Bacterial and fungal responses to drying and rewetting

using bronopol or cycloheximide as inhibitors

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Abstract

Microbial decomposers contribute to carbon dioxide (CO_2) emission through their metabolism, impacting and impacted by climate change. Actions towards regulating soil as a carbon sink will provide one solution to global warming caused by CO_2 emissions. This study investigated bacteria and fungi as the main decomposers in soil and their interactions and contribution to CO_2 release upon drying-rewetting (D/RW). Using a recent study as reference we chose to use the same soil collected from a forest plantation in Wales in October 2016. To examine the interaction between fungal- and bacterial growth rates, bactericide bronopol and fungicide cycloheximide was used to inhibit bacterial- or fungal growth following D/RW. Bacterial- and fungal growth rates as well as respiration rates was measured. To expand this study the half of the soil was pre-treated with beech litter to test whether selection for microbes quick at colonizing new carbon resources would make microbes quicker at responding to D/RW. The results clearly showed the expected competitive release with inhibition of the targeted microbial competitor. Microbial activity increased in the moist soil with litter addition, but the main bacterial responses to D/RW did not shift. Our results showed bacterial and fungal interactions and their responses upon drying and rewetting, identifying correlations between respiration- and growth rates. This thesis confirmed the results of some previous studies within this topic. This thesis also found different bacterial and fungal interactions which could contribute to environmental applications or implementations to fight the global warming.

Contents

Introduction

Soil microorganisms are providing important ecosystem services such as decomposing of organic matter and will be affected by the climate change, such as more frequent drought events (IPCC, 2013). Microorganisms in soil are respiring through metabolism releasing carbon dioxide (CO_2) to the atmosphere, but the same soil could also be used as carbon sink accumulating organic carbon to the soil as microbes grow (Luo and Zhou, 2006). There are believes that promoting and managing agricultural soil as carbon sink could be a solution for the global warming. It is estimated that microbial decomposers are contributing to six times more CO_2 emissions than the human activities (Trivedi et al., 2013). Join Research Centre of the European Commission are currently promoting multiple projects such as CIRCASA, LANDMARK and iSQAPER to promote soil as carbon sink (European Commission, 2018). CIRCASA for example are concerning exchange of international research and knowledge in the field of carbon storage in agricultural soils at global level (Arias-Navarro. C, 2017). Since drought periods and heavy rainfall are more frequent in recent year due to climate change (IPCC, 2013), it is therefore of interest to study how events like drying-rewetting (D/RW) can affect the carbon balance (growth relative to respiration) in different soils.

Following the event of D/RW the labile carbon in soil increases, these are accumulated from dead cells and osmolytes during the drying period (Kim et al., 2012; Yan et al., 2015; Schimel, 2018). Bacterial and fungi are different at capitalizing new resources, with bacteria often having faster responses and quick to use labile carbon (Reischke et al., 2014) while fungi are able to use more recalcitrant carbon (Brant et al., 2006). From previous study we know that there are competitive interactions between bacteria and fungi in soil when the moisture content is stable (Rousk et al., 2008, 2010).

Two distinct bacteria growth responses have been observed during D/RW event (Meisner et al., 2013, 2015; Hicks et al., 2019) (fig. 1). During the period after a rewetting event a “type 1” response have an immediate and linearly increasing bacteria growth (Meisner et al., 2013, 2015). Meanwhile a “type 2” response have a delayed growth or close to zero growth in the beginning then followed by an exponential growth (Meisner et al., 2013, 2015). Fungal growth has previously been unresponsive in soil with a type 1 response where the growth

increase with rewetting and then reside at a level close to control values of a non D/RW sample (Bapiri et al., 2010). In a recent D/RW study fungal growth in soil with type 2 response showed high linearly increasing growth with a growth peak that was 10 times higher than the moist control (Hicks et al., 2019), in the same study bacterial- and fungal interactions during D/RW were tested using a bactericide, however some questions remains to be resolved.

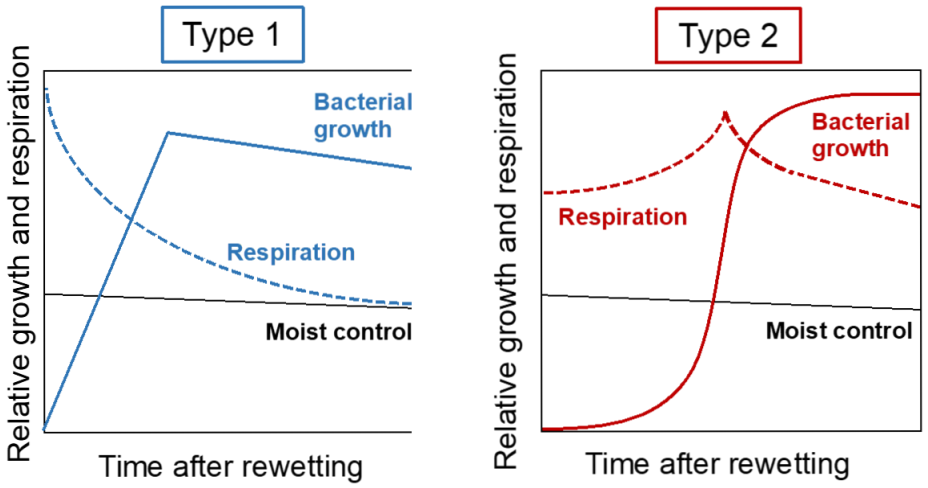

Figure 1 type 1- and type 2 response
Illustration of the 2 observed bacterial growth responses together with their respective soil respiration rate and bacterial growth in moist control during D/RW event (graphs by Ainara Leizeaga).

In this study we verified and expand upon a previous D/RW study (Hicks et al., 2019) which have been looking at bacteria and fungi in soils with type 1 and type 2 response with and without any bacterial inhibition. To expand the previous study, we added a situation where fungi are inhibited as well. The same soil sampled from a forest plantation in Wales, UK that have been stored since October 2016 will be used in this study as T2 soil. Recent study (Nijs et al., 2018) have shown that it is possible for a soil with type 2 response show a type 1 response after it been exposed through several D/RW events, it is believed that the community of microorganisms will adapt to stimulants and shift to those that have more resilience and greater stability (Griffiths & Philippot, 2013; Orwin et al., 2015). Another way to induce a shift in community towards quick colonizers is by adding C resources (Bernard et al., 2007; Cleveland et al., 2007). To test if the selection of fast colonizers by litter addition also results in faster recovering from D/RW (i.e. shift in response from type 2 to type 1) the T2 soil treated once with carbon source (4.5

mg beech litter/ g soil) was used as the T1 soil for this study. The two soils used in this study are named T1 and T2 for simplicity and do not necessarily give a type 1 or type 2 response during D/RW.

To inhibit bacterial growth the bacteria-specific antibiotic bronopol (Bailey et al., 2003) was used, and the fungicide cycloheximide (Rousk et al., 2009) was used to inhibit fungal growth.

The hypotheses:

- That T1 and T2 soil of this study will show different responses in terms of bacteria-, fungal growth and respiration. Due to the addition of litter T1 moist control soil (not dried but constantly moist) should have higher activity than T2 soil. We also hypothesized that addition of litter would select for microbes quicker colonizers, which would make microbes quicker at responding to D/RW (type 2 to type 1 response).
- Since the same Bangor soil that have been stored is used as T2 soil the results with and without bronopol inhibition should imitate the same or similar results from that of the previous study (Hicks et al., 2019), and if differences arise these should be due to the difference in methods used or changes of microbial community composition caused by the long storage time of the soil.
- The inhibition of bacteria would cause a competitive release that would at different stages of growth benefit thus enhance fungi, and vice versa if fungi are the ones inhibited during D/RW.

Through this study we will try to find out if our hypothesis about the bacterial and fungal interactions holds, and what differences adding litter to the soil will do. We will find out what is the main cause that is driving soil respiration during D/RW event and how much the bacteria and fungi contributes to the CO_2 respired during this period.

Material and Methods

Delimitations

The different moments and methods of this study were set to fit within a 7-week time schedule. D/RW observation length, number of timepoints, numbers of samples and the number of replicates was made to fit the study within this period.

Delimitations was made to fit this study to a bachelor thesis. With time as the main limiting factor. Repetition of tests, the numbers of test samples, sampling period as well as test replicates was cut down mainly due to lack of time. Time was also allocated to familiarize with the lab, related literature and the different methods used for the soil analysis. The literature used in this study are mainly from previous studies associated with the Microbial Ecology department of Lund University, or references used by these studies. The references are taken from literature that are available in English, these literatures have been reviewed and originate from public institutions which keep bias to the minimum. Resource was not an issue since the study was issued by Rousk lab of Lund University, equipment and materials was provided by the lab.

Soil selection

The selection of soil samples was decided to mimic previous study (Hicks et al., 2019) using two types of soils. A T2 soil from Bangor (Wales) was selected because of its previously shown response to D/RW event. It was collected ca. 10 cm deep from 4 locations in a beech forest plantation in Bangor during October 2016, this soil was stored in a cold storage room at Lund university and was available for use. As mentioned in the introduction Bangor soil pretreated (2 April 2019) with carbon source (4.5 mg beech litter/ g soil) was used as T1 soil for this study. The soils were well mixed and sieved (< 4 mm) to remove unwanted content like rocks, roots and larger decomposing litter fragments/pieces.

Soil properties

Some basic soil tests were carried out to determine soil property of these 2 soils, these are pH, respiration, electric conductivity (EC), water holding capacity, soil dry weight test and loss of ignition method was used to find out soil organic matter content (600°C). Duplicates or triplicates of both soil types were carried out for soil property tests.

The pH was determined by using a pH meter on 1:5 soil solution (soil/distillated water). EC was measured similar as pH but using a EC meter using 1:5 soil solution. WHC was measured by weighing 5.0 g soil, then covered in a plastic tube with nylon mesh in underside and covered by parafilm on top side (preventing evaporation) and weigh the tube. The tube with soil in it was placed on water allowing the soil to absorb water for 24 h, before putting the tube for 6 h draining. The tubes are weighed again to then determine maximum WHC. Soil respiration was measured by weighing one gram of soil into a 20 ml glass vial, at given timepoint empty the air in the vial by using pressurized air before sealing it for incubation. The amount of CO_2 during the incubation time can be measured by using a gas chromatograph equipped with methanizer and a flame ionization detector.

Dose-response relationships

Dose-response analysis was necessary to find the optimal concentration of bronopol and cycloheximide to use in the following D/RW experiment. It was also possible to detect the non-target effects of bronopol and cycloheximide through dose-response analysis. Concentrations of 1000, 333, 111, 37, 12 µg/g soil for Bronopol and 800, 400, 200, 100, 50 µg/g soil for Cycloheximide were chosen. Each of these concentrations were added to 15 g of T1 and 15 g of T2 soils, these samples plus two untreated control for each type of soil were kept in plastic cans for totals of 5 days.

^{3}H-Leucine (Leu) incorporation

Bacterial growth was determined by measuring the rate of ^{3}H-Leucine (Leu) incorporation in extracted bacteria (Bååth, 2001; Rousk et al., 2009). 1.00 g soil

was mixed with 20 ml distillated waterin a 50 ml plastic tube then mixed by 3 min on vortex and phase separated by 10 min in centrifuge at 1000 g. 1.5 ml of supernatant is put into 2 ml Eppendorf-vials 20 µl then ^{3}H-Leucine solution was added and the vials was incubated in the dark in room temperature for 1´h. The bacterial growth was then terminated by adding 75µl cold 100% TCA (trichloro acid) and mixed by 5 sek vortex. A series of washing steps was performed as described by Bååth et al. (2001). Scintillation cocktail (Ultima Gold; PerkingElmer, USA) was added and mixed by vortex. The radioactivity was then measured using a liquid scintillaton counter. The bacterial growth was then measured by the amount of leucine incorporated into extracted bacteria (pmol Leu incorporation g^{-1} h^{-1}).

acetate-in-ergosterol incorporation

Fungal growth was determined by using the acetate-in-ergosterol (Ac-in-erg) incorporation method (Newell and Fallon, 1991) adapted for soil (Bååth et al., 2001; Rousk et al., 2009). It measures fungal growth by estimating the rate of ergosterol synthesis. 1.00 g of soil was mixed with 1.95 ml distillated water and 50 µl ^{14}C-acetate solution into 10 ml glass tubes. It was then incubated for 2 h at room temperature. Growth was terminated by mixing 500 µg of 10% formalin. The ergosterol and incorporated acetate was measured according to Rousk and Bååth (2007). Scintillation cocktail was added, and the fungal growth was measured by the amount of acetate incorporated into ergosterol (pmol g^{-1} h^{-1}) with a scintillator using UV absorbance at 282 nm compared to external standard.

Dose-response analyze

The growth rates were measured for all the samples at day 1 and day 5. These methods show a high temporal resolution on the growth rates giving us the data needed for analysis. The data collected was used to plot dose-response graphs (see supplementary) to determine the optimal concentration of bronopol and cycloheximide to use. The criteria for these concentrations was to have major inhibition for their target group while not showing any significant inhibition for their non target group. The concentrations should be applicable for both T1 and T2 soils. The concentrations that was selected and used in later D/RW event was 111 µg bronopol/g soil and 100 µg cycloheximide/g soil.

Drying and rewetting experiment

The soils were air-dried for 2 days (15 April – 17 April 2019) under a fan in room temperature (~18°C). The water content of the air-dried soils was then measured. A drying and rewetting schedule was made to keep the laboratory moments smooth, for bacterial- and fungal growth rate as well as for respiration measurement. It was decided to collect data samples for up to at least 150 h after rewetting. As data needs to be collected at different timepoints during this 150 h (12 timepoints) for growth rates and 120 h (8 timepoints) for respiration, it was not possible to collect all of them during regular working hours. The rewetting time for the samples was therefore divided into different batches allowing all the sample to be collected during working hours, this was done between 18 April and 29 April. Since destructive sampling was used each sample (of 1.00 g of dried soil) was prepared in test tubes in advance ready for rewetting. Two solutions, one containing bronopol (concentration calculated to reach 100 µg/g soil when added to 1.00 g of air-dried soil) and one containing cycloheximide (concentration calculated to reach 111 µg/g soil when added to 1.00 g of dried soil) was prepared in advance, these and distilled water was used to rewet soil samples. The amount of bronopol or cycloheximide in the aqueous solution was calculated to reach the concentration derived from the dose-response analysis when adding 370 µl of it to 1.00 g of dried soil reaching about 50% WHC.

Twelve samples were collected for each growth rate timepoints (12 timepoints), these samples are shown in table 1 bellow.

Table 1 12 samples measured at each timepoint.

Type soil 1	Bacteria control	Bacteria with bronopol	Bacteria with cycloheximide
Type soil 1	Fungal control	Fungi with bronopol	Fungi with cycloheximide
Type soil 2	Bacteria control	Bacteria bronopol	Bacteria with cycloheximide
Type soil 2	Fungal control	Fungi with bronopol	Fungi with cycloheximide

The rewetted samples were incubated in a dark space with constant temperature (16°C) before they are sampled. As for methods to determinate growth, Ac-in-erg (Bååth et al., 2001) and leucine incorporation (Bååth et al., 2001)) was used.

The respiration rate of a rewetted T1 and T2 soil (control, Bronopol-treated, cycloheximide-treated) were also measured (6 sample per timepoint) by using the accumulation of CO_2 in vials with 1.00 gram of rewetted soil. The soil samples are incubated during for a set amount of time at respective timepoints to then be measured using a gas chromatograph (GC).

Alongside the main growth and respiration measurements, growth and respiration of control soil from continuously moist T1 and T2 soil (moist control (MC)) was measured for use as reference during later analysis to see the difference between D/RW soil and the same soils but under stable conditions. Only 3 timepoints each was measured for MC growth rates and respiration. pH-test and electric conductivity was conducted twice on D/RW soil during the main D/RW experiment to monitor any changes.

Analysis, data collection and data treatment

Since destructive sampling was used, it was possible to collect and store the samples in waiting for them be further processed and then analyzed. This action was possible because the growth for bacteria or fungus are stopped in the early stages of destructive sampling.

A scintillator was used to measure growth rate for the processed test samples (fungal- and bacterial growth) in units of DPM (counts per minute). The analysis data was printed down on paper and plotted onto graphs using Excel for further result evaluation. Several correlations between respiration and growth rates was tested and plotted using linear regression in Excel, and Pearson correlation coefficient was calculated to find out the R value and the significance.

Results

Soil properties

The following table (table 2) presents soil property data (use as reference) for the soils used in this D/RW experiment. There are some minor differences between type 1 and type 2 soils, most notably higher SOM content for type 1 soil.

Table 2 Soil properties
T1 and T2 soils used for the D/RW event. Mean data with standard error in brackets. WC, SOM, pH and EC measurements of the soils had 3 duplicates each, WHC had 2 duplicates and WC of the dried soil had no duplicates.

Properties	Type 1 soil Treated type 2 soil (4.5 mg beech litter g^{-1})	Type 2 soil Beech plantation, Bangor, UK
WC % (water content)	27.8 (0.06)	27.8 (0.06)
WHC % (water holding capacity)	81.9 (1.79)	74.8 (0.33)
SOM % (soil organic matter)	9.8 (0.10)	9.2 (0.04)
pH (H_2O (1/5 soil to H_2O))	4.9 (0.03)	4.7 (0.01)
EC µS cm^{-1} (electric conductivity)	274.3 (7.13)	279.7 (0.67)
WC % (air-dried soil water content)	1.7	1.9

WC describes the total amount of water in the measured soil. WHC describes how much water the soil can maximum contain relative to same soil with null WC. EC is measured in micro siemens per square centimeter.

D/RW results

All the D/RW results are presented in figure 2 as graphs. Each graph contains data series from dried soil rewetted with water (control), bronopol (B) and cycloheximide (C), there is also a moist control trendline showcasing non-D/RW soil (same soil type) under stable moisture.

T1 bacterial growth graph (Fig. 2A): the bacterial growth in bronopol-treated type 1 soil was close to zero throughout the testing period. After 27 h bacterial growth in control and cycloheximide-treated soil (type 1) started to rise, growth was higher in C-treated soil throughout the rest of the testing period. The bacterial growth in moist control was very high around 400 pmol Leu $g^{-1}h^{-1}$ compared to the control that peak at 37 pmol Leu $g^{-1}h^{-1}$.

T2 bacterial growth graph (Fig. 2b): similar bacterial growth result in B-treated soil (T2) as in graph 1A. After 27 h bacterial growth in control and C-treated soil (T2) started, in general C-treated soil had a higher bacterial growth than control. The growth pattern in C-treated soil (T2) was however different from T1 soil, it peaked just before 100 h to 90 Leu g-1h-1 and then fell 30 Leu g-1h-1 at later stages.

T1 fungal growth graph (Fig. 2C): immediate fungal growth was observed in the control type 1 soil, this growth peaked to 125 pmol Ace-in-erg g-1 h-1 after 6 h. The fungal growth in control then fell to ca. 70 poll Ace-in-erg g-1 h-1 and was kept around that level throughout the remaining time, with an exception at 140 h were the level fell to 14 pmol Ace-in-erg g-1 h-1. Comparing fungal growth in bronopol-treated and cycloheximide-treated soil (T1), growth was more delayed in C-treated soil in the beginning and was much lower at the end, however they still had a similar peak 41 h around 130 pmol Ace-in-erg g-1 h-1 which was their respective highest growth.

T2 fungal growth graph (Fig. 2D): a more delayed overall growth and all treatments peaks at a later timepoint compared to T1 fungal growth (Fig. 2C). B-treated soil (T2) had in general lower fungal growth compared to C-treated soil (T2) as well as to the control soil. C-treated soil (T2) had a high growth peak reaching 200 Ace-in-erg g^{-1} h^{-1} at ca. 70 h, this growth rate was 10+ times higher than fungal moist control growth rate. The fungal moist control values in type 2 soil (Fig. 2D) was ca. one third of the growth rates of type 1 soil (Fig. 2C). Control soil had a much better growth until ca. 40 h compared to the treated type 2 soils.

T1 respiration (E): the control (T1) showed two respiration peaks one early at 8 h reaching 8.5 µg CO_2 g^{-1} h^{-1} and one around 50 h reaching 7.3 µg CO_2 g^{-1} h^{-1}, the control respiration values are above the values for the moist control. C-treated soil (T1) has also 2 peaks, the first one being smaller than in control soil and the second peak appearing later at ca. 68 h. In B-treated soil (T1) only one clear peak at 27 h

was observed, in later stages the respiration rate falls below moist control values and is the lowest among the type 1 soil samples.

T2 respiration (Fig. 2F): in control soil (T2) two respiration peak was observed, with the second peak very similar to the second peak in T1 respiration graph (Fig. 2E). The first peak is different, it peaks around 27 h reaching 5.2 µg CO_2 g^{-1} h^{-1}.

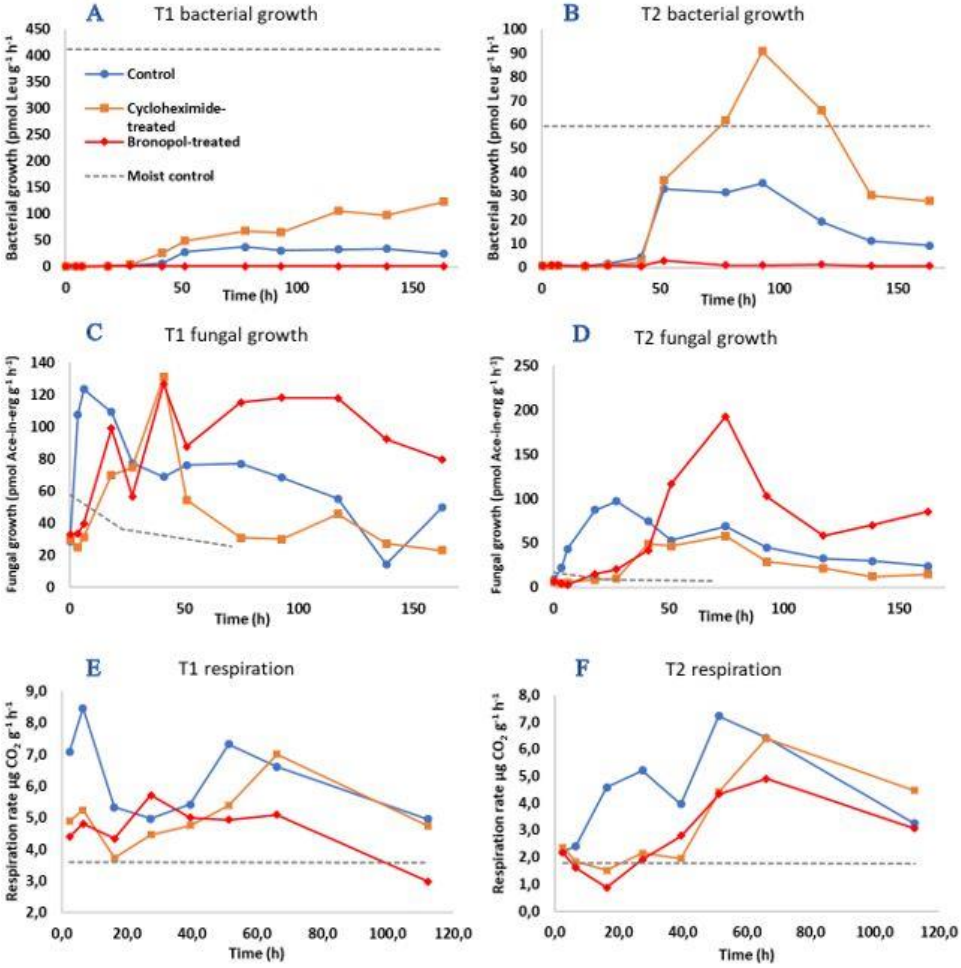

Figure 2 D/RW graphs

Graphs A and B are bacterial growth rate in T1, T2 soil respectively. Graph C and D are fungal growth rate in type 1 respectivtype 2 soi respevtibekyl. Graph E and F are respiration rate in T1 respective T2 soil. Each data point are single values since there is no duplicates. Dashed line represents average rate in a non D/RW T1 and T2 soil, used as reference value and for comparison with D/RW responses. No replicates thus no error bars.

In C-treated T2 respiration the respiration fluctuates around 2 µg CO_2 g^{-1} h^{-1} until 40 h where it starts to increase and peaks at 66 h to 6.4 µg CO_2 g^{-1} h^{-1} with followed slow respiration decline towards the end, it end up with the highest respiration among T2 soils. In B-treated T2 respiration have a decreasing respiration early on and increases at later stages where it peaks at 66 h to 4.9 µg CO_2 g^{-1} h^{-1} with followed slow respiration decline towards the end (112 h). A lower respiration was observed in the T2 moist control graph showing 1.8 µg CO_2 g^{-1} h^{-1} compared to 3.6 µg CO_2 g^{-1} h^{-1} in T1.

Table 3 Soil properties upon D/RW
No duplicates.

		Type 1 soil		Type 2 soil	
	treatment	day 2	day 4	day 2	day 4
pH	Control	4.8	4.8	4.9	4.5
	B treated	4.9	4.9	4.8	4.6
	C treated	4.9	4.8	4.7	4.7
EC	Control	405	420	357	357
(µs cm^{-1})	B treated	393	353	348	384
	C treated	334	879	348	697

Soil properties (pH and EC) measured upon D/RW was presented (Table 3), most of the properties didn't chance except for EC in C-treated soil that increased by double in both T1 and T2 soils.

Respiration Correlation

Through looking at the D/RW results it appears that the respiration responses was correlating with bacterial- and fungal growth peaks. To test this, linear functions was used on between respiration timepoints to fit the fungal timepoint, thus getting us corresponding growth rates and respirations. The data was used to plot linear

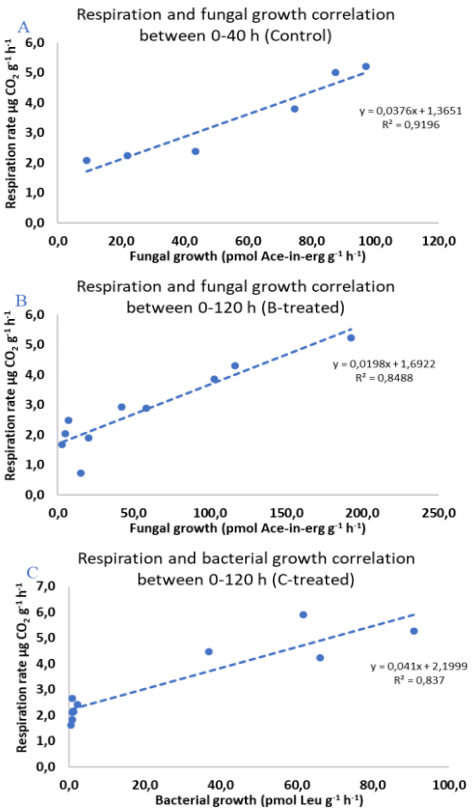

Figure 3 Respiration and growth correlations (A) the correlation between T2 control respiration rate and T2 control fungal growth rate (n=6, linear R^2=0.97, Pearson (r) R=0.96, p<0.01). (B) the correlation between respiration and fungal growth rate in T2 B-treated soils (n=10, linear R^2=0.85, Pearson (r) R=0.92, p<0.01). (C) the correlation between respiration and bacterial growth in T2 C-treated soils (n=10, linear R^2=0.84, Pearson (r) R=0.91, p<0.01).

Correlation is calculated using Pearson Correlation Coefficient Calculator (Retrieved 2019, Maj 22 and June 4). https://www.socscistatistics.com/tests/pearson/default.aspx.

correlation between growth rates and respiration rates between 0 to 40 h (Fig. 3A) and 0 to 120 h (Fig. 3B, 2C) after rewetting. The respiration rate and fungal growth rate of T2 control soils correlates to each other (Pearson (r) R=0.96, p<0.01) from start of rewetting until 40 h after rewetting (Fig. 3A). A correlation was also found (Pearson (r) R=0.92, p<0.01) between respiration and fungal growth rate for T2 B-treated soil (Fig. 3B). Respiration rate and bacterial growth rate in T2 C-treated soil correlates to each other (Pearson (r) R=0.91, p<0.01) until 120 h after rewetting (Fig. 3C).

Discussion

This study showed how bacteria and microbial fungi responds to drying and rewetting under regulated and nonregulated conditions in laboratory settings. We also saw how the soil respiration correlated with microbial responses during this period which could give us ideas for future environmental implementations.

Criteria

In order to address our hypothesis two criteria needs to be met. First the litter addition had an effect on microbial community. The effects of litter addition were clearly shown in the moist controls of bacterial-, fungal growth rate and respiration rate (Fig. 2A-F). Moist control in T1 soil was 6 times higher for bacterial growth rate, 3 times higher for fungal growth rate and 2 times higher respiration rate compared to T2 soil. However, there was no changing in response to drying-rewetting. The bacterial growth rates in the control and B-treated soil between T1 and T2 showed very similar response, they have the same lag period (ca. 20 h) of near zero growth after rewetting to then reach their plateau of growth after approximately 50 h. Thus, we did not see any indication of a shift in bacterial type 2 response towards a type 1 response of immediate and linear growth.

Second criteria were that the inhibitors suppressed the target groups. The intended inhibition by bronopol and cycloheximide was displayed in the results (Fig. 2). In B-treated soils the bacterial growth was very low indicating of an effective inhibition by bronopol. Fungal growth rates were inhibited as well (Fig. 2C, 2D) but not as much. Bacterial growth rate in bronopol-treated soils stayed at bottom close to zero growth rate which was expected and seen in dose-response results (supplementary: Fig. 2A, 2B), since bronopol is an effective bacteria-specific antibiotic (Bailey et al., 2003). Cycloheximide was observed during range finding test to not suppress fungal growth to the same degree as bronopol suppressed bacterial growth (supplementary: Fig. 2C, 2D), which was also shown in the fungal growth graphs (fig. 2C, 2D). Still the suppression was enough to give us a competitive release resulting in a clear increase of bacterial growth in C-treated soils (fig. 2A, 2B).

Bangor soil responses to litter addition

We hypothesized that T1 and T2 soil would show different responses in terms of bacterial-, fungal growth rate and respiration rate during D/RW, the difference would be caused by litter (labile carbon) addition in T1 soil prior D/RW event. More labile carbon was available in T1 soil and this should enhance overall microbial growth and respiration as result of the microbes capitalizing on available resource in response (Bernard et al., 2007; Cleveland et al., 2007). This was verified as T1 soil had higher rates in moist control (Fig. 2A-F). However, our result of litter addition to T1 soil did not shift the bacterial response during D/RW. In contrast our results showed similar bacterial growth in both T1 and T2 control soils (Fig. 2A, 1B). Through this experiment we learned that drying and rewetting process didn't favor or disfavor in that regard fast colonizers, and that there is some other selective pressure leading to a type 1 bacterial response of immediate growth.

Replication of previous drying-rewetting experiment

Our second hypothesis was that we would reproduce the same patterns regarding growth rate and respiration rates in our T2 control soil and bronopol-treated soil. The rates should imitate the previous results (Fig. 4) from Hicks et al., (2019). Since same soil was used any deviation observed could be explained not only by the differences in methods used, but also by difference in soil property or microbial community as a result of prolonged storage in fridge. Soil properties of T2 soil (Table 2 & 3) were similar to the soil properties from the previous study (Hicks et al., 2019). Only difference in methods was the usage of a different sieve size (4 mm) as previously (2.8 mm) to remove unwanted content. The bacterial growth rate results in control and bronopol-treated soil was very similar to previous results (Fig. 2B; Fig. 4), with our control soil having slightly lower moist control values most likely due to depletion of C over time during storage. The fungal growth rate results share some similarities in responses and shapes but with some deviation in the level of growth rates and timepoints for growth peaks (Fig. 2D; Fig. 4).

Our fungal growth rates showed delayed growth peaks in both soils, our fungal growth peak in B-treated soil was higher almost 20-times our moist control level which was even higher than the previous study (Hicks et al., 2019) of 10-times moist control level. The respiration results (Fig. 2F; Fig. 4) showed very different responses. Only similar response found in these 2 graphs is that bronopol-treated T2 soil has reduced CO_2 release, this verifies that enhanced fungal growth in bronopol-treated soil did not compensate for the loss of bacterial growth stated in previous study (Hicks et al., 2019). The respiration rates started at a similar level

that is close to moist control values, control respiration and B-treated soil respiration gave different but expected response (correlates to growth rates) and both after 160 h ended up at a level above moist control level of ca. 1.8 µg CO_2 g^{-1} h^{-1}. However previous respiration results had lower moist control values (ca. 0.3 µg CO_2 g^{-1} h^{-1}) and a different response of declining respiration rates in both soils ending up below the moist control.

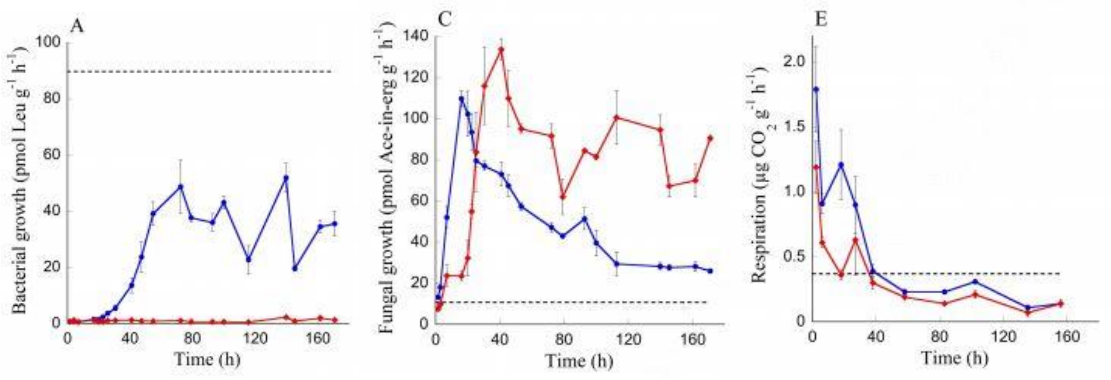

Figure 4 Growth- and respiration rates on soil with type 2 response from previous study (left to right) bacterial growth rate, fungal growth rate and respiration rate in type 2 soil from previous study (Hicks et al., 2019). Blue line represents control and red line represent bronopol treated soil, dashed line presents moist control values.

Our T2 soil displayed a different respiration response compared to the previous study using the same soil. This could imply a difference in the carbon use efficiency (CUE), which can be defined as the ratio of growth to assimilation (Manzoni et al., 2012). A high respiration reflects a high maintenance carbon demand (Anderson and Domsch, 2010), thus we can assume that the extra storage period has driven the soil microbes towards higher maintenance carbon demand and lowered CUE. Development of microbes in ecosystem corresponds to an increase of CUE during the process where the system progresses from a developmental stage towards maturity, when reaching quasi-equilibrium there is low community respiration per unit of biomass (Anderson and Domsch, 1989, 1990). If it´s true we can then assume that the reason for decrease of CUE caused by longer storage indicates changes in microbial community where diversity declined, as the community has to adept to the storage conditions in the cold room.

Further studies are needed to test if other soil types will have a decrease in CUE when exposed to similar conditions. Lower CUE also might be associated with lower quality C (Manzoni et al., 2012), since all easily assessable carbon was

used up during the storage period. From environmental perspective we would like the soil microbial decomposers to display high CUE thus releasing less CO_2 into the atmosphere while building more organic C into the soil (Manzoni et al., 2012).

Bacterial and fungal competition during drying-rewetting event

Our third hypothesis was that inhibition of either bacteria or fungi during D/RW event would cause a competitive release that would benefit the competitor of the inhibited microorganism. This kind of interactions where fungi was constrained by bacteria during D/RW has already been observed in recent study (Hicks et al., 2019). In our study bronopol was selected as bacterial inhibitor and cycloheximide was selected as fungal inhibitor. The dose-response to bacterial and fungal growth was confirmed (supplementary: Fig. 1,2) and the selected doses (111 µg bronopol/g soil, 100 µg cycloheximide/g soil) worked as intended. Both bacteria and fungi showed response that indicates competitive release in soil where the growth of the competitor was suppressed during D/RW (Fig. 2A-2D). Non-targeted effects (suppression) of bronopol on fungal growth have been observed (fig. 2C, 2D), it is believed to have suppressed early fungal growth and early respiration rates in bronopol-treated soils. This fungal suppression was not found at the concentration of 111 µg bronopol g^{-1} soil during dose-response T2 soil day 1 analysis (supplementary: Fig. 2B), making us believe that the sensitivity of fungi to bronopol was increased by D/RW event. The same non-targeted effect was seen in the previous study where an early growth suppression to a less degree was observed in fungal growth rates for B-treated soil (Fig. 4).

CO_2 contribution

The T2 respiration rate (Fig. 2F) could in some cases be correlated to either bacterial- or fungal growth rate (Fig. 3). This displayed wherever it was bacteria or fungus contribution that drove respiration during the different periods after rewetting. The respiration results corresponds well to the growth rates. We can clearly see that bacterial and fungal growth was the main force driving the respiration (Fig. 3). From the control values in figure 2F we can tell that the first respiration peak between 0 to 40 h is result of fungal respiration (linear R^2=0.97, Pearson (r) R=0.96, $p<0.01$), while the second peak is the results of the combined fungal and bacterial respiration. This tell us that fungi community are important at

early stages during D/RW in soil with a type 2 bacterial response, while after ca. 40 h bacteria community will outcompete the fungi community. The second growth peak (fig. 1F) in the control corresponds well with the timepoint where bacteria growth rate reaches its growth plateau at ca. 50 h.

Interesting finding

An interesting finding during D/RW experiment was the EC value of C-treated soil, which increased more than double in C-treated soil between day 2 and day 4 (Table 3). EC is a measurement of ion abundance dissolved in water and an indication of the availability of nutrients in the soil in cases where no salts were added. The EC increase is related to cycloheximide addition since control and B-treated soil didn't show any major changes in EC. There was however no literature found regarding possible explanations.

Looking forward and environmental applications

We saw that soil respiration are stabilizing during later stages after rewetting in the different treatments (Fig. 2F). The resilience of microbial community and its functions in soil could be discussed and exploited for environmental benefits. To evaluate different implementations further studies has to be made and confirmed during those scenarios. Fungi and bacteria have different metabolism and life strategies. Studies have shown that fungal biomass contributes more than bacterial biomass to form organic carbon in soil (Six et al., 2006; Clemmensen et al., 2013). Thus, promoting fungal growth for example by suppressing bacterial growth would promote a better C stabilization in soil and more accumulation of soil organic C (Fig. 2C, 2D) (Bailey et al., 2002). Implementations to for example benefit fungal growth would surely cause economical-, environmental- and ethical debate mainly concerning agriculture and forestry. What such implementations could be will not be further discussed since it is not the aim of the study.

Conclusion

By adding organic matter (beech litter) to Bangor soil a type 2 bacterial response did not shift toward that of a type 1 response. Further D/RW tests by trying other carbon sources or/and with repetitive C addition is however needed to reject this hypothesis.

In this study a soil previously shown to exhibit type 2 bacterial response was used and a type 2 bacterial response was observed together with a pronounced fungal growth response in T2 soil.

The respiration results of our T2 soil showed different responses compared to previous study (Hicks et al., 2019), suggesting that prolonged storage of the soil induced a clear decrease in microbial carbon use efficiency causing more CO_2 to respire after D/RW (0-150 h).

During drying-rewetting event suppressing fungal growth using cycloheximide lead to increased bacterial growth and suppressing bacterial growth using bronopol increased fungal growth, thus confirming the bacterial and fungal interspecies competition.

Respiration peaks were correlated with fungal- and bacterial growth rates, we found that both fungi and bacteria are contributing to the respiration with fungi showing early dominance 0-40 h and bacteria late >40 h.

We believe that regulating soil as a carbon sink by exploiting soil bacterial and fungal interactions could be of environmental benefit and interests. However, more study must be made, and different scenarios must be assessed to validate future implementations.

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Supplementary material

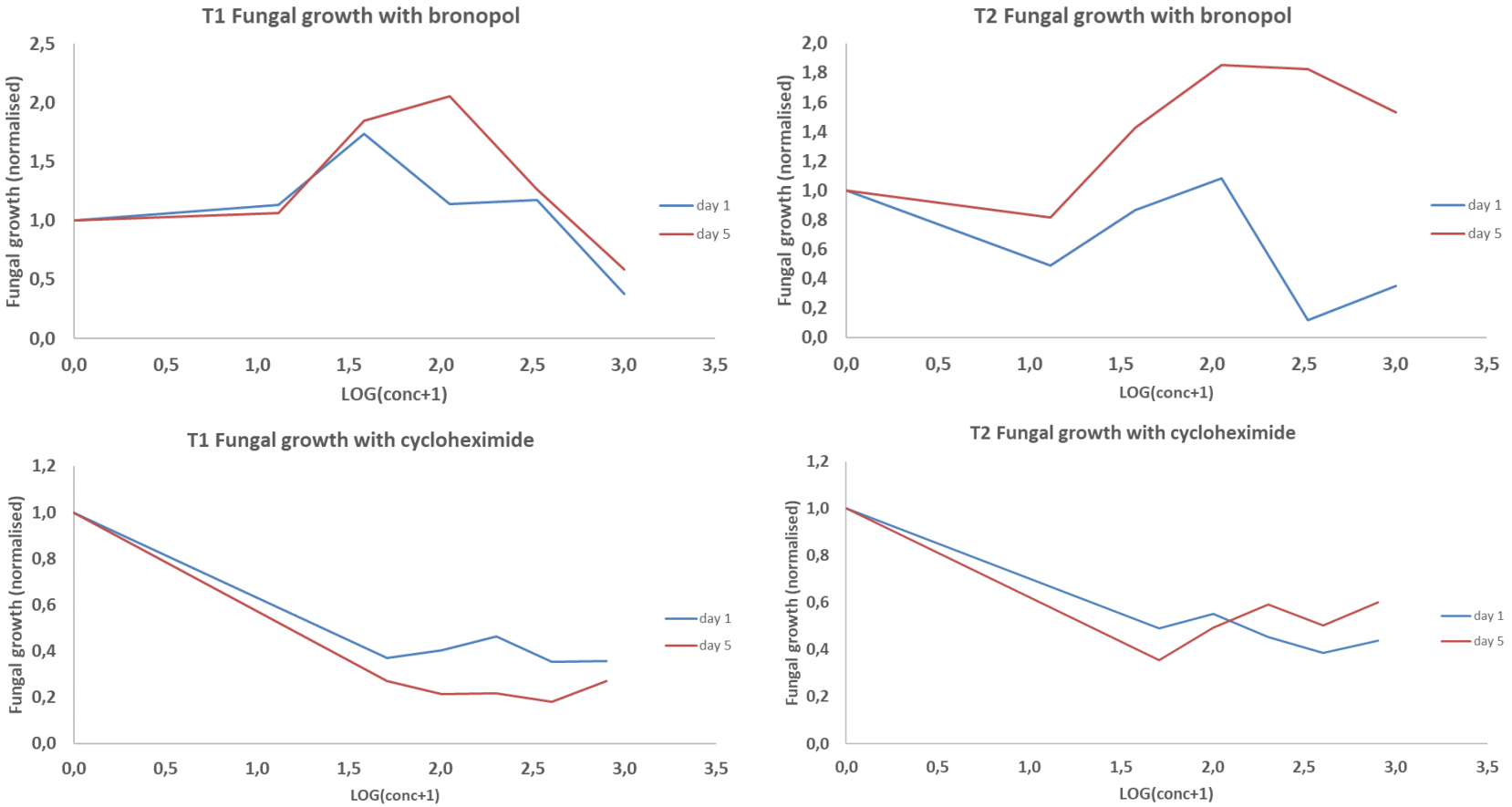

Figure 1 Fungal dose-responses

(A) Fungal growth responses to bronopol concentrations in T1 soil. (B) Fungal growth responses to bronopol concentrations in T2 soil. (C) Fungal growth responses to cycloheximide concentrations in T1 soil. (D) Fungal growth responses to cycloheximide concentrations in T2 soil. Y axis are relative fungal growth rate, X axis are the LOG(conc+1) concentrations of either bronopol or cycloheximide.

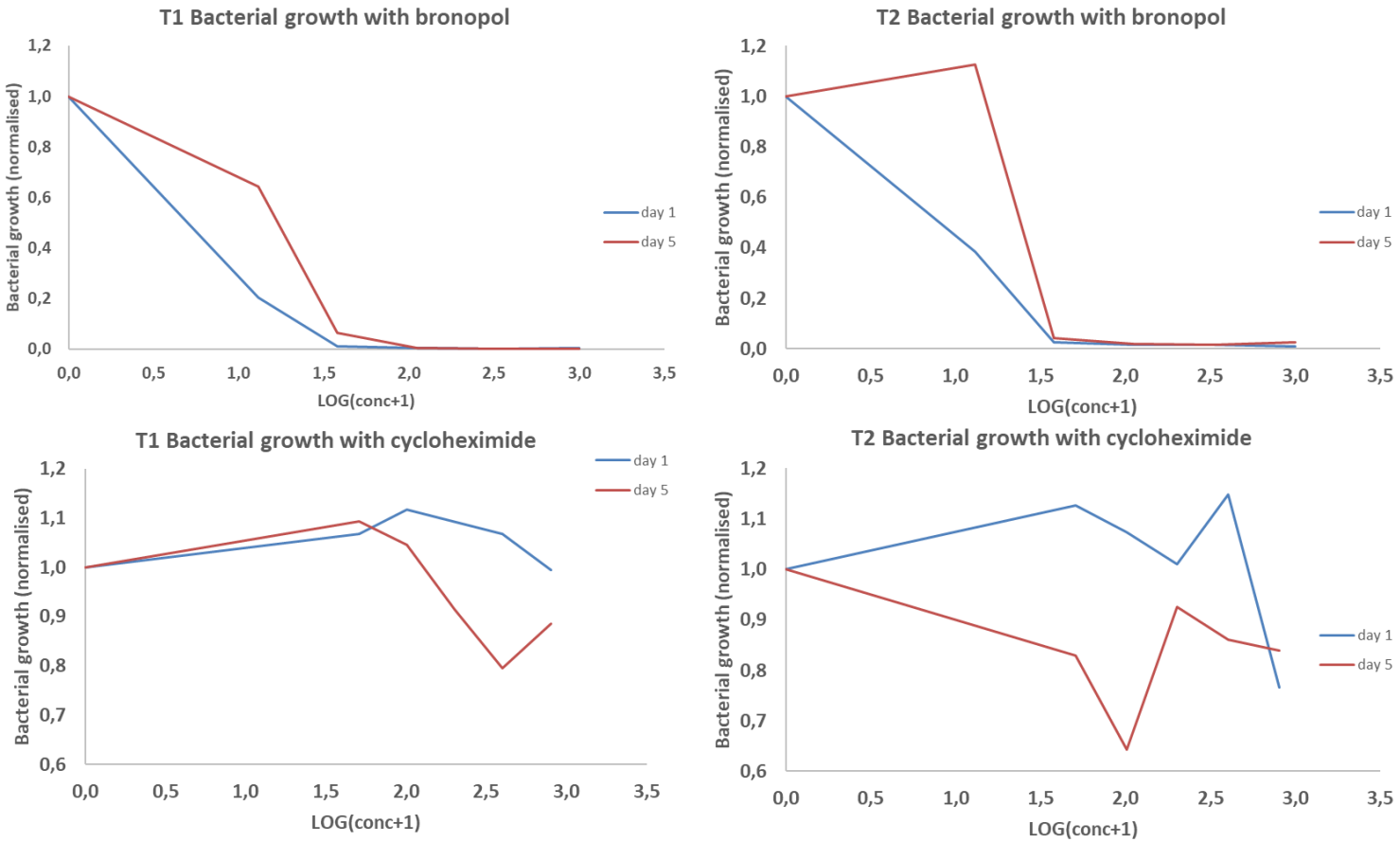

Figure 2 Bacterial dose-responses

(A) Bacterial growth responses to bronopol concentrations in T1 soil. (B) Bacterial growth responses to bronopol concentrations in T2 soil. (C) Bacterial growth responses to cycloheximide concentrations in T1 soil. (D) Bacterial growth responses to cycloheximide concentrations in T2 soil. Y axis are relative bacterial growth rate, X axis are the LOG(conc+1) concentrations of either bronopol or cycloheximide.