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Recovery of forest soil microbial activity after multiyear drought and heavy rainfall event simulations

Master Thesis

By

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Declaration of originality

I hereby declare that the contents of this master's thesis are my own work, supported by the literature references cited and without any assistance from third parties. Furthermore, I confirm that the sources used are acknowledged in the text.

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Abstract

In the context of climate change, an increase in frequency and intensity of severe droughts followed by heavy rainfall events is expected. Soil moisture is one of the major abiotic factors controlling soil microbial activity. Likewise, changes in soil water regime might cause changes in microbially driven processes and in microbial community composition. Although the immediate effects of drying-rewetting cycles have been broadly studied, the long term effects of uneven water availability in natural ecosystems are still uncertain. The aim of this study was to determine the long term effects of repeated drying-rewetting cycles on the forest soil microbial community. For this purpose a field precipitation manipulation experiment was previously performed during 3 years in a pure beech forest (*Fagus sylvatica)*. Two different stress levels were implemented: moderate (MT) and severe treatment (ST) plus a control (CT). During the recovery year, no manipulations were done in the study area and all plots received natural precipitation. In order to see differences between previously treated plots, soil samples were taken regularly from the 3 different treated plots and tested for abiotic parameters (NO_3^-, NH_4^+, DON, DOC, TDN and pH) and biotic parameters (microbial biomass carbon and nitrogen, enzyme activity and PLFAs).

The results showed that 3 years of drying-rewetting cycles have a legacy effect that can be seen 1 year after the cease of the manipulation. Nutrient concentrations showed differences among treatments with higher values of NO_3^- in MT, as well as higher DON and TDN in ST plots. Microbial biomass was higher in the plots under severe stress, while no differences among microbial groups were revealed by the PLFAs analysis. Hence, the results showed that the microbial community has suffered an alteration in its functioning caused by prolonged changes in water availability. At the same time, higher microbial biomass in plots under severe stress indicate that the soil microbial community was able to recover after the cease of the manipulation and even exceed the levels of less stressed plots.

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1. Introduction

The concentration of greenhouse gases (GHG) in the atmosphere has increased in the last centuries due to anthropogenic emissions. Current levels of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have shown an increase since 1750 in 40%, 150% and 20%, respectively (IPCC 2014). The increase of atmospheric GHG concentrations alters the dynamics of natural systems. An increase in the frequency and intensity of extreme weather events, i.e. severe droughts and heavy rainfalls, is expected to occur, causing changes in natural systems (IPCC 2014). Soil is the main reservoir of carbon (C) and nitrogen (N) in terrestrial ecosystems. Microbial nutrient cycling triggers the exchange of GHG between the soil and atmosphere. Turnover of C and N in soils will be affected by the increase of drying/rewetting cycles or modified by water availability due to shifts in precipitation patterns (Borken et al., 1998). As a consequence, changes in precipitation patterns will have a feedback on climate change.

Nutrient cycling in soils is mediated by the activity of the microbial community (Butterbach-Bahl et al., 2011). Likewise, the biochemical processes driven by microorganism are regulated by abiotic factors such as soil moisture and temperature (Franzluebbers et al., 1994). The lack of available water in soils due to prolonged severe droughts will cause a stress on soil microbial communities. Microbial communities will respond to the stress by reducing their activity, being dormant or even dying if the stress situation is too severe (Blagodatskaya & Kuzyakov 2013). Rewetting after drought periods impose a rapid increase of water in the system, producing a stressful situation for microbial communities. In order to survive, microbial communities will have to adapt quickly to the new environmental conditions by osmotic regulation (Mikha et al., 2005). After rewetting, a pulse of N and C is produced by the increased microbial activity and the disruption of previously protected aggregates (Birch 1958).

1.1. Nitrogen cycle

Soils are the main reservoir for N, element that has a key role for terrestrial ecosystems (LeBauer et al., 2008). Nitrogen turnover in soils is mainly controlled by microbial processing through organic matter decomposition (Butterbach-Bahl et al., 2011) (Figure 1). Nitrogen enters the soil phase by either litter decomposition or by fixing organisms that take up N_2 from the atmosphere and reduce it to inorganic forms. Decomposition of organic litter results in an organic N pool in soils, represented by dissolved organic nitrogen (DON). Organic N is reduced to ammonium (NH_4^+) by bacteria through the process known as ammonification or N mineralization (Butterbach-Bahl et al., 2011). Ammonium can be used as an energy source by ammonia-oxidizing microbes resulting in the production of nitrite (NO_2^-) and subsequently converted to nitrate (NO_3^-), process known as nitrification (Jackson et al., 2008). Depending on the nutrient availability in the

environment, ammonium and nitrate can be either taken up by plants or immobilized by microorganisms, however NH_4^+ is mostly preferred by heterotrophic microorganisms as it can be assimilated immediately (Butterbach-Bahl et al., 2011, Schimel et al., 2007). In ecosystems, N can be lost by leaching of nitrite/nitrate due to their hydrophilic form or further reduced to gaseous NO, N_2O and N_2 via denitrification (Butterbach-Bahl et al., 2011). Whether N is found in any of its forms depends on physiological N requirements by plants and microorganisms and environmental conditions (soil temperature and moisture). When the organic N is insufficient to meet microorganisms' nutrient requirements, inorganic N is used from the available pool. This removal of inorganic N (NH_4^+ and NO_3^-) is known as immobilization (Booth et al., 2005).

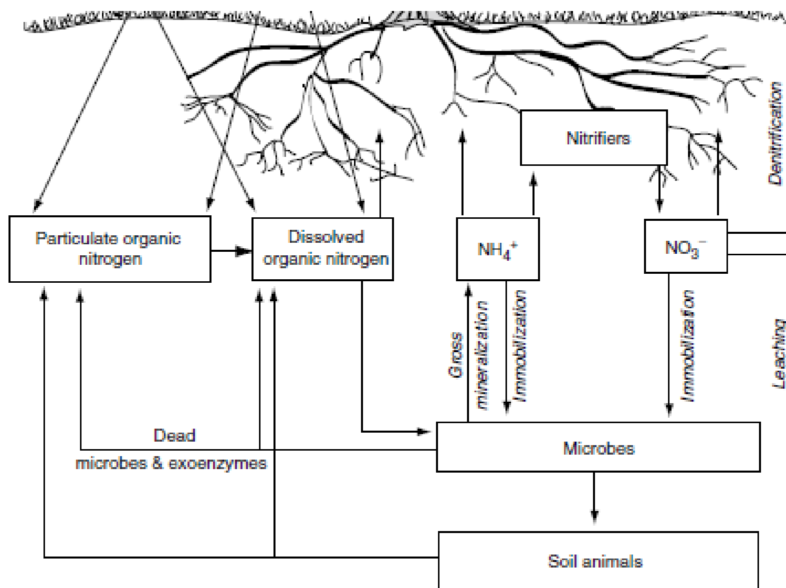

Figure 1*: simplified diagram of the terrestrial nitrogen cycle (from Chapin et al., 2011)*

1.2. Drying and rewetting cycles

Changes in precipitation pattern will modify soil water availability and therefore affect C and N turnover. Soil microorganisms live in water films and balance their cytoplasm water with the soil water phase. When soil is dry, microorganisms have to maintain this equilibrium by dehydration and accumulation of compatible solutes. During dry periods, a reduction in mineralized C and N can be seen compared to moist conditions. This can be due to reduced microbial activity, decreased microbial mobility and/or low nutrient availability, or a combination (Franzluebbers et al., 1994). The rapid rewetting of soils after a drought period involves a readjustment of internal matric potential in microbial cells (Mikha et al., 2005). Hence, the osmotic regulation threshold can be exceeded producing osmotic shock and cell lysis in microorganisms. The shock produced by rapid rewetting can be even more severe than the dry period, as microorganism have a short time to adapt to the water increase. As a consequence, those microorganisms that could not adapt to the new conditions will die and the organic material contained in the cells may be

released into the soil (Fierer and Schimel 2002, Borken and Matzner 2009). This release has the potential to dramatically increase the availability of C and N in the soil, which can be rapidly mineralized by the living community. The pulse of C and N produced after rewetting has been well studied and it is known as the 'Birch effect' (Birch 1958).

Besides the mineralization of released microbial biomass, the C and N pulse has also been assigned to an increased availability of non-microbial substrates after rewetting. Dry and wetting cycles cause physical stress on soil particles which ends up in the disruption of soil miccroagregates (Borken and Matzner 2009). Carbon is stored in soils in form of soil organic matter (SOM) aggregates. This carbon is protected against microbial degradation by chemical and physical mechanisms, however physical disturbances like dry-wet cycles or freeze-thaw events can release the carbon confined making it accessible for microbial degradation (Fierer and Schimel 2002). As a result, C and N mineralization increases during wetting due to the presence of previously unavailable substrate (Borken and Matzner 2009). Furthermore, drying of soils increases the hydrophobicity of soil surfaces (Denef et al., 2011) affecting to the accessibility of organic matter for microorganisms in the short term (Schmitt and Glaser 2011). After two dry-wetting cycles the macro aggregates are more resistant to physical perturbations, thus having no further effects on organic matter (Denef et al., 2001).

In general, wetting of dry soils has been proved to enhance microbial activity in the short term due to the increase in nutrient availability (Birch 1958; Fierer and Schimel 2002).

After some drying-wetting cycles, the size of the pulses decreases. The increase in labile organic matter due to dead microbial biomass and aggregates disruption is limited in time and their concentration decreases as the frequency of drying-rewetting cycles increase. Hence, C mineralization rates have been reported to decrease when increasing the rewetting events (Fierer and Schimel 2002; Mikha et al., 2005). At the same time, a shift in microbial community composition may have happened, altering the previous decomposition rates (Birch 1958; Fierer and Schimel 2002).

Drying-rewetting cycles can also have long term effects on microbial processes. The duration and intensity of drying and rewetting episodes as well as the stress history of the ecosystem play an important role in determining the magnitude of the rewetting CO_2 pulse mediated by microbial activity (Firer et al., 2003).

1.3. Biotic parameters

1.3.1. Soil microbial community

Microbial communities in soils consist of a set of organisms in different physiological states. They are dominated by bacteria and fungi which can be in 4 different states active, potentially active, dormant and dead (Blagodatskaya & Kuzyakov 2013) according to the environmental situation.

The active microorganisms comprise about 0.1-1% of the total microbial biomass in soils. However, there is a fraction of potentially active microorganisms between 10 and 40% of the total microbial biomass which can start growing under favorable conditions (Blagodatskaya & Kuzyakov 2013).

Soil microbial communities are intricately linked to ecosystem functioning, therefore they will be affected by any changes in the environment (Fierer et al., 2003). A rapid response to these changes is required in order to survive. Microbes acclimate to immediate stress by changing the allocation of resources within their cells. In those cases where stress is too extreme, microbial responses vary from dormancy to death (Schimel et al., 2007). Although both responses decrease/supress microbial function from soil microorganisms, better adapted communities will be able to survive in the dormant state and regain activity when conditions improve (de Vries and Shade 2013). Despite the mechanism used to deal with stress, they impose high C and N costs on microbes (Schimel et al., 2007).

Microbial activity is tightly related with soil water content. Drying and rewetting events have been reported to decrease microbial biomass in soils (Gordon et al., 2008; Griffiths and Philippot 2013). Only well adapted microorganism will be able to adapt to changes in precipitation as well as drying-rewetting events. After some time these changes will lead to a community that responds differently to moisture stress (Evans and Wallenstein 2012). The selection for stress tolerant microorganisms take place after a single drying-rewetting event and it has long-term consequences. Hence, changes in the microbial community composition will lead to changes in the community function (Williams and Rice 2007).

Within the microbial community, there are some taxa better adapted to drying-rewetting stress than others. Gram-positive bacteria might better face water stress due to a thicker cell wall and better osmoregulatory capabilities. Conversely, Gram-negative bacteria, with a single layer cell wall, may be more affected by water changes (Fierer et al., 2003; Schimel et al., 2007). Although producing a thicker cell wall is better in terms of survival, it is a costly process in terms of energy. Fungi are also known to be stress resistant due to rapid osmolytes production, a more resistant cell wall and the ability to produce hypha (Fierer et al., 2003). Therefore, fungi and Gram-positive bacteria are usually more favoured in drier than in wetter soils (Zhao et al., 2016). Those

organisms which are not adapted to changes will have to acclimate in order to survive. Acclimation allows microorganism to adjust specific mechanism to the current stress. Whereas resistance is inherent to organisms and imply low energy costs, acclimation requires reallocating energy and nutrients which, might be used for other purposes (Schimel et al., 2007).
In addition, bacterial communities are site dependent therefore not all respond in the same way to drying-rewetting stress. Fierer 2003 showed that after drying-rewetting treatments the bacterial community composition in an oak forest soil was affected but not in grass soils (Fierer et al., 2003). This relates to the stress history due to the fact that bacteria residing in the oak forest soil has been historically less exposed to moisture stress. Furthermore, communities more exposed to extended drought, i.e. in the Mediterranean area, are reported to be more resistant to drought stress than communities present in other ecosystems (Henry 2012).

Changes in microbial community can be seen immediately after perturbations, however adaptations take more time. Lundquist et al., (1999) reported adaptations traits within 3 months of growing season by surface microorganisms. Within a community, responses of different functional groups with different physiological and ecological strategies may be different (Zhao et al., 2016). Likewise, microbial community dominance also fluctuates with seasonality. Hence, it has been seen than summer and winter communities have different physiological capabilities (Koranda et al., 2013).

1.3.2. Enzyme activity in soils

Soil microorganisms need to achieve their metabolic requirements from the transformation of organic substrate present in their living environment. This process is done by the activity of intracellular enzymes as well as by the exudation of extracellular enzymes. Extracellular enzyme activity (EEA) mediates the decomposition of soil organic matter (SOM) by facilitating the breaking down of the complex organic compounds into small assimilable molecules (Caldwell 2005; Sinsabaugh et al., 2008). Likewise, soil enzyme activities are usually related to the chemical composition of SOM and its carbon and nitrogen content (Caldwell 2005). Microbial production of extracellular enzymes has a high nutrient and energy cost, therefore enzyme production only occurs when nutrients and soluble C are scarce (Wallenstein and Weintraub 2008). When a specific nutrient is present in the environment, its associated enzyme production is reduced. Thus, enzyme production strategies may have to minimize carbon and nutrient costs while maximizing associated benefits.
Extracellular enzyme activity can be used as an indicator of microbial nutrients demand and depends on the stoichiometry of microbial biomass regarding to environmental nutrient availability (Waldrop et al., 2000).

The acquisition of nutrients mediated by soil enzyme activities have been related to factors such as soil physico-chemical characteristics, soil microbial community structure, vegetation,

disturbance or succession. Changes in these factors can alter microbial dynamics and in turn change microbial enzyme activities, microbial processes and decomposition of soil organic matter (Schnecker et al., 2014). As a consequence, shifts in microbial communities may happen, having a severe impact at ecosystem level. Some authors state that the ability of soil microbial communities to maintain functional diversity on ecosystems after disturbances could be more relevant to ecosystem productivity than the species diversity itself (Caldwell 2005). In the context of Climate Change, disturbances are expected to happen and might change the enzyme soil pools. Climate effects will not only be seen in short-term changes in activity, but also in long-term enzyme pools changes due to direct effects on microbial production of enzymes (Steinweg et al., 2013; Schimel et al., 2007). Henry 2012 reviewed different climate manipulation experiments concluding that water has the largest effect on the potential activities of hydrolytic and oxidative enzymes. Furthermore, drying of soils due to long summer droughts will reduce the mobility of extracellular enzymes and therefore will impede the acquisition of nutrients (Borken and Matzner 2009).

Although there are many studies relating the EEA as a tool to understand physiological changes of soil community to changes in the nutrient environment, technological limitations and a lack of standardization impede a comparative analysis of the magnitude and distribution of soil EEA. (German et al., 2011).

1.3.3. Phospholipid fatty acids

As most of the soil microorganisms cannot be characterised by conventional cultivation techniques, other methods are needed in order to identify and quantify them. One of the most popular methods is the examination of phospholipid fatty acid (PLFA) from soil microorganism (Frostegård and Bååth 1996). Phospholipids are components of the membrane of all living cells and they can be used as useful biomarkers *(*Zelles 1999; Frostegård and Bååth 1996) because they are degraded fast after cell death, are not present in storage lipids and have a high turnover rate (Piotrowska & Mrozik 2003). Phospholipids consists of a molecule of a 3C glycerol, two of them bonded to two fatty acid chains and one bonded to a phosphate group (Kaur et al., 2005). Due to their unique characteristics, PLFAs have the potential to be used as stress bioindicators. First, they are present in microbial membrane and are sensible to intracellular and extracellular environmental conditions. Secondly*,* responses to environmental disturbance can be seen in changes of the PLFA composition of microbial membrane (phenotypic plasticity) or by altered PLFAs profiles due to shifts in the soil microbial community structure (Kaur et al., 2005). The study of microbial community changes through PLFAs has been extensively reported, i.e. due to heavy metal pollution (Frostegård et al., 1993) or drying-rewetting cycles (Lundquist et al., 1999).

Although the PLFA method has been widely accepted as it is a rapid and inexpensive method to assay the composition of microbial communities in soils, it can be misused (Frostegård et al.,

2011). This method is useful in characterising microbial communities at the phenotypic level, however PLFA profiles do not identify species composition nor microbial biomass. Therefore, the method is useful in characterising changes in communities but not in identifying family groups. Additionally, there is not a clear identification system to correlate determined PLFAs with their correspondent taxa and different authors give different methods of classification (Frostegård et al., 2011; Zelles 1999).

Besides identification of microbial communities, the study of PLFA allows to see environmental stress through PLFA's ratio. For example, the monounsaturated:saturated PLFA ratio has been tied to nutrient availability (Zelles 1999), increasing when increasing organic inputs to soil (Lundquist et al.,1999), whereas ratios of branched chain fatty acids show mechanisms of temperature adaptation (Zelles 1999).

1.4. Ecosystem resilience

Ecosystems have been always under change and constantly adapting to perturbations. Although they are subjected to changes, they tend to remain in a stable state (Shade et al., 2012).These stable states can vary among time, adjusting to the environmental characteristics. Current state of ecosystems not only depend on their ability to thrive under recent conditions but also on historical legacies (Chapin et al., 2011). Legacies are produced by the adaptive capacity of the system after changes over years.

Therefore, ecosystems have to adapt to changes in order to maintain their functioning. Hence, resilience is a key concept in relation to ecosystem responses. Resilience is the ability of the system to sustain its essential function, structure and feedbacks in the face of disturbances (Chapin et al., 2011; Hodgson et al., 2015). It can be seen as the tendency for the system to remain in the same state in face of temporal fluctuations in the environment. Figure 2 represents the concept of resilience with the analogy of a ball in a cup-shaped surface. The range of the environmental and biotic conditions of the system is represented by the basin where the ball is located. If the range is wide, the ecosystem will show high resilience because the system (the ball) remains in the same state (basin) after the perturbation, therefore it will maintain its functions (Hodgson et al., 2015). However, extreme weather events as long droughts or heavy rainfall events cause a notable stress in the ecosystem. If the system is not resilient enough, it will be moved to a new state and will change the former dynamic of the system.

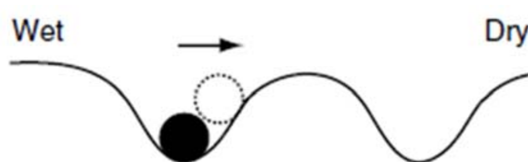

***Figure** 2: representation of the concept of resilience with a ball analogy. Sketch taken from Chapin et al., 2011)*

Diversity in species and populations within functional groups is a key point in order to maintain ecosystem services (Elmqvist et al., 2003). If there is high diversity of functional groups in the system, functionality can be maintained in spite of possible species losses. Belowground communities are dependent on the whole ecosystem community. Plant species diversity positively affect the stability of microbial biomass (de Vries and Shade 2013). At the same time, greater resource availability diversity and heterogeneity within the system will increase community resilience after a disturbance (de Vries and Shade 2013). Therefore, resilience will be enhanced when biogeochemical pools, long-lived organisms and biodiversity are maintained (Gunderson et al., 2006).

2. Objectives and Hypotheses

Changes in the patterns of precipitation noticed by extended summer droughts and heavy rainfall events will have an effect on belowground communities (Fierer 2003). This study investigates the long-term effects of altered precipitation patterns on the soil microbial community of a beech forest. For that purpose, a precipitation manipulation experiment was carried out in an Austrian beech forest during 3 years. Two different drought treatments (moderate stress and severe stress) were applied to different plots. In addition, some control plots that received natural precipitation were set. After the treatment period, no manipulation has been done and the recovery of the ecosystem has been studied. The study presented here is only focused on the period of recovery of the ecosystem.

Therefore, the study aims to answer the following research questions:

1. What are the long-term effects of increased drought-rewetting frequencies on soil nutrient cycling?
2. Will soil microbial communities still be affected after 1 year of recovery?

2.1. Hypotheses

1) Soil nitrate and ammonium concentration will be lower in severely stressed plots than in moderately stressed and control plots

Soil nutrient cycles are tightly related to the abundance of soil microbial communities. At the same time, microbial activity depends on the water availability of the system (Fierer and Schimel 2002). Drying-rewetting cycles cause stress for the microbial community due to rapid changes in water availability (Borken and Matzner 2009).Therefore, it can be expected that communities under severe drought stress will lessen their activity due to the lack of water availability. This shock suffered by microbial communities might be enhanced by a rapid rewetting after a heavy rainfall event. Part of the microbial community might die due to this stress, which consequently will have an effect on nutrient cycling. The effects will be detected by a reduced concentration of nutrients in the soil.

2) Enzyme activity will be higher in severely stressed plots than in the other treatments

In order to acquire nutrients, exoenzymes are produced by microbial communities (Caldwell 2005). Enzyme activity varies according to nutrient availability in soils and microorganisms stoichiometry (Waldrop et al., 2000). Hence, higher enzymatic activity is detected when the presence of nutrients is lower, and it declines once the nutrients requirements are met (Wallenstein and Weintraub 2008). Therefore, we expect that the ratio of nutrient availability and

nutrient demand will be higher in the severely stressed plots thus the enzyme activity will be higher compared to moderately stressed and control plots.

3) Soil microbial population might be lower in severely stressed plots than in moderately stressed and control plots

Extreme weather events shape soil microbial communities by either killing non adapted microorganisms and/or enhancing the population of the well adapted (Borken and Matzner 2009). Water availability determines the activity of the soil microbial community. A decrease of water availability followed by a rapid rewetting causes an osmotic stress in the living microbial community (Fierer and Schimel 2002). Hence, we expect that under severe stress the microbial community might have decreased due to the altered water availability.

4) Microbial community composition will not be different among the different treatments

Soil microbial communities are composed of different microbial taxa. Microorganisms need to adapt to changes in the environment in order to survive (Schimel et al., 2007). Soil moisture is one of the most important factors determining microbial survival (Gordon et al., 2008). Repeated drying-rewetting cycles have been seen to stimulate a more drought tolerant community (Fierer et al., 2003; Fuchslueger et al., 2016). It is expected that one year after 3 years of repeated drying-rewetting cycles, the soil microbial community composition present in the study site might not show differences among the taxonomic groups studied.

3. Material and methods

3.1. Site description

The study has been conducted in the University forest of the University of Natural Resources and Life Science (BOKU, Vienna). The forest is located in the Rosalia Mountains in Southeastern Austria (47° 42' 26'' N / 16° 17' 59'' E). The main forest stand type is a mixture of Norway spruce (*Picea abies)*, silver fir (*Abies alba)* and common beech (*Fagus sylvatica)* with some Scot pine (*Pinus silvestris)*. It extends over an area of 1000 ha and elevation ranges from 400 m up to 900 m asl. The experiment was set up in a pure beech stand on a westward slope with an amplitude of 2 ha located at 600 m asl. The mean annual temperature is 6.5 ºC and mean annual precipitation is 796 mm. The soil at the study site can be classified as Podsolic Cambisol. It is composed by an organic matter O-horizon (0-0.07 m), followed by a humic, slightly eluvial Aeh-horizon (0.07-0.25 m), a cambic, slightly humicsesquioxidic Bhs-horizon (0.25-0.50 m) over weathered granitic rock debris (C-horizon, >0.50 m) (Schwen et al., 2014). The mean soil texture in a depth of 0.10-0.20 m is 0.67 kg kg^{-1} sand, 0.24 kg kg^{-1} silt, and 0.09 kg kg^{-1} clay, classified as sandy loam according to the FAO classification (FAO 1990; Schwen et al., 2014). The mean pH within the Aeh-horizon is 3.8.

Figure 3*: Overview of the experimental area*

3.2. Experimental design

During 2013, 2014 and 2015 an experiment with precipitation manipulation was carried out. Roofs out of plastic were installed above the plots treated in order to exclude natural precipitation on those plots. Roofs were installed approximately 150 cm above the ground and sustained by a metallic structure. The simulation of the heavy rainfall conditions was done through an installed automated sprinkler system beneath the roofs.

In order to create drought and rewetting cycles, two treatments were applied: a “moderate stress”-treatment and a “severe stress”-treatment. The first one received 8 drought-stress cycles from end April until end October, yielding 8 drought periods of 4 weeks each. The severe treatment had 4 drought periods of 8 weeks each. Moreover, control plots were established and they received the natural throughfall. Moderately stressed plots received 75 mm of water at each irrigation time whereas severely stressed plots received 150 mm. Overall, the same amount of water (600 mm) was used in the severe and moderate treatment and it was calculated from the long-term average precipitation of the study site.

In total 12 plots were set up, resulting in four replicate plots for each treatment and control. The size area of the plot was 4x4 m and they were aligned with 4 m distance to each other. To minimize boundary effects, plots were at least at a distance of 2m from large trees (See *Figure 4*).

Roof panels were removed in October to enable undisturbed leaf litter fall and snow fall to the forest floor in the exclusion plots. In addition, gas chambers were located on top of each plot to have a continued measure of the greenhouse gases produced.

Above each plot, trenches (40 cm deep) filled with gravel and a plastic canvas were installed in order to minimize slope-downward water flow.

2016 has been the recovery year, therefore all the roofs had been removed from all plots. No precipitation manipulation treatments were conducted and all the plots received the same amount of water via natural rainfall.

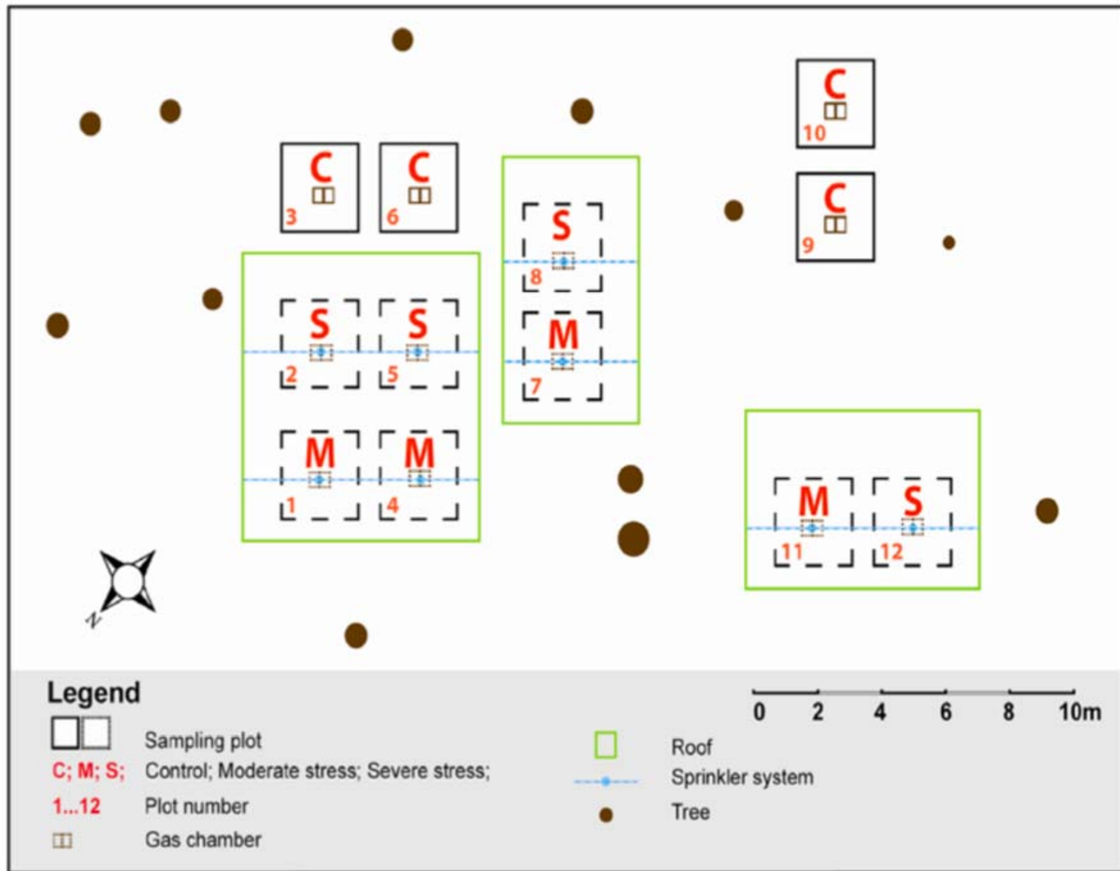

Figure 4*: plot layout of the experimental set up*

3.2.1. Sampling

Soil samples were taken every 4 weeks during the period May-October, following the same date-scheme as previous years. The first soil sampling was called harvest 22 (H22) and the last one harvest 28 (H28).

The sampling of soils was carried out with a soil auger (approx. 10 cm sampling depth, width: 1.75 cm) at each plot. A metallic grid was used to select the sampling points according to a previous scheme. For each plot, soil cores were taken at 3 different spots in the grid and put together in one sampling bag, being considered one sample. Litter lying on the floor was removed prior to the soil excavation. Afterwards the holes were filled with sand and the removed litter was put back on the bare soil.

Samples were labelled according to the harvest number (H22-H28), and plot number (1-12). Samples were taken to the laboratories of the University of Natural Resources and Applied Life Science (BOKU, Vienna).

Moreover, abiotic parameters such as temperature, moisture and precipitation were continuously measured via data loggers. The equipment was already installed at the study site and data was provided by the site management.

3.2.2. Sample preparation and storage

Soil samples were sieved with an analytical sieve (Retsch, DIN ISO 3310-1) of 2 mm mesh-size according to ÖNORM L 1060 (2004). After sieving, samples were stored in the fridge at 4 ºC until the following days where the analyses were performed. Soil samples used for the PLFA analyses were freeze dried after sieving and kept in the freezer at -20 ºC.

To simplify the analysis of the data, harvests were renamed after the month when they were taken:

Harvest	Date of sampling	Renamed as
H22	2/05/2016	Early May
H23	30/05/2016	May
H24	27/06/2016	June
H25	25/07/2016	July
H26	22/08/2016	August
H27	19/09/2016	September
H28	17/10/2016	October

Table 1*: Name assignation to the sampling points*

3.3. Analyses overview

3.3.1. Analysis of NO_3^- and NH_4^+

Nitrate (NO_3^-) and ammonium (NH_4^+) were measured photometrically with an Enspire® Multimode Plate Reader (Perkin Elmer) at absorption spectra of 540 nm for nitrate and 660 nm for ammonium (Schinner et al., 1996).

For the analysis, 2.5 grams of sieved soil were extracted with 25 ml of 1 M potassium chloride (KCl). Samples were then shaken at a GFL 3015 shaker for half an hour at room temperature and afterwards filtered gravimetrically with ashless filter paper (Whatmann ™ #40). Extracts were stored at 4 ºC until the next day when the analysis was performed.

Nitrate was analyzed by the Griess method by reducing it by Vanadium (III) chloride in hydrochloric acid solution to nitrite. The nitrite concentration was coupled with the Griess reaction and the absorbance measured (Hood-Nowotny et al., 2010).Reaction was obtained by pipetting 100 µL of sample extract or standard solution, 100 µL of Griess reagent and 100 µL of

Vanadium(III) chloride solution into a microplate. The Griess reagent was a mix of equal volumes of N-(1-Naphtyl) ethylendiaminedihydrochloride solution and sulphanilic acid solution. Before the measurement the microplate was incubated at 37 °C for 30 minutes.

Ammonium concentration was determined based on the reaction of sodium salicylate with ammonium in the presence of sodium dichloroisocyanurate acid. As a result, a green idophenol molecule was formed. The reaction was enhanced by the addition of sodium nitroprusside as catalyst. 200 µL of sample or standard solution, 40 µL of sodium salicylate solution and 60 µL of oxidation reagent (dichloroisocyanurate acid) were pipetted into a microtitier plate. The plate was incubated for 30 minutes at room temperature and then measured.

For the calculation of NO_3^- and NH_4^+, the absorbance concentration was reduced by the blank absorption. Concentrations were calculated per dry mass (see Eq 1a-c).

Eq. 1a $\quad$ *cs=(absorbance – blank absorbance) * 1 / k*

Eq. 1b: $\quad$ $NO_3^- - N\ (\mu g\ g^{-1}\ dm) = cs * V / dw$

Eq. 1c: $\quad$ $NH_4^+ - N\ (\mu g\ g^{-1}\ dm) = cs * V / dw$

K ...slope of the calibration curve

cs …sample concentration

V…extraction volume (25ml)

dm … sample dry weight (g)

3.3.2. Microbial biomass carbon and nitrogen

Microbial biomass carbon (Cmic) and microbial biomass nitrogen (Nmic) were measured using the chloroform fumigation technique (Schinner et al., 1996). Soil samples (2 g) were placed in aluminum cups and fumigated with chloroform ($CHCl_3$) in an evacuated glass desiccator for 24 h at room temperature. After the removal of the $CHCl_3$ by subsequent vacuum cycles, soluble C was extracted from the fumigated and non-fumigated samples with 1 M KCl for 30 minutes in a horizontal shaker. Extracts were filtered (Whatmann ™ #40) and analyzed for Total Organic Carbon (TOC) and Total Nitrogen (TN) at the Shimadzu TOC/TN analyzer. The analysis is based on the combustion of the extracts at 680 °C and measure of the produce CO_2 with a non-dispersive infrared sensor (NDIR). In order to obtain Microbial biomass C and N, the values obtained from the fumigated samples were subtracted to the values obtained from the KCl extracts performed in the nitrate/ammonium analysis. A proportional factor of 0.35 and 0.54 for Cmic and Nmic respectively was applied to correct for the microbial biomass C and N that was mineralized within the 24h of chloroform fumigation (Schinner et al., 1996).

3.3.3. Soil pH

Soil pH was measured via desorption of protons in the soil solution with a 0.01 M $CaCl_2$ solution. 1 g of sieved soil was put into a plastic beaker and filled up with 10 ml of 0.01 M $CaCl_2$ solution. Samples were placed in the horizontal shaker at room temperature for 30 minutes. The pH was measured with a calibrated pH-meter (Mettler-Toedo, SevenGo DuoTM SG23) at room temperature (ÖNORM L 1083 2006).

3.3.4. Gravimetric water content

Gravimetric water content was determined according to Schinner et al., (1996). 1 g of sieved soil was placed into aluminum cups and weighted. Cups were taken into the oven and dried at 105 °C for 24 hours. After this time, samples were weighted again and the corresponding loss of weight was attributed to the loss of water content.

3.3.5. Enzymes analysis

Potential extracellular enzyme activities were measured fluorometrically and photometrically using a microplate assay. All activities were measured within 48-72 h after sampling of soils, however the analysis had to be repeated for H22 and H23 with frozen soil. 1 gram of sieved soil was suspended in 100 ml of sodium acetate buffer (100 mM, pH 3.8). Samples were homogenized with an ultrasonicator (Bandelin Sonoplus HD 2200, 10 % power) for 40 seconds.

For each enzyme, an appropriate substrate was used. In the study, 7 different enzymes were analyzed: Cellobiohydrolase, ß-Glucosidase, Exochitinase, Phosphatase, Protease, Phenoloxidase and Peroxidase. For the fluorometric assay the following substrates were used respectively: 4-Methylumbelliferyl ß-D-cellobioside, 4-MUF- ß-D-glucopyranoside, MUF-N-acetyl-ß-D-glucosaminid, MUF-phosphate and Leucina-aminomethylcoumarin for the first five enzymes mentioned above. 200 µl of soil suspension and 50 µl of substrate were pipetted into black microtitier plates in three analytical replicates. Moreover, different concentrations of the buffer+substrate were pipetted to obtain a calibration curve. Plates were incubated between 140-200 min at 20 °C in the dark. Before measuring, 10 µl of sodium hydroxide (1 M NaOH) was added to the first 4 enzymes. Fluorescence was measured with a fluorescence spectrophotometer (Perkin Elmer Enspire Plate reader) at 450 nm emission at an excitation at 365 nm and 30 flashes.

Phenoloxidase and peroxidase activities were measured photometrically. 0.9 ml of soil suspension was mixed with 0.9 ml DOPA (3-(3,4-Dihydroxyphenyl)L-alanine,20 mM, final concentration; 10 mM). Samples were shaken for 10 minutes and centrifuged (5 minutes, 5000 rpm). Aliquots were pipetted into microtitter plates (six analytical replicate per sample). Half of the

wells additionally received 10 µl of H_20_2 (0.3%) for measurement of peroxidase. Absorption was measured at 450 nm at the starting time and after 20 hours of incubation at room temperature. Calculations were performed according to German et al., 2011.

3.3.6. Phospholipid fatty acid (PLFA) analysis

PLFAs were extracted from freeze dried soil according to the Bligh and Dyer technique (Bligh and Dyer 1959; Frostegård 1996). 2 g of freeze dried soil was with 6 ml of Blight and Dyer solution (chloroform:methanol:citrate buffer 1:2:0.8), 10 µl of internal standard 1 (C:10, 40 µg/ml), wrapped in alum foil and shaken for 2 hours at room temperature. Then samples were centrifuged and the upper liquid phase was transferred into new tubes. 2 ml of $CHCl_3$ and 2 ml of citrate buffer (0.15 M, pH 4.0) were added and samples were left overnight for separation. After drying, samples were re-dissolved with $CHCl_3$ and the non-polar phase was collected and fractioned into a silica solid phase extraction column (Isolute Si 500 mg 3 ml) by 5 ml of chloroform, subsequently 10 ml of acetone was added in order to get rid of glycolipids. Finally PLFAs were collected by adding 5 ml methanol to the silica column.

The phospholipids were methylated with 1 ml of methanol:toluol (1:1) solution and 1 ml of methanolic KOH. Samples were incubated at 35 °C for 15 minutes and then left to cool down. Subsequently 2 ml of Hexane:Chloroform (4:1), 0.3 ml of 1 M acetic acid and 2 ml dH_2O were added to each sample. Samples were centrifuged and the upper part transferred to a new tube. After drying, PLFA extracts were redissolved with hexane and 100 µl transferred into a GC glass vials. In addition, 10 µl of the Internal Standard (nonadecanoic methylester) were added.

The PLFAs in hexane were then analyzed by gas chromatography with a HP 6980 accompanied by HP-5MS column and detected by flame ionizator detector (FID). Bacterial acid methyl esters (Supelco Bacterial Acid Methyl Ester CP Mix# 47080, Sigma-Aldrich) and Component FAME Mix (CRM47885, Sigma-Aldrich) were used as external standard for comparing peaks and identifying PLFAs by retention times.

PLFAs nomenclature was used as described by Frostegård 1991. They are named by the total number of carbon atoms: number of double bonds, followed by the position of the from the methyl end of the molecule. Suffix “c” states for cis while “t” states for trans configuration. “I” indicates iso and “a” indicates anteiso; “me” indicates midchain methyl branching and “cy” cyclopropyl ring structure. Total bacterial biomass was calculated as the sum of Gram+ bacteria (i15:0, a15:0, i16:0, i17:0) (Hardwood and Russel 1984), Gram- bacteria (15:1ω5, 16:1ω7, cy17:0, 17:1ω7, cy19:0)(Wilkinson 1988) and bacteria (14:0, i14:0, 14:1, 15:0, 16:0, i17:0, 18:0, 20:0) (Frostegård, et al., 1993). Fungal biomass was calculated as the sum of 18:1ω9, 18:3ω3, 18:2ω 6, 9 and 18:2ω6c (Zelles, 1999).

3.4. Statistical analysis

The statistical processing was done with Rstudio. The data set was tested for normality and homogeneity of variance with Shapiro-Wilk-Test and Levene test respectively (car package). Once these 2 prerequisites were checked, a repeated measures analysis of variance, ANOVA, was performed. The drought treatment applied and the different harvests were taken as variables. Harvests were also included in the analysis as the time points when the same soil plots parameters were measured. Furthermore, significant relationships between variables were obtained by a correlation analysis.

Differences between treatments and harvest were tested by a post-hoc Tukey test at a confidence interval of 95%.

Graphical output was done by SigmaPlot 12.0.

4. Results

4.1. Abiotic soil parameter

4.1.1. Nitrate and Ammonium

Nitrate concentrations ranged between from 0.14 to 2.59 µg NO_3^- g^{-1} dw over the observation period and differences between harvests were observed. Moderate treatment concentration (MT) had the highest values compared to the other treatments and it was significantly higher than CT (p-value = 9.6 e-05) and ST (p-value = 0.02), with a peak in early May (2.59 µg NO_3^- g^{-1} dw). Concentration in MT at the beginning of the sampling period was almost 3 times higher than in the other treatment but suffered a decrease in May being closer to the other 2 concentrations. Afterwards all the treatments followed the same trend with some fluctuations among the sampling period.

Ammonium concentrations ranged between 3 µg NH_4^+ g^{-1} dw and 27 µg NH_4^+ g^{-1} dw and showed significant differences between harvests. The values of the 3 treatments tended to follow the same trend, with low values in early May, a peak in May, slightly decrease until July and almost stable values from August until October. No significant differences were found between treatments .ST had its highest value in May (27.42 µg NH_4^+ g^{-1} dw) and the lowest in October (4.73 µg NH_4^+ g^{-1} dw). MT concentrations oscillated around ST and were only higher in June and July. CT values were more variable between May and August, however they were stable from August until October.

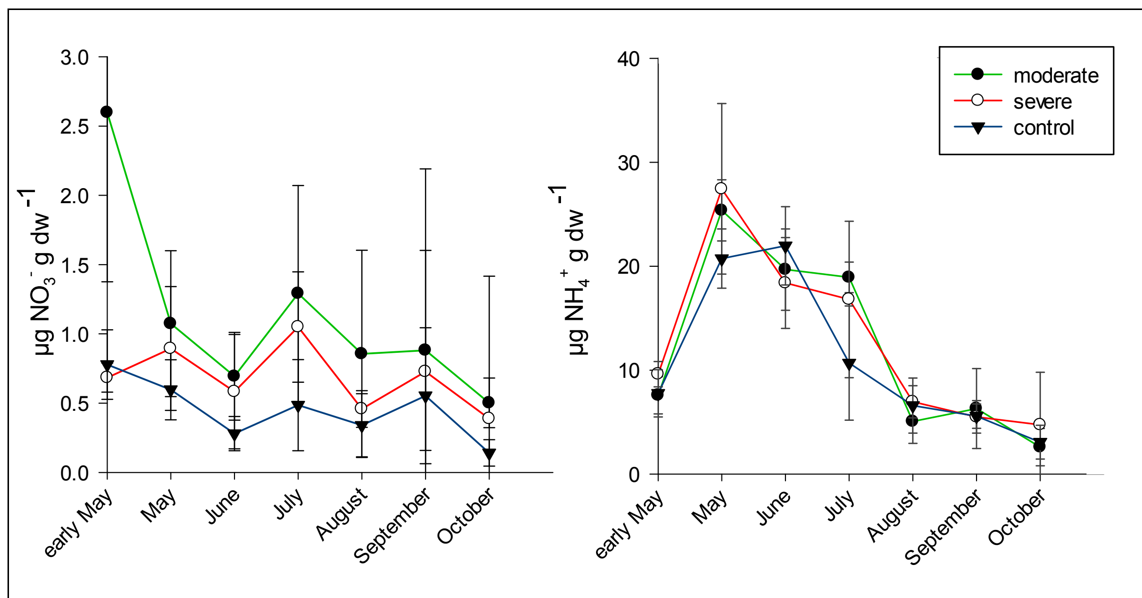

Figure 5: Mean (±SE) values of nitrate (NO_3^-) and ammonium (NH_4^+), over the observation period for the experimental treatments.

4.1.2. Total organic carbon, total organic nitrogen and dissolved organic nitrogen

DOC concentration ranged between 0.07 and 0.28 mg C g^{-1} dw over the observation period, leading to significant differences between harvests. All 3 treatments started with similar concentrations in early May. ST concentration was the highest, with a maximum of 0.28 mg C g^{-1} dw in May, until August. In September it experienced a sharp decreased (0.07 mg C g^{-1} dw) and increased slightly in October. MT and CT followed the same trend with peaks in May and August. Whereas CT decreased at the end of the sampling period, CT stayed in the same levels.

For DON values ranged between 0.01 and 0.04 mg N g^{-1} dw over the observation period and significant differences between harvests were noted. All treatments started with similar concentrations in early May and experienced an increase in May followed by a strong decrease in June. In these months ST concentrations were markedly higher. From July until the end of the harvest period values were more similar. Significant differences were found between ST-CT (p-value = 0.01) and ST-MT (p-value = 0.024).

TDN concentrations ranged between 0.02 and 0.07 mg N g^{-1} dw over the observation period and significant differences between harvests were seen. Concentrations in early May ranged from 0.19 to 0.26 mg N g^{-1} dw and increased 2-3 fold in May. In June, all treatments experienced a decrease followed by a small peak in July. Values were decreasing slightly until October. Concentrations of TDN were also higher in ST plots: significant differences were found between ST-CT (p-value = 0.016) and ST-MT (p-value = 0.075).

4.2. Biotic parameters

4.2.1. Microbial biomass carbon and nitrogen

Values of microbial biomass carbon ranged between 0.4 and 1.7 mg g^{-1} dw over the observation period and differences between harvests were observed. Concentrations were significantly higher in ST plots (ST-CT p-value= 0.02, ST-MT p-value=0.04). In early May values ranged between 0.6-0.9 mg g^{-1} dw and experienced an increase in June, with values up to 1.7 mg g^{-1} dw. Values decreased until August and increased again in September (1-1.3 mg g^{-1} dw). Finally values dropped down in October.

Microbial biomass nitrogen ranged between 0.05 and 0.18 mg g^{-1} dw over the observation period leading to significant differences between harvests. ST presented the highest concentration among the observation period and showed significant differences between ST-MT (p-value = 0.005) and ST-CT (p-value = 0.03). At the beginning of the sampling period the concentration in all treatments ranged between 0.07-0.10 mg g^{-1} dw. After a small decrease in May, Nmic

concentration reached its maximum in June followed by a strong decrease in July. Concentrations slightly increased in August and finally dropped down in October.

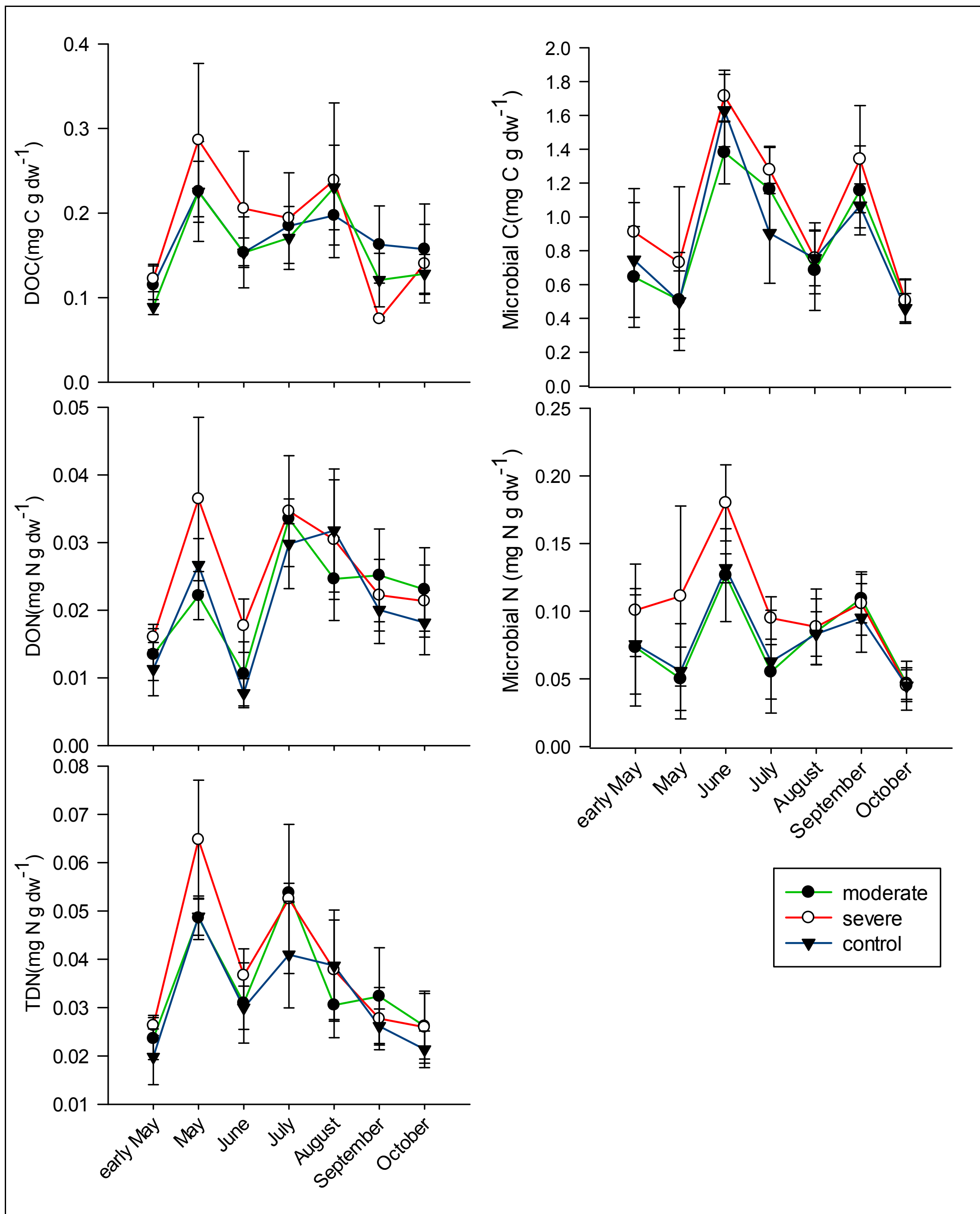

***Figure 6**: Mean (±SE) values of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), total dissolved nitrogen (TDN), microbial biomass carbon (Microbial C) and microbial biomass nitrogen (Microbial N) along the observation period for the experimental treatments.*

4.2.2. Enzyme activities

The activity of Cellobiohydrolase ranged between 11 and 94 nmol g^{-1} h^{-1} over the observation period and significant differences between harvests were observed. Activity was lower in June and July than in the other harvests. The levels in ST were always higher being significantly higher than CT (p-value = 0.075). Its maximum value was in October (94.24 nmol g^{-1} h^{-1}) and the minimum in June (27.74 nmol g^{-1} h^{-1}). MT had always higher values than CT except in August.

ß-Glucosidase activity ranged between 310 and 1953 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. Values were between 310 and 500 nmol g^{-1} h^{-1} for all harvests except in June and July when values were 2-4 times higher. Activity was always higher in ST with significant differences (ST-CT p-value = 0.064, ST-MT p-value = 0.073).

Chitinase activity ranged from 300 and 972 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. Lower activity was observed in June and September, without substantial differences between treatments. In July and August higher activity was found in ST however in October higher activity was found in MT.

Phosphatase activity ranged from 1445 to 6771 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. Higher activities were detected in early May and May followed by lower activities in June-August. For the last months of the observation period, September and October, the activity increased again.

Protease activity ranged between 20 and 171 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. Activity was higher in the CT plots than in the other treatments, resulting significantly higher than MT (p-value = 0.033). All three treatments followed a similar trend, starting in early May around 90 nmol g^{-1} h^{-1}. ST and MT had a small peak in May, followed by a decrease in June and reached their maximum value in July. In the following months they showed a gradual decrease until their minimum value in October.

Peroxidase activity ranged from 0.7 to 3.5 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. The highest activity was observed in early May and May. In June a decrease of the activity was seen (1-1.5 µmol g^{-1} h^{-1}) and those values were kept also in July. August and October showed a slight increase in the activity (up to 2 µmol g^{-1} h^{-1}).

Phenoloxidase activity ranged from 0.09 to 2.1 nmol g^{-1} h^{-1} over the observation period and showed significant differences between harvests. Lowest values were found in early May followed by an activity increase in May, June and July. In August the activity declined again but in September the activity reached its maximum value up to 2 .1 µmol g^{-1} h^{-1}. Finally in October values dropped off. CT was significantly higher than MT (p-value = 0.00023) and ST (p-value = 0.076).

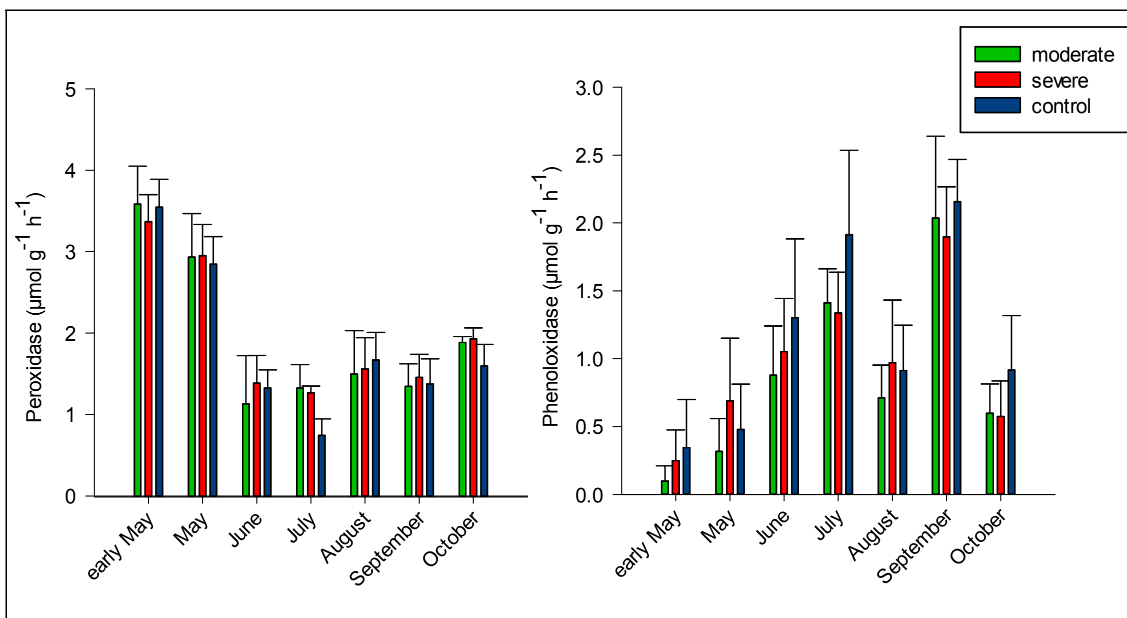

Figure 7: *Mean (±SE) values of enzymatic activity for Peroxidase (left) and Phenoloxidase (right) over the observation period for the experimental treatments.*

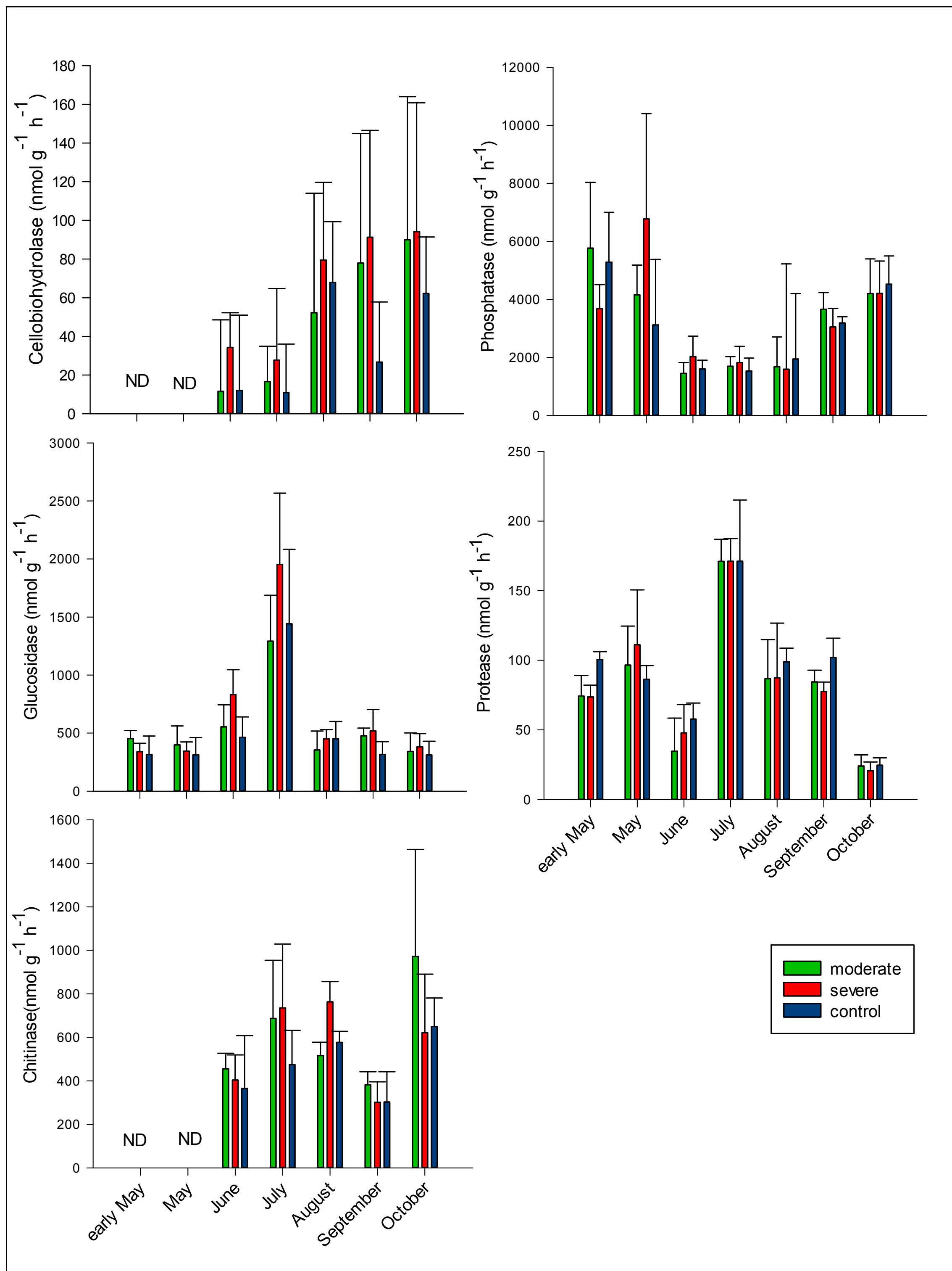

Figure 8*: Mean (±SE) values of enzymatic activity for Cellobiohydrolase, Glucosidase, Chitinase, Phosphatase and Protease, over the observation period for the experimental treatments.*

4.2.3. Phospholipid Fatty Acids

Gram-positive bacteria markers ranged between 1.22 to 2.69 nmol g^{-1} over the observation period and showed significant differences between harvests. Values were very similar for the period early May-July followed by a slight decrease was observed.

Gram-negative bacteria markers ranged between 0.56 to 1.16 nmol g^{-1} over the observation period and significant differences between harvests were noticed. Values were in the same range between early May and June, followed by a decrease in the last months of the sampling period. Notable was the high concentration of CT in September.

Bacteria markers ranged between 1.96 to 6 nmol g^{-1} over the observation period and significant differences between harvests were noticed. Values during the period May-July were the highest and very similar among treatments. In August all the treatments values dropped off to 2 nmol g^{-1}. In September there was a slight increase and finally in October values decreased.

Fungi markers ranged from 1.7 to 4.57 nmol g^{-1} over the observation period and significant differences between harvests were noted. The first 4 months of the sampling period showed similar values among treatments. In August the biomass decreased almost twofold, followed by a small increase in September and finally the lowest values were seen in October.

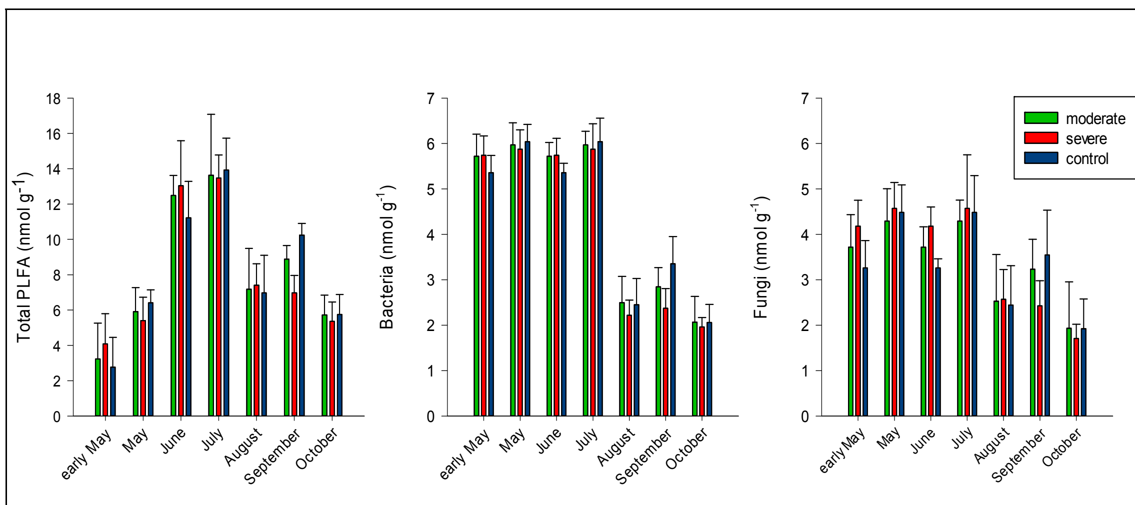

***Figure 9**: Mean (±SE) values of Total Phospholipid Fatty Acids (PLFA) Bacteria and Fungi, over the observation period for the experimental treatments*

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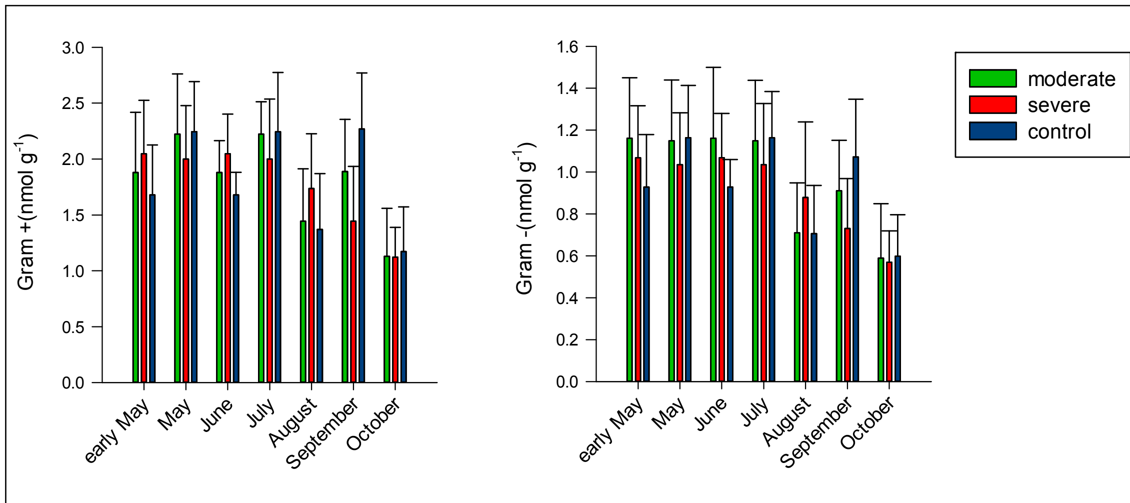

***Figure 10**: Mean (±SE) values of Gram-positive bacteria (Gram+) and Gram-negative bacteria (Gram-), over the observation period for the experimental treatments.*

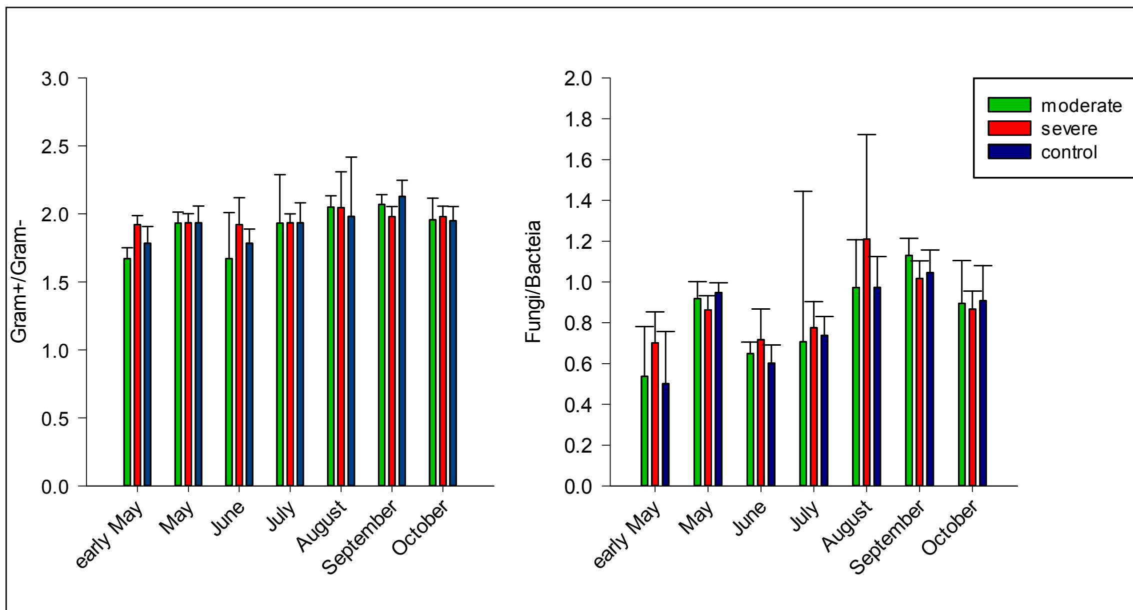

***Figure 11**: Mean (±SE) values of calculated ratios for Gram-positive bacteria and Gram-negative bacteria (Gram+/Gram-) and for Fungi/Bacteria, over the observation period for the experimental treatments.*

4.3. Relationships between investigated parameters

In order to see whether the different parameter studied correlate, a statistical correlation analysis was performed (see Table 2).

Specifically for NO_3^- and microbial biomass nitrogen, statistically significant relationships were found with soil moisture (p-values = 0.0019 and p-value = 0.0050 respectively) (Figure 8). In some cases, the relationships were different according to the treatments.

NO_3^- concentration in soils increased with soil moisture for all treatments (MT: $y = 0.0937x – 1.3852$, $r^2 = 0.178$; ST: $y = 0.0258x – 0.0545$, $r^2 = 0.047$; CT: $y = 0.0442x – 0.7291$, $r^2 = 0.177$) . Most marked increase can be seen in the moderate treatment while severe treatment showed a slow increase of NO_3^- among the moisture values.

Levels of NH_4^+ rose with an increase in moisture except in the moderately stressed plots, where the trend seems to be steady (MT: $y = 0.2786x + 4.4475$; $y = 0.8367x – 10.137$, $r^2 = 8.96\ e^{-4}$; ST: $r^2 = 0.083$; CT: $y = 0.4002x + 0.4405$, $r^2 = 0.023$). Severely stressed plots showed the highest influence of soil mositrure on NH_4^+ concentration.

Microbial biomass nitrogen concentrations also increased with soil moisture. As for ammonium, the strongest influence of soil moisture was seen in the values of severely stressed plots. (MT: $y = 0.0017x + 0.0312$, $r^2 = 0.036$; ST: $y = 0.0019x + 0.0522$, $r^2 = 0.20$; CT: $y= 0.0038x – 0.0215$, $r^2 = 0.095$).

Furthermore, the following relationships between parameters were found interesting:

Soil temperature was related to almost all the parameters studied, specially with the microbial communities identified by the PLFAs analysis (p-value < 0.01).

All enzyme activities except Cellobiohydrolase were related to the microbial groups identified through the PLFA analysis (p-value < 0.05). Moreover, Phenoloxidase and Peroxidase activity had a strong relationship with soil moisture, nitrate and microbial biomass nitrogen, whereas the other enzymatic activities did not show any relationships (p-value < 0.05).

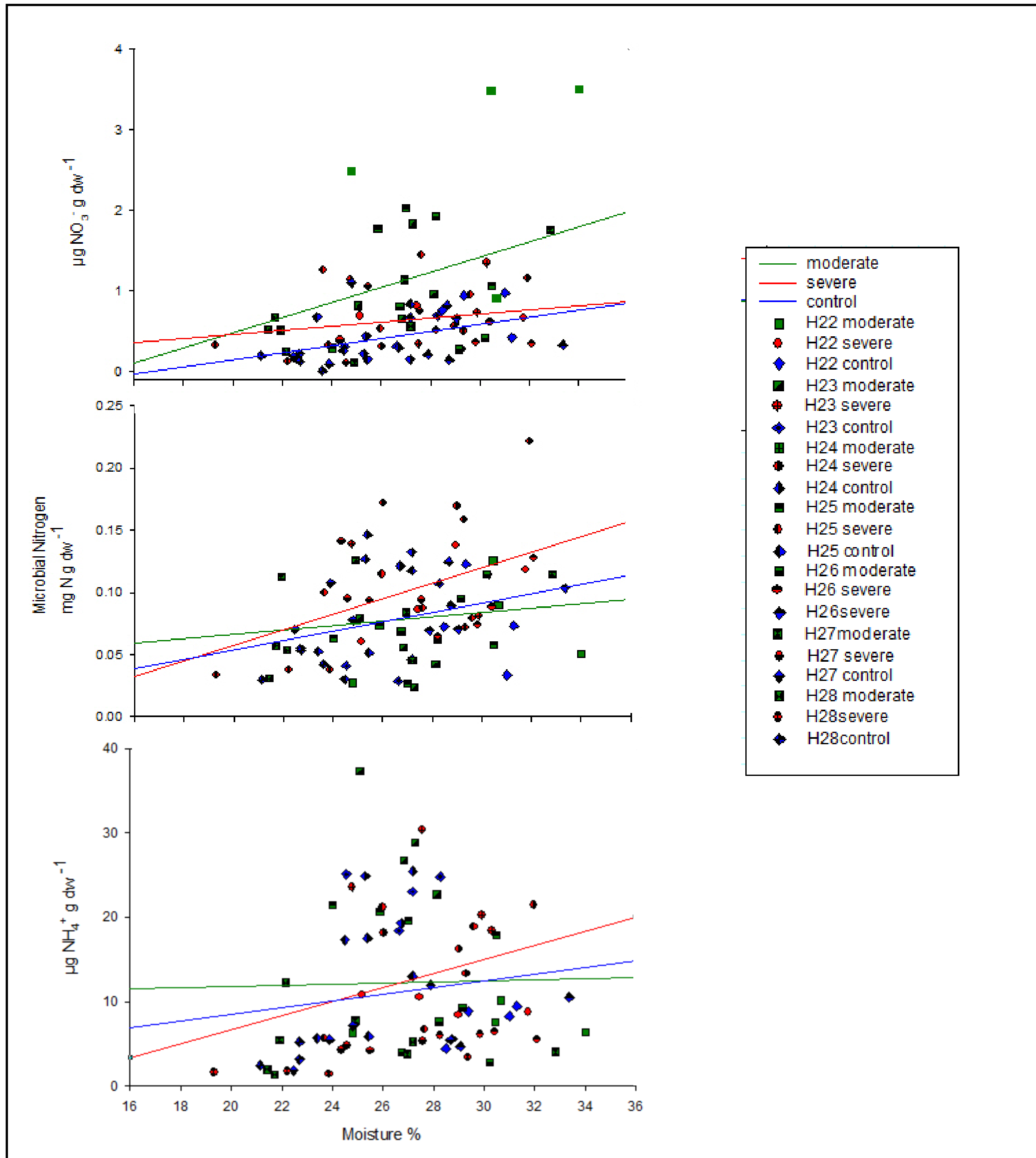

***Figure 12**: Relationships between NO_3^- (upper panel), and Microbial Biomass Nitrogen (middle panel and NH_4^+ (lower panel)) and soil moisture across the investigation period. Regression lines correspond to all the values collected per treatment.*

5. Discussion

While effects of single droughts and rewetting events on soils have been well studied, there is still a lack of understanding of the consequences of repeated droughts and the soil microbial response, as well as the interactions between altered water availability and soil microorganisms in the long run.

Water availability is a key factor regulating microbially driven processes in soils. During soil drying, a clear decrease of soil microbial activity can be seen (Fierer and Schimel 2002). After the drought period, rapid wetting of soils enhances microbial activity which can be detected through pulses of C and N mineralization (Birch 1958; Fierer et al., 2003). These pulses are short-lived and usually exceed the mineralization rate of control (i.e. not subjected to drought) plots (Borken and Matzner 2008). However, frequent drying and rewetting usually lessens the mineralization pulse as the accessible organic matter pool is limited.

Response of soil microbial community to drying-wetting cycles depend on the original state of the soil, previous drought history and the original composition of the microbial community (Bapiri et al., 2010; Fierer et. al., 2003). The combination of these factors will determine the recovery phase of the system.

After 3 years of drying-rewetting cycles, we aimed to see whether there are legacy effects in the forest soil microbial activity. In our experiment, we measured the recovery of the ecosystem after 3 years of drying-rewetting cycles. The results obtained show that there are still differences between the different treatments.

5.1. Abiotic parameters

The nitrogen cycle in soils is mainly biologically driven, however activity of soil microorganisms is known to be highly affected by abiotic parameters as soil moisture and temperature (Chapin et al., 2011; Butterbach-Bahl et al., 2011). Hence, these parameters determine the amount of substrate (e.g. ammonium) transformed by some soil microorganisms which subsequently may affect the amount of substrate (e.g. nitrate) transformed by other soil microbiota.

The results obtained showed a clear correlation between nitrate and soil moisture, however we did not see such correlation for ammonium. During the observation period, ammonium concentration in soils did not show significant differences among treatments. On the other hand, nitrate concentrations differed between treatments and MT had the highest concentration in all sampling points. Moreover, ST was also significantly higher than CT.

Drying-rewetting cycles alter the rate and dynamics of microbial nitrification in soils, reducing it during drying and enhancing it after rewetting (Borken and Matzner 2008). The enhancement of

the microbial activity is favored by the accumulation of microbial and plant necromass, release of solutes caused by the lysis of microbial cells and the exposure of previous protected organic matter (Borken and Matzner 2008). Reduced soil water content is known to cause a decline in ammonia oxidizing bacteria and their activity, further leading to a reduction in nitrification rates and nitrate availability in the soil (Dannenmann et al., 2016). Other authors showed that drying of moist soil can decrease nitrification rates up to 40% (Stark and Firestone 1995), although this rate might be different for different soil types and microbial communities.

It was expected that the plots under severe stress during the preceding 3 years will have lowest concentrations of ammonium and nitrate, as a consequence of the notable stress suffered by the microbial communities. However, the results showed higher concentration of nitrate in MT plots, while there were no significant differences in ammonium concentration among treatments. Therefore, the first hypothesis, i.e. that soil nitrate and ammonium concentration will be lower in severely stressed plots than in moderately stressed and control plots was rejected. Differences were only seen in nitrate concentration and contrary to the expectations, it was higher in MT plots. Nitrate availability in the soil is the net result of several processes, which are also dependent on environmental factors. Differences in nitrate concentrations among the treatments can be the result of a higher nitrification rate, or a lower plant nitrogen uptake. It can be thought that nitrifiers might have been favored under a moderate stress condition (Jackson et al., 2008) and therefore they can be more active in MT plots than in the other two. Further, denitrification may have decreased in the MT compared to the control plots, thereby reducing NO_3^- losses.

At the same time, the highest values of DON and TDN were found under severe stress. DON in soils represents the amount of organic nitrogen that can be mostly used by microorganism. It is used by microbes to fulfill their nutrition requirements and as a result microbes convert the bioavailable DON into ammonium. It might be considered that during the drought stress period, microbial activity might have been lessened and consequently the organic nitrogen pool has not been used, resulting in higher DON values in ST plots.
Values obtained for the DOC analysis showed no differences between our treatments. Carbon has been considered as a nutrient limiting microbial growth in forests (Chapin et al., 2011). Organic matter decomposition has been argued to be more affected by substrate quality and temperature than by soil moisture (Gao et al., 2016), and has been reported to show seasonal differences (Borken et al., 1998). In our case, past drying-rewetting cycles seem not to have affected the amount of available C for microbial communities in the long-term.

5.2. Soil microbial biomass

Microbial biomass carbon and nitrogen followed a seasonal pattern, with higher values in spring compared to autumn, in accordance to previous studies in Austrian beech forests (Hackl et al., 2000). Furthermore, we saw significant differences between treatments, with higher values in ST for both microbial biomass carbon and nitrogen. Microbial biomass nitrogen in ST was two times higher than in the other plots which was quite surprising as we hypothesized that under severe drought stress the microbial community will suffer a decrease in biomass compared to moderately stressed and control plots. Therefore, and according to the results, we have to reject this hypothesis.

During drought periods, soil microbial communities decrease their activity and try to optimize the use of the resources which are still available (Mikha et al., 2005). Some microorganisms turn to inactive growing stages, e.g. dormancy, during the stress situation. Hence, only a relatively small proportion of the soil microbes is active at any given time (Blagodatskaya and Kuzyakov 2013). Consequently, it can be thought that the stress simulated in ST plots was not extreme enough to kill the microbial biomass, so it could survive the stress period in a dormant life stage in the soil (Shade et al., 2012). The subsequent increase in water availability after abandonment of the manipulation might have been used by dormant microbial communities to be active again and increase at a higher rate in the plots under severe stress. According to our results, microbial biomass seems to have achieved or even exceeded the levels observed in the control plots, already during the first vegetation period after the disturbances/water regime alteration has ceased.

5.3. Enzyme activities

Enzyme activities reflect the nutritional status of soil microbial communities (Waldrop et al., 2000). Although they have been widely studied, the method followed only shows the potential activity of the enzymes under adequate conditions and may not reflect actual field conditions. Increases in soil temperatures and more frequent drying and wetting cycles change microbial community composition, which might be linked with an increase in both biomass and enzyme activities (Burns et al., 2013). For all enzymes studied except one, values of their activity correlated with field soil temperature and PLFAs associated with Gram-positive, Gram-negative bacteria and fungi. Correlation between soil temperature and extracellular enzymatic activity (EEA) has been reported in other studies (Bell and Henry 2011), however the relation between EEA and soil moisture is less understood. In our study, only Phenoloxidase and Peroxidase correlated with field soil moisture. These two enzymes are associated to fungal activity (Sinsabaugh 2010) which are considered to be more drought resistant than bacteria (Fierer et al., 2003) and are also known to produce a broader range of extracellular enzymes than bacteria (Romaní et al., 2006).

Although other studies did not observe differences in potential enzyme activities 1 year after a precipitation manipulation experiment (Steinweg et al., 2013), the results obtained from our enzyme activity analyses showed some differences between treatments. The activity of Cellobiohydrolase was significantly higher in plots under severe drought treatments than in the control. At the same time, ß-Glucosidase activity was significantly higher in plots under severe drought treatments than in the other treatments. Cellobiohydrolase is released for the acquisition of cellulose whereas ß-Glucosidase activity reflects glucose requirements (Steinweg et al., 2013). These higher values in those enzymes likely relate with the higher content of microbial biomass in severely stressed plots, thus it is feasible that microbial nutrient demand in severely stressed plots is higher. In other words, the results obtained let us think that the availability of cellulose and glucose in severely stressed plots was lower than the demand, which led to an enhancement of enzyme production. Protease activity also showed differences between treatments, with the highest activity in moderately stressed plots, however it was only significantly higher than CT. In the case of Phenoloxidase, CT showed the highest activity and it was significantly different from the other 2 treatments. Phenoloxidases group different enzymes which oxidize phenols while consuming oxygen (Sinsabaugh 2010). Moreover, Phenoloxidases are known to be produced by fungi and bacteria to diminish the toxicity of phenols, metal ions and as an antimicrobial defense (Sinsabaugh 2010). In our study, Peroxidase and Phenoloxidase were the only enzymes that correlated with soil temperature and soil moisture, as it has been found in other studies (Sinsabaugh et al., 2008). Simultaneously, they also correlate with NO_3^- and microbial biomass nitrogen, a relationship that has not been seen in any of the other enzyme activities. Moreover, enzymatic activities showed temporal dynamics seen by differences between harvests. These differences can be driven by variations in both nutrient demand and nutrient availability.

We expected that enzymes activities would be higher in the plots under severe drought effects, however the results obtained could not be generalized for the investigated enzymes. Therefore, we have to accept our hypothesis for Cellobiohydrolase and ß-Glucosidase, while we have to reject it for Chitinase, Phosphatase, Protease, Peroxidase and Phenoloxidase.

The activity of the soil microbial community is controlled by nutrient availability (Keiblinger et al., 2012) and can be affected by changes in temperature and soil moisture. Once the concentration of the nutrients meets the demand by microorganisms, the production of enzymes declines (Wallenstein and Weintraub 2008). Hydric stress is known to cause a decrease in enzymatic activity in the short time as more resources are allocated into surviving (Daou et al., 2016). Because the production of enzymes has high energy costs, they are only produced when the availability of the target compound is scarce. Therefore in case of highly adverse situations, enzyme production is reduced. Hence, enzymatic production has been shown not to correlate with microbial biomass (Alarcon et al., 2010). According to this argumentation, the results obtained indicate that the net result between demand and availability of some nutrients is lower in ST plots, enhancing the microbial enzyme production to fulfill the nutritional requirements.

5.4. PLFAs

The analysis of PLFAs allows the characterization of the microbial community composition in soils (White et al., 1993; Zelles 1999). By conducting our regular sampling over the vegetation period, we aimed at detecting the temporal patterns of the microbial community composition after cease of precipitation manipulation. Our results showed a seasonal trend with higher abundance of PLFAs in June and July compared to values obtained at the beginning and at the end of the sampling. This is consistent with the higher values of PLFAs found in spring compared to autumn for a beech forest by Hackl (Hackl et al., 2005). Overall, the microbial population was bacteria dominated, as has been showed by other studies in some European beech forests (Zechmeister-Boltenstern et al., 2011).

The results obtained revealed no differences in community composition among treatments, confirming our hypothesis. These results are in line with other studies which did neither observe differences in microbial community composition nor in microbial biomass after a field precipitation manipulation experiment in a subtropical rainforest (Zhao et al., 2016), or after rainfall manipulation treatments (Evans and Wallenstein 2012).

Soil microbial communities previously exposed to drought stress have been observed to be more resistant to new disturbances than those that have not experience stress before (Fierer et al., 2003). Hence, repeated drying and rewetting of soils has been seen to stimulate a more drought tolerant microbial community (Fierer et al., 2003) and seems to alter the microbial functioning (Fuchslueger 2016). It has been seen that the shift of the soil microbial community might happen after a single drying rewetting event, when the more stress tolerant taxa are enhanced (Evans and Wallenstein 2012). In our study, the analysis of PLFAs don't have the accuracy to distinguish between specific taxa and check more subtle changes within the microbial community.

5.5. Ecosystem resilience

An increase in drought intensity and frequency has an effect in on ecosystem dynamics. Soil moisture legacy effects constrain soil nutrients and microbial responsiveness, as it has been shown for mycorrhizal colonization after different dry/wet treatments (Cavagnaro 2016). At the same time, legacy of drought has been shown to modulate microbial response after rewetting of dry forest soils (Göransson et al., 2013) and alter soil microbial function (Fierer and Schimel 2002). In fact, drought also leads to shifts in organic matter quality for organic decomposition, affecting the soil microbial community (Fuchsluegger 2016). Further, former disturbances in the ecosystem might control present rates of microbial processes by modifying the traits of microbial communities which link contemporary abiotic drivers (soil moisture) and microbial function (Evans and Wallenstein 2012). For example, it has been observed that soils previously exposed to

drought had lower respiration rates when subjected again to drought (Evans and Wallenstein 2012). Likewise, seasonality of the precipitation is a key factor in determining soil microbial productivity (Sun et al., 2016).

Resilience is a fundamental trait for the ecosystem in order to be able to face disturbances. A microbial community can be considered resilient if after a change in the environmental conditions, it recovers quickly by growth or by physiological adaptation (Allison and Martiny 2008). Microbial resilience has been seen in studies where microbial communities previously exposed to drought stress were more resistant to new disturbances than those that have not experience stress before (Fierer et al., 2003) and microbial composition can differ from the undisturbed community in a time scale of a few years (Allison and Martiny 2008). In our study, we can see legacy effects after 3 years of repeated drying and rewetting cycles in nutrient cycling and microbial biomass. While we did not see differences in the community composition by the PLFAs analysis, differences in microbial biomass carbon, microbial biomass nitrogen biomass and in nitrate concentration among treatments where evident one year after the manipulation of water regime stopped. Hence, the results obtained show a legacy effect in the soil caused by prolonged droughts and rewetting. These results lead us to think that an increase in the frequency of drying and wetting cycles cause a change in microbial functioning that can be detected in the long term.

6. Conclusion

Repeated drying-rewetting cycles alter microbially driven processes within natural ecosystems. One year after the cease of the precipitation manipulation experiments, the consequences of the stress can still be seen. Nitrate concentration showed higher values in moderately stressed plots whereas no differences in ammonium concentration were detected. At the same time, total dissolved nitrogen and dissolved organic nitrogen concentrations were higher in severely stressed plots. Moisture affects the nitrogen cycle and consequently the amount of available nitrogen in the ecosystem. Nitrate availability in soils is the net result of several processes, consequently differences in nitrate concentrations among the treatments might be the result of a higher nitrification rate or a lower plant nitrogen uptake. Likewise, higher values of organic nitrogen in severely stressed plots indicate a low rate of use due to a likely decrease of microbial activity during the drying periods.

Contrary to the expectations, microbial biomass carbon and nitrogen was higher in the plots affected by severe droughts. Microbial communities in these plots might have survived the drying-rewetting cycles period by surviving strategies such as dormancy. Once the stress conditions have ceased, microbes have likely enhanced their growth and even exceeded the growth rates of the communities under the moderate stress and control. These results show that the microbial community in our site has been able to recover after long term unfavorable conditions. Hence, it seems that repeated drying and rewetting cycles stimulate a more drought tolerant community. On the other hand, the PLFA analysis showed no changes in the community composition. However, it can be possible that the scale of the analysis was too broad to detect differences within specific microbial groups.

Overall, it was shown that repeated cycles of drying and rewetting of forest soils have an effect on ecosystem dynamics which can be seen in the long term.

7. References

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8. Annexes

Table 2: results obtained from the statistical correlation of the parameters studied. Values state for the correlation coefficients and the significance of their p-values (* Indicates significant difference at $p<0.1$; ** indicates significant difference at $p<0.05$). Abbreviations of the parameter are used as following: dissolved organic carbon (DOC)), dissolved organic nitrogen (DON), total dissolved nitrogen (TDN),microbial carbon/nitrogen biomass ratio (Microbial C/N), gram positive bacteria (Gram+), gram negative bacteria (Gram -)

	NH_4^+	NO_3^-	DOC	TDN	DON	Microbial biomass carbon	Microbial biomass nitrogen	Microbial C/N	Soil moisture	pH	Soil temperature	Cellobiohydrolase
NH_4^+	1											
NO_3^-	0.07	1										
DOC	0.47**	-0.08	1									
TDN	0.73**	0.04	0.78**	1								
DON	0.15	-0.08	0.7**	0.79**	1							
Microbial biomass carbon	0.25**	-0.05	-0.04	0.1	-0.07	1						
Microbial biomass nitrogen	0.15	-0.02	0.09	0	-0.13	0.86	1					
Microbial C/N	0.05	0.1	-0.18	0.12	0.12	0.23	-0.14**	1				
Soil moisture	0.15	0.33**	0.29**	0.23**	0.17	0.13	0.3**	-0.21*	1			
pH	-0.09	-0.04	-0.04	-0.05	0.01	0.09	0.07	0	-0.16	1		
Soil temperature	0.46**	-0.1	0.41*	0.55**	0.39	0.49**	0.28**	0.28**	0.05	0.02	1	
Cellobiohydrolase	-0.37**	-0.01	0.07	-0.11	0.17	-0.09	-0.05	-0.1	-0.02	0.07	-0.06	1
ß-Glucosidase	0.24**	0.16	0.15	0.42**	0.38	0.38**	0.15	0.39**	0.08	-0.03	0.53**	0.04
Chitinase	-0.22**	-0.2*	0.26*	0.12	0.39	0.07**	-0.01	0.21*	-0.11	-0.02	0.2*	0.57**
Phosphatase	0	0.18*	0.09**	0.07	0.08	-0.32	-0.16	-0.27**	0.14	0.06	-0.59**	0.07
Protease	0.25**	0.2*	0.2	0.5**	0.47	0.1**	-0.08	0.4**	0.14	0	0.53**	-0.23**
Phenoloxidase	-0.11	-0.31**	-0.03	0.12	0.3	0.43**	0.18*	0.32**	-0.22**	0.22**	0.51**	0.15

Table 2 (cont.)

	NH_4^+	NO_3^-	DOC	TDN	DON	Microbial biomass carbon	Microbial biomass nitrogen	Microbial C/N	Soil moisture	pH	Soil temperature	Cellobiohydrolase
Peroxidase	0.11	**0.32****	-0.03	-0.04	-0.18	-0.42	**-0.19***	**-0.35****	**0.34****	-0.07	**-0.64****	**-0.31****
Gram +	0.12	-0.16	**0.21****	**0.26****	0.28	**0.43****	**0.25****	**0.35****	-0.05	-0.04	**0.66****	0.07
Gram -	**0.21***	-0.14	**0.25****	**0.28****	0.23	**0.44****	**0.27****	**0.34****	-0.1	-0.06	**0.66****	0.05
Bacteria	**0.37****	-0.06	0.11	**0.29****	0.09	0.63	**0.35****	**0.47****	-0.07	-0.06	**0.75****	-0.14
Fungi	**0.23****	-0.13	0.23	**0.34****	0.29	**0.54****	**0.32****	**0.39****	-0.04	-0.02	**0.74****	0.02

	ß-Glucosidase	Chitinase	Phosphatase	Protease	Phenoloxidase	Peroxidase	Gram +	Gram -	Bacteria
ß-Glucosidase	1								
Chitinase	**0.37****	1							
Phosphatase	**-0.3****	-0.22	1						
Protease	**0.54****	-0.03	**-0.22****	1					
Phenoloxidase	0.26	0.23	**-0.26****	0.31	1				
Peroxidase	**-0.37****	**-0.56****	**0.61****	**-0.11****	**-0.71****	1			
Gram +	**0.47****	**0.36****	**-0.47****	0.27	**0.51****	**-0.63****	1		
Gram -	**0.47****	**0.34****	**-0.46****	**0.23****	**0.44****	**-0.59****	**0.95****	1	
Bacteria	**0.69****	**0.29****	**-0.55****	**0.34****	**0.43****	**-0.64****	**0.76****	**0.77****	1
Fungi	**0.65****	**0.4****	**-0.52****	**0.33****	**0.52****	**-0.67****	**0.94****	**0.92****	**0.88****

Table 3: Soil chemical properties in all plots in the study site

Harvest	Date	Plot	Treatment	Soil moisture %	NH_4^+ (µg N /gdw)	NO_3^-(µg N /gdw)	DOC (mg C /g dw)	TDN (mg N /g dw)	DON (mg N /g dw)	C mic (mg C /g dw)	N mic (mg N /g dw)	pH
22	2/05/2016	1	moderate	24.801	6.301	2.490	0.081	0.018	0.009	0.315	0.027	4.135
22	2/05/2016	2	severe	25.175	10.779	0.691	0.116	0.027	0.015	0.611	0.060	4.031
22	2/05/2016	3	control	28.491	4.421	0.764	0.084	0.012	0.007	0.592	0.073	3.989
22	2/05/2016	4	moderate	34.020	6.337	3.508	0.139	0.027	0.017	0.500	0.051	4.035
22	2/05/2016	5	severe	27.442	10.515	0.820	0.122	0.028	0.017	0.787	0.086	3.838
22	2/05/2016	6	control	31.303	9.463	0.429	0.079	0.022	0.012	0.804	0.073	3.811
22	2/05/2016	7	moderate	30.455	7.534	3.485	0.114	0.022	0.011	0.990	0.126	4.254
22	2/05/2016	8	severe	31.748	8.722	0.660	0.108	0.023	0.014	1.148	0.118	4.008
22	2/05/2016	9	control	29.366	8.860	0.943	0.093	0.020	0.010	1.189	0.123	3.846
22	2/05/2016	10	control	31.014	8.276	0.978	0.099	0.025	0.016	0.396	0.033	3.873
22	2/05/2016	11	moderate	30.669	10.108	0.910	0.125	0.027	0.016	0.775	0.090	3.931
22	2/05/2016	12	severe	28.983	8.394	0.567	0.143	0.027	0.018	1.098	0.138	4.338
23	30/05/2016	1	moderate	27.282	28.841	1.840	0.170	0.050	0.019	0.411	0.023	4.237
23	30/05/2016	2	severe	27.549	30.360	0.349	0.213	0.058	0.027	0.123	-0.013	4.326
23	30/05/2016	3	control	28.274	24.814	0.699	0.192	0.048	0.023	0.908	0.107	4.06
23	30/05/2016	4	moderate	28.128	22.685	0.970	0.236	0.046	0.022	0.413	0.042	3.902
23	30/05/2016	5	severe	24.779	23.583	1.147	0.208	0.052	0.027	1.164	0.139	3.998
23	30/05/2016	6	control	27.175	23.035	0.675	0.276	0.051	0.027	0.267	0.046	9.683
23	30/05/2016	7	moderate	26.824	26.768	0.663	0.304	0.055	0.027	0.442	0.056	4.064
23	30/05/2016	8	severe	30.300	18.444	1.353	0.392	0.072	0.052	0.938	0.114	4.091
23	30/05/2016	9	control	26.634	18.415	0.316	0.213	0.044	0.025	0.324	0.029	3.877
23	30/05/2016	10	control	24.537	20.265	0.307	0.221	0.052	0.032	0.500	0.041	3.895
23	30/05/2016	11	moderate	25.074	23.174	0.821	0.193	0.045	0.021	0.765	0.079	4.085
23	30/05/2016	12	severe	29.911	37.327	0.729	0.332	0.078	0.040	0.695	0.081	4.166
24	27/06/2016	1	moderate	14.734	16.632	0.698	0.120	0.023	0.005	1.273	0.099	3.904
24	27/06/2016	2	severe	29.014	16.217	0.589	0.150	0.033	0.017	1.675	0.169	4.051

Table 3 (cont.)

Harvest	Date	Plot	Treatment	Soil moisture %	NH_4^+ (µg N /gdw)	NO_3^- (µg N /gdw)	DOC (mg C /g dw)	TDN (mg N /g dw)	DON (mg N /g dw)	C mic (mg C /g dw)	N mic (mg N /g dw)	pH
24	27/06/2016	3	control	26.721	19.300	0.298	0.138	0.026	0.006	1.533	0.121	3.847
24	27/06/2016	4	moderate	32.004	19.939	0.664	0.154	0.033	0.012	1.369	0.113	4.207
24	27/06/2016	5	severe	31.961	21.481	1.164	0.171	0.035	0.013	1.851	0.221	4.095
24	27/06/2016	6	control	25.298	24.899	0.222	0.148	0.031	0.006	1.468	0.127	3.784
24	27/06/2016	7	moderate	29.951	17.001	1.094	0.128	0.027	0.008	1.646	0.177	4.384
24	27/06/2016	8	severe	29.300	13.298	0.272	0.199	0.033	0.020	1.515	0.158	3.927
24	27/06/2016	9	control	27.183	25.485	0.155	0.179	0.036	0.010	1.570	0.133	3.932
24	27/06/2016	10	control	25.389	18.141	0.445	0.148	0.027	0.009	1.944	0.146	3.811
24	27/06/2016	11	moderate	26.311	25.106	0.320	0.213	0.042	0.016	1.236	0.117	4.026
24	27/06/2016	12	severe	26.044	22.522	0.306	0.302	0.045	0.022	1.815	0.172	4.028
25	25/07/2016	1	moderate	27.011	19.581	2.029	0.200	0.054	0.032	0.798	0.027	4.091
25	25/07/2016	2	severe	29.595	18.860	0.953	0.131	0.054	0.035	1.400	0.079	4.169
25	25/07/2016	3	control	23.385	5.669	0.679	0.127	0.027	0.021	0.897	0.052	3.641
25	25/07/2016	4	moderate	30.475	17.909	1.069	0.189	0.053	0.034	1.286	0.058	3.558
25	25/07/2016	5	severe	23.679	5.642	1.258	0.171	0.031	0.024	1.126	0.100	3.671
25	25/07/2016	6	control	27.169	12.997	0.845	0.206	0.050	0.036	1.289	0.118	3.554
25	25/07/2016	7	moderate	25.871	20.663	1.776	0.177	0.056	0.034	1.333	0.073	3.929
25	25/07/2016	8	severe	26.004	21.217	0.537	0.224	0.059	0.037	1.394	0.115	3.873
25	25/07/2016	9	control	24.482	17.320	0.261	0.152	0.049	0.032	0.854	0.030	3.57
25	25/07/2016	10	control	25.440	6.695	0.157	0.197	0.037	0.031	0.572	0.051	3.579
25	25/07/2016	11	moderate	24.000	17.506	0.294	0.173	0.052	0.034	1.237	0.063	3.792
25	25/07/2016	12	severe	27.640	21.467	1.449	0.251	0.066	0.043	1.193	0.087	3.964
26	22/08/2016	1	moderate	26.751	3.991	0.804	0.151	0.021	0.016	0.491	0.068	3.988
26	22/08/2016	2	severe	28.257	6.002	0.510	0.158	0.030	0.023	0.623	0.065	3.92
26	22/08/2016	3	control	29.058	4.708	0.661	0.203	0.032	0.027	0.585	0.070	3.713

Table 3 (cont.)

Harvest	Date	Plot	Treatment	Soil moisture %	NH_4^+ (µg N /gdw)	NO_3^-(µg N /gdw)	DOC (mg C /g dw)	TDN (mg N /g dw)	DON (mg N /g dw)	C mic (mg C /g dw)	N mic (mg N /g dw)	pH
26	22/08/2016	4	moderate	28.222	7.616	1.927	0.194	0.034	0.025	0.487	0.062	4.147
26	22/08/2016	5	severe	29.842	6.073	0.355	0.176	0.031	0.025	0.610	0.073	3.841
26	22/08/2016	6	control	33.367	10.504	0.334	0.277	0.055	0.044	0.974	0.103	3.539
26	22/08/2016	7	moderate	30.221	2.786	0.414	0.210	0.031	0.028	0.971	0.114	3.78
26	22/08/2016	8	severe	30.422	6.437	0.622	0.265	0.038	0.031	0.728	0.088	3.767
26	22/08/2016	9	control	28.741	5.578	0.148	0.267	0.039	0.033	0.732	0.090	3.708
26	22/08/2016	10	control	27.888	5.517	0.218	0.174	0.029	0.023	0.725	0.069	3.652
26	22/08/2016	11	moderate	29.134	5.855	0.278	0.234	0.036	0.030	0.790	0.095	3.782
26	22/08/2016	12	severe	32.100	9.254	0.347	0.356	0.052	0.043	1.060	0.128	3.668
27	19/09/2016	1	moderate	26.962	3.763	1.142	0.118	0.023	0.018	0.764	0.084	3.983
27	19/09/2016	2	severe	27.590	5.308	0.744	0.075	0.024	0.018	1.136	0.094	4.142
27	19/09/2016	3	control	24.808	7.110	1.106	0.097	0.021	0.013	0.881	0.078	3.912
27	19/09/2016	4	moderate	32.836	4.027	1.756	0.163	0.030	0.024	1.326	0.115	3.82
27	19/09/2016	5	severe	25.492	4.165	1.050	0.072	0.023	0.018	1.195	0.093	4.214
27	19/09/2016	6	control	28.671	5.419	0.824	0.152	0.028	0.021	1.183	0.124	3.725
27	19/09/2016	7	moderate	21.903	5.417	0.515	0.144	0.030	0.024	1.263	0.112	4012
27	19/09/2016	8	severe	24.575	4.766	0.109	0.078	0.026	0.024	1.222	0.095	3.865
27	19/09/2016	9	control	23.883	5.478	0.096	0.144	0.029	0.023	1.118	0.108	3.709
27	19/09/2016	10	control	22.453	4.244	0.189	0.091	0.027	0.023	1.078	0.070	3.735
27	19/09/2016	11	moderate	24.900	11.959	0.110	0.225	0.047	0.035	1.274	0.126	3.886
27	19/09/2016	12	severe	24.349	7.735	0.387	0.075	0.037	0.029	1.813	0.141	3.771
28	17/10/2016	1	moderate	21.421	1.946	0.523	0.102	0.019	0.017	0.367	0.031	3.928
28	17/10/2016	2	severe	29.365	3.397	0.506	0.131	0.028	0.024	0.650	0.072	4.096
28	17/10/2016	3	control	22.690	3.172	0.225	0.104	0.017	0.014	0.545	0.055	3.829

Table 3 (cont.)

Harvest	Date	Plot	Treatment	Soil moisture %	NH_4^+ (µg N /gdw)	NO_3^- (µg N /gdw)	DOC (mg C /g dw)	TDN (mg N /g dw)	DON (mg N /g dw)	C mic (mg C /g dw)	N mic (mg N /g dw)	pH
28	17/10/2016	4	moderate	27.191	5.237	0.566	0.210	0.031	0.026	0.413	0.045	3.974
28	17/10/2016	5	severe	19.316	1.591	0.329	0.081	0.016	0.014	0.352	0.033	3.984
28	17/10/2016	6	control	22.700	5.236	0.130	0.117	0.020	0.015	0.521	0.053	3.732
28	17/10/2016	7	moderate	21.709	1.338	0.669	0.122	0.022	0.020	0.598	0.057	3.953
28	17/10/2016	8	severe	22.211	1.743	0.130	0.156	0.026	0.026	0.469	0.037	3.9
28	17/10/2016	9	control	21.132	2.452	0.198	0.133	0.026	0.023	0.406	0.030	3.802
28	17/10/2016	10	control	23.620	1.381	0.012	0.158	0.023	0.021	0.363	0.042	3.778
28	17/10/2016	11	moderate	22.154	1.818	0.249	0.195	0.032	0.030	0.621	0.053	3.974
28	17/10/2016	12	severe	23.864	12.223	0.329	0.192	0.034	0.021	0.556	0.037	3.872

Table 4: Enzyme activities measured for all plots in the study site

Harvest	Date	Plot	Treatment	Cellobiohydrolase (nmol g^{-1} h^{-1})	ß-Glucosidase (nmol g^{-1} h^{-1})	Chitinase (nmol g^{-1} h^{-1})	Phosphatase (nmol g^{-1} h^{-1})	Protease (nmol g^{-1} h^{-1})	Phenoloxidase (nmol g^{-1} h^{-1})	Peroxidase (nmol g^{-1} h^{-1})
22	2/05/2016	1	moderate	85.640	492.913	72.835	3787.453	87.634	0.181	3.008
22	2/05/2016	2	severe	13.484	380.148	70.915	3413.366	67.785	0.276	3.175
22	2/05/2016	3	control	-72.966	445.654	-16.648	3553.236	92.191	-0.129	3.852
22	2/05/2016	4	moderate	92.578	360.327	74.197	4471.197	85.190	-0.067	3.727
22	2/05/2016	5	severe	-41.513	313.541	5.995	4102.987	81.088	0.480	3.286
22	2/05/2016	6	control	-8.487	457.745	97.368	5413.493	104.838	0.684	3.624
22	2/05/2016	7	moderate	-1.941	517.793	75.082	5899.403	68.003	0.129	3.474
22	2/05/2016	8	severe	-23.885	414.905	45.021	4549.620	80.814	0.303	3.855
22	2/05/2016	9	control	-20.465	202.182	10.862	4570.551	102.604	0.290	3.058
22	2/05/2016	10	control	-4.129	155.320	-31.680	7589.708	102.498	0.533	3.650
22	2/05/2016	11	moderate	2.260	437.529	24.246	8901.684	56.390	0.151	4.120

Table 4 (cont.)

Harvest	Date	Plot	Treatment	Cellobiohydrolase (nmol g^{-1} h^{-1})	ß-Glucosidase (nmol g^{-1} h^{-1})	Chitinase (nmol g^{-1} h^{-1})	Phosphatase (nmol g^{-1} h^{-1})	Protease (nmol g^{-1} h^{-1})	Phenoloxidase (nmol g^{-1} h^{-1})	Peroxidase (nmol g^{-1} h^{-1})
22	2/05/2016	12	severe	-42.308	252.780	77.067	2655.260	64.764	-0.063	3.149
23	30/05/2016	1	moderate	-5.688	314.311	31.645	4579.317	72.095	0.520	2.209
23	30/05/2016	2	severe	47.963	274.425	44.264	5000.253	75.356	1.069	2.427
23	30/05/2016	3	control	-11.153	299.681	59.137	4359.014	91.324	0.223	2.933
23	30/05/2016	4	moderate	43.713	616.641	169.205	4604.266	87.089	0.000	3.442
23	30/05/2016	5	severe	-43.016	282.822	29.749	3353.528	159.542	0.174	3.232
23	30/05/2016	6	control	-18.609	522.066	86.418	5130.159	81.491	0.275	3.220
23	30/05/2016	7	moderate	52.858	420.039	55.835	4813.319	136.994	0.257	3.186
23	30/05/2016	8	severe	33.631	385.528	145.645	11737.475	83.179	1.088	2.897
23	30/05/2016	9	control	-31.102	247.903	34.958	4.233	97.295	0.463	2.827
23	30/05/2016	10	control	-80.978	179.318	-31.847	2981.219	75.328	0.954	2.404
23	30/05/2016	11	moderate	40.432	242.101	112.029	2611.174	90.002	0.492	2.896
23	30/05/2016	12	severe	3.766	435.091	227.690	6996.513	126.652	0.426	3.244
24	27/06/2016	1	moderate	17.665	361.638	361.913	910.369	33.823	0.687	0.941
24	27/06/2016	2	severe	23.971	578.480	293.491	2134.693	71.807	1.229	1.508
24	27/06/2016	3	control	43.777	623.098	672.546	1976.157	60.057	0.680	1.532
24	27/06/2016	4	moderate	-26.629	478.475	489.613	1552.839	1.746	0.492	1.977
24	27/06/2016	5	severe	61.080	1085.704	315.504	2898.420	53.365	0.609	1.775
24	27/06/2016	6	control	45.712	593.898	378.966	1299.849	64.355	0.996	1.490
24	27/06/2016	7	moderate	46.970	810.635	443.164	1546.857	46.425	1.026	1.005
24	27/06/2016	8	severe	26.559	900.395	502.778	1197.567	43.397	1.499	1.285
24	27/06/2016	9	control	-8.858	385.791	329.358	1420.501	65.824	1.549	1.067
24	27/06/2016	10	control	-32.169	253.178	80.780	1703.831	40.958	1.985	1.209
24	27/06/2016	11	moderate	8.378	565.080	527.119	1773.229	56.630	1.311	0.599
24	27/06/2016	12	severe	25.741	764.898	503.795	1898.463	22.842	0.870	0.974

Table 4 (cont.)

Harvest	Date	Plot	Treatment	Cellobiohydrolase (nmol g^{-1} h^{-1})	ß-Glucosidase (nmol g^{-1} h^{-1})	Chitinase (nmol g^{-1} h^{-1})	Phosphatase (nmol g^{-1} h^{-1})	Protease (nmol g^{-1} h^{-1})	Phenoloxidase (nmol g^{-1} h^{-1})	Peroxidase (nmol g^{-1} h^{-1})
25	25/07/2016	1	moderate	39.904	1296.331	1011.393	2113.377	175.608	1.376	1.135
25	25/07/2016	2	severe	-20.286	1781.351	451.540	1538.120	151.843	1.335	1.212
25	25/07/2016	3	control	22.094	1445.417	431.528	883.588	169.945	1.251	0.594
25	25/07/2016	4	moderate	13.728	1600.559	423.791	1424.581	149.200	1.150	1.718
25	25/07/2016	5	severe	20.864	1255.283	513.528	1446.918	190.322	1.173	1.284
25	25/07/2016	6	control	38.198	2344.788	706.304	1817.079	114.032	1.544	1.042
25	25/07/2016	7	moderate	-3.626	1538.308	793.810	1806.843	172.029	1.371	1.092
25	25/07/2016	8	severe	65.849	2731.703	1007.696	2649.351	166.142	1.759	1.189
25	25/07/2016	9	control	3.755	1056.301	354.019	1548.648	220.888	2.260	0.681
25	25/07/2016	10	control	-19.997	919.412	405.517	1864.071	179.691	2.595	0.652
25	25/07/2016	11	moderate	16.551	732.630	519.148	1444.368	187.224	1.752	1.354
25	25/07/2016	12	severe	44.553	2045.763	967.645	1636.269	176.362	1.076	1.375
26	22/08/2016	1	moderate	66.749	412.127	408.014	1412.585	87.075	0.329	1.359
26	22/08/2016	2	severe	20.649	394.095	379.552	1212.315	84.752	0.831	1.575
26	22/08/2016	3	control	36.842	499.936	585.499	1941.633	96.766	0.511	1.796
26	22/08/2016	4	moderate	44.520	317.995	449.825	1298.563	72.727	0.526	1.776
26	22/08/2016	5	severe	58.790	382.359	466.499	1262.539	61.241	0.621	1.646
26	22/08/2016	6	control	72.482	581.146	846.919	2925.360	100.079	0.875	1.723
26	22/08/2016	7	moderate	58.713	441.673	597.515	2341.838	89.796	0.842	1.515
26	22/08/2016	8	severe	119.975	577.174	999.055	1927.062	106.346	1.189	1.745
26	22/08/2016	9	control	82.018	380.672	335.108	1589.655	91.956	1.123	1.651
26	22/08/2016	10	control	80.371	342.801	539.727	1315.726	106.726	1.144	1.511
26	22/08/2016	11	moderate	39.033	247.533	608.696	1642.235	97.347	1.147	1.336
26	22/08/2016	12	severe	118.402	444.790	1206.427	1969.244	97.060	1.241	1.270
27	19/09/2016	1	moderate	79.916	418.835	334.905	3001.647	94.704	1.400	1.741

Table 4 (cont.)

Harvest	Date	Plot	Treatment	Cellobiohydrolase (nmol g^{-1} h^{-1})	ß-Glucosidase (nmol g^{-1} h^{-1})	Chitinase (nmol g^{-1} h^{-1})	Phosphatase (nmol g^{-1} h^{-1})	Protease (nmol g^{-1} h^{-1})	Phenoloxidase (nmol g^{-1} h^{-1})	Peroxidase (nmol g^{-1} h^{-1})
27	19/09/2016	2	severe	31.948	322.400	183.585	2712.742	75.452	1.664	1.754
27	19/09/2016	3	control	59.037	392.434	170.545	2872.351	115.476	1.904	0.956
27	19/09/2016	4	moderate	84.640	566.460	393.455	3377.466	76.433	1.672	1.136
27	19/09/2016	5	severe	151.365	624.934	378.513	3698.230	73.196	1.636	1.510
27	19/09/2016	6	control	45.755	411.653	344.468	3333.490	93.988	1.870	1.625
27	19/09/2016	7	moderate	65.984	439.123	336.903	4229.123	87.658	2.379	1.329
27	19/09/2016	8	severe	59.019	411.064	263.466	2328.773	74.091	2.429	1.489
27	19/09/2016	9	control	-8.311	172.325	216.008	3270.271	111.763	2.394	1.323
27	19/09/2016	10	control	10.606	285.387	480.163	3271.937	86.464	2.456	1.589
27	19/09/2016	11	moderate	81.262	484.389	462.621	4037.105	79.345	2.695	1.173
27	19/09/2016	12	severe	122.807	717.366	377.785	3466.834	87.622	1.858	1.069
28	17/10/2016	1	moderate	66.083	321.974	333.577	2597.500	13.384	0.350	1.967
28	17/10/2016	2	severe	171.956	482.186	935.899	4804.372	25.666	0.886	1.971
28	17/10/2016	3	control	89.640	395.016	751.590	5226.030	23.077	0.816	1.483
28	17/10/2016	4	moderate	174.733	571.192	1264.512	5259.769	32.789	0.800	1.799
28	17/10/2016	5	severe	9.651	226.789	332.655	2606.808	12.491	0.339	1.730
28	17/10/2016	6	control	84.116	381.961	755.260	4193.330	19.124	0.394	1.833
28	17/10/2016	7	moderate	48.262	207.692	853.262	3996.146	24.620	0.486	1.852
28	17/10/2016	8	severe	103.384	362.713	478.490	4323.544	18.574	0.695	1.968
28	17/10/2016	9	control	30.107	326.793	611.942	3290.617	24.445	1.283	1.283
28	17/10/2016	10	control	44.987	137.300	481.969	5379.905	31.863	1.173	1.793
28	17/10/2016	11	moderate	70.760	266.049	1437.348	4945.186	25.485	0.755	1.916

Table 5: Amount of identified PLFAs of soil samples in all plots in the study site

			PLFAs (nmol g^{-1} dw)																				
Date	Plot	Treatment	14:0	14:0	i15:0	a15:0	15:0	15:1ω 5	i16.0	16:0	16:1ω 7	i17:0	17:0	cy17:0	18:0	18:1ω 9 t	18:1ω 9 c	18:1ω 9 t	18:2ω 6,9	19:0	cy19:0	18:3ω 3	20:0
2/05/2016	1	moderate	0.094	0.005	0.624	0.332	0.053	0.017	0.322	0.849	0.513	0.125	0.048	0.218	0.175	0.000	0.629	0.071	0.232	0.838	0.015	0.989	0.095
2/05/2016	2	severe	0.046	0.003	0.285	0.211	0.026	0.012	0.152	0.476	0.260	0.058	0.023	0.082	0.095	0.000	0.349	0.045	0.138	0.836	0.005	0.558	0.040
2/05/2016	3	control	0.069	0.004	0.510	0.275	0.039	0.013	0.235	0.689	0.478	0.107	0.027	0.141	0.141	0.000	0.540	0.102	0.202	0.835	0.009	0.777	0.067
2/05/2016	4	moderate	0.024	0.000	0.136	0.057	0.011	0.005	0.066	0.194	0.108	0.025	0.008	0.040	0.048	0.000	0.138	0.014	0.059	0.835	0.003	0.202	0.039
2/05/2016	5	severe	0.047	0.000	0.304	0.182	0.028	0.009	0.145	0.451	0.268	0.062	0.026	0.088	0.100	0.000	0.316	0.037	0.120	0.835	0.007	0.589	0.063
2/05/2016	6	control	0.014	0.000	0.109	0.071	0.008	0.003	0.046	0.217	0.115	0.026	0.008	0.040	0.057	0.000	0.189	0.028	0.051	0.833	0.003	0.268	0.023
2/05/2016	7	moderate	0.018	0.000	0.133	0.084	0.012	0.003	0.057	0.213	0.122	0.025	0.009	0.038	0.043	0.000	0.150	0.019	0.073	0.840	0.003	0.209	0.009
2/05/2016	8	severe	0.096	0.000	0.678	0.361	0.055	0.017	0.332	0.875	0.529	0.129	0.050	0.225	0.181	0.000	0.652	0.073	0.240	0.870	0.016	1.023	0.099
2/05/2016	9	control	0.019	0.000	0.146	0.096	0.012	0.004	0.064	0.238	0.131	0.029	0.009	0.045	0.044	0.000	0.158	0.022	0.075	0.841	0.003	0.218	0.007
2/05/2016	10	control	0.008	0.000	0.063	0.043	0.006	0.000	0.031	0.130	0.068	0.015	0.006	0.026	0.034	0.000	0.104	0.014	0.042	0.834	0.002	0.120	0.005
2/05/2016	11	moderate	0.020	0.000	0.182	0.109	0.016	0.006	0.085	0.328	0.180	0.038	0.014	0.058	0.066	0.003	0.218	0.032	0.124	0.839	0.004	0.281	0.011
2/05/2016	12	severe	0.018	0.000	0.150	0.107	0.013	0.004	0.086	0.266	0.151	0.035	0.013	0.050	0.052	0.000	0.214	0.031	0.083	0.838	0.005	0.305	0.009
30/05/2016	1	moderate	0.077	0.000	0.577	0.261	0.053	0.015	0.249	0.824	0.443	0.118	0.050	0.171	0.171	0.000	0.536	0.078	0.310	0.835	0.020	0.943	0.092
30/05/2016	2	severe	0.040	0.004	0.271	0.190	0.025	0.009	0.117	0.458	0.235	0.060	0.023	0.079	0.093	0.000	0.326	0.043	0.197	0.835	0.004	0.582	0.037
30/05/2016	3	control	0.088	0.005	0.663	0.375	0.053	0.017	0.278	0.976	0.534	0.135	0.052	0.181	0.203	0.000	0.785	0.144	0.338	0.840	0.013	1.053	0.077
30/05/2016	4	moderate	0.110	0.006	0.698	0.324	0.070	0.017	0.362	1.041	0.946	0.135	0.062	0.224	0.212	0.000	0.762	0.098	0.401	0.834	0.024	1.265	0.112
30/05/2016	5	severe	0.066	0.005	0.451	0.244	0.040	0.013	0.230	0.700	0.378	0.091	0.039	0.129	0.143	0.000	0.488	0.061	0.286	0.837	0.011	0.889	0.072
30/05/2016	6	control	0.067	0.006	0.431	0.288	0.046	0.014	0.224	0.756	0.362	0.091	0.040	0.119	0.158	0.000	0.565	0.111	0.335	0.832	0.008	0.859	0.054
30/05/2016	7	moderate	0.050	0.004	0.330	0.243	0.033	0.010	0.174	0.605	0.299	0.074	0.032	0.114	0.122	0.000	0.457	0.073	0.235	0.832	0.007	0.674	0.041
30/05/2016	8	severe	0.096	0.006	0.678	0.361	0.055	0.017	0.332	0.875	0.529	0.129	0.050	0.225	0.181	0.000	0.652	0.073	0.240	0.870	0.016	1.023	0.099
30/05/2016	9	control	0.086	0.004	0.584	0.361	0.057	0.026	0.277	1.006	0.517	0.113	0.049	0.176	0.208	0.000	0.635	0.075	0.417	0.839	0.009	0.965	0.064
30/05/2016	11	moderate	0.072	0.004	0.510	0.369	0.050	0.019	0.226	0.836	0.427	0.100	0.040	0.149	0.161	0.000	0.555	0.062	0.352	0.838	0.008	0.910	0.047
30/05/2016	12	severe	0.083	0.005	0.517	0.294	0.060	0.042	0.228	1.077	0.521	0.094	0.047	0.168	0.176	0.000	0.653	0.071	0.517	0.841	0.009	0.847	0.045

Table 5 (cont.)

			PLFAs (nmol g^{-1} dw)																				
Date	Plot	Treatment	14:0	14:0	i15:0	a15:0	15:0	15:1ω5	i16.0	16:0	16:1ω7	i17:0	17:0	cy17:0	18:0	18:1ω9 t	18:1ω9 c	18:1ω9 t	18:2ω6,9	19:0	cy19:0	18:3ω3	20:0
27/06/2016	4	moderate	0.134	0.000	0.895	0.368	0.128	0.021	0.571	1.658	0.674	0.236	0.094	0.288	0.356	0.000	0.959	0.100	1.105	3.342	0.037	1.707	0.206
27/06/2016	5	severe	0.135	0.000	1.045	0.501	0.118	0.021	0.637	1.793	0.917	0.215	0.110	0.339	0.341	0.000	1.192	0.151	1.028	3.348	0.035	2.210	0.153
27/06/2016	6	control	0.124	0.005	0.941	0.609	0.132	0.027	0.596	1.855	0.831	0.206	0.096	0.298	0.393	0.000	1.113	0.169	1.024	3.338	0.021	2.081	0.104
27/06/2016	7	moderate	0.068	0.006	0.603	0.351	0.086	0.020	0.393	1.331	0.633	0.138	0.051	0.220	0.296	0.000	0.838	0.138	0.915	3.348	0.017	1.285	0.094
27/06/2016	8	severe	0.117	0.000	0.894	0.544	0.151	0.035	0.592	1.885	0.800	0.221	0.074	0.296	0.329	0.000	1.140	0.133	1.583	3.338	0.023	1.963	0.103
27/06/2016	9	control	0.075	0.007	0.628	0.455	0.096	0.018	0.334	1.431	0.640	0.115	0.048	0.224	0.214	0.000	0.720	0.115	1.105	3.338	0.015	1.226	0.056
27/06/2016	10	control	0.056	0.005	0.454	0.253	0.067	0.016	0.262	0.993	0.448	0.111	0.037	0.175	0.209	0.000	0.538	0.076	0.816	3.352	0.008	1.036	0.064
27/06/2016	11	moderate	0.115	0.006	0.897	0.562	0.115	0.024	0.450	1.765	0.833	0.208	0.090	0.308	0.364	0.000	1.086	0.157	1.135	3.337	0.013	1.893	0.086
27/06/2016	12	severe	0.117	0.000	0.939	0.553	0.130	0.025	0.574	1.904	0.825	0.226	0.109	0.296	0.363	0.000	1.116	0.150	1.711	3.353	0.015	1.923	0.090
25/07/2016	1	moderate	0.098	0.003	0.775	0.462	0.132	0.015	0.444	1.507	0.677	0.186	0.057	0.287	0.297	0.000	0.764	0.121	1.258	3.343	0.014	1.406	0.106
25/07/2016	2	severe	0.131	0.006	1.011	0.597	0.110	0.023	0.582	1.968	0.855	0.225	0.081	0.336	0.391	0.000	1.156	0.181	1.410	3.352	0.015	2.186	0.121
25/07/2016	3	control	0.126	0.000	1.057	0.620	0.139	0.031	0.633	2.175	0.937	0.282	0.134	0.346	0.465	0.000	1.255	0.249	1.571	3.338	0.017	2.001	0.147
25/07/2016	4	moderate	0.242	0.010	1.415	1.073	0.192	0.032	0.709	2.784	1.265	0.241	0.113	0.427	0.419	0.000	1.537	0.203	2.127	3.342	0.008	2.443	0.114
25/07/2016	5	severe	0.092	0.000	0.741	0.421	0.097	0.018	0.448	1.428	0.612	0.196	0.084	0.240	0.310	0.000	0.827	0.113	0.924	3.348	0.013	1.658	0.108
25/07/2016	6	control	0.135	0.005	1.069	0.599	0.127	0.017	0.535	2.075	0.929	0.222	0.088	0.340	0.454	0.000	1.348	0.214	1.638	3.338	0.013	2.029	0.107
25/07/2016	7	moderate	0.095	0.000	0.765	0.477	0.118	0.016	0.441	1.657	0.700	0.178	0.082	0.314	0.323	0.000	1.038	0.140	1.380	3.348	0.013	1.603	0.090
25/07/2016	8	severe	0.096	0.010	0.727	0.442	0.124	0.017	0.421	1.742	0.637	0.184	0.086	0.276	0.318	0.000	0.962	0.116	2.382	3.338	0.013	1.541	0.098
25/07/2016	9	control	0.102	0.007	0.729	0.489	0.113	0.022	0.378	1.488	0.610	0.166	0.066	0.240	0.290	0.000	0.766	0.111	1.432	3.338	0.012	1.301	0.072
25/07/2016	10	control	0.102	0.009	0.895	0.578	0.093	0.017	0.501	1.664	0.823	0.228	0.095	0.285	0.384	0.000	1.146	0.196	0.769	3.352	0.016	1.903	0.125

Table 5 (cont.)

			PLFAs (nmol g^{-1} dw)																				
Date	Plot	Treatment	14:0	14:0	i15:0	a15:0	15:0	15:1ω5	i16.0	16:0	16:1ω7	i17:0	17:0	cy17:0	18:0	18:1ω9 t	18:1ω9 c	18:1ω9 t	18:2ω6,9	19:0	cy19:0	18:3ω3	20:0
25/07/2016	12	severe	0.102	0.000	0.827	0.494	0.162	0.023	0.464	1.844	0.729	0.222	0.096	0.319	0.376	0.000	0.939	0.144	2.077	3.353	0.014	1.670	0.120
22/08/2016	1	moderate	0.043	0.000	0.329	0.188	0.041	0.009	0.187	0.532	0.250	0.083	0.040	0.092	0.114	0.000	0.244	0.038	0.271	0.836	0.014	0.496	0.042
22/08/2016	2	severe	0.059	0.007	0.559	0.395	0.052	0.021	0.324	1.084	0.412	0.125	0.024	0.151	0.192	0.000	0.494	0.141	0.615	0.835	0.023	1.028	0.047
22/08/2016	3	control	0.049	0.000	0.355	0.176	0.045	0.007	0.218	0.607	0.266	0.083	0.041	0.094	0.131	0.000	0.342	0.051	0.423	0.838	0.004	0.501	0.044
22/08/2016	4	moderate	0.108	0.004	0.636	0.276	0.100	0.020	0.386	1.127	0.493	0.141	0.064	0.207	0.240	0.000	0.640	0.059	1.049	0.834	0.017	0.875	0.106
22/08/2016	5	severe	0.090	0.004	0.673	0.314	0.074	0.015	0.443	1.153	0.476	0.152	0.070	0.195	0.237	0.000	0.588	0.070	0.721	0.835	0.026	1.146	0.107
22/08/2016	6	control	0.125	0.005	0.825	0.474	0.133	0.020	0.514	1.554	0.607	0.186	0.089	0.226	0.318	0.000	0.940	0.140	0.819	0.838	0.012	1.500	0.066
22/08/2016	7	moderate	0.118	0.007	0.743	0.415	0.116	0.022	0.437	1.401	0.608	0.186	0.081	0.225	0.286	0.000	0.941	0.022	0.968	0.838	0.021	1.170	0.053
22/08/2016	8	severe	0.096	0.004	0.678	0.361	0.055	0.017	0.332	0.875	0.529	0.129	0.050	0.225	0.181	0.000	0.652	0.073	0.240	0.870	0.016	1.023	0.099
22/08/2016	9	control	0.072	0.005	0.474	0.290	0.091	0.018	0.280	0.992	0.661	0.107	0.051	0.144	0.196	0.000	0.514	0.101	0.898	0.835	0.026	0.850	0.055
22/08/2016	10	control	0.088	0.007	0.671	0.365	0.089	0.025	0.324	1.208	0.508	0.135	0.064	0.192	0.280	0.000	0.603	0.086	0.988	0.840	0.013	1.022	0.053
22/08/2016	11	moderate	0.102	0.006	0.727	0.512	0.104	0.020	0.373	1.410	0.611	0.161	0.066	0.218	0.269	0.000	0.822	0.111	1.031	0.836	0.014	1.375	0.056
22/08/2016	12	severe	0.165	0.008	1.130	0.562	0.185	0.036	0.580	0.000	0.978	0.190	0.089	0.370	0.412	0.000	1.278	0.158	0.215	0.839	0.024	1.844	0.072
19/09/2016	1	moderate	0.127	0.012	1.113	0.556	0.116	0.016	0.607	1.668	0.811	0.244	0.064	0.302	0.329	0.000	0.971	0.113	1.219	0.838	0.042	1.733	0.094
19/09/2016	2	severe	0.079	0.009	0.620	0.400	0.062	0.015	0.293	1.111	0.494	0.134	0.049	0.169	0.236	0.000	0.624	0.086	0.845	0.838	0.018	1.087	0.059
19/09/2016	3	control	0.113	0.009	0.930	0.511	0.129	0.019	0.461	1.514	0.690	0.196	0.079	0.230	0.291	0.000	0.836	0.141	0.638	0.836	0.015	1.240	0.078
19/09/2016	4	moderate	0.115	0.007	0.847	0.340	0.103	0.015	0.478	1.398	0.618	0.159	0.061	0.265	0.273	0.000	0.748	0.063	1.245	0.834	0.022	1.272	0.126
19/09/2016	5	severe	0.126	0.007	0.861	0.455	0.114	0.012	0.476	1.437	0.690	0.185	0.068	0.281	0.254	0.000	0.750	0.087	0.994	0.835	0.026	1.276	0.088
19/09/2016	6	control	0.126	0.005	0.915	0.537	0.126	0.019	0.503	1.633	0.680	0.175	0.053	0.234	0.312	0.000	1.014	0.163	0.768	0.838	0.010	1.569	0.070
19/09/2016	7	moderate	0.082	0.003	0.519	0.332	0.081	0.014	0.272	0.974	0.412	0.110	0.046	0.155	0.182	0.000	0.617	0.080	0.871	0.838	0.008	0.867	0.042

Table 5 (cont.)

			PLFAs (nmol g^{-1} dw)																				
Date	Plot	Treatment	14:0	14:0	i15:0	a15:0	15:0	15:1ω 5	i16.0	16:0	16:1ω 7	i17:0	17:0	cy17:0	18:0	18:1ω 9 t	18:1ω 9 c	18:1ω 9 t	18:2ω 6,9	19:0	cy19:0	18:3ω 3	20:0
19/09/2016	8	severe	0.096	0.003	0.678	0.361	0.055	0.017	0.332	0.875	0.529	0.129	0.050	0.225	0.181	0.000	0.652	0.073	0.240	0.870	0.016	1.023	0.099
19/09/2016	9	control	0.178	0.008	1.334	0.778	0.176	0.030	0.643	2.410	1.035	0.252	0.097	0.390	0.442	0.000	1.271	0.170	1.478	0.837	0.029	2.031	0.094
19/09/2016	10	control	0.112	0.005	0.813	0.425	0.117	0.021	0.440	1.412	0.616	0.163	0.076	0.256	0.324	0.000	0.751	0.137	0.838	0.838	0.015	1.139	0.071
19/09/2016	11	moderate	0.119	0.006	0.925	0.459	0.117	0.016	0.413	1.487	0.683	0.179	0.057	0.248	0.281	0.000	0.918	0.110	0.751	0.837	0.016	1.347	0.064
19/09/2016	12	severe	0.046	0.004	0.357	0.202	0.052	0.015	0.214	0.741	0.288	0.082	0.043	0.122	0.139	0.000	0.434	0.054	0.769	0.842	0.008	0.700	0.026
17/10/2016	1	moderate	0.051	0.004	0.368	0.192	0.034	0.012	0.185	0.515	0.274	0.073	0.031	0.094	0.119	0.000	0.341	0.035	0.189	0.835	0.007	0.580	0.056
17/10/2016	2	severe	0.056	0.004	0.389	0.265	0.035	0.012	0.178	0.699	0.349	0.084	0.034	0.109	0.142	0.000	0.497	0.088	0.261	0.838	0.006	0.758	0.053
17/10/2016	3	control	0.087	0.006	0.598	0.366	0.067	0.019	0.305	1.050	0.541	0.131	0.058	0.183	0.211	0.000	0.741	0.124	0.550	0.834	0.011	1.051	0.069
17/10/2016	4	moderate	0.081	0.005	0.491	0.246	0.050	0.011	0.244	0.794	0.408	0.096	0.044	0.165	0.151	0.000	0.540	0.063	0.396	0.841	0.012	0.742	0.089
17/10/2016	5	severe	0.060	0.003	0.430	0.235	0.039	0.012	0.206	0.598	0.338	0.084	0.036	0.107	0.124	0.000	0.385	0.044	0.183	0.834	0.008	0.701	0.056
17/10/2016	6	control	0.075	0.004	0.547	0.368	0.052	0.015	0.261	0.920	0.449	0.107	0.046	0.158	0.176	0.000	0.564	0.079	0.412	0.839	0.007	0.909	0.048
17/10/2016	7	moderate	0.053	0.004	0.370	0.265	0.034	0.011	0.159	0.575	0.305	0.076	0.030	0.098	0.116	0.000	0.419	0.062	0.256	0.836	0.006	0.692	0.043
17/10/2016	8	severe	0.096	0.004	0.678	0.361	0.055	0.017	0.332	0.875	0.529	0.129	0.050	0.225	0.181	0.000	0.652	0.073	0.240	0.870	0.016	1.023	0.099
17/10/2016	9	control	0.089	0.004	0.660	0.387	0.056	0.016	0.257	0.938	0.496	0.124	0.043	0.176	0.184	0.000	0.612	0.108	0.446	0.837	0.012	1.102	0.066
17/10/2016	10	control	0.040	0.004	0.255	0.164	0.027	0.009	0.116	0.437	0.216	0.048	0.019	0.083	0.092	0.000	0.290	0.036	0.215	0.836	0.005	0.443	0.023
17/10/2016	11	moderate	0.106	0.006	0.741	0.513	0.083	0.014	0.338	1.445	0.675	0.160	0.067	0.248	0.266	0.013	1.005	0.135	0.789	0.832	0.015	1.481	0.070
17/10/2016	12	severe	0.075	0.004	0.491	0.294	0.050	0.019	0.239	0.799	0.395	0.097	0.041	0.129	0.147	0.000	0.546	0.069	0.417	0.843	0.007	0.896	0.041