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FACULTY OF ENGINEERING

Master's Thesis Report

How do rainfall patterns change microbial induced carbon dynamics in soil?

February 2023 – June 2023

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Degree Project in Biotechnology (30 ECTS)

Preface

This master thesis was performed at the Rousk Lab at the Biology department from the Faculty of Sciences, LU . The project spanned 20 weeks, between February and June 2023, and serves as part of the Master of Science in Biotechnology programme offered by Lund University. The examination of this thesis is being conducted at the Applied Microbiology division of LTH.

The goal of this thesis is to shorten the knowledge gap in how perennial crops can be useful in reducing climate change consequences by better understanding soil dynamics under climate change scenarios.

I would like to thank my supervisors, Dr. Albert Brangarí and Professor Johannes Rousk, for the continuous support as well as my examiner, Associate Prof. Catherine Paul, for the helpful guidance and availability throughout this project. I would also like to thank all of those at the MBLU group for their help and assistance and especially for always having time for my questions. An especial thanks is due to Dr. Vanesa Santás that supported me and was a true friend during my time at the Rousk Lab. Obrigada Vanesa, por me ouvires sempre que eu precisava e pelos conselhos que me deste, especialmente o da sandes de nutella. I would also like to thanks Honorine for being not only my lab buddy, but a great friend. You are the best, merci!

Claro que não poderia de deixar de agradecer à minha família, aos meus pais e ao João, sem vocês não tinha conseguido. Obrigada pelos vossos sacrifícios, por me porem sempre em primeiro lugar, pelo apoio e amor incondicional, pela paciência, por tudo! Amo-vos. Sasha, you were the best dog ever, até um dia.

Abstract

Climate change is changing the precipitation patterns around the globe, leading to more extreme weather events like severe drought and heavy rainfalls. These events are intrinsically related to soil moisture fluctuations, which strongly modulates carbon fluxes in terrestrial ecosystems. Studying the behavior of microbial communities present in agriculture soils during drying and rewetting events can help understand soil dynamics under climate change scenarios and help the knowledge gap of how perennial crops can be useful in reducing climate change consequences.

In this project, I compared perennial and annual crop systems in depth, down to 90 cm, by characterizing the soils and measuring their microbial responses to a drying and rewetting event. From this study, it was possible to conclude that both soils present a sensitive response to such event, with both lag and recovery times increasing with depth. Moreover, soils exposed to perennial crops generally showed shorter lag and recovery times than those from annual crops, which suggests that perennial crops might have a higher capacity to withstand the negative effects of droughts.

Based on the results obtained and the intrinsic limitations of the experiment, it is clear that further investigation is needed, namely in the form of respiration studies, more replicates and statistical analysis.

Key words: *drying and rewetting, bacterial growth, fungal growth, annual crops, perennial crops, soil depth, climate change*

Popular science summary

Climate change is causing extreme weather events worldwide, like severe droughts and heavy rainfall, by changing precipitation patterns. These events affect water levels and have a major impact on carbon movement in soils. To understand how soil behaves under changing climate conditions, I studied how microbes in agricultural soils behave after drying and rewetting events, by comparing perennial and annual crops soils to explore their potential in mitigating climate change effects.

I characterized soils from perennial and annual croplands from Skåne, Sweden and conducted drying and rewetting experiments to evaluate the microbial responses. The results showed that both soil types were sensitive to drought, with microbes taking longer to recover in deeper soils. Surprisingly, soils from perennial crops showed to be better adapted to drought conditions, with the potential to help fight climate change.

Future studies should include more replicates, analyze respiration and the microbial communities by sequencing DNA, and apply statistical techniques to support the validity of the conclusions.

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Abbreviation list

AC	Soils under annual cropping
ANOVA	Analysis of variance
C	Carbon
CO_2	Carbon dioxide
CUE	Carbon use efficiency
DRW	Drying and rewetting
EC	Electrical conductivity
GHG	Greenhouse gas
H_2O	Water
IPCC	Intergovernmental panel on climate change
ns	Non-significant
SIC	Soil inorganic carbon
SOM	Soil organic matter
WC	Water content
WHC	Water holding capacity

1. Delimitations

The following delimitations applied to this research project:

- Out of the 20 weeks of the project, 5 weeks were spent learning the techniques, 9 weeks were spent in the laboratory performing the research project, and 6 weeks were spent analyzing data, writing the report, doing the opposition to another student, and preparing the oral presentation.
- Statistical analysis was run only for the soil's characterization.
- Respiration data was lost due to instrument failure.
- Only 2 out of the 4 blocks could undergo microbial analysis due to time constraints.

2. Introduction

In its latest report, the IPCC warned, once again, that human-caused climate change, namely, GHG (greenhouse gas) emissions, are affecting many weather and climate extremes in all regions and that there is a serious risk of surpassing the global warming limit of 1.5 ºC by 2035. This will have serious consequences for, for example, crop yields, species loss or ecosystem's viability (IPCC, 2023).

Climate change is changing precipitation patterns around the globe, leading to more extreme weather events such as severe droughts and heavy rainfalls (Hu et al., 2021; X.-B. Wang et al., 2022). In Skåne, South of Sweden, climate change is expected to result in a warmer and drier summer followed by more precipitation in other seasons (Ministry of the Environment of Sweden, 2009). These alteration of the patterns of soil drying and rewetting (DRW) will strongly affect terrestrial ecosystems and soil carbon cycling (Hu et al., 2021; Meisner et al., 2021; X.-B. Wang et al., 2022).

Soil microbial communities regulate many soil ecosystem functions, including cycling of organic matter and nutrients, and GHG emissions making them important actors in mitigating the effects of climate change (Bardgett et al., 2008; Tecon & Or, 2017) as seen in the right side of Figure 1. Water availability affects the activity of soil microorganisms and thus the microbial capability to perform these functions (Meisner et al., 2021). The exposure of soil microorganisms to moisture fluctuations strongly modulates carbon fluxes in soil (Birch, 1958; Manzoni et al., 2012; Schimel et al., 2007) .

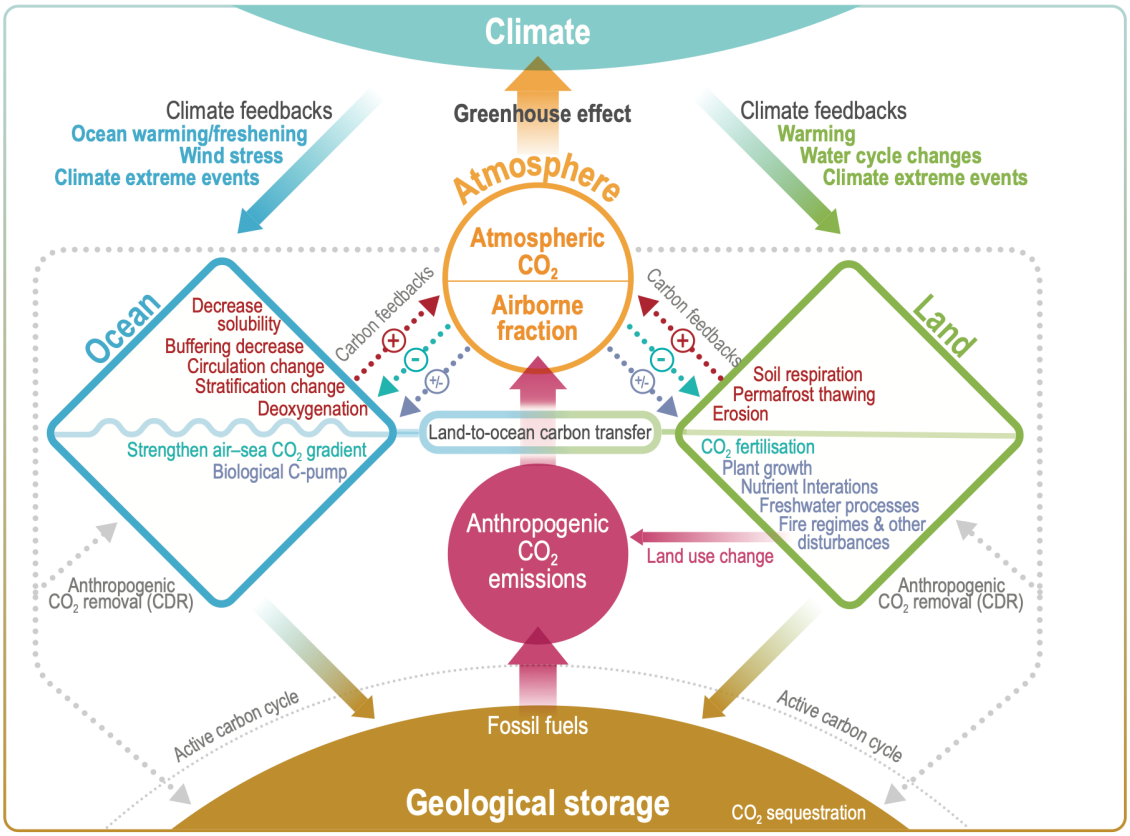

Figure 1 – Key compartments, processes and pathways that govern historical and future CO2 concentrations and carbon–climate feedbacks through the coupled Earth system. Reproduced from (Canadell et al., 2021).

Literature suggests that abnormal DRW events affect microbial growth and respiration rates in soil, as microbes must acclimate immediately to stress by switching their resources from growth to survival pathways (Schimel et al., 2007). An example is the Birch effect, which is a pulse of CO2 release to the atmosphere after a rewetting event (Birch, 1958; Kim et al., 2012). This induces a soil efflux that can account for a significant part of the annual ecosystem's emissions (Rousk & Brangarí, 2022).

Microbial growth is strongly decoupled from respiration during these events (Brangarí et al., 2020; Göransson et al., 2013). The responses of microbial communities to DRW events can follow two patterns: “type 1” and “type 2”, that can be seen in Figure 2. The so-called “type 1” or “resilient” responses are characterized, for bacterial growth, by a linear fast recovery of microbial growth rates from low values to a peak, that then decreases to pre-disturbed levels, and for respiration, by a sudden peak at the moment of rewetting followed by a decrease to a steady state (Leizeaga et al., 2022; Meisner et al., 2017). Communities exhibiting this pattern usually display a relatively short recovery time. The “type 2” responses, associated with the communities on the “sensitive” end of the response spectrum, display, for bacterial growth, an exponential-like growth recovery after a lag period up to 20 hours of no growth, then peak and end up decreasing to a steady state. The respiration rates associated with this pattern peak and remain sustained at high levels for up to several hours after rewetting,

ending up increasing even further and generating a second peak (Brangarí et al., 2022; Meisner et al., 2017). When it comes to fungal growth, the growth rates can exhibit a pattern similar to the bacterial type 1 response, or a smooth logarithmic transition to the steady state values, however they have not yet been categorized into specific response types (Brangarí et al., 2022). It has also been suggested that the interaction between bacteria and fungi complicates the recovery patterns and that there is a competitive interaction between the two microbial groups (Brangarí et al., 2022).

These responses are molded by the environmental conditions the communities have experienced. One key factor is the history of water availability. In general, microbial communities that have experienced frequent drying-rewetting cycles can subsist better to water stress and display a more resilient type 1 response pattern when exposed to similar perturbations (Leizeaga et al., 2022). Nonetheless, the exposure to prolonged dry periods can shift the community's response pattern from type 1 to type 2 (Meisner et al., 2017).

One element that can help determine the pattern type is the recovery time. The recovery time is the time taken by the community to recover to moisture control levels; in this study, since all data were normalized, it will be the time between the DRW and the community reaching 0.5 (50% of the moist control rates). It is relevant to mention that there is a whole spectrum of responses between the two extreme patterns that can exhibit different speeds of recovery. However, shorter recovery times are usually associated with type 1 patterns while on the opposite end, longer recovery times are associated with type 2 patterns. This suggests that the recovery time can be an indicator of microbial resilience (Brangarí et al., 2022).

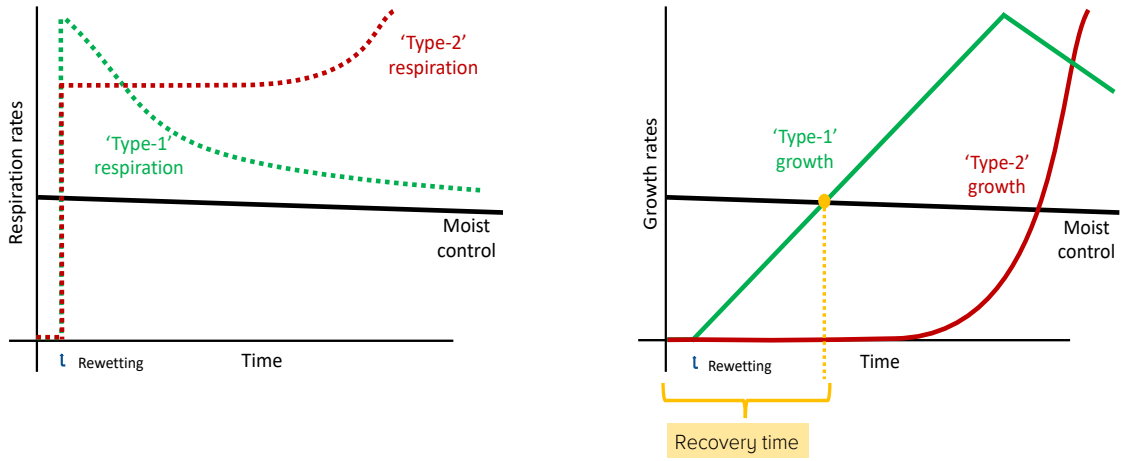

Figure 2 – Schematic overview of the two response patterns to a drying and rewetting event for (A) respiration rate and (B) growth rate. Adapted from (Meisner et al., 2017).

Water availability is influenced by climate, soil depth, and land management (Tecon & Or, 2017). Microbial presence and functions vary with the strong vertical gradients and stratification of nutrients, water, oxygen, pH, and temperature (Tecon & Or, 2017). Topsoil horizons experience extreme moisture changes, while deeper profiles are more protected, suggesting that communities would be less resilient

to water stress with depth. Microbial communities in agriculture soils are particularly vulnerable to DRW cycles due to the soil being regularly exposed to such events (X.-B. Wang et al., 2022). These soils are usually tilled, which has a big impact on microbial behavior during DRW events since it alters the soil stratification which reshuffles resources and microbial communities (Brangarí et al., 2022).

In order to grow, plants require, among other things, water, oxygen, and nutrients provided by the soil. The soil structure and its properties are determinants of the plant's well-being since they are related to heat flow, water retention, soil organisms, available nutrients and carbon stocks, all parameters that are extremely important to the survival of flora (Azevedo et al., 2023).

The influence of DRW on soils have been studied thoroughly (e.g., (Hicks, 2023; Zhang et al., 2022)), however there is still a knowledge gap on the effect that different agricultural systems would have on C cycling along the soils vertical profile, hence this study.

Agriculture soils can be the media for annual crops like wheat and perennial crops (PC) like and Kernza (*Thinopyrum intermedium*, Family: *Poaceae*), an intermediate wheatgrass (de Oliveira et al., 2020; Peixoto et al., 2022). While annual crops (AC), as stated by their name, have an annual cycle of life; perennial crops tend to have longer growing seasons, which for Kernza can be seven to eight years (Zhang et al., 2011). The life cycle of the plant influences the type of tillage conducted in the soils. For AC the tillage process, which can go down to 40 cm, is performed annually before each growing season. This process, alters the soil's stratification, as said before, but is also known for disrupting soil aggregates which has consequences for crop yield because of the release of the carbon and nitrogen previously stabilized in the soil by such aggregates (Chantigny et al., 1997; Means et al., 2022). Due to the longer life cycle of perennial crops, the tillage of the soil and its disturbance is reduced when compared to annual crops, reducing the disruption of soil aggregates, and maintaining a more structured soil with its stabilized nutrients (Azevedo et al., 2023; Ledo et al., 2020).

However, these are not the only differences between these two types of crops. PC have deeper root systems which allow them not only to use, retain and intercept more precipitation (Zhang et al., 2011) but also to exploit water from previous rain seasons stored in deeper horizons, thus being more tolerant to current drought seasons and increasing and stabilizing crop yield (de Oliveira et al., 2020).

Perennial crops have been studied for their potential to mitigate climate change (Ledo et al., 2020). Deeper roots with significant activity below one meter can enhance subsoil carbon storage, offsetting the CO_2 emissions occurring at the surface (Peixoto et al., 2022). This is particularly relevant because subsoils are considered to store carbon more permanently than top soils (Peixoto et al., 2022). Furthermore, the extended life cycle and continuous ground cover can help sequester more carbon and decrease nutrient leakage (de Oliveira et al., 2020). Additionally, the continuous photosynthesis increases the biomass that will be

converted to organic matter, which can be sequestered or maintained by the soils (Ledo et al., 2020).

Studying microbial responses to DRW of communities present in agriculture soils of annual and perennial crops can help understand soil behavior under climate change scenarios and how perennial crops can help reducing climate change consequences. In addition, it offers an opportunity to study the ecological behavior of soil microbial communities and evaluate how they recover and adapt to environmental stress (Brangarí et al., 2022; Meisner et al., 2017).

In this study the impacts of drying-rewetting events on microbial activity at different depths were investigated, from topsoil down to 90 cm in depth, and in agricultural soils with different types of crops (Wheat (Annual or AC) and Kernza (Perennial or PC)). To do so, bacterial growth, fungal growth and respiration were measured at high temporal resolution in the lab during an event of drying and rewetting, where the soils were air-dried to around 2/3% of their water holding capacity (WHC) (to simulate an intense dry period) and dried to 10% of their WHC (to simulate a less intense dry period) and rewetted to optimal moisture (50% WHC). The soil's water potential was also investigated.

The hypotheses for this project were: *H1 a)* microbial communities from deeper horizons will exhibit a and less resilient response to a DRW event, while *H1 b)* communities from shallower horizons will exhibit a more and resilient response to a DRW event. In addition, *H2)* microbial communities from agricultural soils with annual crops will be less sensitive to a DRW events than those with perennial crops due to the annual life cycle of the plant leaving the soil unprotected and more exposed to moisture fluctuations.

3. Material and methods

6.1 Soil sampling

Soil samples were collected from the Lönnstorp SITES Agroecological Field Experiment (SAFE) in Lomma (Skåne, South Sweden) at the end of February 2023 from eight different adjacent sites divided into 4 blocks (Figure 3): four consisting of soil from wheat crops (annual) and 4 consisting of soil from Kernza crops (perennial), here called Annual (AC soils), and Perennial (PC soils), respectively. The SAFE experiment is an ongoing experiment that has had those crops for 8 years. In each site, a column of soil was collected from topsoil down to 90 cm, and the soil was divided into 3 depths: 0-20 cm, 20-50 cm and 50-90 cm.

The soil subsamples were homogenized and sieved through a 4 mm sieve, with grass, rocks and roots being handpicked. Samples were then stored at 5 °C in the dark until used in the laboratory.

Figure 3 – Overview of the four systems of SAFE: reference (conventional) system (REF), Organic system (ORG), agroecological intensification system (AI), and perennial system (PER); repeated in four blocks (A-D). Reproduced from (Barreiro & Albertsson, 2022).

6.2 Characterization of the soil

Soil characterization was performed following standard procedures (as in (Brangarí et al., 2022)). Soil electric conductivity and pH were measured using electrodes in a mixture of 1:5 soil:water. The soil's water content (WC) at the moment of sampling and the percentage of soil organic matter (SOM) were estimated by drying the soils for 12 hours at 105 °C followed by dry loss on ignition at 500 °C for 12 hours followed by the estimation of the percentage of soil inorganic carbon (SIC) by dry loss on ignition at 800 °C for 12 hours (as in (Wang et al., 2011)).

The water holding capacity (WHC) was determined gravimetrically following the protocol described in (Hicks et al., 2018). To do so, around 10 g of soil were compacted into a column that was then left to soak water for 24 hours, after which the soils were drain for 6 hours. The soils were then dried at 105 °C overnight. The weight was measured before soaking, after draining, and after drying which allowed for the WHC calculations.

The influence of agricultural systems and depth were analyzed by performing a 2-way analysis of variance (ANOVA), where agriculture system (annual or perennial), and soil depth (0-20 cm, 20-50 cm, or 50-90 cm) were used as the two factors. The data used were the values obtained for each of the measurements in Table 1 for the 4 blocks. The statistical analysis was conducted using the Past software, version 4.13 (as in (Brangarí et al., 2022)).

6.3 Drying and rewetting experiment

The drying and rewetting experiment was adapted from well-tested standard protocols (Brangarí et al., 2022; Meisner et al., 2017).

With the goal of testing the resilience of the microbial communities, the soil subsamples were first placed on microcosms and slowly dried down to (1) air-dry conditions (~3-4% WHC) or (2) 10% of their WHC, for 1 week, while gently mixing and taking subsamples for the measuring of water potential. When the desired moisture conditions were achieved, subsamples of 2.0 g of dry soil were rewetted to 50% of WHC. The microbial responses to rewetting, including bacterial growth, fungal growth, and microbial respiration, were measured at high temporal resolution for 3 days (see "Microbial analysis" below). The rates of moist controls (1.0 g) were also determined in parallel by keeping samples continuously at 50% WHC and performing the measurements three times (on the three days during which the experiment lasted).

6.4 Microbial analysis

6.4.1 Microbial respiration

Microbial respiration rates were calculated as previously described in literature (Hicks et al., 2019; Meisner et al., 2017) by using a gas chromatographer and measuring the CO_2 production. Incubation periods of 1-24 hours were used. The respiration rates were expressed as micrograms of CO_2 produced per gram of organic matter and per hour (µg C/g OM/h).

6.4.2 Bacterial growth

Bacterial growth was estimated by the incorporation on ^{3}H-Leucine (Leu) into extracted bacteria (Bååth et al., 2001). 2.0 grams of soil (1.0 grams for the controls) were mixed with 20 mL of deionized water, vortexed and centrifuged at low speed. 20 µL of Leu were incorporated into aliquots of 1.5 mL of the bacterial suspension. After a 2-h incubation in the dark the bacterial growth was ended by the addition of 75 µL of trichloroacetic acid. The samples were washed, and the amount of radioactive Leu incorporated into the new biomass was measured on a liquid scintillator. Bacterial growth rates were transformed to units of microbial-C through a conversion factor (Soares & Rousk, 2019) and were expressed as microgram of incorporated carbon per gram of organic matter and per hour (µg C/g OM/h).

6.4.3 Fungal growth and biomass

Fungal growth was estimated by the incorporation of ^{14}C-Acetate into ergosterol (Rousk & Bååth, 2007). 2.0 grams of soil (1.0 g for the controls) were mixed with 1.05 mL of deionized water and 50 µL of a solution containing ^{14}C-Acetate (ratio 2:3 of labeled acetate and non-labeled acetate), vortexed and incubated for 2 h in the dark. 500 µL of 10% formalin were added to terminate growth. The ergosterol were extracted by adding 10% KOH dissolved in methanol, sonicating for 15 min and heating for 60 min, after which 1 mL of deionized water and 2 mL of cyclohexane were added followed by vortexing and centrifuging to separate the two phases.

The fungal growth rates and biomass were estimated by HPLC and scintillation. Fungal growth rates and biomass were transformed to units of microbial-C through a conversion factor (Soares & Rousk, 2019) and were expressed as micrograms of incorporated carbon per gram of organic matter and per hour (µg C/g OM/h).

7 Results and discussion

7.1 Soil characterization

A summary of the soil's characteristics can be seen in Table 1.

*Table 1 – Soil's main characteristics in perennial and annual crops at three depth intervals (0-20, 20-50, and 50-90 cm), expressed as mean ± standard error (n=3 for 0-20 cm; n=4 for 20-50 cm and 50-90 cm). The table includes water holding capacity (WHC), soil organic matter (SOM), soil inorganic carbon (SIC), soil water content (WC) at the moment of sampling, pH and electrical conductivity (EC) and a summary of the 2-way ANOVA results. The p-values are the values of significance for differences between agricultural system, depths, and the integration between them (a = 0.05; ns: non-significant, *: p < 0.05; **: p < 0.005; ***: p < 0.001). See section 6.2 "Characterization of the soil" for more details.*

sample	depth	WHC (g H_2O/g dry soil (%))	SOM (g/g dry soil)	SIC (g/g dry soil)	Water Content (g H_2O/g dry soil)	pH	EC (µS)
Perennial	0-20 cm	46.52 ± 0.05	0.027 ± 0.011	0.005 ± 0.002	0.145 ± 0.057	7.2 ± 0.3	84.8 ± 4.5
	20-50 cm	44.14 ± 0.05	0.021 ± 0.005	0.009 ± 0.004	0.148 ± 0.034	7.5 ± 0.4	73.2 ± 3.6
	50-90 cm	35.37 ± 0.05	0.012 ± 0.003	0.034 ± 0.013	0.138 ± 0.032	8.7 ± 0.2	114.2 ± 2.6
Annual	0-20 cm	53.09 ± 0.03	0.037 ± 0.011	0.007 ± 0.002	0.199 ± 0.059	7.4 ± 0.2	91.4 ± 5.5
	20-50 cm	51.71 ± 0.02	0.021 ± 0.005	0.009 ± 0.002	0.150 ± 0.034	7.9 ± 0.1	107.1 ± 3.7
	50-90 cm	47.81 ± 0.03	0.010 ± 0.003	0.036 ± 0.012	0.137 ± 0.032	8.6 ± 0.1	114.1 ± 2.4
p - Agricultural System		**	ns	ns	ns	ns	**
p - Depth		ns	***	**	***	***	***
p - Interaction		ns	ns	ns	ns	ns	**

Water holding capacity (WHC) describes the ability of soil to retain water. There is a significant difference between agricultural systems ($p < 0.005$), with soils subjected to annual cropping exhibiting slightly higher values than soils under perennial cropping. It is also noticeable that the WHC of the soils diminishes with depth, with PC soils having a difference of around 10% with depth, and AC soils having a 6% difference, however, there are not significant differences between depths or related to the interaction.

Soil organic matter significantly decreased with depth ($p < 0.001$), dropping ~15% and a ~27% between the top layer (0-20 cm) and the bottom layer (50-90 cm) on PC and AC soils, respectively. It is worth mentioning that although statistically non-significant, the middle and bottom layers show virtually identical SOM contents across agricultural systems, however, the top layer of the AC soils exhibits a 10% higher SOM content compared to the top layer of the PC soils. SOM originates from the decomposition of plant (and animal) residues, which explains why topsoils have larger organic pools compared to deeper layers. Opposite to the top layer

which has a frequent input of SOM mainly in the form of plant necromass, deeper layers don't, hence, the lower content of SOM (Zhou et al., 2019). Regarding the differences across agricultural systems, annual crops are planted and harvested every year, while perennial crops have a longer life cycle that lasts 7-8 years. An annual harvest translates into large amounts of plant material being left on the soil every year. That plant material is converted to SOM therefore explaining why AC soils have more SOM than PC soils.

Regarding soil inorganic carbon, an opposite trend can be found. Both soils start with smaller SIC contents on the top layers which significantly increase in the deepest layer ($p < 0.005$). The difference between the top and bottom layers, in SIC content is about 0.030 g/g of dry soil for both types of soil. SIC is mainly stored in the form of rocks and minerals that derive from the formation of carbonates (Zhou et al., 2019). The deeper in the soils profile the more rocks are found, and higher the SIC content. Furthermore, SOM and SIC are two sides of the same coin, the higher one is, the lower the other tends to be.

The differences in the water content at the moment of sampling are only significant for depth ($p < 0.001$) but are non-significant between agricultural systems or the interaction between agricultural systems and depth.

pH and EC both increasing with depth. EC shows statistically significant differences in all the factors analyzed, nevertheless, pH only shows significant differences for the depth factor ($p < 0.001$). On the top layers of the soil there is more SOM, and its decomposition leads to the production of organic acids, which lower the pH. Higher pH in deeper profiles can be associated with the presence of high amounts of SIC, which acts like a pH buffer due to the reaction between the carbonates and protons (Zhou et al., 2019).

7.2 Water potential

Water potential quantifies the propensity of water movement from one area to another via osmosis, gravity, pressure, and capillary action. Due to being a measure of energy of the water in the soil, its value can be correlated with water movement and availability for plants and microorganisms. A water retention curve describes the relationship between soil water potential and the amount of water in the soil, indicating how soil properties affect water availability and the stress to which soil microorganisms are exposed.

The results from the water potential measurements can be seen plotted against the WHC in percentage in Figures 4 and S1.

From the plot it is noticeable that with the decrease of WHC, the water potential also decreases. It is also noticeable that, with depth, there is a shift in water potential towards the right for the PC soils, while for the AC soils the shift is in the opposite direction.

From water retention curves one can extract the value of the wilting point, which is defined as the minimum amount of water in the soil that the plant requires not to wilt. Conventionally is the value of water moisture at which the pressure is 1.5 MPa,

which since the plot as its y axis as a logarithmic axis, is the value of moisture that corresponds on the y axis as 0.176 MPa.

The shift mentioned earlier is very clear when analyzing the wilting point. Roughly the wilting point is 32, 35, and 43 percent of WHC for the PC soils from topsoil, middle soil, and bottom soil, respectively; and it. Is 25, 21, and 19 percent of WHC for the AC soils from topsoil, middle soil, and bottom soil, respectively.

Water potential can be correlated with soil organic matter. The more SOM, the higher the water potential (Manzoni et al., 2012). That is the relationship seen by the AC soils, the deeper the profile, the lesser the SOM thus, the lower the value for water potential.

Surprisingly, that is the opposite of what is seen for the PC crops. It is clear that the long roots of perennial crops influence water potential in a way that requires further exploration.

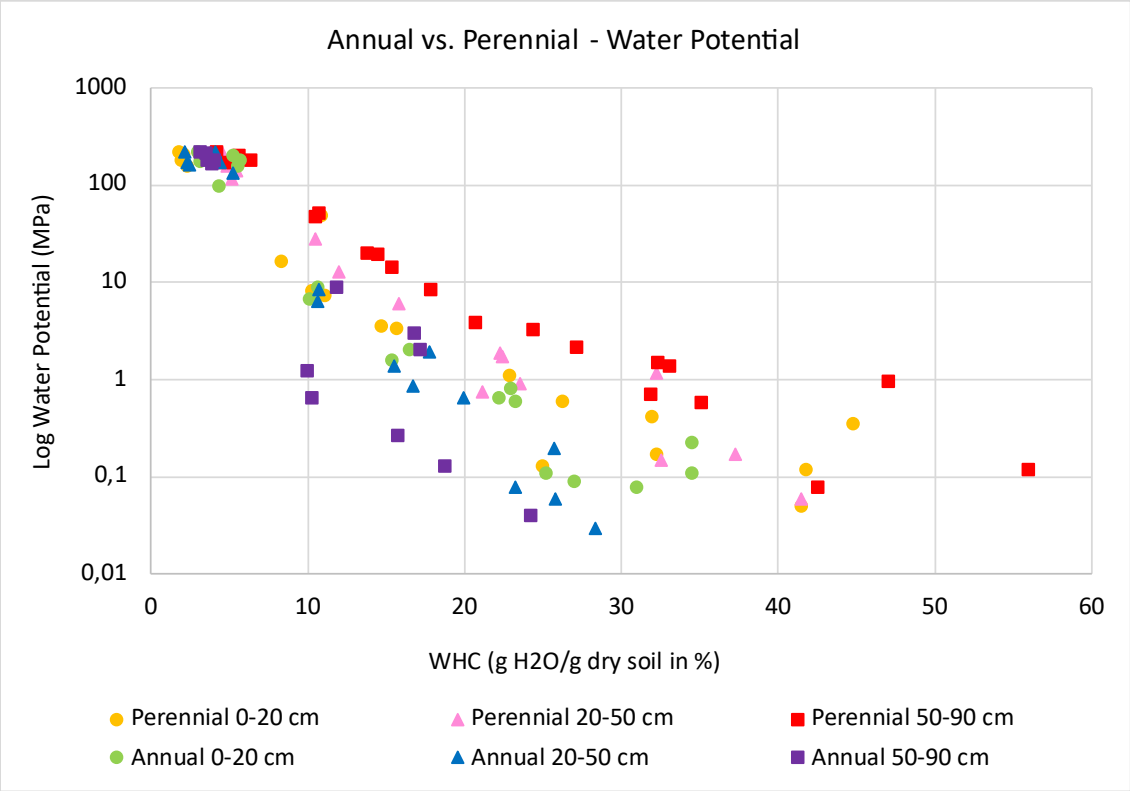

Figure 4 – Relation between water potential displayed as MPa in a logarithmic axis and soil's water holding capacity as grams of water per grams of dry soil in percentage.

7.3 Microbial analysis – responses to drying and rewetting

7.3.1 Steady-state rates

By looking at Figure 5 (additionally, S2 and S3), which shows the (A) bacterial and (B) fungal growth rates for the bacterial growth steady states, used to normalize the values displayed in Figures 6 and 8 (respectively), it is possible to infer that for the top layers, the bacterial communities of both soils grow on a similar rate of around 1000 µg C/g OM/h, while for the other layers the communities

from PC soils grow almost at a double rate from the communities of the AC soils (1500 µg C/g OM/h vs. around 700 µg C/g OM/H). It is also noticeable that while for the AC soils, the rates decrease with depth, for the PC soils the rates increase from the 0-20 cm layer to the 20-50 cm and 50-90 cm layers, in which the rates are similar.

When comparing the fungal growth steady state rates between type of soil and depth, it is evident that the rates for the PC soils are fairly constant with depth, while in the AC soils there is a clear decrease in rate with depth.

The decrease with depth in growth rates at the moisture control level was expected. It was unexpected thought that only the communities living in AC soils decreased their growth rates with depth. A possible explanation for this could be because the long roots of perennial plants might be providing resources along their vertical profile, leaving communities present in those soils more adapted. In contrast, annual crops have smaller roots, so the microbial communities living in deeper profiles on those soils do not have the same input of resources and are not so well adapted.

Another possible explanation could be that in depth there is a more anaerobic environment, at which the microbial community is adjusted (Naylor et al., 2022). However, during the sampling and handling of the soils, they were kept in an aerobic environment, which could lead to changes of the microbial communities.

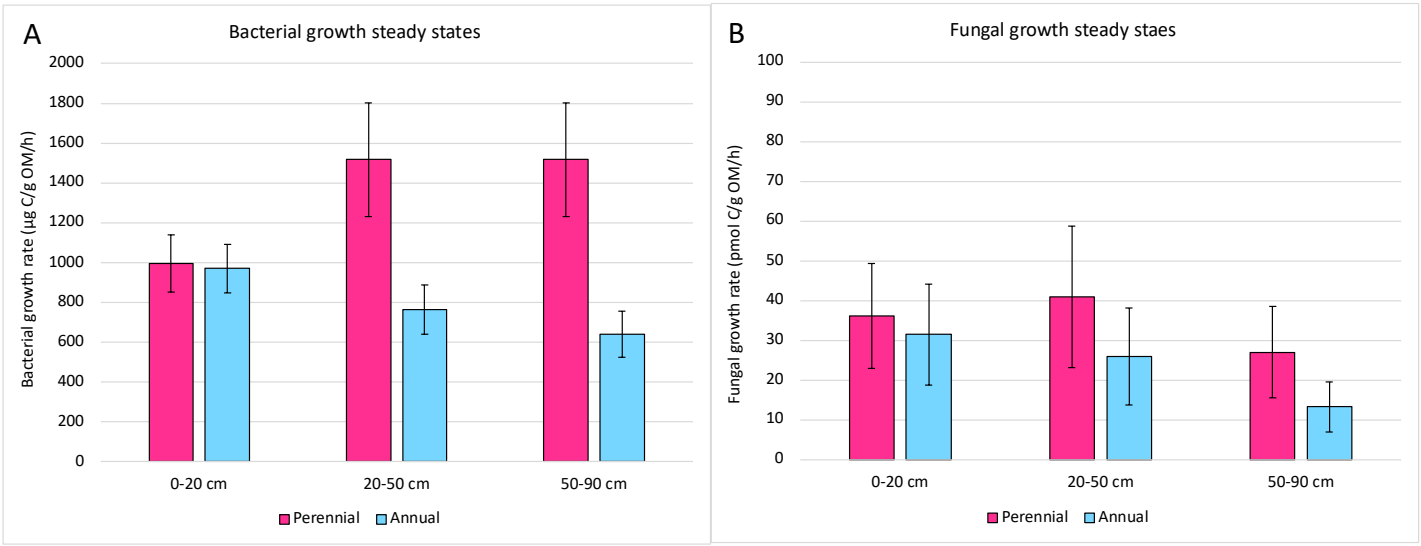

Figure 5 – Bacterial growth rates (A) and fungal growth rates (B) for the steady states of PC and AC soils grouped by depth.

7.3.2 Responses to rewetting

Figure 6 shows the normalized bacterial growth rates for AC and PC soils under the two different DRW conditions.

During the 80 hours of the experiment, all bacterial communities increase their growth rates after a lag time period of no apparent growth, which is typical in a

type 2 response to rewetting. The patterns change between moisture treatment and soil type. While for the rewetting from 10% WHC there's a peak around the 48 hours and 54 hours for PC and AC soils, respectively, for the rewetting from air dried, the peaks are around the 54 and 55 hours for PC and AC soils correspondingly. There also the factor of the height of the peak that also changes, not only between soil type and drying treatment but also with depth.

For the rewetting from 10% WHC, the peaks are higher for the AC soils, with heights of around 13 and 2.8 (50-90 cm, and both 0-20 cm and 20-50 cm, respectively), while for the PC soils the peaks round about 2.5, 2.4, and 1 from the top to bottom layers.

For the rewetting from air dried treatment, the amplitude of the peak of the AC soils is almost double of the one from PC soils, with heights of 8, 2.5, 1 (for the layers: AC 50-90 cm, AC 0-20 cm, and AC 20-50 cm, accordingly), and 4.4, 2.9, and 1.9 (for the layers: PC 20-50 cm, PC 50-90 cm, and PC 0-20 cm, accordingly).

The bacterial community of the deepest depth (50-90 cm) on the AC soils are very markedly higher than all the other bacterial growth rates, however this sample also has the higher standard error bars. Since only two out of the four replicates were feasible of being conducted and analyzed, a possible justification for the higher growth rates could be that one of the replicates had wrong measurements leading to an overall wrong analysis.

Figure 7 shows the recovery and lag times for each soil and treatment for the bacterial communities.

Starting with the recovery time, the PC soils increase their recovery time with depth for the air-dried treatment while being relatively constant for the rewetting from 10% WHC treatment. AC soil decrease their recovery time with depth for the air-dried treatment while increasing it for the rewetting from 10% WHC treatment. However, it is important mentioning that this study, as previously said, was conducted with only two replicates, and for the AC soil layer 20-50 cm, one of those replicates didn't recover, which might be the reason why the recovery time for that soil is so distinct from the other soils; the same can justification is given for the lag time of the same soil.

Overall, the bacterial communities from PC soils have bigger growth rates than the ones from AC soils. Bigger growth rates could be translated into more biomass which goes accordingly to (Chantigny et al., 1997).

Moving to the lag time analysis, it seems like PC soils 0-20 cm and 20-50 cm have the same lag time, around 3.5 hours, and PC 50-90 cm and the AC soils have similar lag times around the 10 hours for the air-dried treatment. For the rewetting from 10% of the WHC treatment, the top layer of the PC soil has the shorter lag time around the 2 hours, followed by the deepest layer of the AC soils around the 5 hours. AC 20-50 cm has the higher lag time around 15 hours, while all the other soils display similar lag times of around 8-9 hours.

H1 stated that the communities would become less resilient to a DRW event with depth, which could be translated into a type 2 recovery with longer lag and recovery times. That was partially proved. Although the type 2 behavior was seen

and that, overall, the recovery times increase with depth, the lag times don't show such a clear of a trend.

From the fungal growth responses to the rewetting event (Figure 8) one can see that between the two types of soils and the different depths, the responses are very similar. Neither of the communities fully recovers, which is shown by neither of the datasets crossing the value 1. It is also observable that despite not fully recovering, the fungal communities do not demonstrate a lag period, which was expected (De Vries et al., 2012; Hicks et al., 2019). A possible reason for the fungal communities not to recover could be because the resources are being overtaken by the bacterial communities since when comparing the growth rates, bacteria have a growth rate that is two to three time higher. Another possible explanation could be that fungi tends to thrive in more acidic pH, which is not the pH of the soils being studied (Neurauter et al., 2023).

H2 affirmed that communities present in AC soils would be more resilient than the ones from PC soils. Again, this could be seen by communities from AC soils posing shorter lag and recovery times. This was not what was observed. In fact, following the results, one could suggest that the opposite to be true instead. Following the results of the water potential and the wilting point, perennial crops soils behave the opposite of what is described in the literature, which might explain why microorganisms in perennial crops are better adapted to drought.

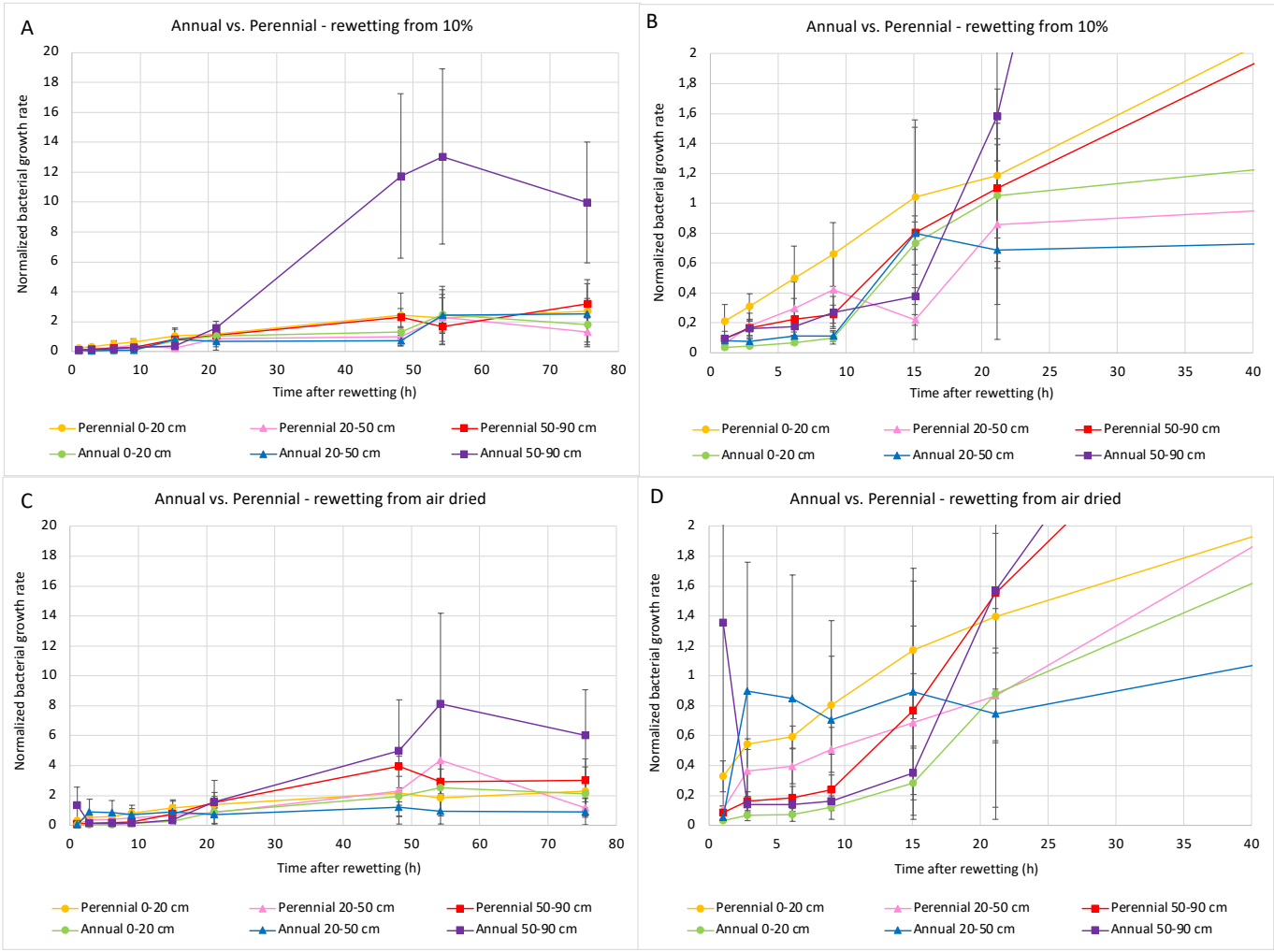

Figure 6 – Normalized bacterial growth rates from AC versus PC soils rewetted from A) 10% water holding capacity and C) air dried versus time after rewetting (h). B) and D) are zoomed in views from the first 40 hours after rewetting from plots A and C, respectively. Values were normalized by dividing each value by the average of the steady states.

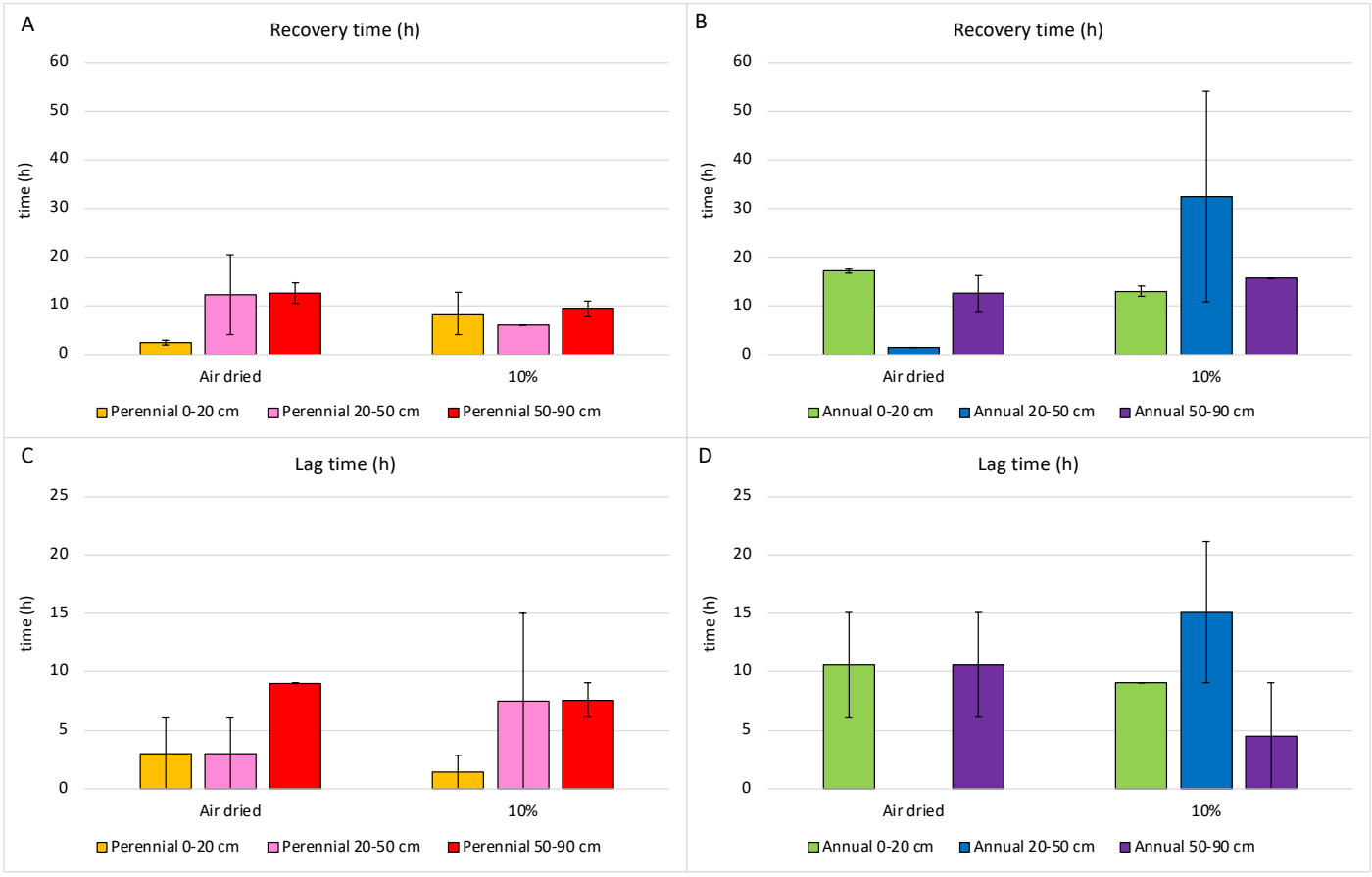

Figure 7 – A) and B) Recovery times and C) and D) lag times for the bacterial communities grouped by dried condition of the soils.

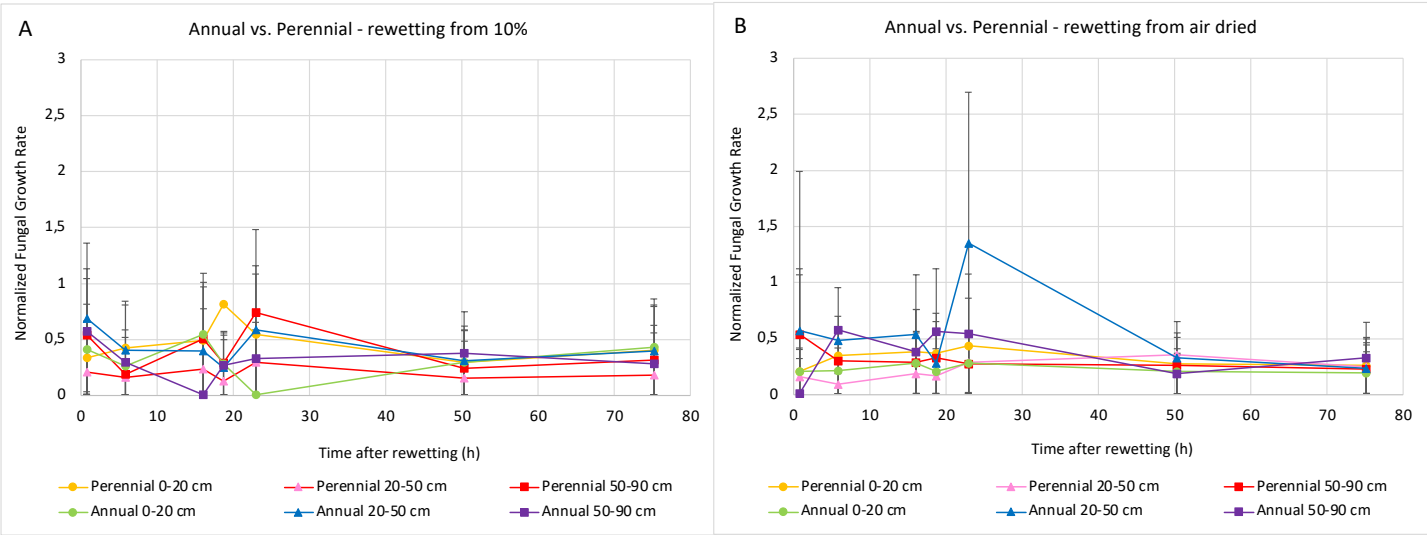

Figure 8 – Normalized fungal growth rates from AC versus PC soils rewetted from A) 10% water holding capacity and B) air dried versus time after rewetting (h). The values were normalized by dividing each value by the average of the steady states.

4. Reflection about the respiration results

Unfortunately, due to a failure of the GC instrument where the respiration samples were run, there is no available data to present regarding the respiration part of the study. However, this was still a learning moment that deserves the same reflection as the results presented above.

One of the reasons why my data was lost was because, after having to delay the experiment for a couple of weeks, my supervisors and I decided to analyze two blocks at the same time instead of doing them separately as originally planned. This decision would have allowed me to have the same replicates for respiration as for the rest of the microbial analysis but also, would allow for a stronger analysis of the results. However, by trying to analyze the two blocks simultaneously I risked losing the data for all samples, which was what ultimately happened due to unforeseen circumstances.

With this I learned that it would've been preferable to have performed the technique for each block separately, with the risk of losing just one of the replicates and presenting just one block. Another possible route would have been testing the instrument after it had been repaired to check the reparation's success instead of loading it with all my samples without doing the due diligence it required; and from that deciding if the experiment should or not proceed.

It could've also been possible to ask for permission to use the GC of another lab while the instrument available was being repaired, that way the project would not have been delayed and possibly more replicated could have been analyzed.

Lastly, I learned that one should never leave their whole set of samples unattended in an instrument that had just been repaired and especially one should never load the said instrument and leave it to run overnight without having a backup plan in case it fails.

5. Conclusion

When this study started, there was a knowledge gap regarding the behavior and characteristics of microbial communities living in PC soils and how the long roots of perennial crops could affect the microbial communities living in deeper profiles compared to the communities living at the same depth but without the presence of such long roots, as is the case in annual crops. It was thought that microbial communities living in AC soils experience different conditions that the ones living in PC soils, thus behaving differently when faced with the same perturbation. That can be seen in the first experiment of this study related to water potential. While AC soils behaved as predicted, with their wilting point decreasing with the SOM content, and consequently, with depth; PC soils, surprisingly behaved in the opposite way. However, regarding the conclusions about the hypothesis theorized at the beginning of this study it doesn't seem that simple.

Hypothesis 1 was partially proven true, as the microbial communities exhibited a type 2 behavior with recovery times increasing with depth; however, no conclusions can be drawn from the recovery times.

Hypothesis 2 was not sustained, since results indicated that microbial communities from soils with perennial crops are more resilient than the ones from soils with annual crops.

From the results obtained, it could be said that perennial crops soils might help mitigate climate change consequences due to the higher growth rates exhibited by those soils, when compared to the annual crops soils.

6. Future perspectives

Firstly, since time only allowed for two out of the 4 replicates to be analyzed and knowing the importance of having more than 2 replicates, the other 4 soils should be investigated.

It would also be relevant to redo the respiration analysis and obtain the results for all the soils since it would provide a better picture of the whole microbial community. By studying respiration, it would also allow for carbon use efficiency (CUE as is known) calculations, which could provide an insight into how efficient the communities are at using carbon, meaning, how efficient they are at converting carbon to biomass.

Another attractive topic that could be studied would be the ergosterol content of the fungal growth samples. Since the samples are run in the HPLC before counting their radioactivity, ergosterol content is measured, however, due to time constraints, it was not possible to analyze these results and that would have been interesting because ergosterol is a membrane lipid that is characteristic to fungi, so by measuring ergosterol we could have an idea of how much fungi are in the samples.

It could also be relevant to sample and sequence DNA from the soil samples to have a better understanding of the composition of the microbial communities and how they interact, and compare them between agricultural systems.

Lastly, once all of the above is accomplished, it would be interesting to perform statistical analysis on the results to have a better idea of their significance.

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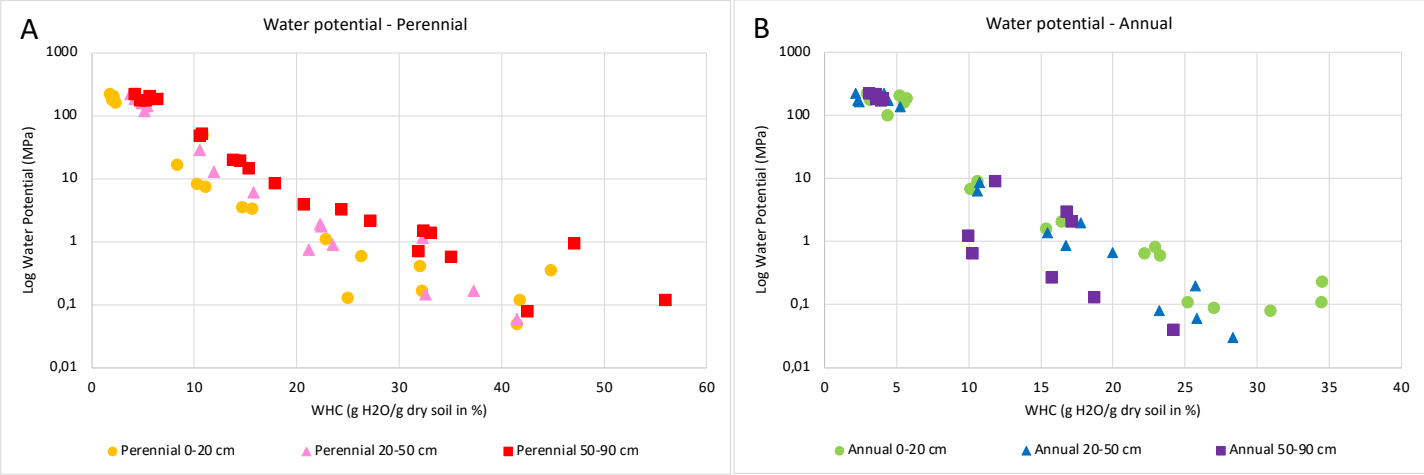
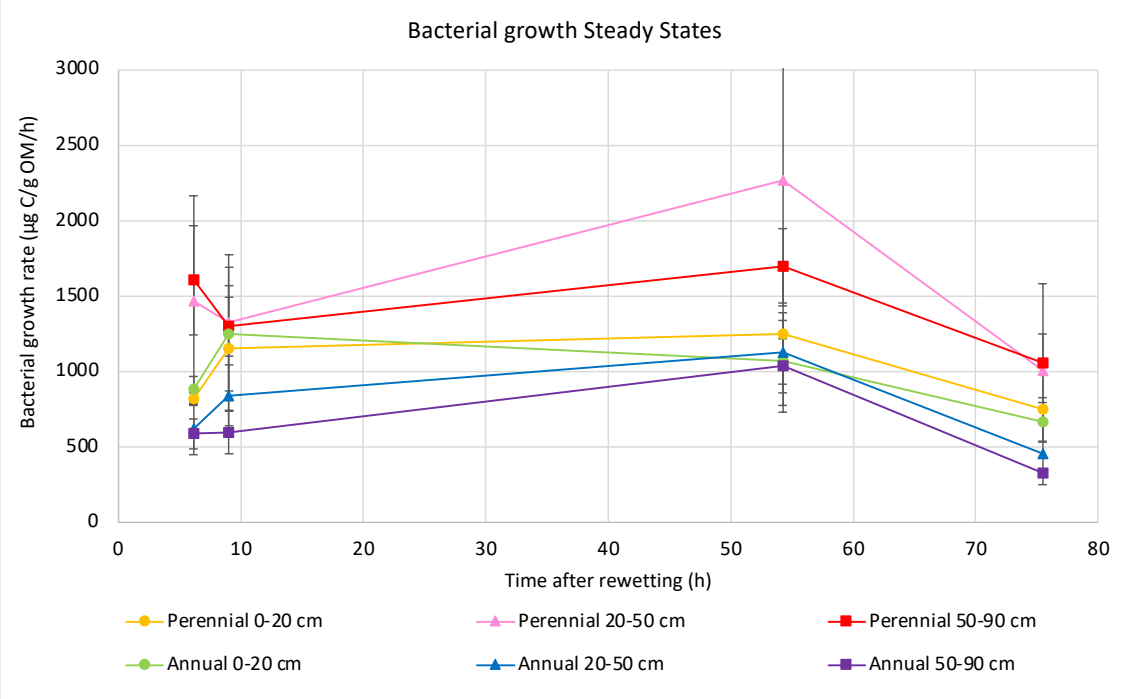
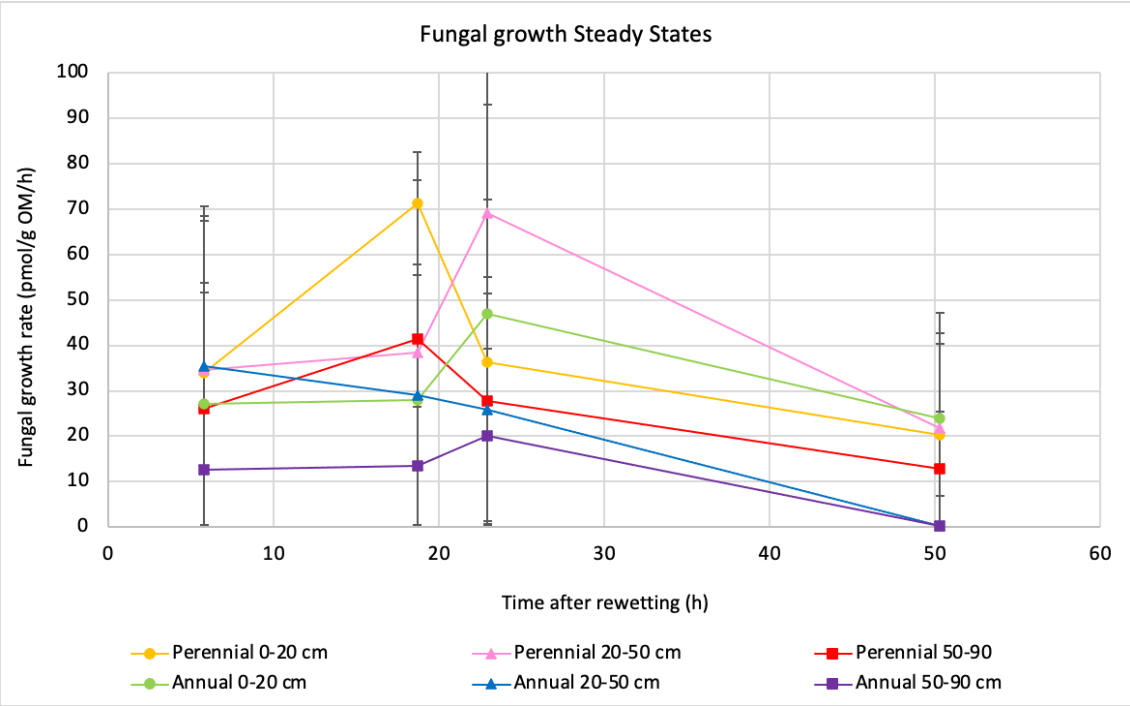
8. Supplementary material



S 1 – Relation between water potential displayed as MPa in a logarithmic axis and soil's water holding capacity as grams of water per grams of dry soil in percentage for A) PC crops and B) AC crops.



S 2 – Bacterial growth rates for the steady states of PC and AC soils.



S 3 – Fungal growth rates for the steady states of PC and AC soils.