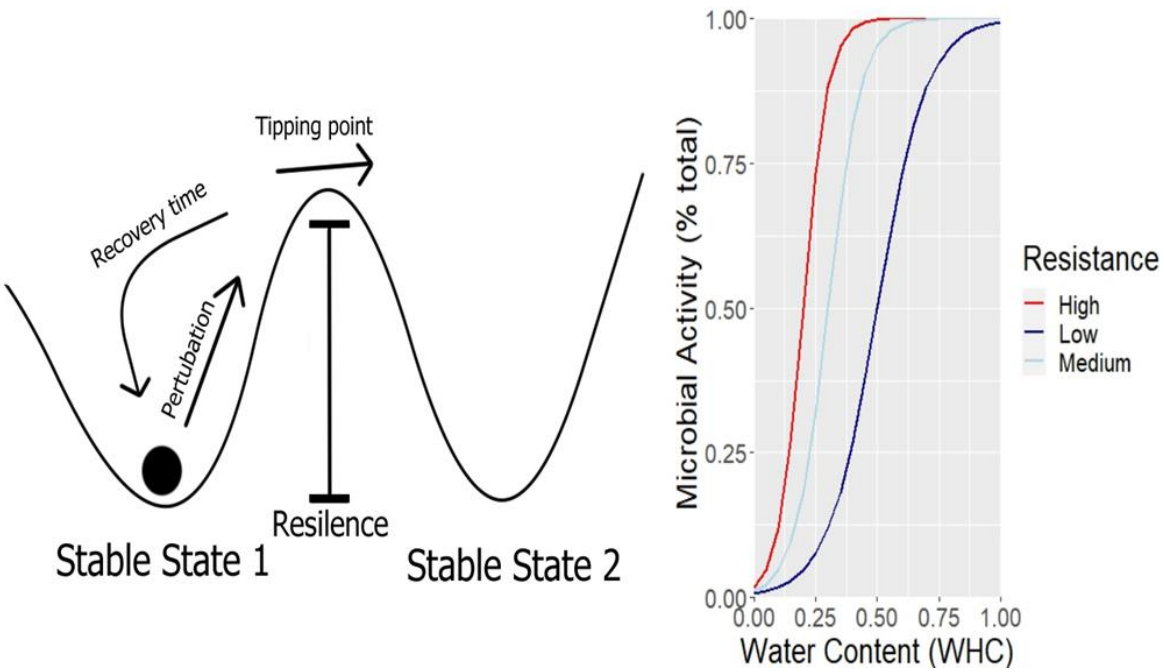
Microbes have various ecological strategies to cope with cycles of perturbation. Conceptually these are broadly represented in terms of resistance and resilience (Griffiths & Phillipot 2013). Resistance to perturbation would mean retaining functionality in such conditions. Resilience would be the ability to recover to prior conditions following perturbation. In this thesis, I will explore the differences in microbial resilience and resistance under altered regimes of moisture-related perturbations.

2.2 Drought and drying of soils

Temperature has two effects which limit moisture availability in soils. First, high temperatures can evaporate water and accelerate evapotranspiration in soil and second low temperatures can freeze water in soil. Both freezing and drought cause water to be inaccessible to microbes and thus can have similar effects on the soil ecosystem. However, the scale of these likely fosters different adaptations. Previous observations have shown that being exposed to a drought event does not prime microbes for freezing nor vice versa (Schimel 2018, Sierra et al. 2015, Mikan et al. 2002). On top of the effects of temperature, climate change is predicted to affect precipitation regularity and quantity (Alexander 2016). Notably, in many arctic and sub-arctic ecosystems, there is a predicted decreased frequency and increased quantity of precipitation which will likely

affect soil processes (Wrona et al. 2016). The decreased frequency of precipitation will likely lead to a more frequent occurrence of drought conditions. Snow plays a vital role in the activity of microbes in winter, as it can insulate soil and in melting provide accessible water. If snow melts and is followed by extreme cold it can cause osmotic stress through freezing without the snow's insulating layer (Wilson et al. 2020). However, in some cases when warming induces snow melt during winter, additional moisture could become available to soils, which may be water limited, reducing their drought stress (Petersky & Harpold 2018). In many systems it is not clear if the insulation or moisture addition properties of snow are more relevant.

Microbial resistance and resilience to altered moisture can vary throughout high latitude systems based on exposure to, the quantity of, and frequency of precipitation, which will all affect the C dynamics of such a system (Griffiths and Phillipot 2013; Crowther et al., 2019). C dynamics in high latitude systems have important differences in historically wet versus dry



conditions (Lara et al. 2020). For instance, drought in a historically wet arctic ecosystem can

Figure 1: Conceptual graph displaying resistance and resilience to moisture stress. On the resistance graph Y axis higher is higher activity, lower is lower activity. On the X axis left is lower moisture and right is higher moisture. The different lines represent different drought histories with the left most curve being the most exposed to drying and the right most curve being the least exposed.

alter its sensitivity to changes in temperature by changing its specific heat capacity, potentially leading to higher CO_2 emissions and less fixed C (Webster et al. 2013). According to the Koppen climate model most subarctic and arctic ecosystems are considered without seasonality in terms of precipitation (Beck et al. 2018). Thus, moisture differences between sites would likely be locally specific and a consequence of other site factors like topography or parent material characteristics (Lara et al. 2020). Since there is low metabolic activity due to cold there is an accumulation of C in organic matter, thus these ecosystems currently act as a C sink. However, as temperature changes the stability of sequestered carbon may be altered, and the severity of these temperature alterations may be a moisture-driven function due to the interaction of moisture and temperature (Illeris et al. 2014). Microbial responses to moisture have been the subject of much scholarly pursuit. When water in the soil is low, it can lead to microbes being water-limited (Sierra et al. 2015). Trying to quantify the effect of moisture on microbial communities in soil has been attempted by many studies usually resulting in a similar Dependance. As moisture increases microbial responses tend to be logistic; they often increase exponentially at a minimum quantity of moisture before stagnating at an optimum quantity of moisture (Figure 1). This is reversed when soils dry, they move from a constant rate of activity to a logistic decline at a moisture level determined by their drought resistance, until the activity flatlines (Figure 1). Moisture curves additionally are affected by the soil microbial community's legacy or what they have been previously exposed to, which is highlighted below (Evans et al. 2021). Resilience on the other hand can be described as how well the microbial community can rebound to states before drying. This is often described as a stable equilibrium, where resilience can be defined as the quantity of water stress that it would take to push the community to an alternative stable state and the rate at which it returns to its original state (Griffiths and Philppot 2013, Shade et al, 2012)(Figure 1)

2.3 Importance of moisture periodicity

The temporal aspects of soil moisture matter for ecosystem functionality and greenhouse gas (GHG) emissions (Jarvis et al., 2007). Upon rewetting soil, growth rapidly increases and there is a pulse of GHG emissions, notably CO_2 (Birch 1958). The pulse of emissions found after the rewetting of a soil is known as the Birch effect. This was first observed by HF Birch in 1958 who noticed it in savannah soils in Africa (Birch 1958). During the rewetting period, emissions from

the soil can produce up to a year's worth of CO_2 over a few days in some systems, particularly if they are arid (Xu et al. 2004). Studies have shown that upon rewetting dried soils there is a decoupling between soil respiration (Iovieno and Bååth, 2008) and microbial growth which can be reflected by decreased carbon use efficiency (CUE). This decoupling varies from system to system, while mechanistically it has yet to be definitively explained, it can be shown to depend on historical conditions as described in the traits and legacy section of this paper.

While there is not a consensus on what causes the Birch effect, there are a few mechanisms which have been theorized to explain the observed pulses of emissions during rewetting dried soil. One proposed explanation is the disintegration of soil aggregates which could release previously sequestered labile matter (Denef et al. 2001). When this material is released, microbes quickly break it down for energy resulting in increased emissions (Denef et al. 2001, Zhang et al. 2022). How much this potential mechanism affects emissions post-rewetting is likely a matter of what historical conditions the soil has been subjected to. One example of this could be how microbes use carbon in their activities. Respiration pulses following rewetting would look different from an r-strategist vs a K-strategist. R strategists may focus on growing or reproducing as quickly as possible, while K strategists will focus on surviving for a longer period. These differences will likely result in a different pattern of respiration, thus altering the dynamics of the pulse as well as their resistance and resilience (de Vries and Shade 2013). K strategists may be more able to adapt, increasing their efficiency and lessening the pulse they produce (Brangari et al 2021). R strategists, however, would respond by increased growth and less efficient use of carbon, possibly resulting in a bigger pulse (Brangari et al 2021). Additionally, other functional traits like the secretion of extracellular polysaccharides (EPS) could also play a role in forming soil aggregates by helping the soil to clump together via EPS's adhesive properties. Either way community dynamics can alter how quickly carbon is used and where microbes use this carbon (Monson et al. 2006). This community effect should be considered with the soil's innate physical properties such as its cation exchange capacity or ability to sequester labile organic material to better understand the context surrounding this phenomenon (Zhu et al. 2020).

Previous studies have attempted to better understand the proposed soil aggregate mechanism and its ecological implications. The physical properties of the soil are often altered

during drought and rewetting (Zhang et al. 2022). Theoretically, prior to drought, biological and physical processes form soil aggregates via mechanisms like secretion of extracellular polysaccharides or enzymes, and soil cohesion. During this destabilization aggregates usually are not disrupted or separated (Denef et al. 2001, Navarro-Garcia at al. 2011). Following these hydraulic forces during rewetting can physically disrupt soil aggregates and microbes can access the previously occluded organic matter (Zhang et Al. 2022, Denef et al. 2001). These alterations vary in scale and severity based on two factors: the length of the drying period and the number of drying and rewetting cycles (DRW) (Zhang et Al. 2022). In this interpretation, microbes would take advantage of the newly available substrate and the result of this would be the observed pulse. Modelling approaches have shown that depending on soil history the disruption of soil aggregates can produce up to 50% of the emissions from rewetting (Brangari et al. 2021)

Historically, the predominant theory has been that a significant portion of emissions observed in the Birch effect could result from organisms utilizing nutrients from recently lysed dead cells (Scheu and Parkinson 1994). Osmotic lysis occurs when the water potential between the soil and the cell ruptures the cell membrane, releasing resources for other cells to take up. However, this is likely not the case in many soils given that several studies have shown that cellular lysing contributes only a very small amount to nutrient pools following a drought (Halverson et al. 2000, Salazar et al. 2018, Aanderud et al. 2015). Ostensibly, cells tend to go into a partial dormancy and often do not burst from osmotic stress. When parameterized in models, this also fits observational data better than without these parameters (Salazar et al. 2018). One consideration that should be noted is the potential priming effect even a small amount of cell lysing could have. The priming effect occurs when labile carbon is introduced to a system and the small amount of energy stimulates the decomposition of more recalcitrant carbons (Kuzyyakov et al. 2000). This means that lysing would be indirectly responsible for the emissions, but still a key feature of this potential mechanism. However, this is arguably system-dependent and lysis as a source of C cannot be ruled out of our current possibilities for mechanisms of the Birch effect.

Recently, the theory has shifted in focus to examine the mineral interactions of microbes in soil (Wider et al. 2014, Fierer and Schimel 2002). While lysing may not be a significant source of emissions, the microbial mechanism to avoid this may be the culprit. To keep from

desiccation, cells in drought conditions may accumulate osmolytes (Warren 2014). When water is reintroduced to a system, cells may metabolize or flush these osmolytes out so that the observed pulse results from them (Warren 2020). Additionally, some extracellular enzymes may continue to be active during drought or may reactivate with cells when water is reintroduced (Alister et al. 2013, Acosta-Martinez et al. 2014). It is important to remember that all these concepts have yet to be tested, and there is no scientific consensus on how the pulse observed in the Birch effect is formed. Indeed, it could be a combination of all three mechanisms and some models have predicted how much each mechanism might contribute to the pulse of emissions following rewetting is also largely affected by the soil history (Brangari et al. 2021).

2.4 History, legacy and traits of microbial communities

A microbial community's history of evolutionary shaping based on moisture legacy can be used in the framework of trait-based ecology for soil microbes. Malik et al. (2020) as well as Malik and Bouskill (2022) reviewed microbial responses across studies and posited that microbial traits in regard to microbial growth and cycling of resources can be characterized in terms of three strategies: high yield (Y), resource acquisition (A), and stress tolerance (S) or (YAS) which is then used to refer to the combination of these three factors. In this study, I would expect the distribution of traits to be shifted towards stress tolerance in soils with legacies that microbial communities would have experienced as extreme. By this framework soils that have not been exposed to stress should be better at either acquiring resources or have higher activity than those subjected to additional water stress (Malik and Bouskill 2022). This can be used to link responses to broader ecological concepts such as resilience and resistance by conceptualizing why all microbes do not have these traits, as well as help modellers develop more precise ways to model the legacy of soil microbes.

Evans et al. (2022) theorized about the shapes of microbial responses to changes in moisture based on exposure to previous drought or DRW events. For instance, soil microbes exposed to drought would generally have lower activity but tolerate lower levels of moisture and still function (Evens et al., 2022). Similarly, communities used to a condition closer to optimal moisture should be more productive but may shut down sooner when drying occurs (Evens et al., 2022). Finally, Evans et al (2022), theorized that if conditions have been extremely variable that

microbial activity may stay at a consistently low level that is less active across differing moistures in soils. These observations align with data in several experiments, although less well studied and modelled at extreme ends of the spectra for drought and saturation (Sierra et al. 2015).

Looking at the above concepts, a crucial concept for understanding soil activity during drought conditions emerges the legacy effect. The legacy effect of a soil microbial community is the collection of traits, functions, and responses that a community has gained from being exposed to prior conditions. Soil microbes on a community scale change in accordance with their environment when experiencing repeated events. Thus, a soils microbial community's response to a single event will be a result of previous events. In terms of GHGs, this can be a crucial predictor that is left out of current models. Because systems that have experienced drought in the past have different responses in terms of growth, respiration and turnover of nutrients, dynamics resulting from differences in processes between systems with varied legacies must be understood in depth before they can be adequately modelled. In Evans et al. (2020) this approach can be demonstrated by the shifts in activity of a community given its exposure to varied moisture. However, Malik and Bouskills (2022) approach it would cause a shift in the distribution of relevant traits. Additionally, a study in 2016 found that microbial enzymatic activity taken from soil microbes that had been exposed to different legacies had predictably different dependencies based off what stresses they have experienced (Averill et al. 2016).

Early measurements of the Birch effect assumed a constant rate of growth proportional to the respiration of a microbial community. This, however, is not the case as it has been shown that bacterial growth and respiration decouple during rewetting (Ioveino and Bååth 2008). This decoupling leads to the application of C use efficiency (CUE), which explains how much C is used for respiration and how much is used for growth during a rewetting event. The degree to which the Birch effect decouples growth and respiration varies based on the microbial legacy (Nijs et al. 2019, Goransson et al. 2013, Fierer et al. 2003). Bacterial growth response to drying

and rewetting can be considered a gradient between type 1 and type 2 responses (Leizeaga et al. 2021, Blazewicz et al 2014, Schimel et al 2007). Type 1 responses have an immediate linear increase in growth rate following a rewetting event. After this burst in growth there is a short decline to a stable state (Leizeaga et al. 2021). During this growth period, respiration also increases linearly until it peaks and drops to a stable state (Leizeaga et al. 2021). On the other end of the spectrum, type 2 soils when rewetting show a lag period before their growth rate increases exponentially (Leizeaga et al. 2021). Unlike growth, respiration for type 2 soils starts immediately after rewetting and remains linearly increasing until it returns to a stable state (Leizeaga et al. 2021). In the time after the rewetting, respiration decreases to levels observed prior (Meisner et al. 2013, Leizeaga et al. 2021). Bacterial response to drying and rewetting is malleable and can shift between type 1 and type 2 (Leizeaga et al. 2021). Exposure to DRW cycles often causes soil to shift directionally to type 1, where its lag and recovery time decrease (Leizeaga et al. 2021). This suggests that after a first DRW bacteria experience a second DRW event as less harsh. This has been observed in a study which exposed type 2 soils and type 1 soils to drying and rewetting cycles. When exposed the type one soil decreases its recovery time further and the two type two soils shifted towards type 1 soils in terms of decreasing the lag time before growth and in reduced recovery time (Leizeaga et al. 2021). These two responses have been linked to R and K strategies; type 1 being a rapidly recovering R-strategist and type 2 being a hardy but slow K-strategist (Blazewicz et al 2014).

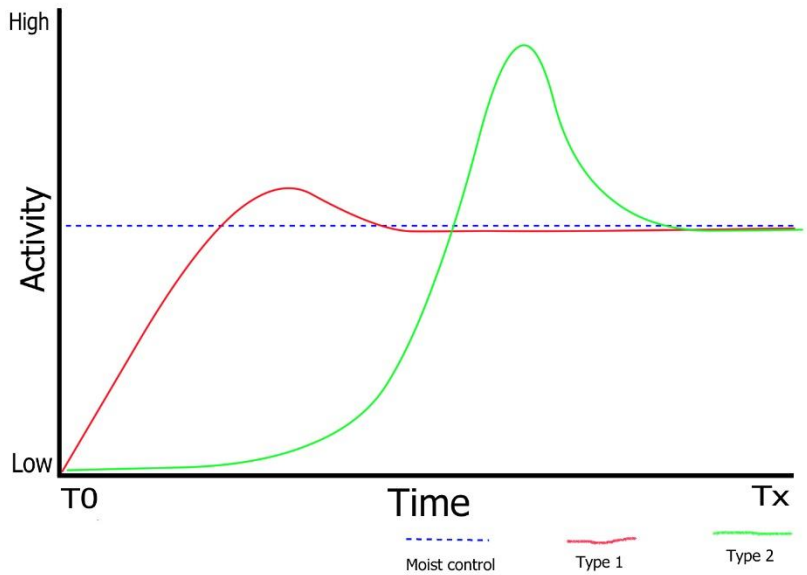


Figure 2: Theoretical figure demonstrating the differences between type 1 and type 2 responses to rewetting. Adapted from Leizeaga et al. (2021). Type 1 is the resilient soil while type 2 is the vulnerable soil

Modelling has also been used to explore the possible microbial community changes and their effect on the Birch effect. The process-based model EcoSMMARTS shows the capacity to not only model the pulse of emissions and decoupling of respiration and growth rate but also proposes an explanation for type 1 and type 2 extrapolated from soil history (Brangari et al. 2020). Brangari et al. (2021) explored the variation in response, duration, and severity of the possible mechanisms using EcoSMMARTS. The results provided from these modelling experiments suggest that trait changes in communities scaled to the severity and duration of DRW events. Despite not being able to explain which microbial mechanism was responsible for trait change (phenotypic plasticity, evolution, or community species shift) the traits entering a DRW cycle had a significant effect on the microbial response.

Phenomena similar to the Birch effect have been observed with freezing and thawing (Meisner et al. 2021). While the effects of freezing might ostensibly prepare soil microbes to easily adapt to drought conditions, studies have shown that the two effects are not necessarily directly linked in terms of microbial response and perhaps mechanism (Meisner et al. 2021). While exposure to rewetting seemed to reduce emissions from thawing, less effect was observed when thawed soils were dried and subsequently rewetted (Meisner et al. 2021). However, the same study showed that exposure to freezing and thawing might have an effect on a soil's response to drying and rewetting (Meisner et al. 2021).

2.5 Respiration, growth, and the global carbon budget

Modeling, as well as observation, has shown that these drying-rewetting events can impact estimates of the global C budget. In Rousk and Brangari (2022), there is an argument that many of the initial pulses are ignored as noise in models but likely can affect the actual C balance. Additionally, Jarvis et al. (2007) measured fluxes via eddy covariance following rewetting in Mediterranean soils postulating that under the right conditions, these could constitute a globally important phenomenon. Both drying and rewetting as well as freezing and thawing can have important dynamics for GHG emissions in the arctic (Meisner et al. 2021). Since there is the possibility of interaction between the two, in the form of the snow functioning to either insulate the soil or melting to provide moisture, investigating this can provide important insights to modellers. Additionally, the emissions pulse of the Birch effect, CO_2 release is proportional to

the availability of C in agriculture (Barnard et al. 2020). With altered plant communities and possibly more C input in the arctic, it is possible that more CO_2 emission from Birch pulses will occur. To better understand ecosystem dynamics modern models must account for the C dynamics of soil (Tang et al. 2022). Following this, there is a gap as many models do not account for the pulses from rewetting and do not account for soil microbial trait changes based on their legacies (Rousk and Brangari 2022).

Drought effects may also have major implications for how the arctic will respond to climate change, understanding the interaction that precipitation will have will likely be a major factor in determining the C budget (Xu and Shang 2016). This is still a large unknown for researchers, as on one hand drought can limit plant productivity while microbes remain active, yet under some circumstances, water may limit the growth of both plants and microbes (Evans et al. 2022).

2.6 Hypothesis

We expected soil legacies to have a significant impact on the microbial response both in terms of how and when soils were warmed and the moisture levels that they were exposed to. Specific questions were: (1) How have warming treatments impacted moisture legacies of subarctic birch soils? (2) How have these legacies played out in microbial responses in an experimental drying and rewetting perturbation under controlled conditions? (3) What consequences will these responses have for environmental changes and GHG emissions? I hypothesized that samples from the two-year legacy of summer warming sites (1) will show less sensitivity to drier conditions (greater resistance) and (2) recover faster (be more resilient) and recover with a greater CUE when being rewetted. These effects will be more pronounced with the extreme warming. (3) Winter warming will have the opposite effect causing soils to be less resistant, therefore requiring more moisture to be fully active and (4) will recover slower (be less resilient) in response to rewetting when compared to control and summer warmed soils.

3. Methods and materials

3.1 Site description and field treatments

In this study, I examined soils from a subarctic birch forest. The site was located at around 68°21'16.6"N 18°49'13.6"E near the Abisko scientific research center. The Koppen climate model is a widely used system to classify zones of vegetation based of temperature and moisture (Beck et al. 2018). This area in Abisko is classified by the Koppen climate model as subarctic with cool summers and year-round precipitation (Dfc)(Beck et al. 2018). The station had a mean annual precipitation (MAP) of around 350 mm and a mean annual temperature (MAT) of around -0.5° C (Abisko Site Background, 2022). The soil was a Histosol, rich in organic matter, and the parent material was a base-rich schist (Global Soil Biodiversity Index, 2016). Vegetation consisted of *Betula nana* in the overstory, with *Vaccinium ulinosum* dominating the understory which is consistent with recent (Malmer et al., 2005). Year-round moisture levels and temperatures were measured via Tomst thermos-moisture sensor (hereby referred to as TOMST) and this was used to test how the legacy of these soil communities was altered by heat treatment (Wild et al., 2019). Field experiments consisted of five treatments: control, summer warming, winter warming, chronic warming, and extreme warming. Each treatment was replicated 4 times in 4 blocks thus each block had 1 replicate of each treatment. Each block was at least 10 meters apart and contained one $1m^2$ plot per treatment. Control plots had no warming, summer warming plots were warmed by 2° C only in the summer, winter warming plots were warmed by 2° C only in the winter, extreme warming was warmed by 3-4 degrees C only in the summer and chronic warming was warmed by 2° C year-round. Warming in sites was achieved continuously via IR-heaters 1.2 m from the soil surface (PAS 2, 250W for summer warming/winter warming / chronic warming and 650W for extreme warming, Backer BHV AB, Sweden) (Kimball 2005). Each site was established two years prior in June of 2020 to the study with the exception of extreme warming which was established one year prior in June of 2021. Sampling of sites was conducted August 16-19^{th} 2022 at the end of two years of warming, during which 250 g was taken from the top 5 cm of soil and processed into multiple composite cores. Soils were first sieved with 4mm mesh and then kept cold (5° C) until they were processed. Normalized

difference vegetation index (NDVI) was measured using an NDVI meter (SpectroSense2+, Skye, UK), which was held above each plot during the 2022 sampling season (Verhulst et al., 2009).

The treatments in this study were the same as those used in a Cruz-Parades et al. (2021) and thus the justification of them is twofold, first using the same treatments allows easy comparison, second the necessity from cost and time for site setup as there was not enough time to set up a 2-year warming experiment for this thesis. The use of heaters in this fashion mirrors previous studies on the same site, allowing for easier comparison between studies (Kimball 2005. Cruz-Parades et al. 2021). Open top chamber would not have been an option given the need for winter warming in the experimental design and other heating methods have been shown to significantly bias a study (Bai et al., 2013). Sieving soils is a common practice to homogenize the soil, while there is some debate about the importance of soil structure in its function, the inability to account for soil structural heterogeneity makes it necessary (Schumecher et al., 1990,). NDVI was used as a quick way to assess plant functions. Because this was not a plant-centered study, extra time and cost were not allocated to plant biomass, diversity or phenology. NDVI is a quick, time-efficient way to evaluate photosynthetic activity and as such, it was used instead of metrics like plant biomass or root biomass (Hill and Donald, 2003).

3.2 Lab treatments

To examine moisture dependance which I used to characterize drought resistance, samples of soils were placed in microcosms and had their WHC measured as described below, then the amount of water to adjust soils to 50% was calculated

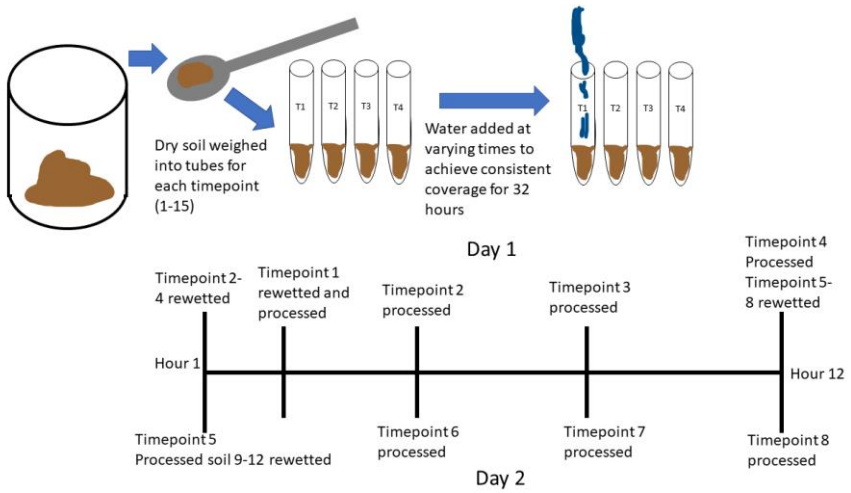


Figure 3: Sampling scheme for rewetting portion of the experiment. On the top half is what occurred on day 1, on the bottom is what occurred on day two. Sampling occurred at the same time each day, the left side showing the start of the 12 hours and the right the end of the 12 hours. This scheme was repeated up to day 3 but differed as sampling occurred less often thereafter.

and added to the soils. Soils in microcosms without lids were then progressively dried at room temperature (25° C) with a fan. Every 4 hours during the drying phase subsamples were taken. Each subsample included a water content (WC) sample which was taken by weighing the sample and then heating it in a 105° C oven till the next day when it was weighed again. Bacterial growth, fungal growth and respiration samples were also taken from each subsample. At the end of each day, lids were put on the microcosms, and they were stored at 5° C. After the first days' WC samples were examined, sampling frequency was adjusted to ensure that samples were evenly distributed over the full range of WC for each treatment. Subsamples for bacterial growth, fungal growth and respiration were stored at 5° C until they could be processed together. Once all subsamples reached below 5%-1% WHC, a final moisture dependance sample was taken and samples were stored while subsamples were processed within 2 days after the soil was completely air dry. Next, I examined how soils would respond to rewetting as a metric of resilience. First, the WC from the last subsample of the moisture dependance was used to calculate how much water would need to be added to 1g of soil to bring it to 50% WC. Soils from microcosms were weighed into separate tubes for each subsampled time point. Once all tubes were weighed, timepoints 2-4 were rewetted to 50% WC, after that timepoint 1 was rewetted and immediately processed for respiration, bacterial growth and fungal growth (Figure 3). Following this, time points 2-4 were processed every 4 hours for 12 hours leading to 12 hours of semi-continuous samples (Figure 3). At the end of the day, timepoints 5-8 were rewetted and then 12 hours later sample 5 was processed (Figure 3). This pattern was repeated for 32 hours after which all remaining samples were rewetted and processed less frequently.

This section of the study was novel and designed to study specifically the moisture response in this environment. 50% WHC has been shown to most often be around the optimum for microbial activity (Evans et al. 2021). This is why I opted to adjust soils to that, as well as why when rewetting occurred, I rewetted to that level. Sub 5% WHC conversely is shown to be an area where microbes generally show no activity which is why it was chosen as an endpoint (Evens et al 2021). I dried soils at room temperature to avoid the confounding variable of temperature which has been shown to alter microbial growth rates (Barnard et al. 2020). Additionally, the timeframe of rewetting measurements was done logistically because most changes that happen at the beginning of rewetting sampling were weighted towards the first 33

hours (Jarvis et al., 2007). After this, the rest of the time was to ensure that the full duration of the reaction and return to basal rate was captured (Jarvis et al., 2007).

3.3 Organic matter content, Water holding capacity and water content

Water holding capacity (WHC) was estimated from gravimetric measurements, where soils were first weighed, then saturated with water for 24 hours, and then weighed again, defining 100% of WHC. Next soils were dried for 24 hours at 105° C and weighed again, defining 0% WHC. After the weight of the soil was dried, it was placed in a 550° C oven overnight to burn off organic material and was weighed again the next day to quantify organic matter. When measuring the water content (WC) of a sample, the sample was weighed, dried for 12 hours at 105° C and then weighed again (Reynolds 1970). This weight was then converted into %WHC.

Water holding capacity in lab testing was used because it is easier to compare how much water is relatively available between soils and the percentage change makes it easy to see how water availability was affected (Jones 2007) For the field measurements this was converted to volumetric water content for the background because it is easier to visualize a quantity of water that per quantity of soil with this metric (Jones 2007). Soil organic matter is generally measured using an oven as I did, other methods can be costly and would provide details not crucial to this study (Nelson and Sommers, 1996).

3.4 Electrical conductivity and pH

Soil pH and Electrical conductivity (EC) were measured with a pH electrode and EC electrode after 1 g of soils were mixed with (5 mL) of distilled water and shaken on a reciprocating shaker for 1 hour. The electrode was then inserted into the solution and the reading was taken once it stabilized.

Soil pH is well established as a driver of soil microorganisms, as such, making sure that it is relatively consistent between treatments is important to discount it as a confounding variable (Thomas 2018). Using electronic probes for pH is cost and time efficient compared to titration. It is also more accurate than litmus paper (Thomas 2018). EC is standardly measured to detect salinity or nutrient content. Using a probe for this is generally standard practice (Rhoades and Manteghi, 1989).

3.5 Respiration

0.5 g if soil was inserted into 20 mL GC vials for each data point. During moisture dependance subsampling, respiration tubes were stored for up to 3 days in a cold room and incubated for up to 24 hours in the dark at room temperature before being processed (Parkin et al., 2015). During rewetting, samples were taken at the time point they represented and then incubated for 2-4 hours. Differing incubation times were accounted for by dividing each sample by the incubation time. Prior to incubation, tubes for samples were aired with pressurized gas and then sealed via a butyl rubber septum held by a metal ring bent onto the tube by a crimper to ensure the previously produced CO_2 was purged and that the environment was closed (Parkin et al., 2015). Blank vials were used to account for the background quantity of CO_2. Respiration was measured via gas chromatography on an Agilent 7697A Headspace Sampler equipped with a thermal conductivity detector (Parkin et al 2015). The front SSL inlet was at 12.8 psi and 200° C, the column was at 35° C with a rate of 6.5 mL/min and the front detector for the thermal conductivity detector was at 200° C.

Gas chromatography of CO_2 production was used because of its specificity meaning it will be less likely to give a false positive and its ability to pick up small amounts of CO_2 given the size of subsamples (Anderson 2015). While some methods like cavity-enhanced absorption spectrometry may be more sensitive, they can also be less selective (Anderson 2015). Additionally, gas chromatography has been used previously in studies examining the same site, making results comparable with previous studies (Cruz-Parades et al., 2021).

3.6 Bacterial growth via Leucine incorporation

The leucine incorporation method can be examined in detail from Bååth 1994a, first 20 mL of water was added to 0.1-0.5 g of the soil sample, and then the water and soil were mixed into a solution. Next bacteria were physically separated from the soil solution via pipetting from a known position after centrifugation (Bååth 1994b). The pipetted portion was added to a new 2 mL tube. 20 µl of a ratio of 8 water, 1 leucine, and 1 ^3H labelled leucine leading to a final concentration of 100 nM/L of ^3H labelled leucine was added to the bacterial solution and incubated for 1-2 hours. The concentration level is below the saturation level of 500 nM/L. Afterwards, 75 µl of 100% TCA was added to the solution to terminate the growth. Bacterial

solutions were vortexed with 1.5 mL 5% TCA and then 1.5 mL of 80% ETOH while centrifuging and removing of supernatant between each of these steps. After the ETOH was removed 200 µl of 1.0 M NaOH was added, and the samples were vortexed until the bacterial pellet was solubilized. Then the sample was put in a 90° C oven for 60 minutes. After this 1 mL of scintillation cocktail was added and vortexed. Samples were then scintillated and disintegration per minute (DPM) was measured which was later converted to C units (Bååth 1994a).

Leucine incorporation is one of the only growth measurements for bacteria that can directly measure bacterial protein synthesis (Alden et al., 2001). While cell counting and biomass measurements both would be biased by potentially dormant cells or species which are active on a longer time scale and DNA incorporation or ATP-centered measurements may be correlated with protein synthesis, they do not directly measure it (Blagodatskaya and Kuyakov, 2013). Since I did not conduct any community analysis, which would possibly use DNA and thus make rates of DNA synthesis more important to examine, Leucine was a more reasonable choice (Blagodatskaya and Kuyakov, 2013).

3.7 Fungal growth via Ergosterol in Acetate

The following method is explained in detail in Bååth 2001, first 1.95 mL of distilled water was added to 0.1 – 0.25 g of soil, then 50 µl of 3 parts acetate and 2 parts ^{14}C labelled acetate solution leading to a final concentration of 220 ul/L ^{14}C labelled acetate was added to the mixture. This concentration was also below the saturation level which occurs at about 3-4 mM/L. After this, the solution was vortexed and incubated for 2-4 hours before being terminated with 500 µl of 10% formalin. Samples were then centrifuged and the supernatant was removed, leaving the soil mixture. Next, 5 mL of 10% KOH dissolved in methanol was added and samples were sonicated for 15 minutes then heated in a water bath to 70° C for 60 minutes before being vortexed. After this, 1mL of distilled H_2O and 2 mL of cyclohexane were added to the sample, the sample was vortexed, and then centrifuged, and the supernatant (cyclohexane with dissolved ergosterol) was then pipetted into a new tube. This step was repeated without the H_2O. Following this, the cyclohexane was evaporated with N_2 gas at 40° C. The ergosterol now dried to the bottom of the tube was dissolved in 200 µl of methanol and filtered into a 250 µl insert before the ergosterol

was extracted via high-pressure liquid chromatography (HPLC). Finally, 3mL of scintillation cocktail was added and the sample DPM was measured via scintillation (Bååth 2001).

Many of the arguments that justify using Leucine incorporation also apply here; ergosterol synthesis from acetate is a relatively direct measurement for cell growth and protein synthesis (Bååth 2001). Additionally, the use of ergosterol allows for measurement to be exclusive to fungi, which many other methods would not allow (Bååth 2001).

3.8 Data analysis and statistics

All data that was growth or respiration related was converted to C units to compare respiration, fungal growth and bacterial accurately. To convert from Leucine to C units I first converted the leucine to thymidine equivalents which were done in Cruz-Parades et al. (2021) by multiplying the leucine data by 0.096628. Following this I then used a conversion factor in Soares and Rousk (2019) to convert thymidine to C units by multiplying the thymidine data by 0.0055. To convert from Ergosterol to C units I used a conversion factor of 0.0026 (Soares and Rousk 2019). Data was then analyzed in R-studio using DPLYR, minpackNLM, vegan, ggplot2 and easynlm packages (Dixon 2003). R is a reliable and versatile program for statistics; its many available packages make it a good fit for this study (Ihaka and Gentleman 1996). Additionally, other tools like MATLAB and Python take more manual input for statistical tests (Ozgur et al. 2021). This may not be an issue for experienced users but can increase sources of error.

Data were normalized for accurate comparisons between groups and to account for background rates not related to moisture dynamics (Weiss et al., 2015). Data for the moisture dependency curve was normalized by dividing by a maximum value resulting in a scale of 0-1 (Rousk et al., 2012). When the moisture data were fitted to a curve, the data was divided by the estimated asymptote in the regressed equation causing the curve to converge at the asymptote of 1 (Rousk et al., 2012). Normalizing the curves in this manner allowed the EC50 and EC10 to be more easily visualized on the graph. This caused data points to shift from 1 being the maximum to data having a slightly larger range. Drying and rewetting data were normalized by dividing by the moist control value. Moist control values were obtained from moist samples that were never dried down. This allowed the background rate of any given sample to be at 1, which made for an easy comparison of recovery time.

Winter and summer temperatures were analyzed separately in terms of background data to account for treatments having considerably different effects during these seasons. A yearly average for temperatures and moisture during these times would not have captured the temporal aspects of legacy effects I wanted to observe. The time series analysis used was a moving average and this was conducted for all moisture and temperature plots to examine the variability and quantity of these aspects. The moving average was simple and could give detailed results with a small bin size of 24 hours (Katz and Skaggs, 1981). Given that data was taken on the sensor every 15 minutes, this still yielded a good amount of data to average.

To determine if there was a difference between groups a one-way ANOVA was conducted between treatments for each of the following variables: moisture, temperature, electrical conductivity, pH, soil organic matter, and NDVI. Some samples had datasets that did not meet the test assumptions of homogeneity of the variance and homoscedasticity for ANOVA, in these cases data was log transformed, which is shown to make data better meet these assumptions (Berry 1987; Eisenheart 1947). If ANOVA tests returned significant value, a post-hoc Tukey HSD was conducted between all treatments in the ANVOA to determine which pairwise differences were present.

$$y = \frac{C}{1 + e^{b(WHC-a)}}$$

Equation 1: Logistic equation used for bacterial growth or respiration during moisture dependance. Y is the growth or respiration rate at a WHC level , C is the max growth rate, b is the rate of decrease and a is point where the x value for halfway down the curve which is the EC50. In the case of EC10 the Y was put as 0.1 and then the x value for WC was calculated.

For moisture dependance samples, data was fitted to a logistic curve (Equation 1) and then normalized using the maximum rate of change value. This was then repeated to make curves converge to a single point allowing for a clearer comparison of EC50, where growth of a sample has halved, thus it can be linked to the resistance of drought stress. EC50 was extracted by taking variable “a” and an ANOVA was used to compare the various EC50 values and determine if there is a statistically significant difference between microbial growth rates/respiration across difference treatments and thus if different treatments did indeed have different resistances to drought stress. This was repeated for EC10, the point where a curve reached 10 percent of its initial growth rate or respiration, however this needed to be solved algebraically.

The response of microbial life to moisture stress has been shown to generally follow a logistic curve (Evans et al. 2021). As such, a logistic curve was seen as the default option. This was furthered by the fact that previous studies that glimpsed the surface of moisture response also followed a logistic pattern (Cruz-Parades et al. 2021). EC50 and EC10 are metrics that have been widely used in literature that is related to stress tolerance (Cagon et al., 2017). In studies that focus on stress tolerance of environmental controls, EC is used, however, in toxicology studies IC50 functions the same way (Cagon et al., 2017). EC50 is a common metric because there is generally a significant effect on system functionality around the 50% mark, however, I also used EC10 to supplement that as that is where many more complex ecosystem functions cease to occur (Bapiri et al., 2010).

$$y = d + ae^{-e^{b-(cx)}}$$

Equation 2: Gompertz curve used for modeling bacterial response to rewetting, a is the time value where growth rate stops increasing, b is the initial growth rate at rewetting, c is the rate of change. X is the time from rewetting and Y is the growth rate at time x.

$$y = ae^{-bx}$$

Equation 3: Negative exponential equation used for respiration during the rewetting period where a is the initial pulse of respiration, and b is the rate of decay for the respiration as it decreases.

Rewetting points were plotted onto a time series and fitted to a Gompertz curve (Equation 2)(Bapiri et al., 2010; Leizeaga et al., 2022). Following this, the recovery time was defined as the time taken until levels similar to its respective moist control were reached and its lag time was defined as the time after rewetting occurs but before the curve begins (Leizeaga et al., 2022). Lag time was calculated by taking the Log of the above formula and examining when its rate of change differed from zero, while recovery time was solved algebraically (Leizeaga et al., 2022). These were both compared between treatments to examine the community's resilience. If treatments did not reach previous activity levels, the point at which they reached an asymptote was measured. If treatments did not have a lag time, they were excluded from the analysis. Respiration was fitted to a negative exponential curve (Equation 3)(Leizeaga et al., 2022; Brangari et al., 2021).

Removing zero values from lag time analysis was to reduce the possibility of a type 1 error about the differences in response. While these samples may have had no lag time, when comparing lag time, I decided that the risk of biasing results by inserting a zero value into the data was greater than the risk of biasing the data by removing the treatments with no lag time. This reasoning also follows the fact that these values are extracted from data that fit to a curve, and not direct observations. The Gompertz curve has been used to plot bacterial growth responses to soil rewetting in many past studies (Iovieno and Rousk, 2008). The Gompertz curve highlights the lag time as well as the point where it crosses the moist control line (Leizeaga et al., 2022). As such while it does not fall after the peak, it is still a useful function for analysis.

CUE was calculated by using the integral for the first 15 hours of respiration and growth curves to find areas under the fitted curve (Equation 4). From there, a ratio of areas was calculated using equation 5. From here the ratios were compared using ANOVA. 15 hours was chosen because the focal point of the analysis for CUE was the decoupling of respiration and growth rate; this usually returns to its normal ratio during the first 12-24 hours following rewetting (Jarvis et al., 2007; Leizeaga et al., 2022).

$$\int_0^{15} f(x)dx = y$$

Equation 4 : The integral is taken for hour 0 through 12 after the rewetting. F(x) in this function represents either equation 2 or 3, while Y is the area under that curve.

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

Equation 5 : CUE equation where G represents area under the bacterial growth curve, R represents area under the respiration curve and CUE is the resulting carbon use efficiency.

If data did not show a response to moisture or to rewetting, it was analyzed as multiplicative levels. This allows there to be a potential difference in how treatments affected growth, even if moisture was not involved. To do this, averages of the growth rate across time in the case of DRW and moisture levels in the case of moisture dependency were used. This has been shown as an effective way to compare time series when they do not change much (Correll et al., 2012). Following this a one-way ANOVA was used to search for differentiation between treatments.

4. Results

4.1 Site background data

The one-ways ANOVA showed no statistically significant differences in pH, water holding capacity, and soil organic matter between treatments (Table 1). Years did not significantly differ from each other, however winter and summers did. Temperatures between treatments differed significantly ($p<0.001$)(Figure 4-6). Moisture levels between treatments were significantly different ($p<0.001$) (Figure 5-7). While variation was difficult to measure, qualitatively, moisture was slightly more variable in the extreme and summertime treatments (Figure 7). Electrical conductivity was different between treatments (Table 1 and Figure 8). NVDI was significantly different between treatments ($p=0.04$) respectively(Figure 6). A subsequent Tukey HSD showed that summer and extreme treatments were significantly different in NDVI. (Figure 6).

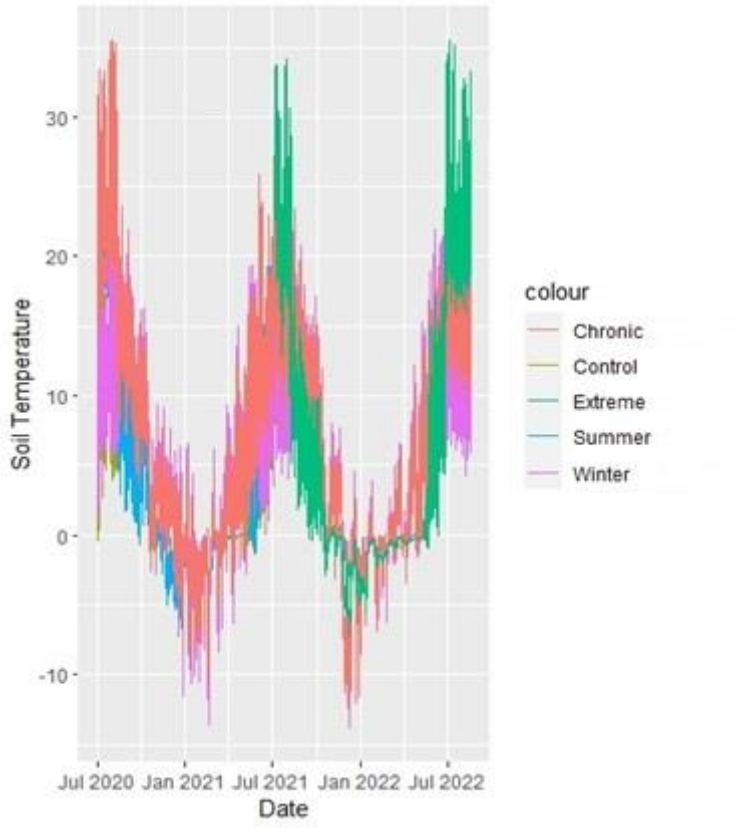


Figure 4: Temperature values for all two years, averages of all replicates for each treatment. (n=4)

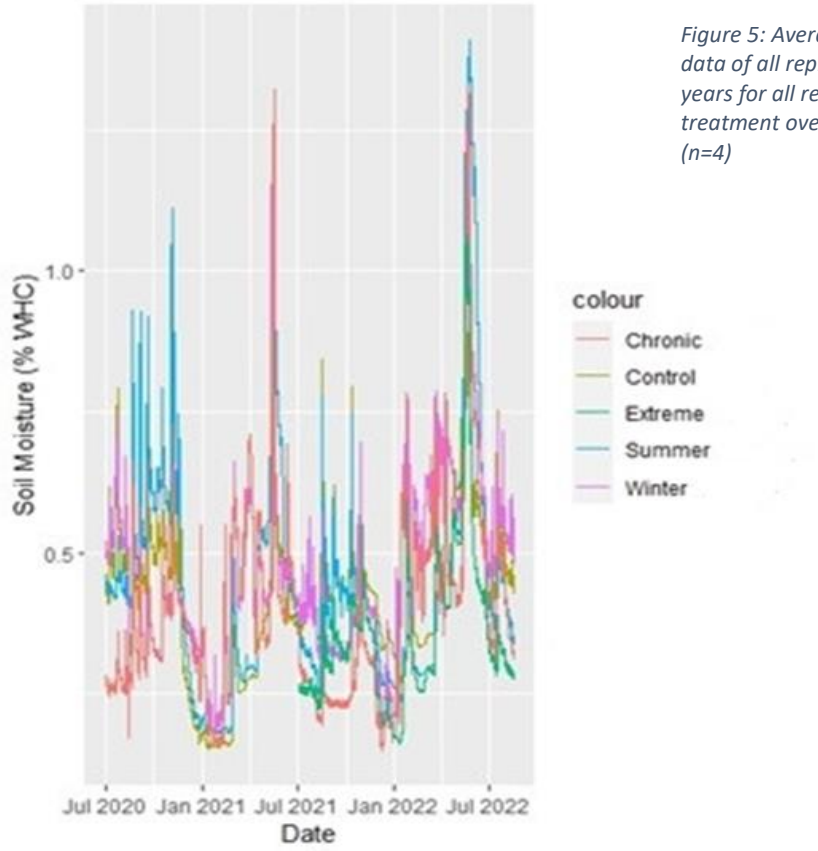


Figure 5: Average moisture data of all replicates over two years for all replicates each treatment over two years. (n=4)

Table 1: Site background data average values and p-values between for an ANOVA conducted between treatments.

Soil characteristic	ANOVA P-value for treatment	DoF	F-Value	Average
Soil pH	0.77	4	0.443	4.72
Soil organic matter (g SOM/g dry soil)	0.68	4	0.577	13.44
Water holding capacity (mL/g dry soil)	0.67	4	0.598	8.07
Electrical conductivity (mS/m)	0.0108	4	4.793	109

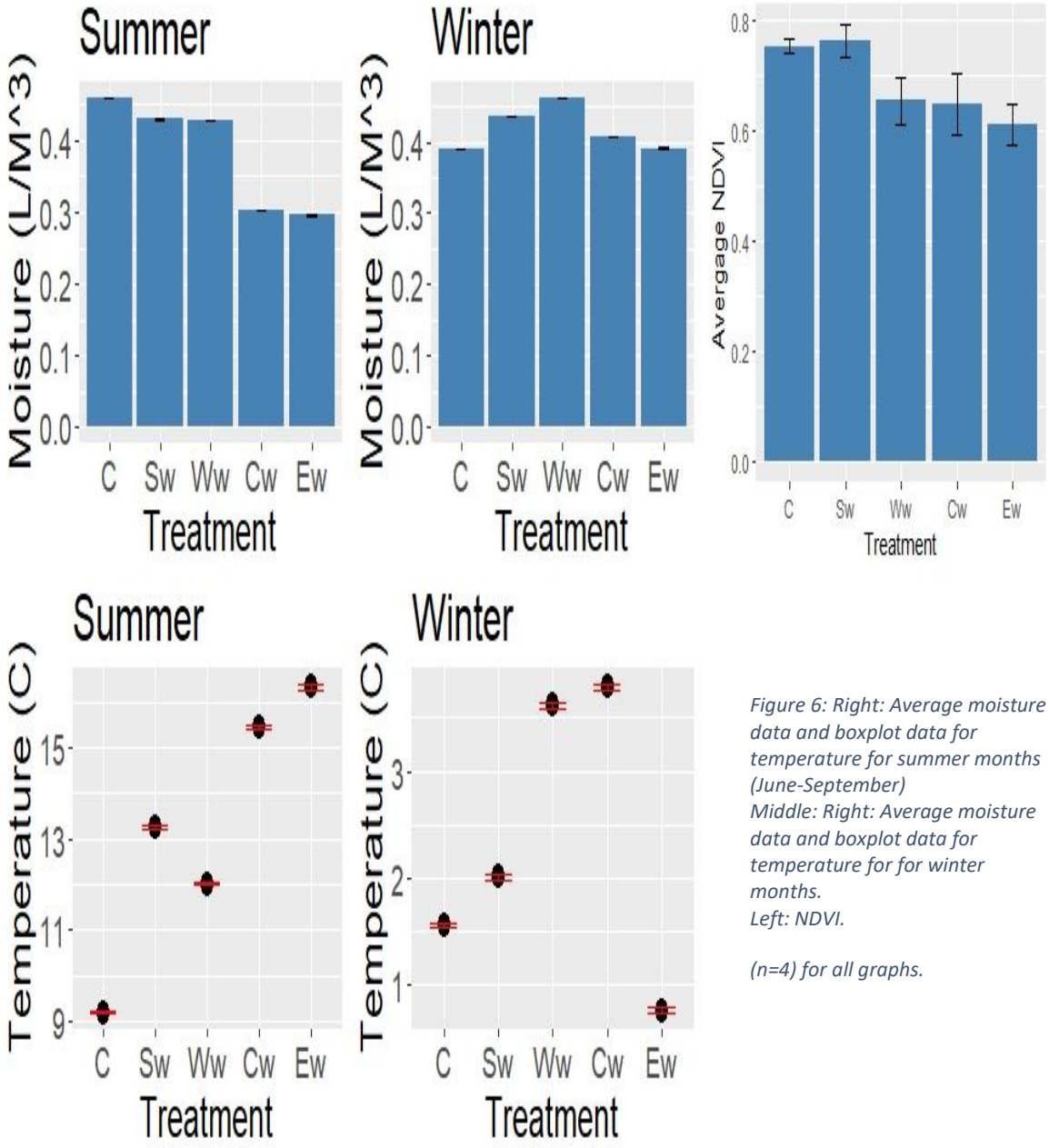


Figure 6: Right: Average moisture data and boxplot data for temperature for summer months (June-September)
Middle: Right: Average moisture data and boxplot data for temperature for for winter months.
Left: NDVI.

(n=4) for all graphs.

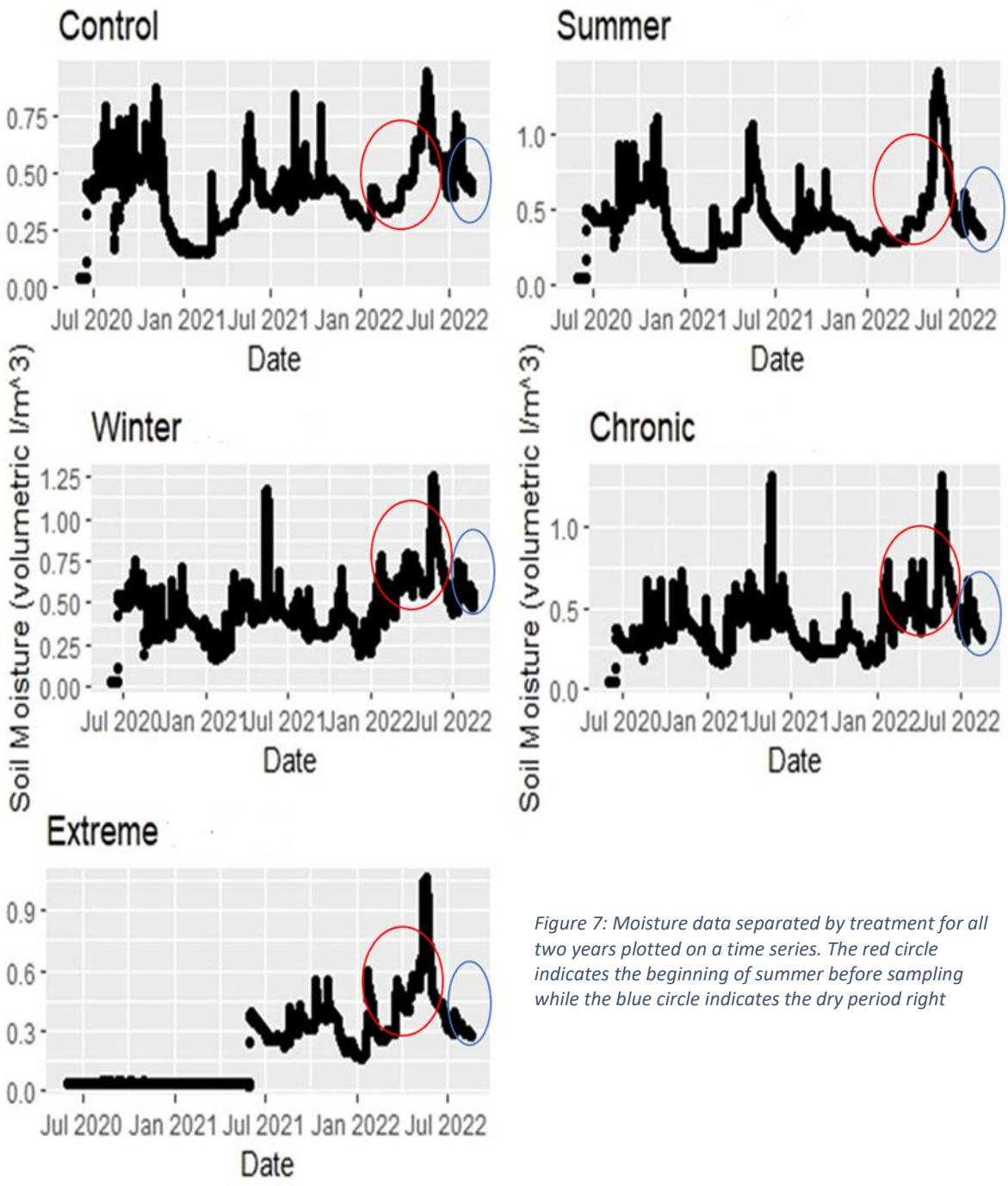


Figure 7: Moisture data separated by treatment for all two years plotted on a time series. The red circle indicates the beginning of summer before sampling while the blue circle indicates the dry period right

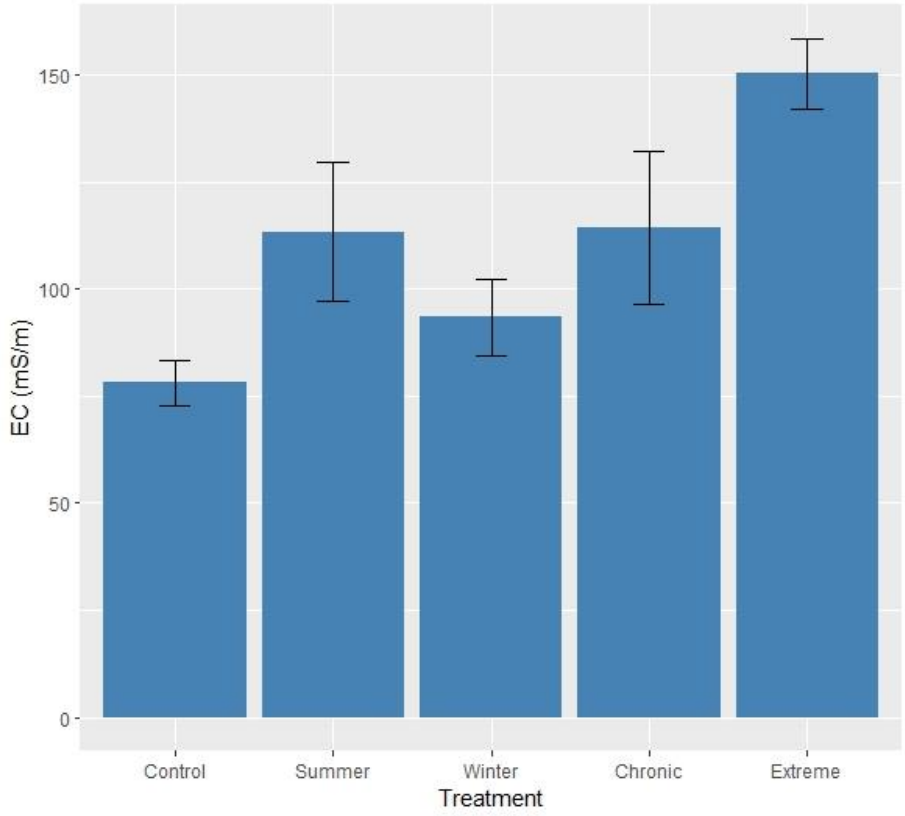


Figure 8: Electrical conductivity data for treatments, error bars are standard error of four replicates. There was a significant difference between extreme and control; and extreme and winter (n=4).

4.2 Moisture dependance

An ANOVA test of EC50 from the moisture dependance curves showed no significant difference between the treatment's resistance to drought ($p = 0.26$). Of the fitted bacterial curves, control and winter warming consistently grew at low levels of moisture while other treatments seemed less consistent with their response. Summer warmings growth rate decreased much sooner compared to other treatments (Figure 9). All EC50s however were fairly low with WC level being in the 10-20% range for most treatments (Figure 10). EC10 was also not significant between treatments ($p=0.72$)(Figure 11). Fungal treatments did not respond to moisture, as such, I measured their growth multiplicatively, with no statistically significant difference between treatments observed in the ANOVA ($p = 0.66$) (Figure 12). EC50s of respiration curves also had no statistically significant difference between treatments ($p = 0.58$)(Figure 13). Respiration curves tended to be more clustered than bacterial growth curves (Figure 14). Some respiration curves had a decrease after a peak, indicating a possible decrease with increasing moisture (Figure 14).

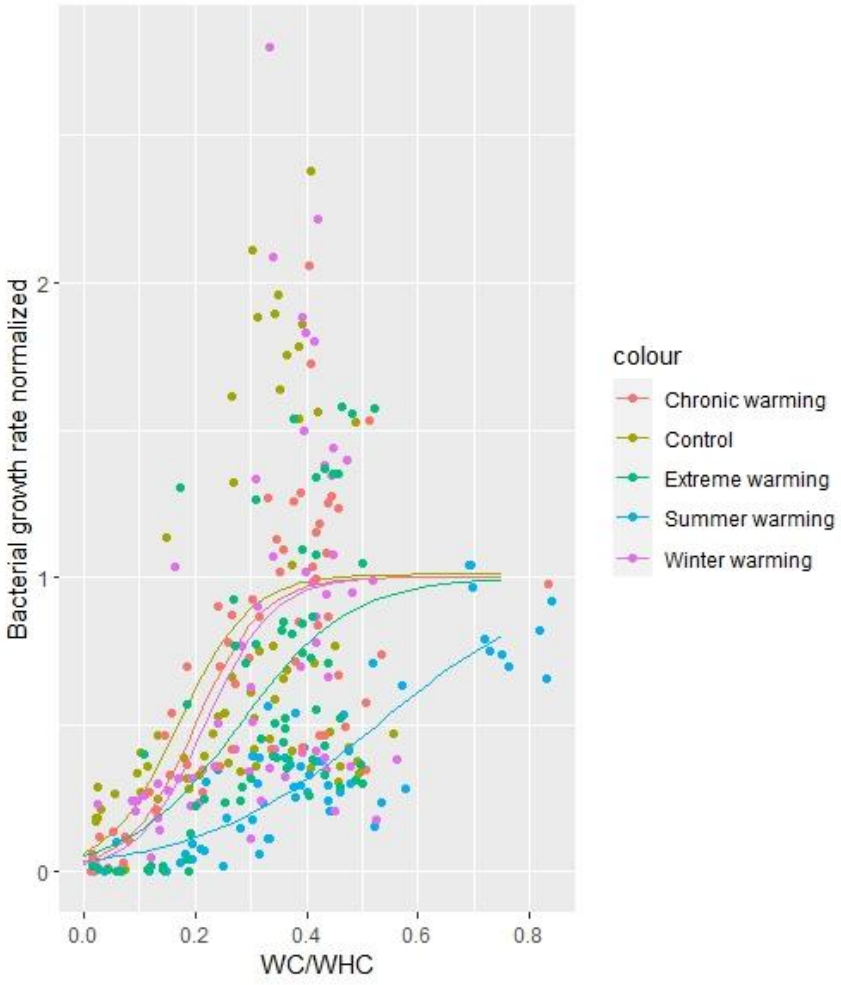


Figure 9: Consensus curves for all moisture dependency treatment. Each curve is made up of the average coefficients for the fit of the four replicates. Curves fitted using the logistic regression in R. Error bars excluded for clarity. For each curve (n=4).

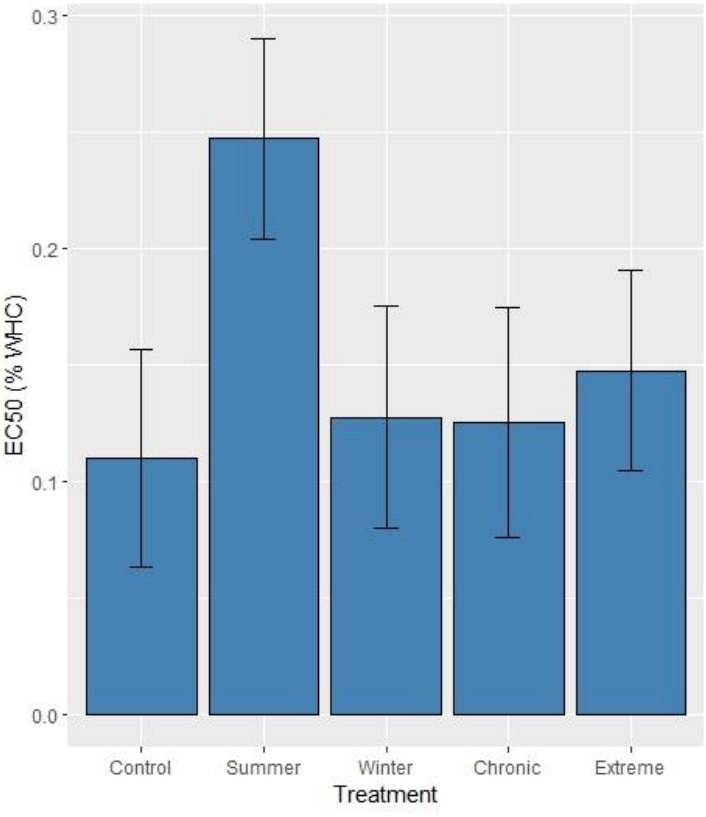


Figure 10: EC50 values for moisture dependency. Each bar is an average value of the EC50 from each replicate. Error bars is the standard error from those 4 values.(n=4).

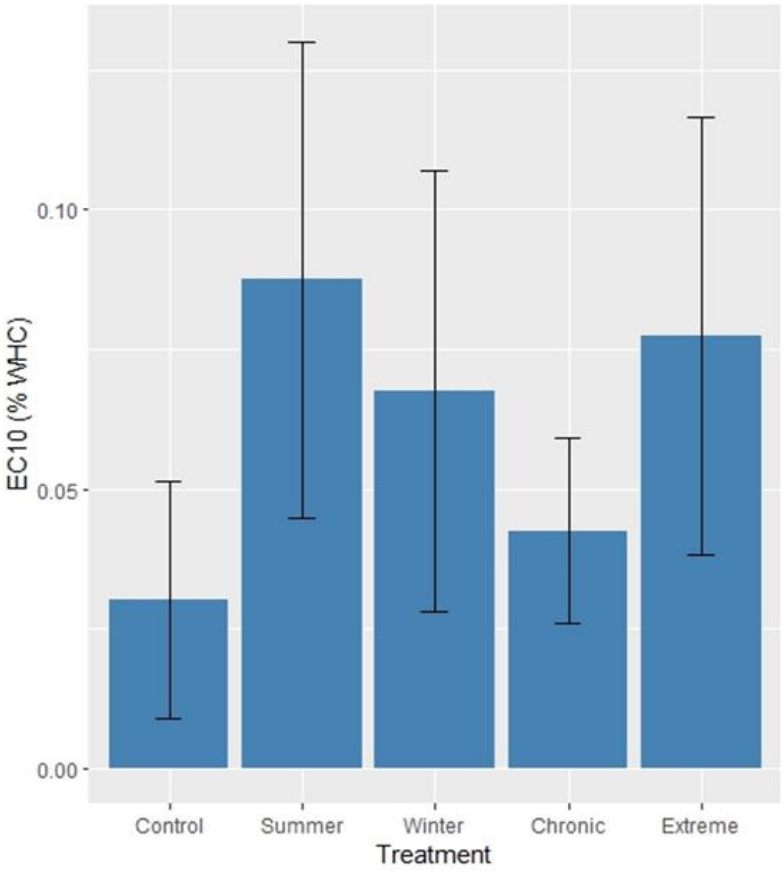


Figure 11: EC10 values for moisture dependency. Each bar is an average value of the EC10 from each replicate. Error bars is a standard error from those 4 replicates .(n=4).

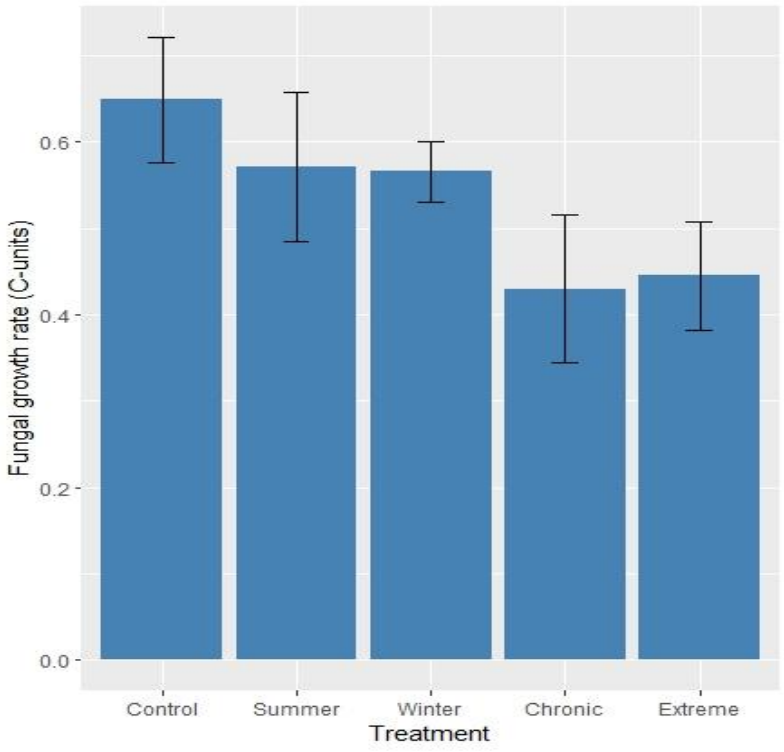


Figure 12: Average growth rate of fungal samples across all time points used as a metric to see what effects treatments had on fungal growth. Error bars are standard error of the four replicates (n=4).

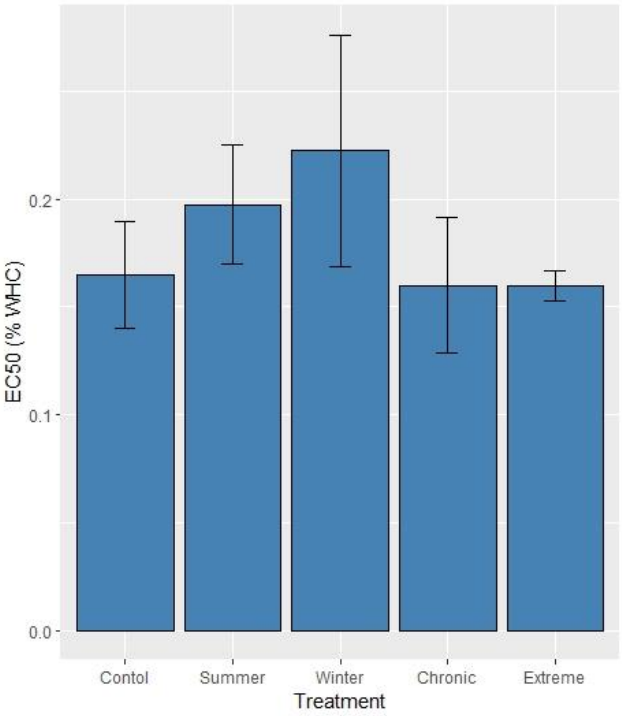


Figure 13: Average values of the EC50 for respiration samples calculated from the 4 replicates of each bar. Error bars are standard error from the same replicates (n=4).

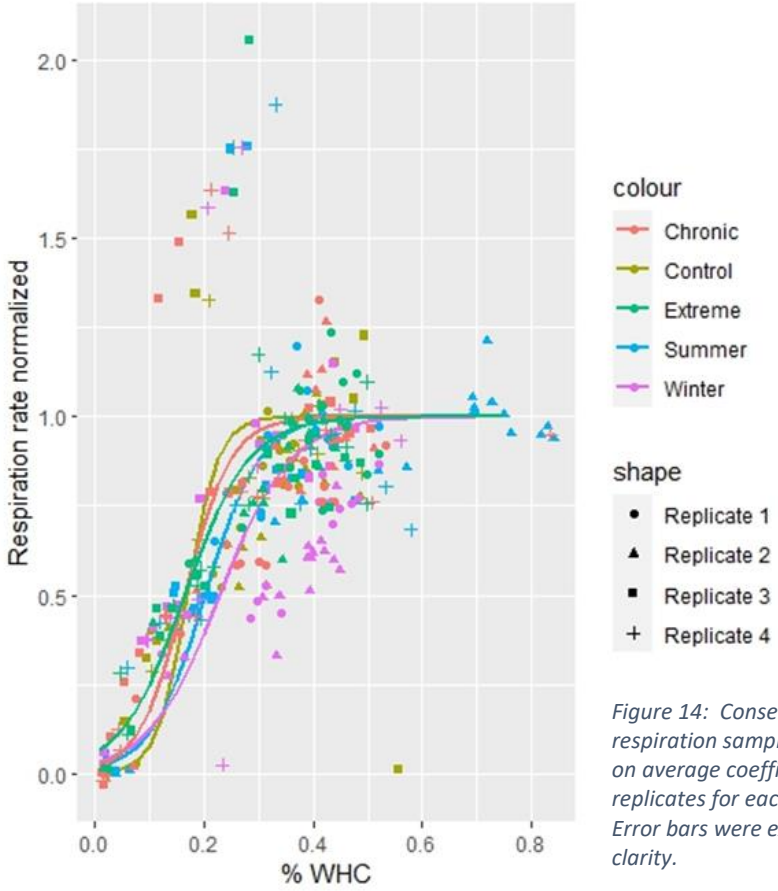


Figure 14: Consensus curves for respiration samples. Curves based on average coefficients from all 4 replicates for each treatment. Error bars were excluded for clarity.

4.3 Drying and rewetting

Rewetting had well defined results in terms of bacterial response. An ANOVA test returned a significant result between treatments for recovery time with extreme being significantly longer than summer warming and control treatments ($p = 0.047$)(Figure 15). However, lag time was not significantly different ($p = 0.53$)(Figure 16). Summer warming treatments returned to levels observed before DRW first in half of the treatments with the other two replicates having control and extreme as returning first (Figure 15). Chronic warming and extreme warming treatments were slow to respond in 3 out of the 4 replicates, in some cases not even recovering to the moist control (Figure 17). Fungal growth, which did not seem to be affected by lack of moisture seemed to have multiplicative levels to them as they were stratified by treatment without change over time. However, these multiplicative levels did not have a significant relationship to treatments ($p = 0.44$)(Figure 18). As expected, respiration in most samples had a large initial peak of emissions tapering into a asymptote as respiration stabilized (Figure 19). Of these peaks, winter warming treatments generally had the highest pulse, with extreme warming, summer warming and chronic warming often having the lowest (Figure 19).

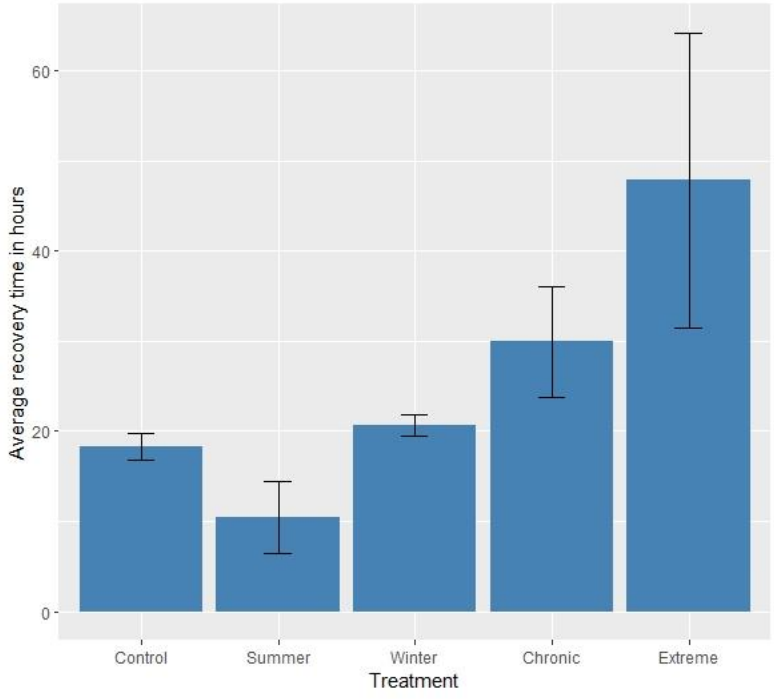


Figure 15: Average time before the curve reaches the moist threshold (recovery time). The values were calculated individually and then averaged. Error bars are standard error of the four replicates (n=4). Extreme warming was significantly different compared to summer warming and control.

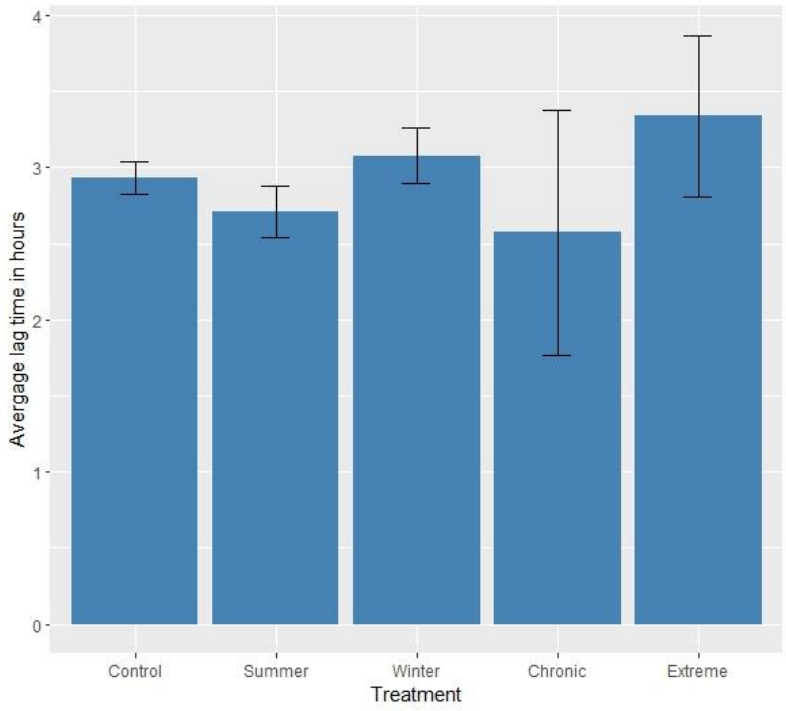


Figure 16: Average time in hours, this is the time before a response occurs. Samples with not lag time were excluded to avoid false positive results. Error bars are standard error of the four replicates (n=4).

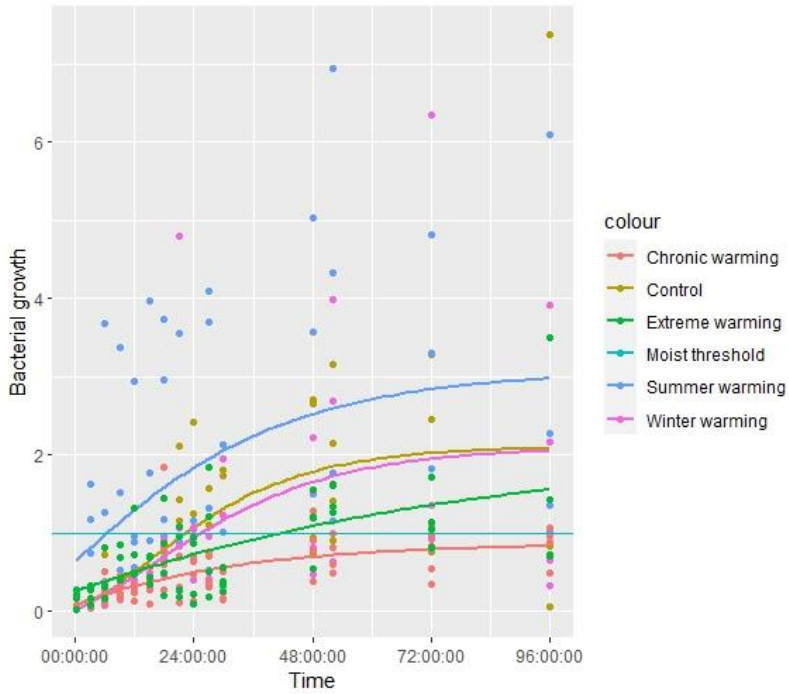


Figure 17: Bacterial growth consensus curves following rewetting at 00:00:00. The moist threshold was set at one when data was normalized by dividing by its control. Thus resulting in 1 being the level of growth without rewetting, (n=4)

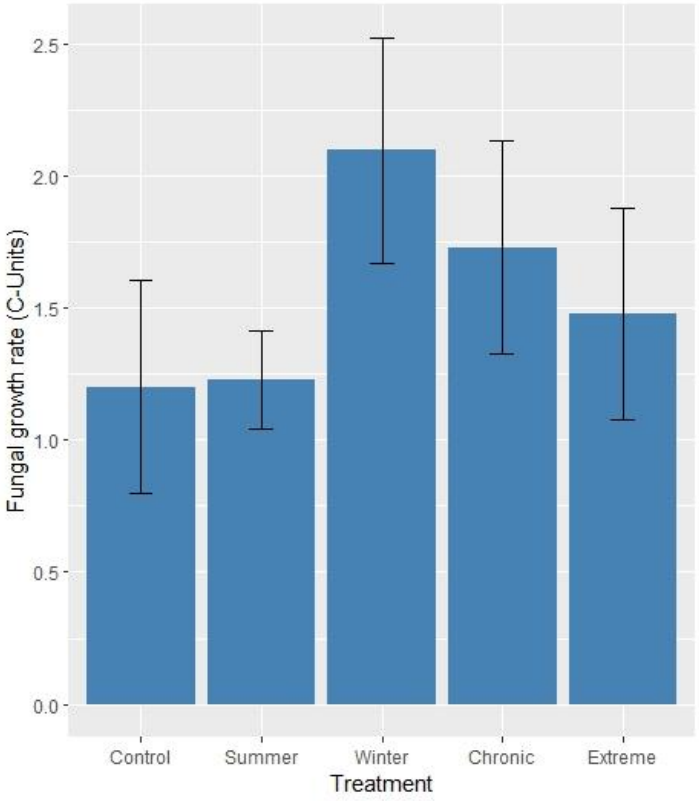


Figure 18: Values are average of the 4 fungal replicates for each treatment taken over the period of the study. Error bars are standard error of each respective treatment. (n=4).

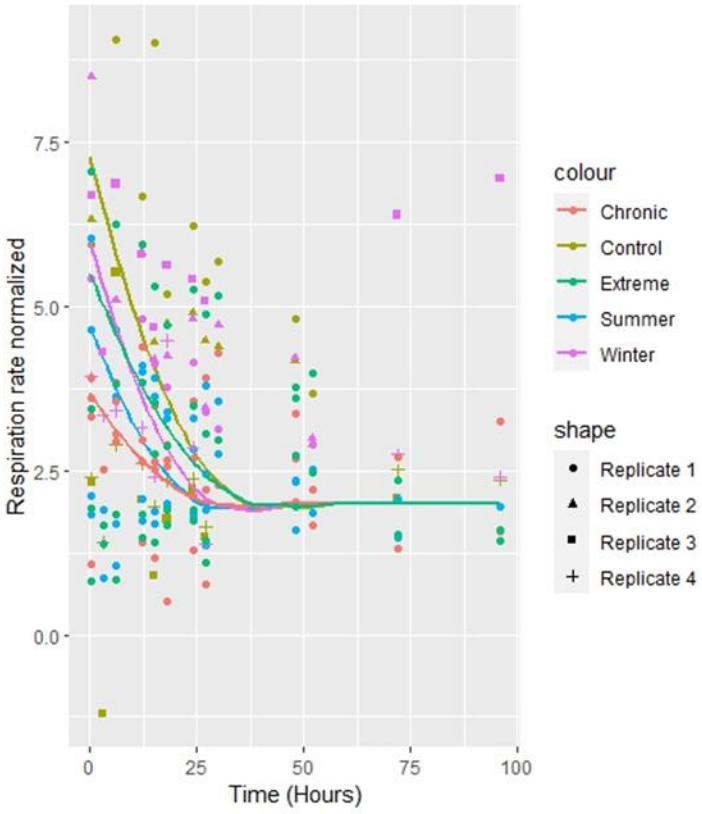


Figure 19: Respiration consensus curves following rewetting of soils. Data is an average of coefficients from all 4 replicates (n=4)

4.4 Carbon use efficiency

ANOVA of the CUE after the first 15 hours of rewetting showed that there were significant differences between groups (p = 0.0008)(Figure 20). A subsequent Tukey HSD demonstrated that the extreme treatments were different from control and winter warming treatments. The values of CUE ranged from 10% to 28% CUE and with there being a significant difference between summer and control (p=0.006) as well as summer warming and winter warming (p=0.003) (Figure 20).

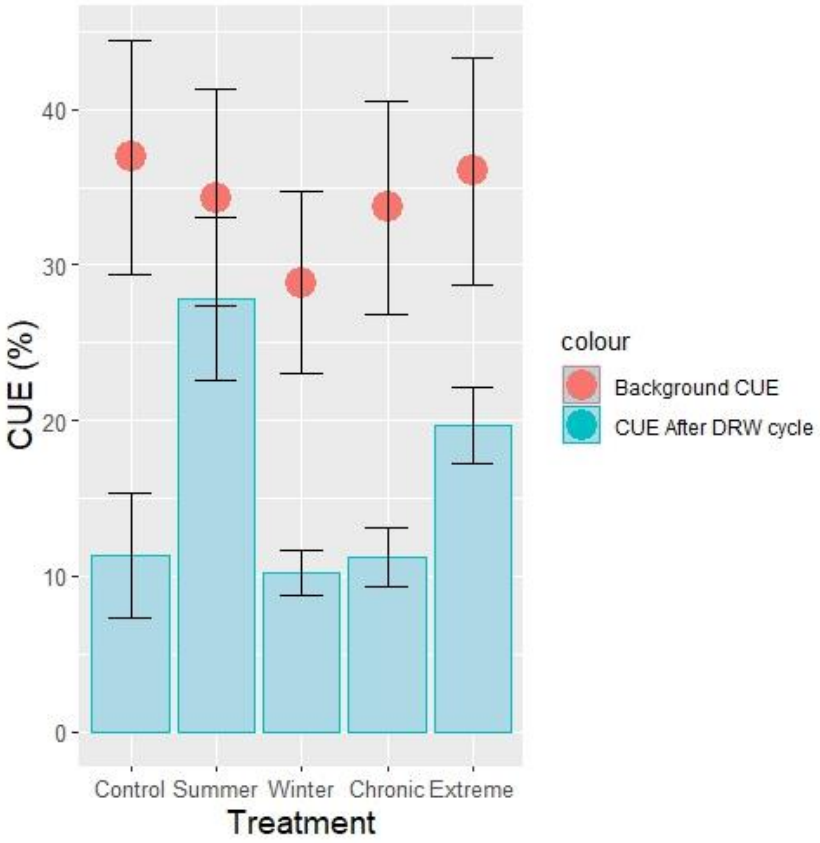


Figure 20: Average carbon use efficiency data for each treatment. Error bars are standard error for the four replicates of each treatment. Values above the thermodynamic maximum were excluded in the analysis. Orange dots represent background rates which are the same soils that have not undergone DRW (n=4). There was a significant difference between summer warming and winter warming as well as summer warming and control.

5. Discussion

5.1 Addressing moisture legacy

To address the underlying assumptions of our hypothesis, there was an impact on the moisture legacies when they were warmed. While the magnitude of this impact was fairly small, there was still a statistically significant difference between treatments (Figure 6). A crucial part of

interpreting these results is looking at the legacy itself, however, sensor data from the field only shows a small magnitude of difference in the moisture levels between treatments in these sites. There are two potential explanations for this, first that the sensor at 8cm was not weighted adequately capture surface phenomenon at 0-5cm (Wild et al., 2019). Heating tapers off at lower depths in soil and it is possible that the decrease in heat, lowered evapotranspiration and thus the moisture difference caused at the surface of the soil by the heating IR heaters was not mirrored in the field measurements (Deng et al. 2023). Despite these issues, the difference between treatments still seemed to have an effect on how microbes responded to stress.

5.2 Microbial resistance

For hypothesis (1) I must falsify the hypothesis, as resistance was not increased by a drier moisture legacy. The results from the moisture dependance portion of this study showed that soils did not resist drying down differently between treatments as all soils had similar EC50 and EC10 values for respiration and bacterial growth (Figures 9-13). The multiplicative levels of fungal growth also had no relationship with treatment during the drying down. However, the lack of change in fungal samples may indicate that in comparison to bacteria, fungi had a more K-adapted strategy of being able to stay active even in extremely dry conditions (Figure 12 and Figure 16). The bacterial measurements would indicate that resistance was not something that was selected for in these treatments. It is possible that in the field no treatment was dry long enough and thus there was no advantage to being active while drought stressed. This could be due to low differences in water content between treatments, as it usually takes a large amount of stress to push this adaptation, especially in regions where carbon is limited (Allison and Goulden 2017). Resistant traits are more frequently observed where operating at a low level is advantageous, the variation observed here likely either favors the ability to respond to moisture or dormancy because dry periods were short (Malik and Bouskill 2022). Additionally, these short periods of moisture stress may mean dry periods did persist long enough to select for resistant microbes (Evans et al., 2022).

5.3 Microbial resilience

We cannot falsify hypothesis (2) as resilience and CUE was increased by a drier moisture legacy. the microbial ability to recover to its former level of activity was evident in the response to

rewetting. Fungal growth again had no significant difference over time nor between treatments (Figure 18). Bacterial lag time also had no significant difference between treatments (Figure 16). Contrarily, bacterial recovery time responded with significant differences between treatments. Summer warming had a significantly faster recovery time compared to the extreme and chronic warming treatments (Figure 15). The results regarding resistance and resilience above echo many of the findings in Nijs et al. (2018). In this paper, soil from an 18-year drought was examined in a similar method to this one. In Nijs et al (2018) there was a preferential selection for resilience over resistance, despite the field being in a relative drought for 18 years. Additionally, most of the soils in this study responded similarly to our summer warming treatment by demonstrating no change to EC50 but a clear type 1 response to rewetting. While there is a differing response between treatments and the commonality is exposure to drier conditions, the response is not uniform regarding recovery. However, in terms of the CUE, there is indeed a slightly uniform response of increased for all treatments exposed to drier conditions in summer compared to control. This builds on past studies which show that soils exposed to DRW will have a smaller decoupling in respiration and bacterial growth (Nijs et al., 2019). This could be a result of microbes using carbon more efficiently in their stress response (Malik and Bouskill 2022).

5.3 Microbial sensitivity to winter warming and extreme warming

We must also falsify hypothesis (4) as winter warming was not significantly less resilient than controls. In terms of rewetting winter warming treatments were generally slower than summer warming, but still in line with control. This could be because the winter temperatures did not affect the snowpack leading to there not being enough of a difference in moisture (Schimel et al, 2007: Meisner et al., 2021). It could also mean that the effects of increased moisture were nullified by freeze-thaw cycles that occurred, leading to a neutral response from microbes (Li et al., 2023).

Hypothesis (3) must be partially falsified, while CUE was greater in the extreme warming, resilience was not. The quick response of summer warming contrasted with the slow response of chronic warming and extreme warming indicate that they were not adapted to rewetting, yet all three of these were exposed to drier conditions with extreme warming and chronic warming being the driest of the treatments in the summer. This is opposed to what

previous studies have shown in terms of moisture response (Nijs et al., 2019; Leizaga et al., 2022). This is further perplexed by the CUE being greater in extreme and summer than other treatments which also is in line with previous studies examining CUE responses (Nijs et al., 2019). To solve this problem, other site variables and theoretical concepts must be considered.

5.4 Site data, microbial resistance, and microbial resilience

Given the above, if only based on the moisture and temperature data, I would have expected extreme warming followed by chronic warming to have the highest resilience due to them having experienced the driest and most varying conditions. They would be followed by summer warming, then by control and winter warming. However, the pattern observed is not consistent with this. As such there is a need for further explanatory variables that may describe the data.

There were other factors linked to plant responses differing between treatments that could help explain our observations. NDVI was significantly different between summer and extreme treatments (Figure 6). As these are both indicators of photosynthetic activity, it is likely a reflection of the extreme warming reaching a tipping point to where the heat has a negative impact on vegetation. A tipping point occurs when an abrupt shift pushes an ecosystem to an alternative state (Dakos et al. 2019). This can reduce functionality in ecosystem services like nutrient cycling or carbon sequestration (Dakos et al. 2019). In the background section of this thesis, one can see that a tipping point is when the perturbation forces the system into a differing stable state. In this case scenario, I saw a loss of plant function evidenced by lowered NDVI. If this is the case, then this occurred somewhere between 3° C and 6° C above the ambient temperature as the summer warming treatment had a significantly different response than the extreme warming treatment. This could indicate a lack of plant inputs and thus a lack of available energy to respond to the rewetting event (Bradford et al., 2013; Prommer et a., 2020; Griffiths and Phillipot, 2013). This could further be supported by the accumulation of nutrients indicated by the EC results (Figure 8)(Jonasson 1999). Since Abisko's soils have no source for salinity, the amount of EC is used to measure nutrients. A pooling of nutrients would imply that the bacteria in the soil are not nutrient limited in terms of N-P-K but instead maybe C limited (Jonasson et al., 1999; Hicks et al., 2020). Since soil microbes often rely on plant input for labile C the lack of vegetation may have damaged the ability to recover in the extreme and chronic treatments. Many studies have shown bacterial communities shifting in both composition and

structure along with plant changes in the arctic usually leading to increased respiration as plant species shift towards greater leaf and root inputs (Shi et al. 2015; Mekonnen et al 2021). A study by Hicks et al. (2022) showed that C and C-N inputs to an arctic system primed more activity in mining N as well as mineralizing C. In this study the goal was to try to replicate plant inputs, following this it is likely that lack of plant inputs may cause the inverse, that being less organic matter priming and thus less growth and respiration. This lines up with De Vries and Shade (2013) prediction that with plants resilience will increase.

Temperatures direct effects could have been involved in the differences between these treatments. In the extreme warming and summer warmings samples, the increased temperature could have altered the metabolic rate in which bacteria used carbon (Cruz-Parades et al. 2021). In the summer warming, the used labile carbon may have been replaced via plants, yet perhaps the lack of plants in the extreme treatment could not replace the carbon consumed by the increased metabolism of the microbes.

5.5 Conceptual explanations of observed phenomena

One way to interpret these results is through the trait-based model provided by Malik et al. (2020). As stress of heat and drought combined with lack of carbon may shift microbial communities toward a place between stress tolerance and resource acquisition-centered strategies. Looking at the data through this framework allows one to account for the different responses without solely relying on previous variations in moisture displayed in the TOMST data. In arctic and subarctic soils, carbon availability can limit the growth of many microbial communities (Neurauter et al. 2023). Following this, because the production of secondary metabolites can be a taxing process, it is also possibly, a carbon needy one. This can be supported in the findings of Fierer and Schimel (2002); authors found that C-and N mineralization was significantly impacted by drying/rewetting cycles. While there is a large amount of recalcitrant carbon, this takes more energy and time to access, thus making it only preferable when labile carbon from easily accessible input is harder to come by (Zhou et al. 2012). This would mean that under these circumstances, resilience was sacrificed for the ability to break down more complex recalcitrate carbons.

If I view the control as centred in the YAS triangle, I can think about what would move microbial traits to different strategies. Stress like drought would lead microbes to shift to a stress tolerance strategy over traits for resource acquisition or high yield strategy. However, in the case of extreme or chronic treatments, it is possible that lack of access to labile carbon pushed these microbes to adopt a more resource acquisition-focused strategy so that in a carbon-limited environment they could still access C.

On the other hand, control and winter warming treatments are likely to be situated closer to the high growth yield point of the triangle, explaining their type 2 response, although the freezing of the soil still would provide them with some stress tolerance. Lastly, since summer warming was exposed to stress without the resource limitation of carbon, it may have shifted towards a stress tolerance strategy, informing its rapid type 1 response.

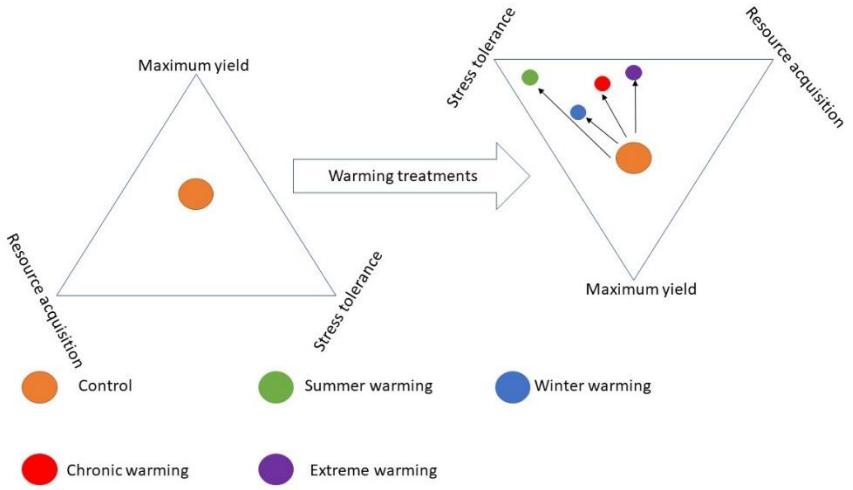


Figure 21: The YAS interpretation applied to our results would show that the extreme warming would split the traits between resource acquisition and stress tolerance, leading to a less efficient response. However, summer, which did not have additional issue with lack of carbon and thus could focus on allocating traits to resisting stress. Adapted from Malik et al 2020.

Bardgett and Caruso (2020) offer an alternative way to conceptualize these results. In their paper, they focus on how resistance and resilience function and how underlying variables can cause different responses to the same stress. I can use their framework of the threshold of a system to theorize what may have happened in our experiment. In this theory, ecosystems generally have a certain tendency towards a stable state. The more resilient a system is the more it will take to alter the return to its prior state. However, even a resilient community can undergo repeated patterns of disturbance, or factors amplifying a disturbance that throw it to an alternative stable state (Bardgett and Caruso 2020). In our experiment, I saw a quick rebound of summer warming treatments to their functionality prior to drying. This is evidenced by the bacterial growth rate

and respiration returning to comparable levels to before the drying event (Figure 17). This was not the case with the extreme treatment, as it took significantly longer to recover and, in some cases, never fully recovered to pre-drying levels. This may indicate that past a certain quantity of warming, the metabolic increased rate of consuming carbon and lack of input from plants could exacerbate the effects of drying and rewetting pushing the microbial community into a new stable state. It is possible that our extreme treatment arrived at an alternate state due to the external factors it underwent prior to our experiment, while the summer warming treatment was resilient and had the conditions it needed to return to the state before the DRW. Bardgett and Caruso (2020) indicate that more resilient systems will be less nutrient efficient. Our results show the opposite of this as summer warming had the highest carbon use efficiency followed by extreme warming. In terms of Evens et al. (2020), the extreme treatments would be undergoing a shift to a low activity threshold soil may indicate a movement to the highly variable moisture history state which is characterized by consistent yet low levels of activity.

Microbial diversity is also often tied to resilience in microbial ecosystems, often through functional redundancies in microbial communities (Shade et al. 2012). A final possibility is that the alteration of plant inputs with the warming treatment altered microbial diversity, making the microbial community less resilient (Shade et al. 2016; Allison and Martiny 2008). Microbial diversity has long been tied to plant species diversity, and as shown on many of the diversity by productivity gradients, this can have functional effects (Stefan et al. 2021). However, total NDVI has little to no correlation to diversity, so there is no way in this study to determine if diversity or mere input caused a decrease in microbial function (Martinez and Labib., 2023). Despite this, there was a clear shift in vegetation productivity. Since much of the plants interaction with microbes is focused on carbon exchange, this reduction, even if it did not affect plant diversity, may have been the reason for change in microbial resilience (Bradford et al., 2013; Prommer et a., 2020; Griffiths and Phillipot, 2013). In this case it is a possibility that the extreme warming was enough to affect the plants which are theoretically much more H_2O needy than the microbes as plants need water for their photosynthesis and their cellular activities while microbes do not need to undergo photosynthesis. Through the lack of plant inputs microbial diversity was decreased and thus the extreme warming treatment was set towards an alternate state.

Carbon use efficiency is of particular importance for the GHG aspect of our question. All treatments had lower CUE during the rewetting aspect of our experiment (Figure 20). Interestingly enough, the extreme and summer warming which were different in most aspects of their response were most similar here (Figure 20). A lower carbon use efficiency means more carbon respirated and less sequestered (Sinsabaugh et al., 2013). In this case, treatments had a significant impact on where carbon was allocated. It is possible that carbon use efficiency increased during perturbation with the summer warming, extreme warming and chronic warming treatments, till a point. Following this, treatments that had plants die off saw a decrease in CUE as microbes had to switch their strategy to access less available carbon, thus using more energy on metabolic activities and not growth (Malik et al., 2020;). Interestingly the highest pulse of emissions was from the winter warming and control treatments (Figure 19). These two treatments also had a low CUE conferring with the theory that they had to put more energy into metabolic activities (Manzoni et al., 2018). Future studies may wish to link the conceptual traits involved with CUE directly to pulses and inputs during DRW cycles.

If I am to accurately model soil respiration which accounts for a significant portion of the global carbon budget, I would need to understand how the alteration of moisture regimes affects growth (IPCC 2021). While respiration and microbial growth can be relatively predictable, how the magnitude of these pulses will change with warming is more complex than one set of variables (Rousk and Brangari, 2022). Additionally understanding the drivers of resilience must include legacy but is not limited to it. It is likely that most stochastic variables in terms of CO_2 emissions cannot be accurately modelled as climate changes without a thorough understanding of how these systems function (Rousk and Brangari, 2022; Brangari et al., 2020).

6. Conclusions, implications, and outlooks

In this thesis, it was shown that there were no differences between resistances to moisture stress for any of the temperature treatments (1). However, resilience and CUE during the rewetting of soil did differ between temperature treatments (2). While treatments in the winter did not cause any change in resilience (3), the extreme treatment did not show more resilience in either CUE or recovery compared to summer warming treatments (4). Following this, there was an attempt to explain some of the discrepancies where the observations did not line up with prior studies.

NDVI and EC were examined as possible factors indicating that lack of plant inputs of labile carbon could be responsible for these shifts. I proposed that differences observed in treatments may have to do with shifting traits, plant inputs or carbon limitations in addition to the direct stress of water deficit. I then situated these results into the trait triangle put forth by Malik et al (2020). Further studies could examine these theories directly in similar subarctic soils. Studies could also attempt to parse out the various factors affecting resilience along with the soil community's legacy. Furthermore, linking this to emission pulses would be a logical next step to interpreting how altered moisture regimes will play on an ecosystem scale.

7. Bibliography

Aanderud, Z. T., Jones, S. E., Fierer, N., & Lennon, J. T. (2015). Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.00024

Abisko Background Information based on MAPS and GIS Data. (n.d.).

Acosta-Martinez, V., Moore-Kucera, J., Cotton, J., Gardner, T., & Wester, D. (2014). Soil enzyme activities during the 2011 Texas record drought/heat wave and implications to biogeochemical cycling and organic matter dynamics. *Applied Soil Ecology*, *75*, 43–51. https://doi.org/10.1016/j.apsoil.2013.10.008

Aldén, L., Demoling, F., & Bååth, E. (2001). Rapid Method of Determining Factors Limiting Bacterial Growth in Soil. *Applied and Environmental Microbiology*, *67*(4), 1830–1838. https://doi.org/10.1128/AEM.67.4.1830-1838.2001

Alexander, L. V. (2016). Global observed long-term changes in temperature and precipitation extremes: A review of progress and limitations in IPCC assessments and beyond. *Weather and Climate Extremes*, *11*, 4–16. https://doi.org/10.1016/j.wace.2015.10.007

Allison, S. D., & Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*(supplement_1), 11512–11519. https://doi.org/10.1073/pnas.0801925105

Anderson, J. P. E. (2015). Soil Respiration. In A. L. Page (Ed.), *Agronomy Monographs* (pp. 831–871). American Society of Agronomy, Soil Science Society of America. https://doi.org/10.2134/agronmonogr9.2.2ed.c41

Averill, C., Waring, B. G., & Hawkes, C. V. (2016). Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture. *Global Change Biology*, *22*(5), 1957–1964. https://doi.org/10.1111/gcb.13219

Bååth, E. (2001). Estimation of fungal growth rates in soil using 14C-acetate incorporation into ergosterol. *Soil Biology and Biochemistry*, *33*(14), 2011–2018. https://doi.org/10.1016/S0038-0717(01)00137-7

Bååth, E. (1994a). Measurement of protein synthesis by soil bacterial assemblages with the leucine incorporation technique. *Biology and Fertility of Soils*, *17*(2), 147–153. https://doi.org/10.1007/BF00337747

Bååth, E. (1994b). Thymidine and leucine incorporation in soil bacteria with different cell size. *Microbial Ecology*, *27*(3). https://doi.org/10.1007/BF00182410

Bai, E., Li, S., Xu, W., Li, W., Dai, W., & Jiang, P. (2013). A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, *199*(2), 441–451. https://doi.org/10.1111/nph.12252

Bapiri, A., Bååth, E., & Rousk, J. (2010). Drying–Rewetting Cycles Affect Fungal and Bacterial Growth Differently in an Arable Soil. *Microbial Ecology*, *60*(2), 419–428. https://doi.org/10.1007/s00248-010-9723-5

Bardgett, R. D., & Caruso, T. (2020). Soil microbial community responses to climate extremes: Resistance, resilience and transitions to alternative states. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1794), 20190112. https://doi.org/10.1098/rstb.2019.0112

Barnard, R. L., Blazewicz, S. J., & Firestone, M. K. (2020). Rewetting of soil: Revisiting the origin of soil CO2 emissions. *Soil Biology and Biochemistry*, *147*, 107819. https://doi.org/10.1016/j.soilbio.2020.107819

Beck, H. E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., & Wood, E. F. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data*, *5*(1), 180214. https://doi.org/10.1038/sdata.2018.214

Berry, D. A. (1987). Logarithmic Transformations in ANOVA. *Biometrics*, *43*(2), 439. https://doi.org/10.2307/2531826

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*(1), 9–31. https://doi.org/10.1007/BF01343734

Blagodatskaya, E., & Kuzyakov, Y. (2013). Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biology and Biochemistry*, *67*, 192–211. https://doi.org/10.1016/j.soilbio.2013.08.024

Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., & Strickland, M. S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. *Biogeochemistry*, *113*(1–3), 271–281. https://doi.org/10.1007/s10533-012-9822-0

Brangarí, A. C., Manzoni, S., & Rousk, J. (2020). A soil microbial model to analyze decoupled microbial growth and respiration during soil drying and rewetting. *Soil Biology and Biochemistry*, *148*, 107871. https://doi.org/10.1016/j.soilbio.2020.107871

Brangarí, A. C., Manzoni, S., & Rousk, J. (2021). The mechanisms underpinning microbial resilience to drying and rewetting – A model analysis. *Soil Biology and Biochemistry*, *162*, 108400. https://doi.org/10.1016/j.soilbio.2021.108400

Brown, P. (n.d.). *Basics of Evaporation and Evapotranspiration*.

Cagnon, C., Cravo-Laureau, C., Duran, R., & Lauga, B. (Eds.). (2017). *Microbial Ecotoxicology* (1st ed. 2017). Springer International Publishing : Imprint: Springer. https://doi.org/10.1007/978-3-319-61795-4

Climate Change 2021: The Physical Science Basis. (n.d.).

Correll, M., Albers, D., Franconeri, S., & Gleicher, M. (2012). Comparing averages in time series data. *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*, 1095–1104. https://doi.org/10.1145/2207676.2208556

Crowther, T. W., van den Hoogen, J., Wan, J., Mayes, M. A., Keiser, A. D., Mo, L., Averill, C., & Maynard, D. S. (2019). The global soil community and its influence on

biogeochemistry. *Science*, *365*(6455), eaav0550. https://doi.org/10.1126/science.aav0550

Cruz-Paredes, C., Tájmel, D., & Rousk, J. (2021). Can moisture affect temperature Dependances of microbial growth and respiration? *Soil Biology and Biochemistry*, *156*, 108223. https://doi.org/10.1016/j.soilbio.2021.108223

Curiel Yuste, J., Baldocchi, D. D., Gershenson, A., Goldstein, A., Misson, L., & Wong, S. (2007). Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. *Global Change Biology*, *13*(9), 2018–2035. https://doi.org/10.1111/j.1365-2486.2007.01415.x

Curtin, D., Beare, M. H., & Hernandez-Ramirez, G. (2012). Temperature and Moisture Effects on Microbial Biomass and Soil Organic Matter Mineralization. *Soil Science Society of America Journal*, *76*(6), 2055–2067. https://doi.org/10.2136/sssaj2012.0011

Dakos, V., Matthews, B., Hendry, A. P., Levine, J., Loeuille, N., Norberg, J., Nosil, P., Scheffer, M., & De Meester, L. (2019). Ecosystem tipping points in an evolving world. *Nature Ecology & Evolution*, *3*(3), 355–362. https://doi.org/10.1038/s41559-019-0797-2

Daniel, R. M., & Danson, M. J. (2013). Temperature and the catalytic activity of enzymes: A fresh understanding. *FEBS Letters*, *587*(17), 2738–2743. https://doi.org/10.1016/j.febslet.2013.06.027

de Nijs, E. A., Hicks, L. C., Leizeaga, A., Tietema, A., & Rousk, J. (2019). Soil microbial moisture Dependances and responses to drying–rewetting: The legacy of 18 years drought. *Global Change Biology*, *25*(3), 1005–1015. https://doi.org/10.1111/gcb.14508

De Vries, F. T., & Shade, A. (2013). Controls on soil microbial community stability under climate change. *Frontiers in Microbiology*, *4*. https://doi.org/10.3389/fmicb.2013.00265

Denef, K., Six, J., Bossuyt, H., Frey, S. D., Elliott, E. T., Merckx, R., & Paustian, K. (2001). In¯uence of dry±wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biology*.

Deng, L., Huang, L., Zhang, Y., Li, A., Gao, R., Zhang, L., & Lei, W. (2023). Analytic model for calculation of soil temperature and heat balance of bare soil surface in solar greenhouse. *Solar Energy*, *249*, 312–326. https://doi.org/10.1016/j.solener.2022.11.030

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

E. Evans, S., D. Allison, S., & V. Hawkes, C. (2022). Microbes, memory and moisture: Predicting microbial moisture responses and their impact on carbon cycling. *Functional Ecology*, *36*(6), 1430–1441. https://doi.org/10.1111/1365-2435.14034

Eisenhart, C. (1947). The Assumptions Underlying the Analysis of Variance. *Biometrics*, *3*(1), 1. https://doi.org/10.2307/3001534

Fierer, N., & Schimel, J. P. (2002). Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777–787. https://doi.org/10.1016/S0038-0717(02)00007-X

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Influence of Drying-Rewetting Frequency on Soil Bacterial Community Structure. *Microbial Ecology*, *45*(1), 63–71. https://doi.org/10.1007/s00248-002-1007-2

Global soil biodiversity atlas. (2016). Publications Office of the European Union.

Göransson, H., Godbold, D. L., Jones, D. L., & Rousk, J. (2013). Bacterial growth and respiration responses upon rewetting dry forest soils: Impact of drought-legacy. *Soil Biology and Biochemistry*, *57*, 477–486. https://doi.org/10.1016/j.soilbio.2012.08.031

Griffiths, B. S., & Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews*, *37*(2), 112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x

Halverson, L. J., Jones, T. M., & Firestone, M. K. (2000). Release of Intracellular Solutes by Four Soil Bacteria Exposed to Dilution Stress. *Soil Science Society of America Journal*, *64*(5), 1630–1637. https://doi.org/10.2136/sssaj2000.6451630x

Hicks, L. C., Leizeaga, A., Rousk, K., Michelsen, A., & Rousk, J. (2020). Simulated rhizosphere deposits induce microbial N-mining that may accelerate shrubification in the subarctic. *Ecology*, *101*(9). https://doi.org/10.1002/ecy.3094

Hill, M. J., & Donald, G. E. (2003). Estimating spatio-temporal patterns of agricultural productivity in fragmented landscapes using AVHRR NDVI time series. *Remote Sensing of Environment*, *84*(3), 367–384. https://doi.org/10.1016/S0034-4257(02)00128-1

Ihaka, R., & Gentleman, R. (1996). R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, *5*(3), 299–314.

Illeris, L., Christensen, T. R., & Mastepanov, M. (2004). Moisture Effects on Temperature Sensitivity of CO2 Exchange in a Subarctic Heath Ecosystem. *Biogeochemistry*, *70*(3), 315–330. https://doi.org/10.1007/s10533-003-0855-2

Iovieno, P., & BÃ¥Ã¥th, E. (2008). Effect of drying and rewetting on bacterial growth rates in soil: Rewetting and bacterial growth in soil. *FEMS Microbiology Ecology*, *65*(3), 400–407. https://doi.org/10.1111/j.1574-6941.2008.00524.x

Jarvis, P., Rey, A., Petsikos, C., Wingate, L., Rayment, M., Pereira, J., Banza, J., David, J., Miglietta, F., Borghetti, M., Manca, G., & Valentini, R. (2007). Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: The "Birch effect." *Tree Physiology*, *27*(7), 929–940. https://doi.org/10.1093/treephys/27.7.929

Jonasson, S., Michelsen, A., & Schmidt, I. K. (1999). Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil Ecology*, *11*(2–3), 135–146. https://doi.org/10.1016/S0929-1393(98)00145-0

Jones, H. G. (2006). Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany*, *58*(2), 119–130. https://doi.org/10.1093/jxb/erl118

Katz, R. W., & Skaggs, R. H. (1981). On the Use of Autoregressive-Moving Average Processes to Model Meteorological Time Series. *Monthly Weather Review*, *109*(3), 479–484. https://doi.org/10.1175/1520-0493(1981)109<0479:OTUOAM>2.0.CO;2

Kimball, B. A. (2005). Theory and performance of an infrared heater for ecosystem warming. *Global Change Biology*, *0*(0), 051006062331001-??? https://doi.org/10.1111/j.1365-2486.2005.1028.x

Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quanti®cation of priming e€ ects. *Soil Biology*.

Lara, M. J., McGuire, A. D., Euskirchen, E. S., Genet, H., Yi, S., Rutter, R., Iversen, C., Sloan, V., & Wullschleger, S. D. (2020). Local-scale Arctic tundra heterogeneity affects

regional-scale carbon dynamics. *Nature Communications*, *11*(1), 4925. https://doi.org/10.1038/s41467-020-18768-z

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, *528*(7580), 60–68. https://doi.org/10.1038/nature16069

Leizeaga, A., Meisner, A., Rousk, J., & Bååth, E. (2022). Repeated drying and rewetting cycles accelerate bacterial growth recovery after rewetting. *Biology and Fertility of Soils*, *58*(4), 365–374. https://doi.org/10.1007/s00374-022-01623-2

Li, J.-T., Xu, H., Hicks, L. C., Brangarí, A. C., & Rousk, J. (2023). Comparing soil microbial responses to drying-rewetting and freezing-thawing events. *Soil Biology and Biochemistry*, *178*, 108966. https://doi.org/10.1016/j.soilbio.2023.108966

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., & Li, B. (2013). Responses of ecosystem carbon cycle to experimental warming: A meta-analysis. *Ecology*, *94*(3), 726–738. https://doi.org/10.1890/12-0279.1

Malik, A. A., & Bouskill, N. J. (2022). Drought impacts on microbial trait distribution and feedback to soil carbon cycling. *Functional Ecology*, *36*(6), 1442–1456. https://doi.org/10.1111/1365-2435.14010

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal*, *14*(1), 1–9. https://doi.org/10.1038/s41396-019-0510-0

Malmer, N., Johansson, T., Olsrud, M., & Christensen, T. R. (2005). Vegetation, climatic changes and net carbon sequestration in a North-Scandinavian subarctic mire over 30 years. *Global Change Biology*, *0*(0), 051006062331004-??? https://doi.org/10.1111/j.1365-2486.2005.01042.x

Manzoni, S., Čapek, P., Porada, P., Thurner, M., Winterdahl, M., Beer, C., Brüchert, V., Frouz, J., Herrmann, A. M., Lindahl, B. D., Lyon, S. W., Šantrůčková, H., Vico, G., & Way, D. (2018). Reviews and syntheses: Carbon use efficiency from organisms to ecosystems – definitions, theories, and empirical evidence. *Biogeosciences*, *15*(19), 5929–5949. https://doi.org/10.5194/bg-15-5929-2018

Martinez, A. D. L. I., & Labib, S. M. (2023). Demystifying normalized difference vegetation index (NDVI) for greenness exposure assessments and policy interventions in urban greening. *Environmental Research*, *220*, 115155. https://doi.org/10.1016/j.envres.2022.115155

Meisner, A., Bååth, E., & Rousk, J. (2013). Microbial growth responses upon rewetting soil dried for four days or one year. *Soil Biology and Biochemistry*, *66*, 188–192. https://doi.org/10.1016/j.soilbio.2013.07.014

Meisner, A., Snoek, B. L., Nesme, J., Dent, E., Jacquiod, S., Classen, A. T., & Priemé, A. (2021). Soil microbial legacies differ following drying-rewetting and freezing-thawing cycles. *The ISME Journal*, *15*(4), 1207–1221. https://doi.org/10.1038/s41396-020-00844-3

Mekonnen, Z. A., Riley, W. J., Berner, L. T., Bouskill, N. J., Torn, M. S., Iwahana, G., Breen, A. L., Myers-Smith, I. H., Criado, M. G., Liu, Y., Euskirchen, E. S., Goetz, S. J., Mack, M. C., & Grant, R. F. (2021a). Arctic tundra shrubification: A review of mechanisms and impacts on ecosystem carbon balance. *Environmental Research Letters*, *16*(5), 053001. https://doi.org/10.1088/1748-9326/abf28b

Mekonnen, Z. A., Riley, W. J., Berner, L. T., Bouskill, N. J., Torn, M. S., Iwahana, G., Breen, A. L., Myers-Smith, I. H., Criado, M. G., Liu, Y., Euskirchen, E. S., Goetz, S. J., Mack, M. C., & Grant, R. F. (2021b). Arctic tundra shrubification: A review of mechanisms and impacts on ecosystem carbon balance. *Environmental Research Letters*, *16*(5), 053001. https://doi.org/10.1088/1748-9326/abf28b

Mikan, C. J., Schimel, J. P., & Doyle, A. P. (2002). Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology and Biochemistry*, *34*(11), 1785–1795. https://doi.org/10.1016/S0038-0717(02)00168-2

Monson, R. K., Lipson, D. L., Burns, S. P., Turnipseed, A. A., Delany, A. C., Williams, M. W., & Schmidt, S. K. (2006). Winter forest soil respiration controlled by climate and microbial community composition. *Nature*, *439*(7077), 711–714. https://doi.org/10.1038/nature04555

Navarro-García, F., Casermeiro, M. Á., & Schimel, J. P. (2012). When structure means conservation: Effect of aggregate structure in controlling microbial responses to rewetting events. *Soil Biology and Biochemistry*, *44*(1), 1–8. https://doi.org/10.1016/j.soilbio.2011.09.019

Nelson, D. W., & Sommers, L. E. (2018). Total Carbon, Organic Carbon, and Organic Matter. In D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, & M. E. Sumner (Eds.), *SSSA Book Series* (pp. 961–1010). Soil Science Society of America, American Society of Agronomy. https://doi.org/10.2136/sssabookser5.3.c34

Neurauter, M., Yuan, M., Hicks, L. C., & Rousk, J. (2023). Soil microbial resource limitation along a subarctic ecotone from birch forest to tundra heath. *Soil Biology and Biochemistry*, *177*, 108919. https://doi.org/10.1016/j.soilbio.2022.108919

Oelbermann, M., English, M., & Schiff, S. L. (2008). Evaluating carbon dynamics and microbial activity in arctic soils under warmer temperatures. *Canadian Journal of Soil Science*, *88*(1), 31–44. https://doi.org/10.4141/CJSS07060

Ozgur, C., Colliau, T., Rogers, G., & Hughes, Z. (2021). MatLab vs. Python vs. R. *Journal of Data Science*, *15*(3), 355–372. https://doi.org/10.6339/JDS.201707_15(3).0001

Panikov, N. S. (1999). Understanding and prediction of soil microbial community dynamics under global change. *Applied Soil Ecology*, *11*(2–3), 161–176. https://doi.org/10.1016/S0929-1393(98)00143-7

Parkin, T. B., Doran, J. W., & Franco-Vizcaíno, E. (2015). Field and Laboratory Tests of Soil Respiration. In J. W. Doran & A. J. Jones (Eds.), *SSSA Special Publications* (pp. 231–245). Soil Science Society of America. https://doi.org/10.2136/sssaspecpub49.c14

Petersky, R., & Harpold, A. (2018). Now you see it, now you don't: A case study of ephemeral snowpacks and soil moisture response in the Great Basin, USA. *Hydrology and Earth System Sciences*, *22*(9), 4891–4906. https://doi.org/10.5194/hess-22-4891-2018

Poppeliers, S. W. M., Hefting, M., Dorrepaal, E., & Weedon, J. T. (2022). Functional microbial ecology in arctic soils: The need for a year-round perspective. *FEMS Microbiology Ecology*, *98*(12), fiac134. https://doi.org/10.1093/femsec/fiac134

Prommer, J., Walker, T. W. N., Wanek, W., Braun, J., Zezula, D., Hu, Y., Hofhansl, F., & Richter, A. (2020). Increased microbial growth, biomass, and turnover drive soil

organic carbon accumulation at higher plant diversity. *Global Change Biology*, *26*(2), 669–681. https://doi.org/10.1111/gcb.14777

Rantanen, M., Karpechko, A. Yu., Lipponen, A., Nordling, K., Hyvärinen, O., Ruosteenoja, K., Vihma, T., & Laaksonen, A. (2022). The Arctic has warmed nearly four times faster than the globe since 1979. *Communications Earth & Environment*, *3*(1), 168. https://doi.org/10.1038/s43247-022-00498-3

Reynolds, S. G. (1970). The gravimetric method of soil moisture determination Part I A study of equipment, and methodological problems. *Journal of Hydrology*, *11*(3), 258–273. https://doi.org/10.1016/0022-1694(70)90066-1

Ritchie, M. E. (2018). Reaction and diffusion thermodynamics explain optimal temperatures of biochemical reactions. *Scientific Reports*, *8*(1), 11105. https://doi.org/10.1038/s41598-018-28833-9

Rousk, J., & Brangarí, A. (2022a). Do the respiration pulses induced by drying–rewetting matter for the soil–atmosphere carbon balance? *Global Change Biology*, *28*(11), 3486–3488. https://doi.org/10.1111/gcb.16163

Rousk, J., & Brangarí, A. (2022b). Do the respiration pulses induced by drying–rewetting matter for the soil–atmosphere carbon balance? *Global Change Biology*, *28*(11), 3486–3488. https://doi.org/10.1111/gcb.16163

Rousk, J., Frey, S. D., & Bååth, E. (2012). Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Global Change Biology*, *18*(10), 3252–3258. https://doi.org/10.1111/j.1365-2486.2012.02764.x

Salazar, A., Sulman, B. N., & Dukes, J. S. (2018). Microbial dormancy promotes microbial biomass and respiration across pulses of drying-wetting stress. *Soil Biology and Biochemistry*, *116*, 237–244. https://doi.org/10.1016/j.soilbio.2017.10.017

Scheu, S., & Parkinson, D. (1994). Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. *Soil Biology and Biochemistry*, *26*(11), 1515–1525. https://doi.org/10.1016/0038-0717(94)90093-0

Schimel, J., Balser, T. C., & Wallenstein, M. (2007). MICROBIAL STRESS-RESPONSE PHYSIOLOGY AND ITS IMPLICATIONS FOR ECOSYSTEM FUNCTION. *Ecology*, *88*(6), 1386–1394. https://doi.org/10.1890/06-0219

Schimel, J. P. (2018). Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 409–432. https://doi.org/10.1146/annurev-ecolsys-110617-062614

Schumacher, B. A., Shines, K. C., Burton, J. V., & Papp, M. L. (1990). Comparison of Three Methods for Soil Homogenization. *Soil Science Society of America Journal*, *54*(4), 1187–1190. https://doi.org/10.2136/sssaj1990.03615995005400040046x

Schuur, E. A. G., McGuire, A. D., Schädel, C., Grosse, G., Harden, J. W., Hayes, D. J., Hugelius, G., Koven, C. D., Kuhry, P., Lawrence, D. M., Natali, S. M., Olefeldt, D., Romanovsky, V. E., Schaefer, K., Turetsky, M. R., Treat, C. C., & Vonk, J. E. (2015). Climate change and the permafrost carbon feedback. *Nature*, *520*(7546), 171–179. https://doi.org/10.1038/nature14338

Schwingshackl, C., Hirschi, M., & Seneviratne, S. I. (2017). Quantifying Spatiotemporal Variations of Soil Moisture Control on Surface Energy Balance and Near-Surface Air

Temperature. *Journal of Climate*, *30*(18), 7105–7124. https://doi.org/10.1175/JCLI-D-16-0727.1

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Bürgmann, H., Huber, D. H., Langenheder, S., Lennon, J. T., Martiny, J. B. H., Matulich, K. L., Schmidt, T. M., & Handelsman, J. (2012). Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in Microbiology*, *3*. https://doi.org/10.3389/fmicb.2012.00417

Shi, Y., Xiang, X., Shen, C., Chu, H., Neufeld, J. D., Walker, V. K., & Grogan, P. (2015). Vegetation-Associated Impacts on Arctic Tundra Bacterial and Microeukaryotic Communities. *Applied and Environmental Microbiology*, *81*(2), 492–501. https://doi.org/10.1128/AEM.03229-14

Sierra, C. A., Trumbore, S. E., Davidson, E. A., Vicca, S., & Janssens, I. (2015). Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. *Journal of Advances in Modeling Earth Systems*, *7*(1), 335–356. https://doi.org/10.1002/2014MS000358

Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., & Richter, A. (2013). Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. *Ecology Letters*, *16*(7), 930–939. https://doi.org/10.1111/ele.12113

Soares, M., & Rousk, J. (2019). Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. *Soil Biology and Biochemistry*, *131*, 195–205. https://doi.org/10.1016/j.soilbio.2019.01.010

Stefan, L., Hartmann, M., Engbersen, N., Six, J., & Schöb, C. (2021). Positive Effects of Crop Diversity on Productivity Driven by Changes in Soil Microbial Composition. *Frontiers in Microbiology*, *12*, 660749. https://doi.org/10.3389/fmicb.2021.660749

Tang, J., Zhou, P., Miller, P., Schurgers, G., Gustafson, A., Makkonen, R., Fu, Y., & Rinnan, R. (2021). *High latitude vegetation changes will determine future plant volatile impacts on atmospheric organic aerosols* [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-1143422/v1

Thomas, G. W. (2018). Soil pH and Soil Acidity. In D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, & M. E. Sumner (Eds.), *SSSA Book Series* (pp. 475–490). Soil Science Society of America, American Society of Agronomy. https://doi.org/10.2136/sssabookser5.3.c16

Unger, S., Máguas, C., Pereira, J. S., David, T. S., & Werner, C. (2010). The influence of precipitation pulses on soil respiration – Assessing the "Birch effect" by stable carbon isotopes. *Soil Biology and Biochemistry*, *42*(10), 1800–1810. https://doi.org/10.1016/j.soilbio.2010.06.019

Verhulst, N., Govaerts, B., Sayre, K. D., Deckers, J., François, I. M., & Dendooven, L. (2009). Using NDVI and soil quality analysis to assess influence of agronomic management on within-plot spatial variability and factors limiting production. *Plant and Soil*, *317*(1–2), 41–59. https://doi.org/10.1007/s11104-008-9787-x

Warren, C. R. (2014). Response of osmolytes in soil to drying and rewetting. *Soil Biology and Biochemistry*, *70*, 22–32. https://doi.org/10.1016/j.soilbio.2013.12.008

Warren, C. R. (2020). Pools and fluxes of osmolytes in moist soil and dry soil that has been re-wet. *Soil Biology and Biochemistry*, *150*, 108012. https://doi.org/10.1016/j.soilbio.2020.108012

Webster, K. L., McLaughlin, J. W., Kim, Y., Packalen, M. S., & Li, C. S. (2013). Modelling carbon dynamics and response to environmental change along a boreal fen nutrient gradient. *Ecological Modelling*, *248*, 148–164. https://doi.org/10.1016/j.ecolmodel.2012.10.004

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 27. https://doi.org/10.1186/s40168-017-0237-y

Wieder, W. R., Grandy, A. S., Kallenbach, C. M., & Bonan, G. B. (2014). Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model. *Biogeosciences*, *11*(14), 3899–3917. https://doi.org/10.5194/bg-11-3899-2014

Wild, J., Kopecký, M., Macek, M., Šanda, M., Jankovec, J., & Haase, T. (2019). Climate at ecologically relevant scales: A new temperature and soil moisture logger for long-term microclimate measurement. *Agricultural and Forest Meteorology*, *268*, 40–47. https://doi.org/10.1016/j.agrformet.2018.12.018

Wilson, G., Green, M., Brown, J., Campbell, J., Groffman, P., Durán, J., & Morse, J. (2020). Snowpack affects soil microclimate throughout the year. *Climatic Change*, *163*(2), 705–722. https://doi.org/10.1007/s10584-020-02943-8

Wrona, F. J., Johansson, M., Culp, J. M., Jenkins, A., Mård, J., Myers-Smith, I. H., Prowse, T. D., Vincent, W. F., & Wookey, P. A. (2016). Transitions in Arctic ecosystems: Ecological implications of a changing hydrological regime: TERRESTRIAL AND FRESHWATER ECOSYSTEMS. *Journal of Geophysical Research: Biogeosciences*, *121*(3), 650–674. https://doi.org/10.1002/2015JG003133

Xu, L., Baldocchi, D. D., & Tang, J. (2004). How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature: RAIN, GROWTH, AND RESPIRATION. *Global Biogeochemical Cycles*, *18*(4), n/a-n/a. https://doi.org/10.1029/2004GB002281

Xu, M., & Shang, H. (2016). Contribution of soil respiration to the global carbon equation. *Journal of Plant Physiology*, *203*, 16–28. https://doi.org/10.1016/j.jplph.2016.08.007

Zhang, Z., Wang, D., & Li, M. (2022). Soil respiration, aggregate stability and nutrient availability affected by drying duration and drying-rewetting frequency. *Geoderma*, *413*, 115743. https://doi.org/10.1016/j.geoderma.2022.115743

Zhou, J., Xue, K., Xie, J., Deng, Y., Wu, L., Cheng, X., Fei, S., Deng, S., He, Z., Van Nostrand, J. D., & Luo, Y. (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nature Climate Change*, *2*(2), 106–110. https://doi.org/10.1038/nclimate1331

Zhu, Y., Merbold, L., Leitner, S., Xia, L., Pelster, D. E., Diaz-Pines, E., Abwanda, S., Mutuo, P. M., & Butterbach-Bahl, K. (2020). Influence of soil properties on N2O and CO2 emissions from excreta deposited on tropical pastures in Kenya. *Soil Biology and Biochemistry*, *140*, 107636.https://doi.org/10.1016/j.soilbio.2019.107636