Publizierbarer Endbericht

gilt für Studien aus der Programmlinie Forschung

A) Projektdaten

Allgemeines zum Projekt	
Kurztitel:	DRAIN
Langtitel:	Impact of droughts and heavy rain on greenhouse gas emissions and soil microbial communities
Zitiervorschlag:	Díaz-Pinés E., Leitner S, Keiblinger KM, Saronjic N, Zimmermann M, Zechmeister-Boltenstern. 2018. Impact of droughts and heavy rain on greenhouse gas emissions and soil microbial communities, ACRP Project, B368577. Final Report
Programm inkl. Jahr:	6th Call for Proposals, 2013
Dauer:	01.03.2014 bis 31.12.2017
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Projekt- und KooperationspartnerIn (inkl. Bundesland):	
Schlagwörter:	Drying-wetting cycles, forest soils, CO2, N2O, CH4
Projektgesamtkosten:	384,745 €
Fördersumme:	278,825 €
Klimafonds-Nr:	B368577
Erstellt am:	30.03.2018

B) Projektübersicht

1 Kurzfassung

Laut aktuellen Klimaprognosen werden Wetterextreme wie Dürre und Starkniederschläge in den kommenden Jahrzehnten deutlich zunehmen. Wie sich diese Wetterextreme auf biogeochemischen Prozesse im Waldboden auswirken, ist jedoch noch weitgehend unerforscht. Deshalb wurden im vorliegenden Projekt die Auswirkungen von Trockenstress und Starkregen auf Bodenemissionen von Treibhausgasen sowie mikrobielle Gemeinschaften in einem österreichischen Buchenwald untersucht. Dafür wurden folgende zwei Szenarien mittels Regenmanipulation simuliert: mäßiger Trockenstress (sechs aufeinanderfolgende Dürreperioden von je vier Wochen, unterbrochen von 75 mm Niederschlagsereignissen) und starker Trockenstress (drei achtwöchige Dürreperioden, unterbrochen von 150 mm Niederschlagsereignissen). Eine natürliche Kontrolle ohne Regenmanipulation wurde parallel dazu geführt. Die Trockenstressszenarien wurden jeweils in den Vegetationsperioden von 2014 und 2015 simuliert, gefolgt von einer Erholungsperiode im Jahr 2016 und einer zusätzlichen Manipulationsperiode (8 Wochen) im Jahr 2017.

Die Emissionen von Kohlendioxid (CO_2), Methan (CH_4) und Lachgas (N_2O) wurden während des gesamten Beobachtungszeitraumes hochaufgelöst mit einem automatischen Kammermesssystem gemessen. Rund um die Messkammern wurden während der Vegetationsperiode alle vier Wochen Bodenproben gezogen, und Nährstoffkonzentrationen (NO_3^-, NH_4^+, gelöster organischer Kohlenstoff (C) und Stickstoff (N)), mikrobielle Biomasse (mikrobieller C und N), mikrobielle Gemeinschaften und deren ökologische Funktionen (Phospholipid-Fettsäureprofil und Metaproteomik) bestimmt. Zusätzlich wurden die Bodenaggregatstabilität und die Hydrophobizität (Benetzbarkeit) des Bodens gemessen und ihre Auswirkungen auf den Bodenwasserfluss mit Hydrus3D modelliert.

Durch die Regenmanipulation nahmen in den stark gestressten Parzellen die CO_2- und N_2O-Bodenemissionen ab. Methan wurde in der Regel aus der Luft aufgenommen und die CH_4-Aufnahme in den Boden stieg in den Dürreplots deutlich an. Während der Vegetationsperiode nahmen die CO_2-Emissionen um ~30 % ab (Starkstress vs. Kontrolle), dieser Effekt verschwand aber während der Manipulationspause im Winter. Die N_2O Emissionen wurden in den stark trockengestressten Parzellen im Jahr 2014 um bis zu 60 % reduziert, waren dann im Jahr 2015 aber ähnlich wie in den Kontrollflächen, da diese wiederum durch einen sehr trockenen Sommer ebenfalls gestresst waren. Die CH_4-Aufnahme in den Boden stieg während der Manipulationsperioden um etwa 30 %, und dieser Effekt konnte auch noch im Winter während der Manipulationspause festgestellt werden.

Die Bodennährstoffkonzentrationen waren sehr variabel, hier kam es zu keinen konsistenten Unterschieden zwischen den experimentellen Behandlungen während der Manipulationsperiode. Zwei zusätzlich durchgeführte zeitlich hochaufgelöste Experimente zeigten jedoch, dass die Wiederbefeuchtung durch ein Niederschlagsereignis nach der Trockenperiode zu einer kurzfristigen (<10 h)

Freisetzung von NO_3^- und Aminosäuren führte. Die Stickstofffreisetzung war dabei nach der achtwöchigen Dürreperiode höher als nach der vierwöchigen. Außerdem konnte durch die Untersuchung der Hydrophobizität festgestellt werden, dass längere Dürreperioden die Benetzbarkeit des Bodens reduzierten, wodurch sich dessen Wasseraufnahmefähigkeit reduzierte. Dies beruht wahrscheinlich auf einer chemischen Veränderung der Aggregatoberflächen. Diese Ergebnisse (hohe N-Mobilisierung und verringerte Benetzbarkeit) deuten darauf hin, dass durch eine längere Dürre mit darauffolgendem Niederschlagsereignis der Stickstoffverlust durch Auswaschung dramatisch ansteigen kann, während sich die Wasseraufnahmefähigkeit des Bodens verschlechtert.

Auch die mikrobiellen Parameter zeigten eine starke räumliche und zeitliche Variabilität im Verlauf des Experiments. Mikrobielle Biomasse und PLFA-Konzentration (gruppenspezifische mikrobielle Fettsäuren) waren nach einigen Niederschlagsereignissen in den gestressten Parzellen erhöht. Es konnte jedoch keine Änderung im relativen Anteil von grampositiven und gramnegativen Bakterien oder Pilzen mittels PLFAs durch Trockenstress nachgewiesen werden. Die hochauflösenden Ergebnisse der Metaproteomikanalyse zeigten jedoch schon nach einem Jahr Regenmanipulation einen erhöhten Anteil der copiotrophen Phyla (schnell wachsend) und einen Rückgang der oligotrophen Phyla (langsam wachsend). Darüber hinaus schien die funktionelle Diversität nach den Niederschlagsereignissen zuzunehmen, was zum Anstieg der Proteine für Proteinsynthese, Translation, ribosomale Struktur und Biogenese führte. Außerdem kam es nach Ende der Niederschlagsmanipulationen im Jahr 2016 zu einem Trägheitseffekt bei der Wiederherstellung des natürlichen Gleichgewichts. Interessanterweise war die mikrobielle Biomasse zwischen manipulierten Behandlungen und Kontrolle während der Manipulationsperiode nicht signifikant unterschiedlich, jedoch hatten die Parzellen unter starkem Wasserstress während des Erholungsjahres eine erhöhte mikrobielle Biomasse. Auch die Nährstoffkonzentration unterschied sich in der Erholungsphase zwischen den gestressten Parzellen und der Kontrolle. Dies deutet auf eine Verschiebung in der Funktionalität der mikrobiellen Gemeinschaft des Bodens durch die anhaltende Veränderung der Wasserverfügbarkeit hin.

Zusammenfassend wurde gezeigt, dass natürliche Ökosysteme einen gewissen Puffer gegen Wetterschwankungen und Extremwetterereignisse haben, da die Reaktionen in den moderat gestressten Parzellen (die immerhin sechs aufeinanderfolgenden einmonatigen Dürreperioden ausgesetzt waren) sich im Schnitt nur wenig von der natürlichen Kontrolle unterschieden. Gleichzeitig wurde aber auch gezeigt, dass extreme Dürre-Wiederbefeuchtungsereignisse die THG-Emissionen und den N-Kreislauf stark beeinflussen können. Auch die mikrobielle Gemeinschaft in Boden sowie ihre Funktionen wurden durch die Regenmanipulation stark beeinflusst. Insgesamt konnten wir zeigen, dass viele biogeochemische Prozesse vom Intensität und Häufigkeit der Trocken-Wiederbefeuchtungszyklen im Boden abhängen. Daher empfehlen wir bei Modellierungen von C- und N-Ökosystembilanzen unter veränderten klimatischen Bedingungen die Dauer und Häufigkeit von Dürreperioden und Niederschlagsereignissen zu berücksichtigen.

2 Executive Summary

The intensity and frequency of drying-wetting cycles is forecasted to increase in the future, and the consequences for biogeochemical processes in the soil are still unclear. In this frame, a precipitation manipulation experiment was conducted in a beech forest site. Two different levels of water-stress were simulated in 2014 and 2015, followed by a recovery year in 2016, and one additional manipulation period (8 weeks) in 2017. The treatments comprised moderate and severe water stress, involving either 6 cycles of 4-week long drought followed by 75 mm single precipitation event or 3 cycles of 8-week long drought followed by 150 mm single precipitation event, respectively. An environmental control without manipulation was also included in the experimental design.

Soil-atmosphere exchange of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) were assessed semi-continuously (sub-daily resolution) during the whole observation period by means of an automatic chamber measurement system. Periodic soil sampling was conducted, and assessment of nutrient concentrations (NO_3^-, NH_4^+, dissolved organic carbon (C) and nitrogen (N)), microbial biomass and microbial community composition (phospholipid fatty acid (PLFA) profile and metaproteomics) were determined from 2014 to 2016. Water repellency by soil as affected by experimental drought was assessed and its effects on the soil water flux were modeled.

During the manipulation period, we observed a decrease in soil CO_2 and N_2O efflux and an increase in soil CH_4 sink strength in the plots subjected to severe stress. Average reduction in CO_2 fluxes over the vegetation period was 30 % (control vs. severe-stress) but no differences were observed in winter when all plots received natural precipitation. Fluxes of N_2O, which were of minor importance for the total global warming potential of the site, were reduced upon severe water-stress by 60 % in 2014, but no differences were observed for 2015, when the control plots also experienced a natural drought. Soil CH_4 sink strength was enhanced by roughly 30 %, and the effects persisted also in winter, when the precipitation was not manipulated.

Nutrient content was highly variable and no overall differences were observed between experimental treatments and controls during the manipulation period. However, specific experiments using soil microdialysis showed that rewetting led to a short-term flush of NO_3^- and amino acids, with the rewetting flush being higher if the preceding drought period had been longer. Hydrophobicity investigations revealed that prolonged drought periods increased the soil water repellency and caused differences between intrinsic and actual hydraulic properties, which was probably driven by changes of soil organic matter characteristics. In combination, these results (high N mobilization plus increased soil water repellency) suggest that N leaching loss can dramatically increase when a heavy rainfall event follows a severe drought.

Microbial indicators (microbial biomass C and N, microbial PLFA) were highly variable in both space and time. After some irrigation events, microbial PLFA concentration was enhanced, although the response was not consistent for all

events. The rewetting response of different microbial groups (Gram positive and Gram negative bacteria, fungi) was similar. However, in-depth metaproteomics analysis showed a higher abundance of copiotrophic phyla (fast-growing) and a decline of oligotrophic phyla (slow-growing) in response to water stress after one year of manipulation. Further, the functional diversity seemed to increase due to manipulation of precipitation, resulting in a rise in proteins assigned to protein synthesis and translation, ribosomal structure, and biogenesis.

After cease of the manipulation, we observed a legacy effect on specific soil parameters. Interestingly, microbial biomass was not significantly different between treatments during the manipulation period, but increased during the recovery period in plots that had been exposed to severe stress. Furthermore, nutrient contents also differed among treatments during the recovery year, suggesting that the microbial community had suffered an alteration in its functioning caused by prolonged changes in water availability. The effects of altered precipitation on the soil CO_2 fluxes were transient, however some legacy effect regarding CH_4 uptake was observed, with severe-stress treatments still showing higher capacity to absorb atmospheric CH_4.

In conclusion, we were able to show that natural ecosystems are buffered against weather fluctuations and climatic extremes to some extent, as the responses of the moderate-stress were in general not very different from the natural controls. After all these plots were subjected to six subsequent cycles of 4-week long drought periods, whereas the controls only experienced natural (shorter) drying-rewetting cycles. At the same time, we could show that extreme events like severe droughts and heavy rainfalls can strongly affect soil GHG emissions and N cycling. Soil microbial communities were further affected by precipitation redistribution and, despite the fact that some traditional techniques such as fumigation-extraction or PLFA assays were not able to uncover differences between stressed and control plots, novel techniques such as metaproteomics and soil microdialysis allowed us to reveal how soil microbial functionality was strongly affected by more intense drying-wetting cycles. Overall, we showed significant changes in soil biogeochemical cycling, some of them were even evident during the recovery period. Therefore, duration and frequency of drought periods and rainfall events should be considered in models which aim at predicting C and N ecosystem budgets under changing climate conditions.

3 Hintergrund und Zielsetzung/Background and Objectives

Climate change research anticipates an increase in the frequency and intensity of extreme weather events like severe droughts and heavy rainfalls. In this context, a change of summer rainfall patterns in northern mid-latitudes is expected, which will lead to extended summer drought periods followed by stronger rainfall events. The precipitation regime determines soil moisture regimes and thus affects microbiological formation and consumption of soil-borne greenhouse gases (GHG) [1]. Whereas drought generally reduces fluxes of CO_2, N_2O, and CH_4 from temperate forest floors, the effects of rewetting dry soil are still a matter of debate [2]. After rewetting, disproportionately high pulses of GHG have been reported [3,4]. However, the extent of these wetting pulses, and whether frequency and intensity of drying-rewetting cycles influences total soil emissions, is not well documented. Soil-borne GHG are produced and consumed to a large part by soil microorganisms that form highly diverse communities with various synergistic and antagonistic effects. Altered precipitation patterns can change microbial biomass and microbial community structure, therefore affecting key soil functions like nutrient cycling, litter decomposition and production and consumption of GHG. Therefore, understanding the response of soil microorganisms to changed precipitation patterns is probably the key to predict reliably how GHG emissions from soil will react to changed climatic conditions. In recent years, molecular techniques have made rapid advances. In this context, soil metaproteomics, a technique which permits to study the collective protein complements produced by soil microbial communities [5], is a very promising tool for addressing changes in microbial community structures and functions.

So far, most studies that dealt with drying-wetting cycles are laboratory studies [6,7], while field studies that focus on the response of various climate-relevant gases and soil microbiology are still scarce. In our study, we aim at increasing our understanding to that regard, by addressing the impact of more frequent and more severe drought-rewetting cycles on soil nutrient cycling, emissions of soil GHGs and soil microbiology.

Objectives of the project

Within the project, it was our aim to address the following research questions:

- What are the effects of increased drought-rewetting frequencies on soil nutrient cycling and the availability of forest soil organic C?
- Will total soil emissions of greenhouse gases be reduced by extended drought periods or will potential pulses during rewetting periods compensate or even outweigh this reduction, thereby leading to increased overall fluxes?
- How do soil microbial communities respond to increased frequency and intensity of drying-rewetting cycles in a beech forest?

The response of soil emissions of climate-relevant gases to drying and rewetting and the underlying mechanisms are still not well understood. To explain gas pulses after rewetting, a temporary increase of substrate availability has been proposed, which might occur either due to (1) dying of microorganisms during drought or rewetting, (2) release and metabolizing of osmotically active substances such as amino acids or polyols during rewetting, or (3) breaking of soil aggregates which are physically protecting soil organic matter (SOM), thereby making formerly protected substrates available to microorganisms [8].

Hypothesis 1: Higher frequency of drought-rewetting cycles leads to access of additional organic matter, which alters as well the C-cycle as the N-cycle in soils and results in an increase of total climate-relevant gas emissions from soils.

Microbial community composition can alter soil processes [7] and specific microbial groups that are responsible for certain soil processes (e.g. autotrophic nitrifiers, saprotrophic fungi) might exhibit different sensitivities to soil moisture. Filamentous fungi, for example, that can access deeper and moister soil regions or ground water with their mycelium are more tolerant against water stress than bacteria [9]. Other microorganisms might allocate resources to protective osmolytes [10] or building structures like thick peptidoglycan-walls in gram-positive bacteria [11] to increase their stress resistance, or survive drought periods by forming endospores [12]. Taxa that are resilient to water stress might be susceptible to cell death but compensate losses with rapid population growth from surviving cells. Although microbial adaptation to changing environmental conditions is a known phenomenon that occurs across diverse habitats, the adaptation of microbial communities and its effects on process rates in different soils still contain large uncertainties and require further research [2].

Hypothesis 2: Soil microbial communities respond to changes in environmental conditions through (i) physiological adaptions on the cellular level in the short term, and (ii) restructuring of the community composition in the long term, and can be detected by soil metaproteomics. Altered microbial community structures might result in process rates that have a different equilibrium than the same processes in undisturbed soil.

4 Projektinhalt und Ergebnis(se) /Project Content and Results

4.1. Presentation of the project: Main objectives and general experimental set up

The main objective of the project was to address what are the effects of increased drying-wetting cycles on the soil nutrient cycling, the total emission of greenhouse gases (GHG) and the potential alteration of soil microbial communities are their functions. Whereas some useful information has been gained to that regard under laboratory conditions, it was our priority to conduct our investigations under field conditions. Therefore, we performed a manipulation experiment in a mature beech stand where the incoming rain into the soil was manipulated over consecutive years. Briefly, the experimental set up encompassed a 1) control (no manipulation); 2) moderate stress treatment and a 3) severe stress treatment. The moderate stress treatment included simulation of drying-wetting cycles, each involving 4 weeks of precipitation exclusion followed by a single precipitation event of 75 mm; such cycles were repeated six times along the vegetation period (may to October). The severe stress treatment involved drought periods of 8 weeks followed by a precipitation event of 150 mm; this was repeated three times along the vegetation period. Specific details on the implementation of the plots and the configuration can be found in the methods section of this document.

4.2. Presentation and description of work packages

The project was structured in four work packages

Work Package 1. Emissions of greenhouse gases

Objective

Determine whether total emissions of GHGs (CO_2, CH_4, N_2O) will be reduced or increased by extreme events (repeated drought and rewetting).

Description

The response of soil emissions of climate-relevant gases to drying and rewetting and the underlying mechanisms are still not well understood. To explain soil gas pulses after rewetting, a temporary increase of substrate availability has been proposed, which might occur due to the breaking of soil aggregates caused by physical stress of repeated drying and rewetting, and the subsequent release of formerly protected soil organic matter.

We hypothesize that higher frequency of drought-rewetting cycles leads to faster breakdown of aggregate structures. Thus, increasing frequency of drought periods and heavy rainfalls leads to access of additional organic matter, which results in an increase of total climate-relevant gas emissions from soils.

Methodology
Field experiment with permanent online determination of soil gas emissions via an automated gas measurement system coupled to soil moisture and –temperature loggers; determination of soil aggregate stability in the laboratory.

Work Package 2: Soil nutrient-cycling

Objective
Investigate the impact of extreme events on soil nutrient-cycling processes.

Description
Repeated drought and rewetting events markedly influence soil moisture and thus C- and nutrient-cycling. Episodic heavy rainfall can lead to runoff or rapid infiltration, and thus to reduced soil moisture compared to smaller and long-lasting precipitation. Long drought periods have been reported to result in increased hydrophobicity of soils and thus might enhance water runoff. Drought and rewetting cycles are also responsible for C destabilization within soil aggregates. Finally, soil cracks formed due to extreme desiccation represent corridors for rainwater, which quickly drains through these preferential flow paths. Fast runoff of rainwater can result in leaching of mobile substances (e.g. NO_3^-) from the soil, which can cause serious problems as pollutant of groundwater and rivers.

We hypothesize that longer drought periods followed by heavy rainfalls lead to changed soil aggregate stabilities and thus increased losses of mobile substances like nitrate and decreased soil moisture in the long term

Methodology
Field experiment with application of two water stress treatments (WP1); sampling of soil and soil water and chemical analysis in the laboratory.

Work Package 3: Response of the microbial community

Objective
Examine changes in the microbial community composition in response to extreme events; determine microbial stress resistance and resilience.

Description
Specific microbial groups that are responsible for certain soil processes (e.g. autotrophic nitrifiers, saprotrophic fungi) might exhibit different sensitivities to soil moisture. Filamentous fungi, for example, that can access deeper and moister soil regions or groundwater with their mycelium are more tolerant against water stress than bacteria. Other microorganisms might allocate resources to protective osmolytes or building structures like thick peptidoglycan-walls in gram-positive bacteria to increase their stress resistance, or survive drought periods by forming endospores. Taxa that are resilient to water stress might be susceptible to cell death, but compensate losses with rapid population growth from surviving cells.

We hypothesize that soil microbial communities respond to changes in environmental conditions through (i) physiological adaptions on the cellular level in the short term, and (ii) restructuring of the community composition in the long term. Altered microbial community structures might result in process rates that have a different equilibrium than the same processes in undisturbed soil.

Methodology
Field experiment with application of two water stress treatments (WP1); analysis of PLFAs (phospholipid fatty-acids) before, during and after stress treatments to determine microbial stress resistance and resilience. Metaproteomic analysis of a subset of samples to track metabolic pathways will enable us to link microbial diversity (community) to ecosystem function (process rates).

Work Package 4: Project coordination and data synthesis

Objectives
• Project management, coordination of the workgroups, supervision of compliance with the project plan, financial monitoring
• Data integration & synthesis of results, writing of interim and final reports

Description:
Besides the main due of allowing an appropriate workflow between the rest of the work packages, the project coordination aims at actively communicating with internal and external groups, in a way to stimulate the initiation of new projects (Bachelor-, Master- and PhD-theses). The field site will be stablished internationally as a highly-instrumented long-term research site.

Methodology
• Arrangement of regular project meetings.
• Formulation and distribution of protocols of the meetings.
• Ensuring that all work packages adhere to their timetable.
• Keeping contact with the project-specific contact person at the granting agency.
• Organize payments to subcontractors, financial monitoring.

4.3. Description of results

4.3.1. Work package 1: Emissions of greenhouse gases

The site was prepared with wooden walk boards to minimize impact on the plots while walking around for collecting samples. Wooden structures were also installed, onto which the precipitation-exclusion roofs were accommodated. Further, an irrigation system was mounted, equipped with two sprinklers at each of the stressed plots (both moderate and severe). A sheet plate hut was installed at the centre of the site to accommodate the analytical devices, and the chambers

were installed. The site was fully operational by the scheduled beginning of the measurements (Figure 4.1)

Figure 4.1. Overview of the experimental site with precipitation-exclusion roofs and automatic measurement chambers installed. Spring 2015. Credit: Leitner, Sonja

Basic environmental parameters at the different plots are shown in Figure 4.2. Soil temperature was not affected by the manipulation. In contrast, and as expected, soil water content was strongly influenced by the ongoing precipitation manipulation. It was noteworthy that, during winter periods, moderate-stress treatment plots remained more humid than the control plots, whereas severe-stress plots showed usually lower levels of soil water content.

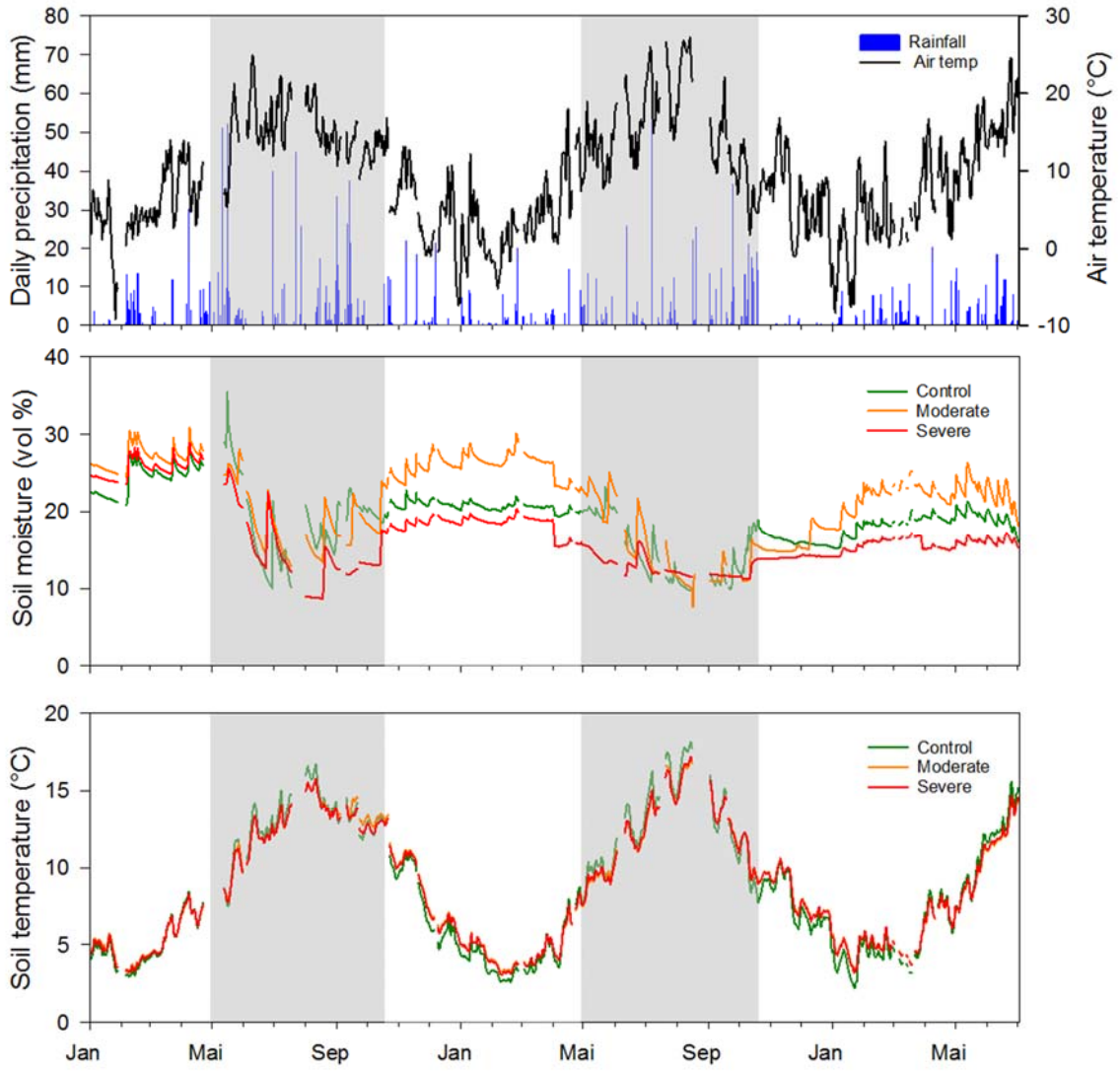

Figure 4.2. Precipitation and air temperature (top), soil moisture (middle) and soil temperature (bottom). Grey-shaded areas indicate the periods in which precipitation was manipulated.

Soil respiration (SR)

During the vegetation period, control plots emitted on average 109 ± 3 mg C m^{-2} h^{-1} in 2014 and 79 ± 3 mg C m^{-2} h^{-1} in 2015 (Figure 4.3). Emissions from moderately stressed plots were in the same range as control plots, with 97 ± 9 mg C m^{-2} h^{-1} in 2014 and 77 ± 4 mg C m^{-2} h^{-1} in 2015. In severely stressed plots, the precipitation manipulation led to a significant decrease in average SR during the vegetation period in both 2014 (77 ± 7 mg C m^{-2} h^{-1}) and 2015 (53 ± 12 mg C m^{-2} h^{-1}). This decrease did not persist during the winter when roofs where removed.

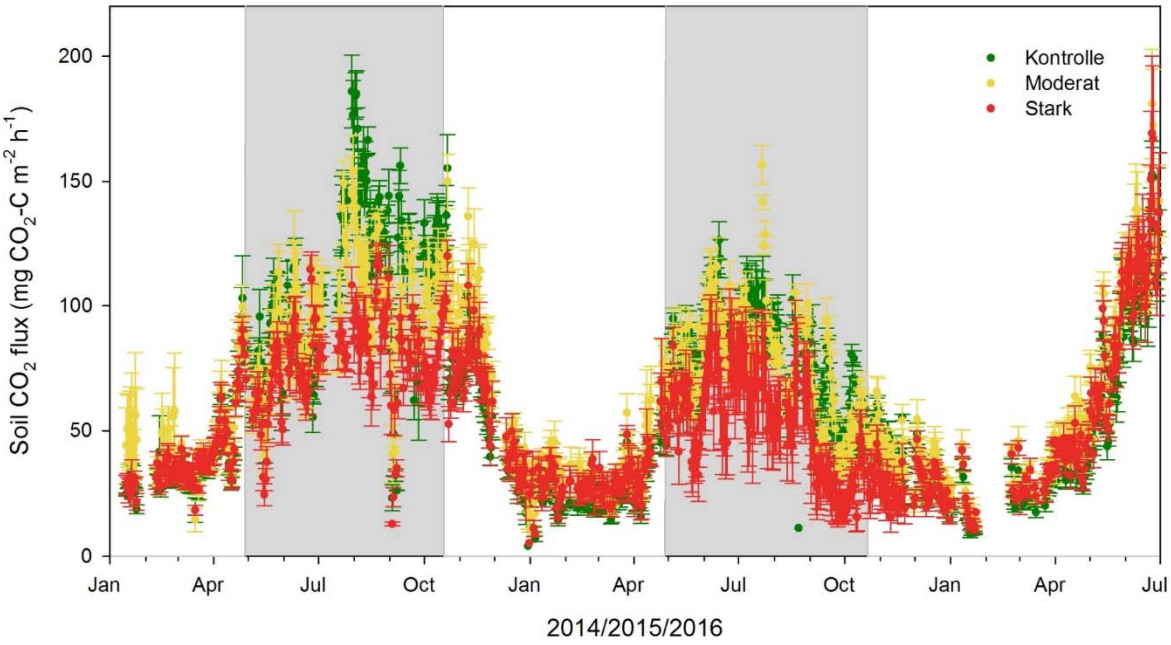

Figure 4.3. Soil CO_2 efflux in Rosalia Lehrforst from January 2014 to July 2016. The grey-shaded areas indicate the periods when the precipitation was manipulated.

To describe the temperature sensitivity of SR, a Gauss model was fit to the SR data of each treatment (Fig. 4.4). Inside a temperature window between 1-15 °C, this Gauss model fit the SR data well, but at Tsoil >15 °C, the data showed two distinct groups: SR measurements on days where soil water-filled pore space (WFPS) was >20 % were underestimated by the Gauss model, while SR measurements on days with soil WFPS ≤20 % were overestimated by the Gauss model.

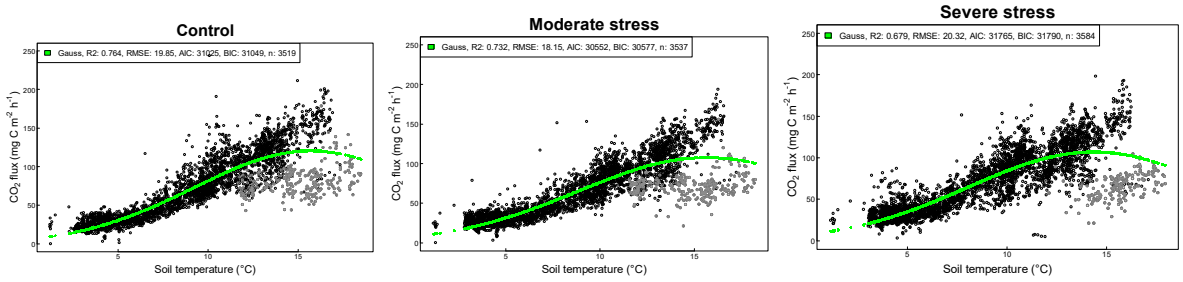

Figure 4.4. Soil CO_2 flux (black circles, mg C m^{-2} h^{-1}) plotted against soil temperature (10 cm depth) of control, moderately stressed and severely stressed plots (3-hourly flux measurements averages, n = 4). Gray dots indicate soil CO_2 flux values measured at WFPS ≤ 20%. Green dots show the relationship between soil CO_2 flux and soil temperature (*RMSE*, root mean square error; *AIC*, Akaike Information Criterion; *BIC*, Bayesian Information Criterion; *n*, number of cases).

Soil-atmosphere CH_4 exchange

The soil was a net CH_4 sink over the whole observation period. Regardless of the treatments, there was a clear seasonal pattern, with higher CH_4 uptake rates during the vegetation period, which were markedly reduced in the winter and early-spring time (Figure 4.5). During the first project year (April 2014-March 2015), cumulative CH_4 uptake rates were lower in the control plots (3.0 ± 0.5 kg CH_4-C ha^{-1} a^{-1}) compared to the moderate stress (3.7 ± 0.3 kg CH_4-C ha^{-1} a^{-1}) and the severe stress (4.2 ± 0.3 kg CH_4-C ha^{-1} a^{-1}) treatments. For the period April 2015-March 2016, differences were no longer evident, and cumulative uptake rates showed a narrower range (i.e. from 3.8 to 4.1 kg CH_4-C ha^{-1} a^{-1}).

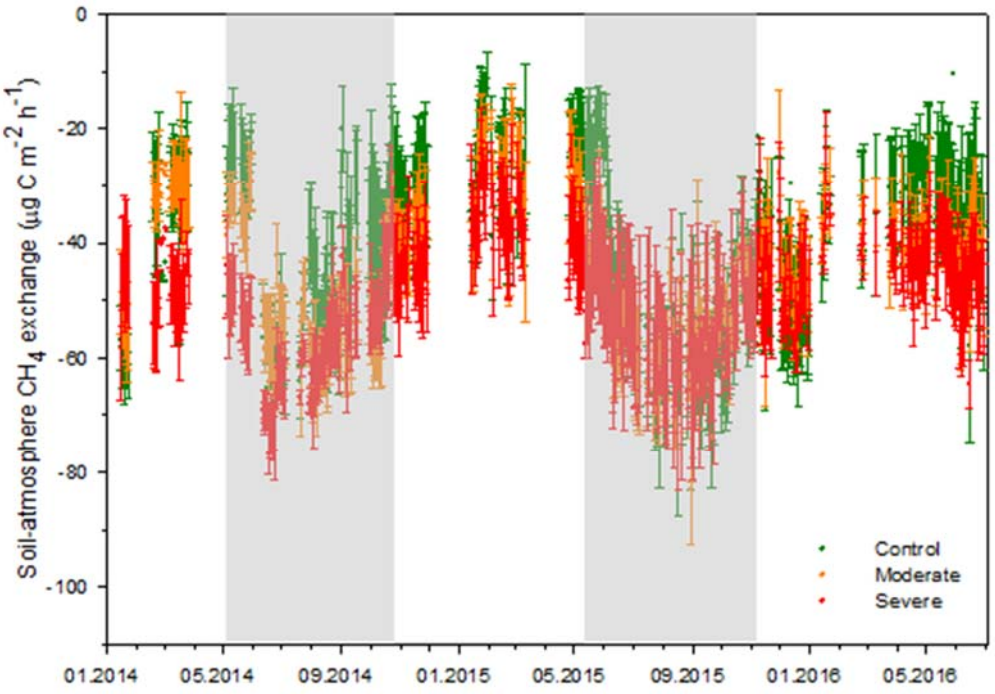

Figure 4.5. Soil-atmosphere CH_4 exchange rates from January 2014 to September 2016.

Interestingly, we observed a different response pattern of CH_4 uptake rates to soil temperature and soil moisture for the different manipulation treatments (Figure 4.6): whereas CH_4 uptake rates in control plots were virtually driven by soil moisture of the uppermost 10 cm only, the influence of soil moisture on CH_4 uptake rates decreased gradually for the moderate and severe stress treatments, with soil temperature showing increasing importance as the level of stress was higher. This may suggest that substantial CH_4 uptake may take place at deeper layers, thus supporting high soil CH_4 uptake rates when the soil moisture near the soil surface is still high.

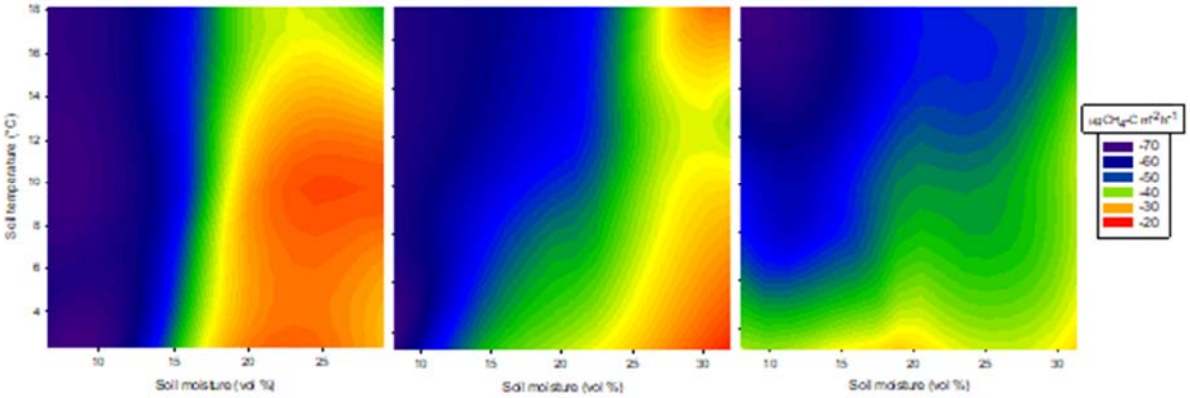

Figure 4.6. Surface contour plots for soil CH_4 fluxes as affected by soil moisture and soil temperature for control (left), moderate stress (middle) and severe stress (right) treatments.

Soil N_2O fluxes

Observed soil N_2O fluxes were low and therefore near the analytical detection limit along the whole observation period (Fig 4.7). Average values for the first rain exclusion period (April-October 2014) were 5.4 ± 2.1, 5.5 ± 1.1 and 1.7±1.3 µg N_2O-N m^{-2} h^{-1} for control, moderate-stress and severe-stress treatments, respectively. During the second observation period in 2015 fluxes were even lower, (1.2 ± 0.4, 0.2 ± 0.4 and 0.9 ± 0.3 for control, moderate-stress and severe-stress, respectively). This yielded cumulative soil N_2O fluxes of < 200 g N_2O-N ha^{-1} a^{1}, below the range of average values reported for beech forests in temperate ecosystems (e.g. 5 kg N_2O-N ha^{-1} a^{-1}, [13];450 g N_2O-N ha^{-1} a^{-1} [14]; 520-790 g N_2O-N ha^{-1} a^{-1} [15]).

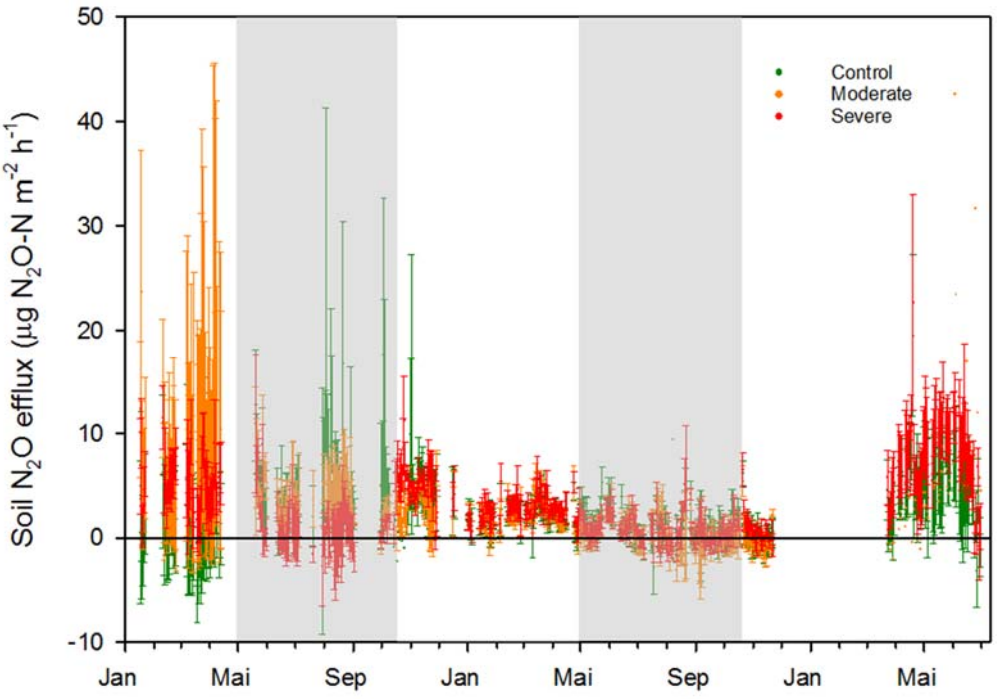

Figure 4.7. Mean soil N_2O fluxes from January 2014 to September 2016.

Since rewetting of soils can lead to a short-lived N_2O pulse which might be gone already after several hours, we could not exclude that our time resolution was not enough to capture such emission peaks. Even if we carried out automatic chamber measurements, they are still of a semi-continuous nature, since it takes three hours from one measurement round to the following one. To find out the potential bias generated by this, we conducted a specific irrigation campaign in 2017. This time, we coupled the automatic chamber system with a CH_4/N_2O Laser (Los Gatos Research Inc, Santa Clara, CA; USA) and we adapted the sampling sequence in order to measure the soil-atmosphere exchange of GHGs within 15 minutes after precipitation. We also reduced the chamber closing time so that time-resolution of the measurements was reduced to 90 minutes (instead of 180 minutes with the normal procedure). This enabled us to detect a N_2O emission pulse from both moderate and severe treatments (Figure 4.8), which lasted for approximately 3 days. However, the magnitude of the pulse was very low, with peak emission values of roughly 5 µg N_2O-N m^{-2} h^{-1} in the water-stress treatments, compared to < 1 µg N_2O-N m^{-2} h^{-1} in the control treatment.

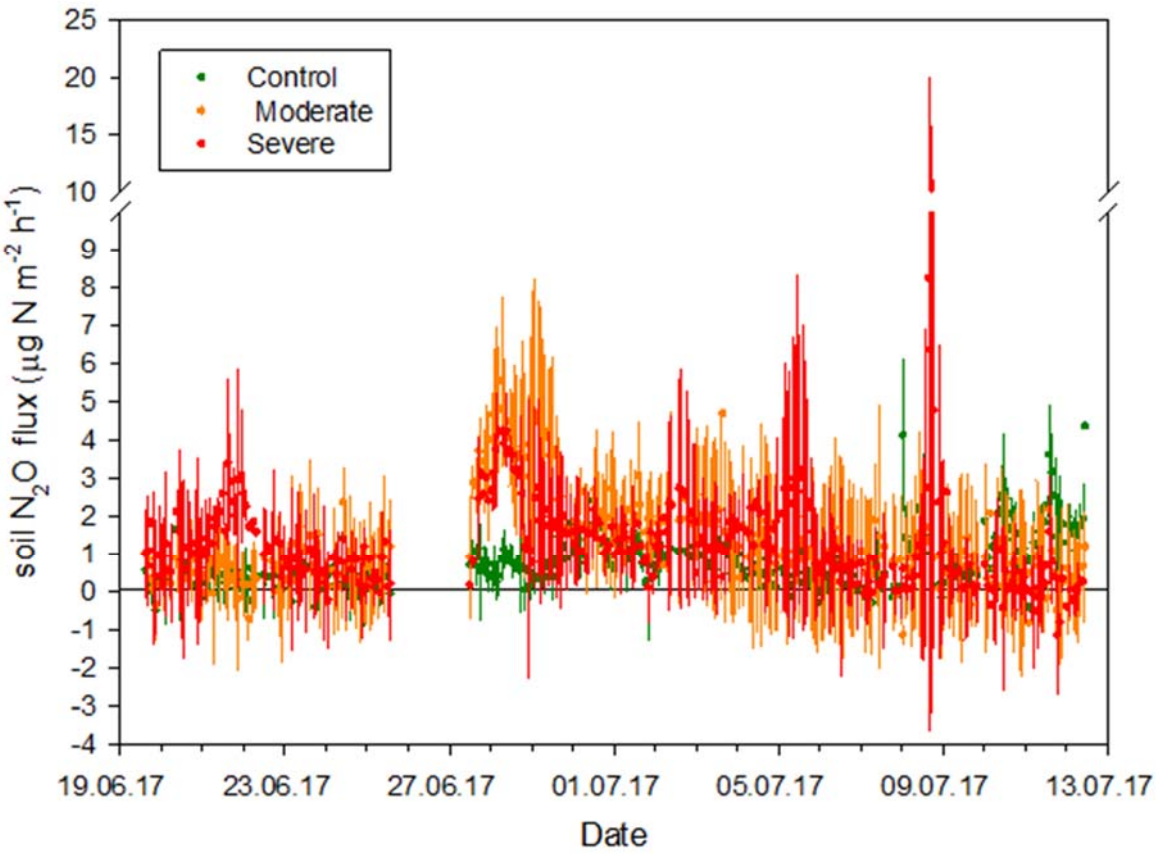

Figure 4.8. Mean soil N_2O fluxes in early summer 2017. An artificial precipitation event was carried out on June 27th (moderate-stress treatment: 75 mm; severe-stress treatment: 150 mm)

Work package 2: Soil nutrient cycling

Rainfall partitioning and input of nutrients from the canopy to the soil

Throughfall, stemflow and interception during the growing season accounted from 272 ± 15 mm (60%), 48 ± 9 mm (10 %) and 138 mm (30 %). The chemical composition of each precipitation component as well as litter percolate and soil water can be seen in (Fig. 4.9). Nutrient concentration in throughfall or stemflow compared to bulk precipitation was higher only for NO_3^-, SO_4^{-2} and PO_4^-, suggesting that the wash off and/or leaching from the vegetation surfaces was only relevant for those nutrients. Nutrient concentrations were highest in the litter percolate, with values dropping again in the mineral soil water. This suggests that, while the litter layer actively releases nutrients through the decomposition of fresh organic matter, those nutrients are rapidly taken up by plants and microorganisms in the mineral soil.

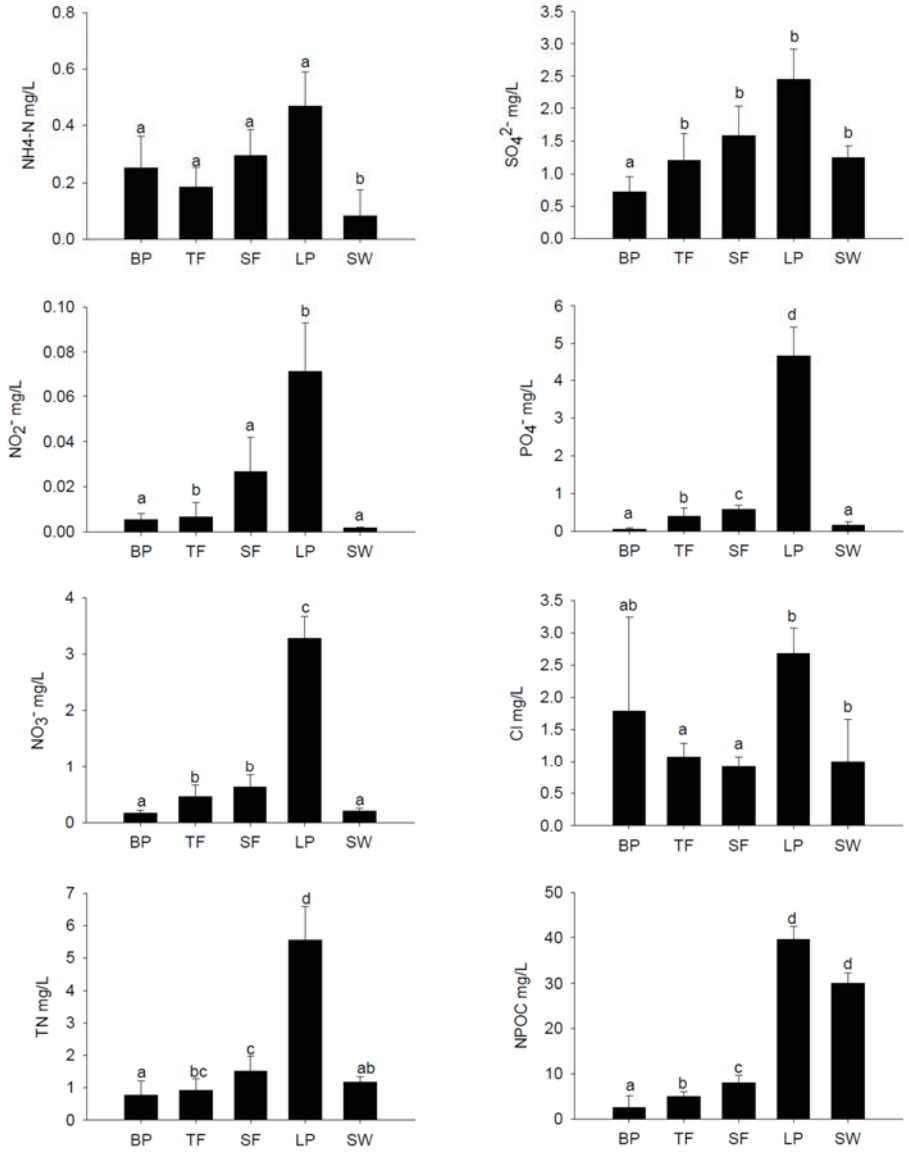

Figure 4.9. Mean concentrations of nutrients in bulk precipitation (BP), throughfall (TF), stemflow (SF), litter percolate (HL) and soil water (BW) from 1st of April to 31st of October.

Mean N wet deposition rates onto the site were also calculated for the vegetation period. Total (throughfall and stemflow) NH_4^+ and NO_3^- deposition was 0.7 and 1.6 kg N ha^{-1} $season^{-1}$, respectively. Bearing in mind the uncertainty of having measured only 6 months over a year, our N deposition estimates are lower than the most actual averages at the European level (5.0 and 4.8 kg N ha^{-1} a^{-1} for NH_4^+ and NO_3^- respectively, period 2011-2015, [16]).

Soil nutrient cycling during the manipulation and recovery period

Inorganic N pools in the soil were highly variable during the investigation period. Average NH_4^+ concentrations ranged between 5 and 40 mg N kg^{-1}, whereas average NO_3^- concentrations varied between <1 and 6 mg N kg^{-1}. During the manipulation period, average values of inorganic N were not significantly different between treatments. Some irrigation events led to an increase of NH_4^+ 72 hours after rewetting (e.g. October 2014) but the responses were in general not consistent. Nitrogen pools in soils are highly dynamic and depend on various processes including enzymatic depolymerization of proteins, input via root exudation, N transformation (e.g. ammonification, nitrification), uptake by plants [17] and immobilization by microorganisms [18]. As a result, soil extraction usually integrates N concentrations over a soil volume in the range of a couple of cm^3 that are relatively static over hours or days. Therefore, we wanted to investigate the short-term dynamics of soil N availability with soil microdialysis, a novel technique with high spatial and temporal resolution. Our microdialysis results showed large differences in NH_4^+ and amino acid flux between the moderate and severe treatment with flux rates that varied by a factor of 10 (Fig. 4.10). When evaluating temporal dynamics, the high flux of NH_4^+ and total amino acids in the moderate-stress treatment was not sustained but dropped after 1 h to fluxes similar to those in the severe-stress treatment. Irrigation of the soil led to a mobilization of NO_3^- and some neutral hydrophilic amino acids. Overall, more N was mobilized in the severe-stress treatment than in the moderate-stress treatment, suggesting that during the longer drought period more labile N had accumulated.

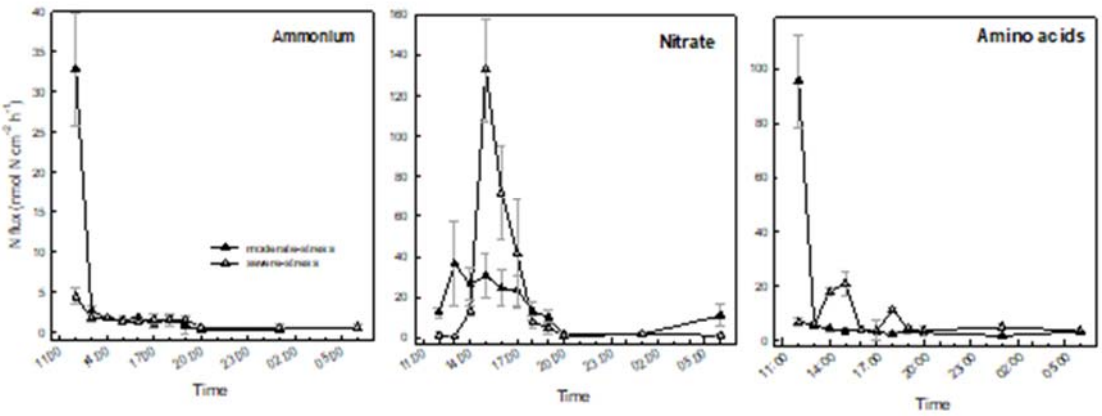

Figure 4.10. Ammonium, nitrate and amino acids fluxes in the topsoil during a precipitation event as measured by microdialysis

Inorganic N content in the soil was further investigated in 2016, when the manipulation stopped, to assess the capacity of the soil to recover after a two-year long disturbance period. Interestingly, while no difference in NH_4^+ content was found, the NO_3^- concentrations were higher in the stress treatments (Fig. 4.11), indicating either reduced N uptake (by plants of microorganisms) or enhanced nitrification rates. This suggests that a legacy effect of extreme drying-wetting cycles affecting NO_3^- transformation processes is still evident one year after stopping the manipulation.

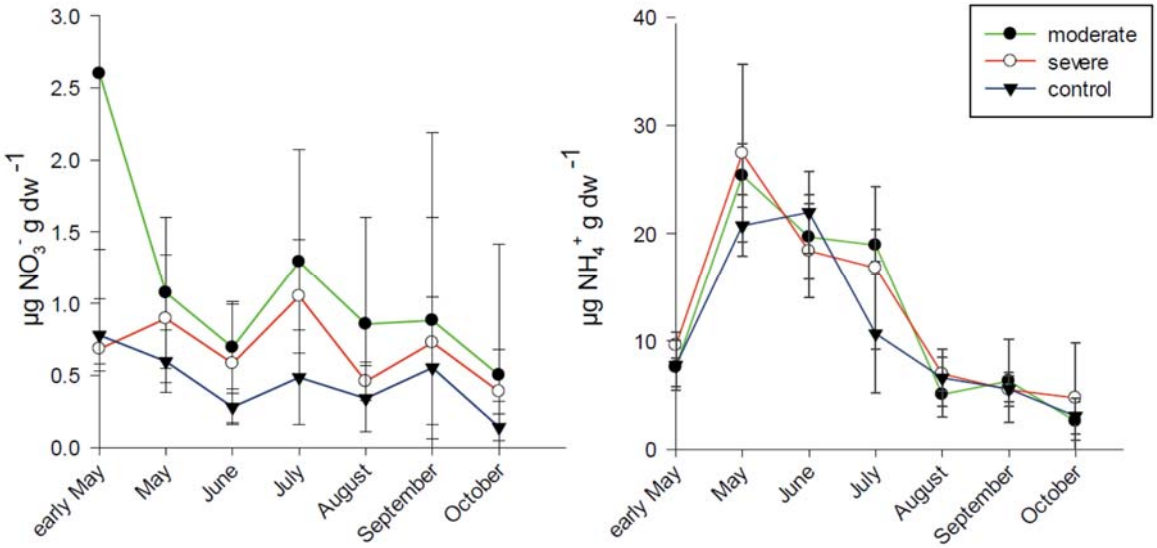

Figure 4.11. Mean (±SE) values of nitrate (NO_3^-) and ammonium (NH_4^+) in the experimental treatments during the "recovery" observation period

Aggregate stability and soil organic matter water repellency as affected by drying-wetting cycles

After drought, soil aggregate stability decreased most likely due to the absence of organic binding agents. However, the short duration of these effects suggested that the disturbance of drying-wetting occur only at early stages of the year and that micro-aggregates have the ability to adapt to changing environmental conditions, explaining the stabilization of organic C and the low release of DOC after rewetting.

At the surface of the A horizon and a 10 cm soil depth, γ (soil-water contact angle) showed higher and more stable values in water-stressed plots compared to the control treatment (Fig. 4.12). Thus, rainfall redistribution likely resulted in a considerable increase of potential SWR at the soil surface with critical γ. The prolonged drought exposure also resulted in a reduction of the horizontal spatial γ variability at the soil surface, probably due to a more homogeneous distribution of water that reaches the soil surface by the artificial irrigation compared to the natural rainfall that reaches the soil surface more erratically due to a redistribution

by the forest canopy. Furthermore, modeling of soil water fluxes showed that higher water repellency increases surface runoff in non-structured soils at hillslopes leading to lower water infiltration rates in the soil.

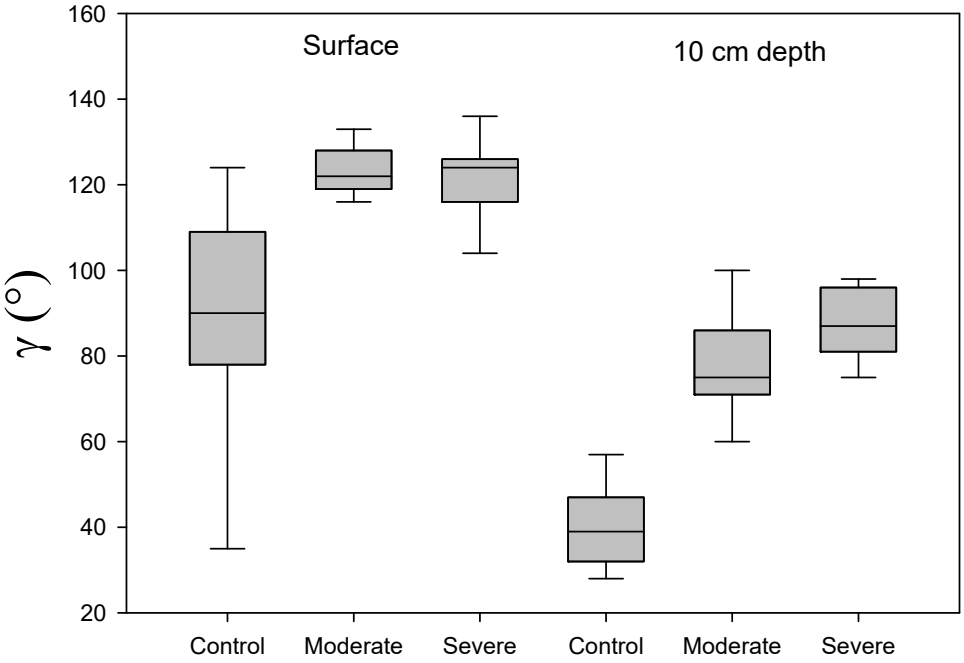

Figure 4.12. Box and whisker plot of the contact angle g between air-dry soil and water determined with the modified sessile drop method in control (C, blue), moderate (M, orange), and extreme rainfall redistribution (E, red) treatments, in the mineral soil surface (left panel) and at a depth of 0.10 m (right panel).

Work package 3: Response of the microbial community

Microbial C and N during and after the manipulation period

Microbial biomass C and N were determined on a regular basis in soil samples with the fumigation—extraction technique. Strong temporal dynamics were observed during the manipulation period (2014 and 2015). Biomass C and N were highest at the beginning of our experiment (April 2014: mean 1.2 µg C g^{-1} and 0.12 µg N g^{-1}) probably due to the moist and warm conditions in the soil after a rainy early-spring. During the vegetation periods of 2014 and 2015 (Fig. 4.13), microbial biomass contents were usually in the range of 0.4-0.6 µg C g^{-1}, as the microbial biomass was probably limited by water availability. We did not observe a consistent response of the microbial C and N to soil rewetting, or to water-stress. This lack of differences suggests that the soil microbial biomass was probably able to adapt to changing water conditions without substantially altering its total C and N content.

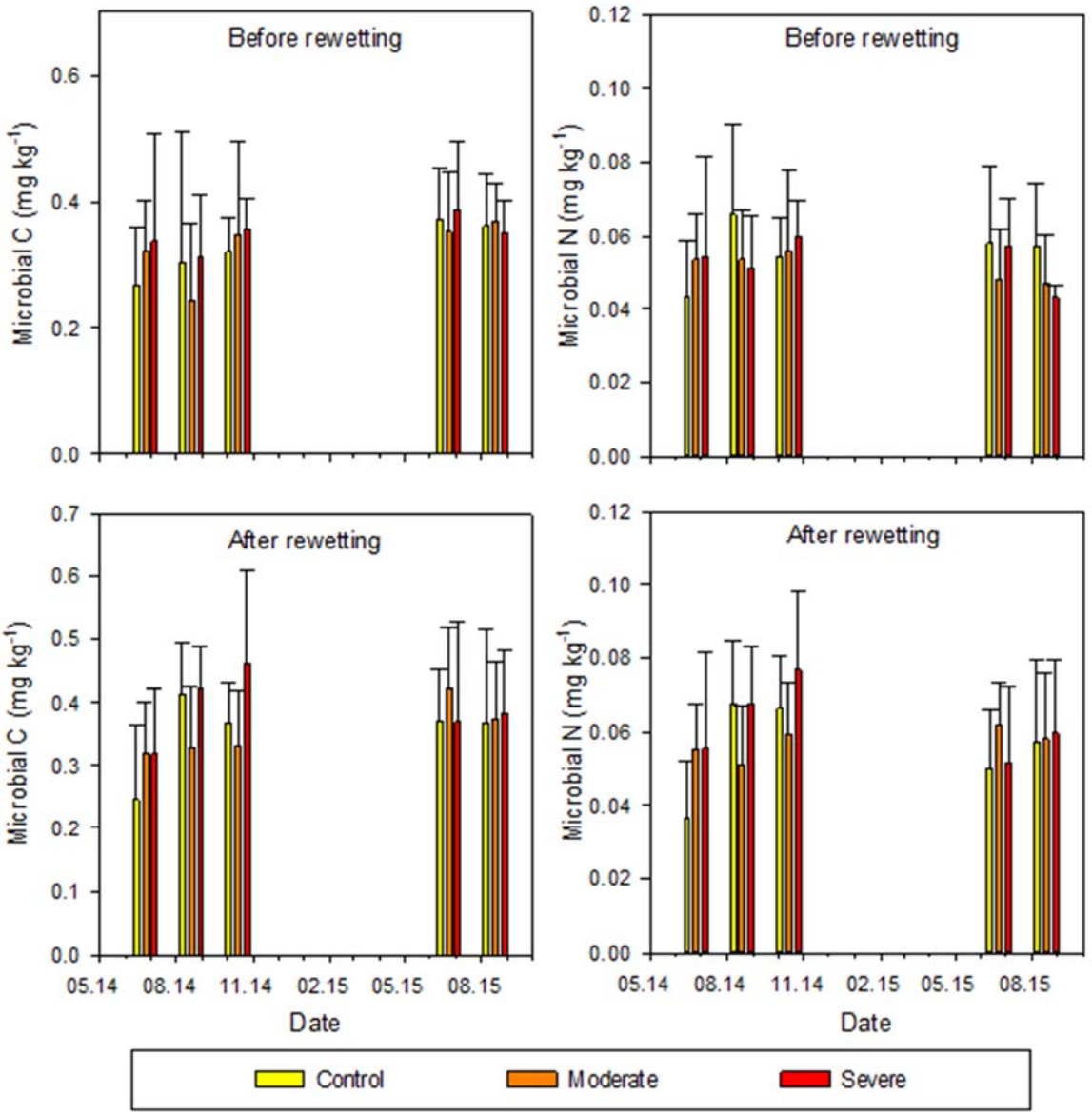

Figure 4.13. Mean microbial C and N contents in the mineral soil before (upper panels) and after (lower panels) artificial rewetting.

Interestingly, differences between control and water-stressed plots in microbial biomass C and N arose during the recovery period in 2016, and they were more evident for microbial biomass N, with the severe-stress treatment showing two-fold higher N contents in the microbial biomass that moderate-stress or control. This suggest that the subsequent increase in water availability after cease of the manipulation might have been used by dormant microbial communities to be active. While this was expected according to existing literature [e.g. 2], we find it surprising that the microbial biomass even exceeded the levels observed in the control plots during the first non-manipulated vegetation period.

Soil microbial community as determined by PLFA during and after the manipulation period.

Results of microbial PLFAs showed a high temporal variability, both between and within observational years (Figure 4.14). Overall, PLFA values in the control plots were lower in 2014 (around 15 nmol PLFA g^{-1} dm) than in 2015 (ca. 22 PLFA g^{-1} dm). A similar temporal pattern was also evident for the moderate and severe-stress treatments.

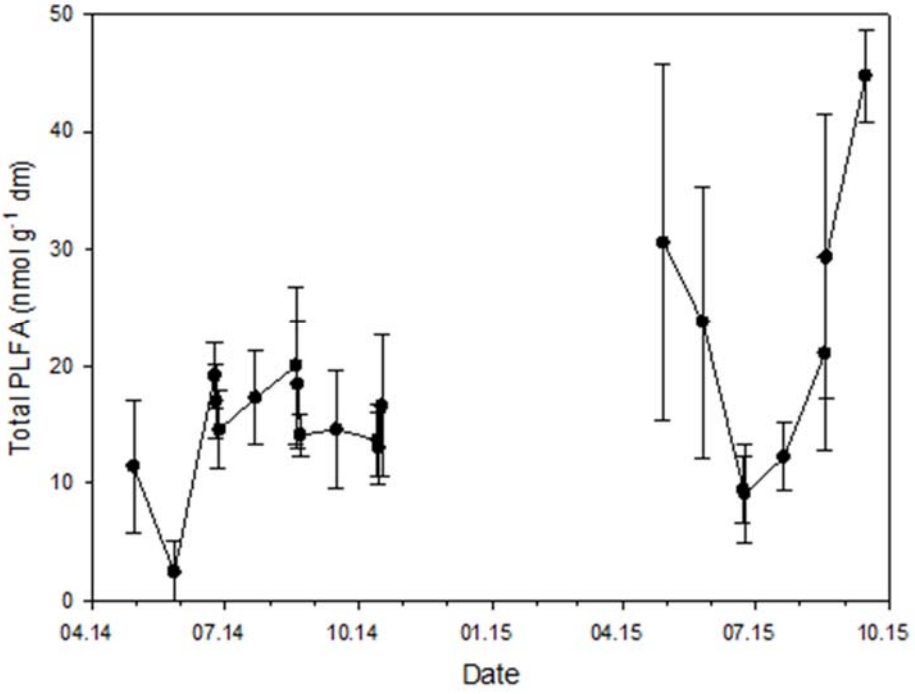

Figure 4.14. Average (± SD, n = 4) microbial PLFA in the control plots over the observation period.

PLFAs of microbial origin were measured in the three treatments at the beginning of each vegetation period. In Spring 2014, total PLFA content was higher in the severe-stress treatment compared to the moderate-stress or control treatments, but differences gradually disappeared with the onset of the artificial drought period. While total PLFA values between treatments were similar after the first drought period, the water-stress treatments showed lower PLFA values after the second (August 2014) and third (October 2014) drought periods. By the beginning of the vegetation period in 2015, PLFA values were overall higher than in the previous year, probably due to wetter conditions.

The response of PLFAs upon rewetting was also highly variable and varied across the artificial irrigation events (Table 1). In the moderate-stress treatment, total PLFA content increased from 11.3 to 15.8 nmol PLFA g^{-1} dm (40 %) and from 7.3 to 12.3 nmol PLFA g^{-1} dm (68 %) in the irrigation events from October 2014 and June 2015, respectively (Table 1), whereas total PLFA did not seem to be affected in the remaining irrigation treatments (i.e. June and August 2014, August 2015). With regard to the severe-stress treatment, an evident increase of total PLFA was

appreciated following irrigation in August 2014 (from 14.0 to 20.2 nmol PLFA g^{-1} dm, 44%), October 2014 (from 8.6 to 15.6 nmol PLFA g^{-1} dm, 80 %) and June 2015 (from 9.1 to 12.1 nmol PLFA g^{-1} dm, 33 %). Concomitantly with the sampling in the water-stress treatments, we also estimated PLFA in the control plots, to account for temporal variations independent from the artificial irrigation. Interestingly, total PLFAs showed a strong increase in August 2015 between the samplings before and after the irrigation, coinciding with a natural precipitation event of about 22 mm between both sampling dates.

According to our results, responses of the different microbial groups (i.e. fungi, Gram positive and Gram negative bacteria) were similar upon rewetting, with response ratios of each microbial group within the range of the overall total PLFA response ratio.

Date	Event	Total PLFA (nmol g^{-1} sdm)			Fungal PLFA (nmol g^{-1} sdm)			Gram+ PLFA (nmol g^{-1} sdm)			Gram - PLFA (nmol g^{-1} sdm)		
		Con	Mod	Sev	Con	Mod	Sev	Con	Mod	Sev	Con	Mode	Sev
4/2014	Begin Veg P	11.4 (5.7)	13.6 (5.5)	16.1 (4.8)	5.9 (3.2)	7.0 (3.0)	8.9 (3.2)	3.0 (1.5)	3.6 (1.3)	3.9 (0.9)	1.9 (0.8)	2.3 (1.0)	2.5 (0.7)
6/2014	Bef Rew	19.2 (2.9)	17.7 (2.3)	18.4 (7.0)	10.4 (1.2)	9.3 (1.5)	9.8 (4.2)	4.7 (1.0)	4.7 (0.5)	4.8 (1.6)	3.2 (0.7)	2.9 (0.3)	3.0 (1.0)
	After Rew	17.0 (3.1)	17.4 (6.0)	15.2 (4.0)	8.9 (1.3)	8.6 (3.1)	7.7 (2.1)	4.4 (1.1)	4.8 (1.6)	4.1 (1.3)	2.9 (0.6)	3.2 (1.1)	2.6 (0.5)
8/2014	Bef Rew	20.0 (6.7)	12.6 (6.8)	14.0 (6.4)	12.9 (3.5)	6.9 (3.9)	8.5 (3.6)	3.7 (2.0)	3.1 (1.6)	2.9 (1.6)	2.6 (0.8)	2.0 (1.1)	2.0 (1.0)
	After Rew	18.4 (5.5)	14.4 (7.0)	20.2 (5.2)	11.4 (3.4)	8.8 (4.7)	11.8 (2.9)	3.5 (1.1)	2.9 (1.2)	4.5 (1.5)	2.7 (0.8)	2.1 (1.0)	3.1 (0.7)
10/2014	Bef Rew	13.7 (3.0)	11.3 (1.6)	8.6 (1.6)	7.0 (1.6)	6.1 (0.8)	4.7 (0.9)	3.5 (0.9)	2.6 (0.4)	2.0 (0.4)	2.5 (0.5)	2.0 (0.2)	1.6 (0.3)
	After Rew	13.0 (3.1)	15.8 (7.4)	15.6 (10.0)	7.0 (1.9)	8.4 (3.9)	8.0 (5.4)	3.0 (0.7)	3.9 (2.0)	4.2 (2.8)	2.4 (0.4)	2.9 (1.3)	2.6 (1.5)
4/2015	Begin Veg P	30.6 (15.2)	28.2 (14.3)	30.0 (10.1)	15.6 (7.3)	14.6 (7.8)	14.6 (5.1)	7.9 (4.4)	7.1 (3.8)	8.2 (2.9)	5.2 (2.6)	4.9 (2.2)	5.5 (1.9)
6/2015	Bef Rew	9.5 (2.9)	7.3 (1.8)	9.1 (3.5)	4.1 (1.0)	3.4 (0.9)	4.2 (1.8)	3.1 (1.1)	2.1 (0.6)	2.8 (1.0)	1.8 (0.6)	1.2 (0.3)	1.6 (0.6)
	After Rew	9.1 (4.1)	12.3 (4.7)	12.1 (3.5)	4.3 (2.1)	5.8 (2.2)	5.6 (1.8)	2.6 (1.1)	3.7 (1.5)	3.7 (1.0)	1.5 (0.6)	2.0 (0.7)	2.1 (0.5)
8/2015	Bef Rew	21.1 (8.3)	20.9 (3.8)	20.8 (8.3)	9.5 (4.2)	9.6 (1.9)	9.2 (3.6)	6.8 (2.5)	6.7 (1.2)	6.7 (2.6)	3.6 (1.2)	3.4 (0.5)	3.7 (1.6)
	After Rew	29.4 (12.2)	22.8 (5.4)	23.6 (5.1)	14.1 (5.9)	11.0 (2.8)	11.1 (2.0)	9.0 (3.8)	7.0 (1.7)	7.4 (1.8)	4.8 (2.0)	3.7 (0.9)	3.9 (0.9)

Table 1. Mean (SD) total, fungal, Gram+ and Gram- PLFA in the control (con), moderate-stress (mod) and severe-stress (sev) treatments over the observation period. Bef rew indicates one day before artificial rewetting" and "After rew" indicates one day after artificial rewetting. Please note that the controls did not receive any artificial rewetting.

Metaproteomics

Change in microbial community structure in response to moderate (M) and severe (S) stress when compared to the control was more pronounced for the fungal community. A shift from Basidiomycota to Ascomycota with stress, which resulted in an increased ratio of Asco-/Basidomycota. In our study, within the phylum of *Ascomycota* an increase in *Sordariomycetes* (M, 314%; S, 348%), *Saccharomycetes* (M, 343%; S, 240%) and *Eurotimycetes* (M, 133%; S, 40%) was observed. However, *Leotiomycetes* and *Agaricomycetes* (Basidiomycota phylum) declined in response to re-wetting (Figure 4.15). Within the most abundant proteins of bacterial origin, the phyla of *Proteobacteria* (M, 21%; S, 35%), *Actinobacteria* (M, 18%; S, 57%) and *Bacterioides* (M, 111%; S, 109%) increased in response to stress. In general, the relative abundance and prevalence of copiotrophic phyla such as *Bacteroidetes*, *Actinobacteria*, *α-* and *δ-Proteobacteria* increased in stressed soils while the abundance of oligotrophic *Acidobacteria* declined (Figure 4.15). A larger number total proteins were expressed in both stressed treatments compared to control, which suggests that water stress induced a higher functional diversity. The largest increase of functional proteins was related to "Lipid transport and metabolism" in moderate-stress while in severe-stress "Carbohydrate transport and metabolism" was highly stimulated. Furthermore, in both stress treatments a rise in proteins assigned to "Translation, ribosomal structure and biogenesis" and "Protein synthesis" suggests a boost in microbial cell growth after rewetting. The changes within intracellular functions could be associated to specific phyla via metaproteomics, indicating that the microbial adaptation to drought-rewetting stress primarily shifted microbial functions. Therefore, soil microbial communities seem to respond to different levels of drying-rewetting stress by developing their functional potential, and may therefore feedback to biogeochemical cycles.

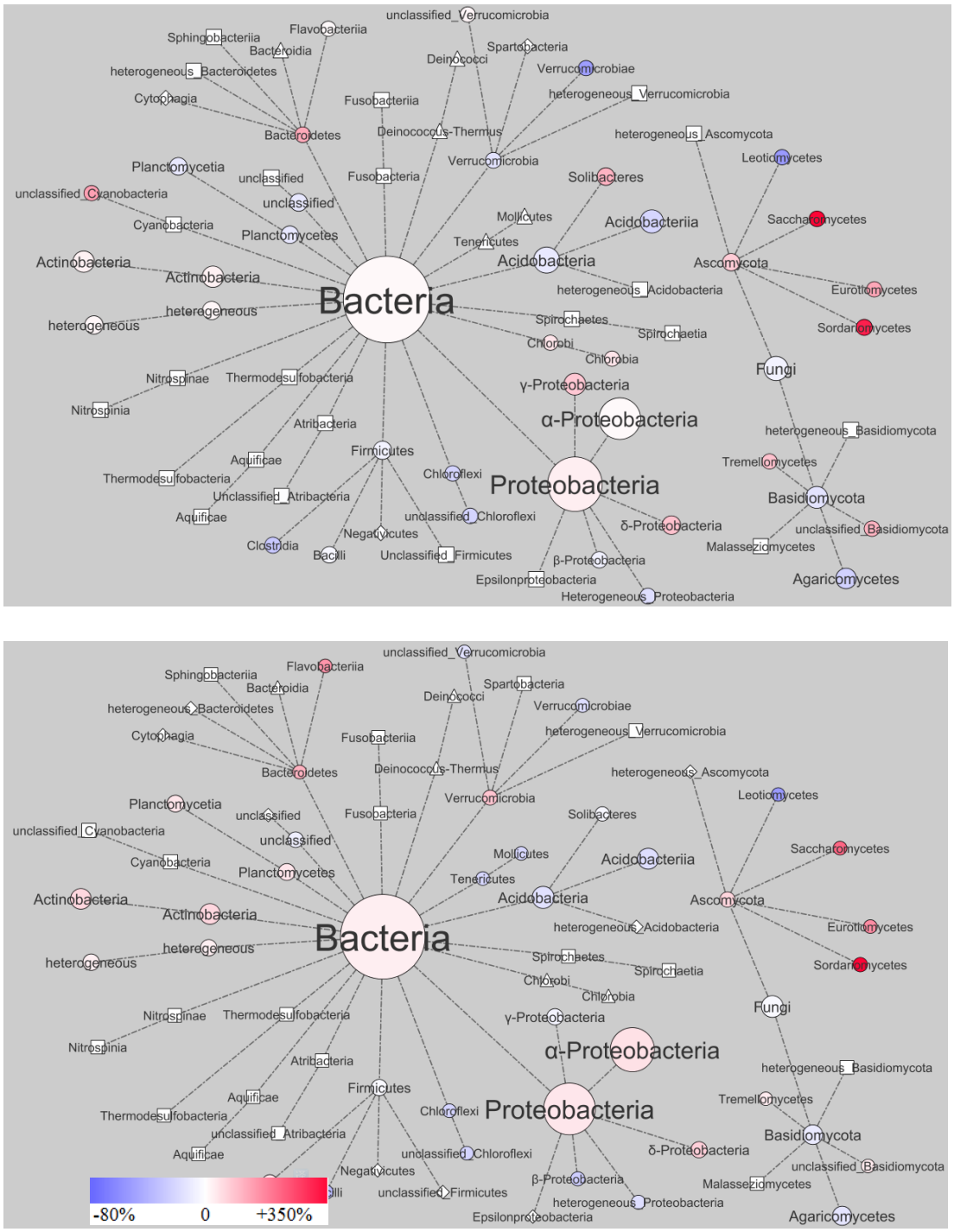

Figure 4.15. Metaproteomics one year after beginning of the manipulation in the moderate stress (upper panel) and sever stress (lower panel) treatment.

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5 Schlussfolgerungen und Empfehlungen / Conclusions and Recommendations

As explained throughout the description of activities and results obtained, we were able to substantially increase our understanding on the consequences of increased frequency and intensity of drying-wetting cycles on biogeochemical cycling of a forest soil at our beech site. We observed a broad range of responses of key aspects of soil functioning, which have been presented and discussed in the description of activities. Below, we have highlighted the main findings according to the different topics under investigation, and also summarized the key results in Table 1:

Parameter	Effect of enhanced soil drying-wetting cycles	
	During disturbance (2014-2015)	After disturbance (2016)
Soil CO_2 emissions	30 % decrease	No effect
Soil N_2O emissions	60 % decrease (2014) to no change (2015)	No effect
Soil CH_4 uptake	30-40 % increase	30% increase
Soil nutrient cycling	Rapid NO3- mobilization following rewetting	More soil N available
Hydrophobicity	Increased	n.a.
Microbial biomass	No evident change (highly variable)	Increased
Microbial composition	No changes in PLFA profile. Trend for increase in copiotrophic; bacterial phyla, decline in oligotrophic.	No change in PLFA profile
Microbial function	A rise in proteins assigned to "translation, ribosomal structure and biogenesis", and "proteins synthesis"	n.a.

Table 1. Summary of main effects observed due to artificially created drying-wetting cycles in the Rosalia forest. Results are divided according to the precipitation manipulation period (2014-2015) and the recovery period (2016). Control vs. severe stress treatments are considered for comparison. Only data from the vegetation period (May to October) have been included. n.a. denotes not available.

Soil-atmosphere greenhouse gas exchange:

Severe manipulation of precipitation induced a clear response on soil CO_2 emissions, which were reduced by approximately 30 % during the vegetation period (control vs. severe-stress treatments). We had hypothesized that increased frequency of drying-wetting cycles would increase the total amount of CO_2 released by the soil, due to the anticipated CO_2 pulse following rewetting. We did observe such a post-rewetting pulse, which was up to 200 % higher than the control baseline. However, the pulse was short-lived (three days) and therefore, the decrease in CO_2 effluxes during the drought period (eight weeks) clearly overruled the pulses after rewetting of the soil. In the case of the moderate treatment, the soil experienced six rewetting events (instead of three for the severe stress treatment) and the drought was not as severe as it lasted less time. Therefore, it seems that both opposed effects (pulse after rewetting and decreased emissions during drought) were annulled by each other, resulting in no significant difference of the moderate-stress plots compared to the control. We were further able to show how temperature-dependent modelling approaches fail in predicting CO_2 fluxes if the constrain induced by water scarcity is not considered. This has strong implications for predicting CO_2 fluxes in future climate scenarios, highlighting the necessity of including the distribution of precipitation for accurate modelling.

A similar response was observed in the case of soil N_2O fluxes, although differences were more variable from year to year (differences between control and severe-stress ranged from 0 to 60 % reduction). In this well-drained beech forest, N_2O fluxes seem to be dominated by episodic events since the baseline emission is virtually absent and, therefore, the frequency and intensity of drying-wetting cycles is also determinant in the total N_2O budget at the year level.

The picture obtained in our experiment for soil CH_4 uptake was slightly different than for the rest of the GHGs. While methanotrophic bacteria might have been constrained by water limitation, higher CH_4 diffusion rates under drought conditions may have been responsible for higher net uptake. However, we speculate that substantial methanotrophic activity may take place in deeper soil layers, since we observed a clear decoupling between CH_4 uptake rates and soil moisture at the uppermost 10 cm in both moderate and severe-stress treatments. Further, CH_4 uptake rates remained higher during the recovery period, which suggests a legacy effect favoring methanotrophic activity. Thus, and in view of our results, we recommend investigating what is the relative contribution of each soil layer to the oxidation of atmospheric CH_4, and to which extent is the methanotrophic activity in each soil layer sensitive to changing environmental conditions. For this purpose, targeted experiments combining soil microbiology and gas soil profile measurements could be highly useful.

Soil water repellence

Our hydrophobicity experiments point towards multiplying effects of drying-wetting cycles on the surface runoff, as it has been shown here by both field and laboratory experiments and modelling. The relative contribution of hydrophobic

functional groups increased due drying-wetting cycles, which subsequently affected the soil water repellency Water flows were further modeled and our results show that higher water repellency increases surface runoff in non-structured soils at hillslopes leading to lower water infiltration rates into the soil. According to this, positive feedbacks between increased frequency and intensity of episodic events and the soil will further increase flooding risks in the region.

Soil nutrient dynamics

Our results suggest that traditional sampling and extraction of soil samples may miss a substantial part of the rapid-occurring N cycling, which can however be captured by techniques able to monitor soil N fluxes in (pseudo) real time. We obtained two different pictures from our investigation: there are no substantial differences in nutrient pools due to drying- rewetting cycles along the vegetation period, but there is a potentially important N mobilization right after rewetting. The latter observation was possible by applying novel soil microdialysis techniques. The observed mobilization following rewetting was dominated by NO_3^- and neutral hydrophilic amino acids. Further, we observed that the flush of N upon rewetting was larger with increasing drought duration. Those results suggest that at our temperate forest site plant-available N was dominated by amino acids, a fraction of N that might be missed using conventional soil extraction methods. We further observed that N levels were higher in the recovery year suggesting that the microbial communities responsible for N transformation processes were still influenced by the already past manipulation of precipitation.

Soil microbial communities

Microbial indicators (microbial biomass C and N, microbial PLFA) were highly variable in both space and time. Still, a reduction of the microbial PLFA content after some artificial drought periods was observed. Likewise, in selected rewetting events a rapid increase (within 24 hours) of the microbial PLFA content was appreciated. In general, differences in PLFA contents between stressed treatments and control were somehow less evident in 2015, which was related to the natural drying-wetting cycles experienced by the control plots in this relatively dry year.

Changes in the microbial community structure in response to drying-wetting cycles were more pronounced for the fungal community, where an increase ratio of Aso/Basidomycota was observed. In general, the relative abundance and prevalence of copiotrophic phyla such as *Bacteroidetes*, *Actinobacteria, α- and δ-Proteobacteria* increased in stressed soils while the abundance of oligotrophic *Acidobacteria* declined. A larger number total proteins were expressed in both stressed treatments compared to control, which suggests that water stress induced a higher functional diversity. Furthermore, in both stress treatments a rise in proteins assigned to "Translation, ribosomal structure and biogenesis" and "Protein synthesis" suggests a boost in microbial cell growth after rewetting. The changes within intracellular functions could be associated to specific phyla via

metaproteomics, indicating that the microbial adaptation to drought-rewetting stress primarily shifted microbial functions.

In conclusion, we were able to show that natural ecosystems are buffered against weather fluctuations and climatic extremes to some extent, as the responses of the moderate-stress, which after all was subjected to 6 subsequent cycles of 4-week long drought periods, were in general not very different from the natural controls, that experienced natural (shorter) drying-rewetting cycles. At the same time, we could show that extreme events like severe droughts and heavy rainfalls can strongly affect soil GHG emissions and N cycling. Soil microbial communities were further affected by precipitation redistribution and soil microbial functionality was strongly affected by more intense drying-wetting cycles. Overall, we showed significant changes in soil biogeochemical cycling, some of them were even evident during the recovery period. Therefore, duration and frequency of drought periods and rainfall events should be considered in models which aim at predicting C and N ecosystem balances under changing climatic conditions.

C) Projektdetails /Project Details

6 Methodik/Methods

6.1. *Site description:*

The study was conducted in the Rosalia Lehrforst, of the University of Natural Resources and Life Sciences (BOKU Vienna). The forest is located in southeastern Austria and extends over an area of ~1000 ha. Mean annual temperature is 6.5°C and mean annual precipitation is 800 mm. The main forest stand type is a mixture of Norway spruce (*Picea abies*), silver fir (*Abies alba*) and common beech (*Fagus sylvatica*) with some Scots pine (*Pinus silverstris*). For our experiments we selected a pure beech stand on a westward slope that measures 2 ha and is located at 600 m asl (47° 42′ 26″ N**.** /16° 17′ 59″ E). Soils are dystric cambisols over siliceous bedrock. Water and electricity supply is available at the study site. The site was fully equipment for automated measurement of microclimate, soil-atmosphere exchange of CO_2, CH_4, and N_2O, as well as soil temperature and soil moisture. The site now belongs to the Austrian nexwork for long-term ecological research (LTER-Austria).

6.2. *Experimental design:*

To address our research questions, we carried out in total a 4-year field experiment combining precipitation exclusion with artificial precipitation. We simulated two types of drying-wetting stress: a "moderate stress" and a "severe stress" treatment, along with a control treatment as reference. Each treatment was replicated four times in a total of 12 experimental plots of 2 m x 2 m in size each. Precipitation exclusion was conducted by covering the plots with 4 m x 4 m transparent acrylic roofs 1.2 m above the ground surface. Irrigation was accomplished by sprinklers with axial-flow full cone nozzles (Series 460, Lechler GmbH) that were installed at the bottom side of the roofs and uniformly distributed the water in a diameter of 1.0 m. In each plot, soil sensors were buried in 10 cm depth to measure soil volumetric water content (VWC, TDR theta.ML2x probes, UMS, Germany) and temperature (Tsoil, thermistor Th2-f probes, UMS, Germany).

The moderate-stress treatment consisted on 6 drought-stress cycles from May until October. Each stress cycle involved four weeks of precipitation exclusion followed by irrigation with 75 mm decalcified tap water. The severe stress treatment plots were subject to three drying-wetting cycles, each of which had eight weeks of precipitation exclusion followed by 150 mm irrigation. Note that the total amount of precipitation received by both treatments is 450 mm, which agrees with the long-term net precipitation that reaches the soil surface The control plots were not covered and received natural precipitation. Because plots were positioned on a slope, trenches were dug above stressed plots to minimize lateral water flow between plots during simulated-drought periods.

The manipulation experiment was extended over two years to account for inter-annual variability. In the third year, no treatments were applied but all measurements continued to monitor the resilience (return to original state) of the ecosystem. During the fourth year (project extension period) the measurements continued and involved one 8-week cycle + 150 mm rain and one 4-week cycle + 75 mm rain for the severe and moderate stress plots, respectively.

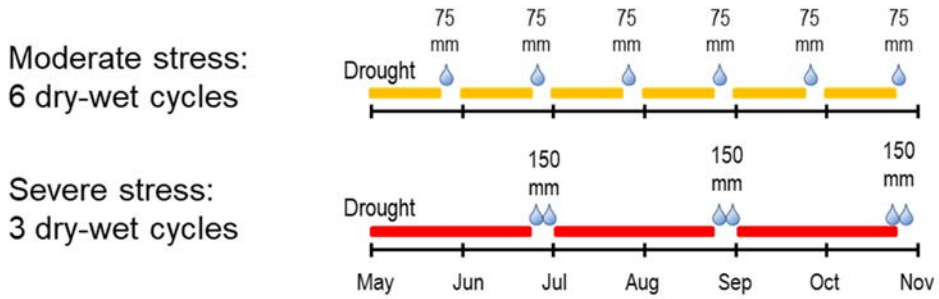

Figure 6.1. Scheme of the precipitation manipulation treatments.

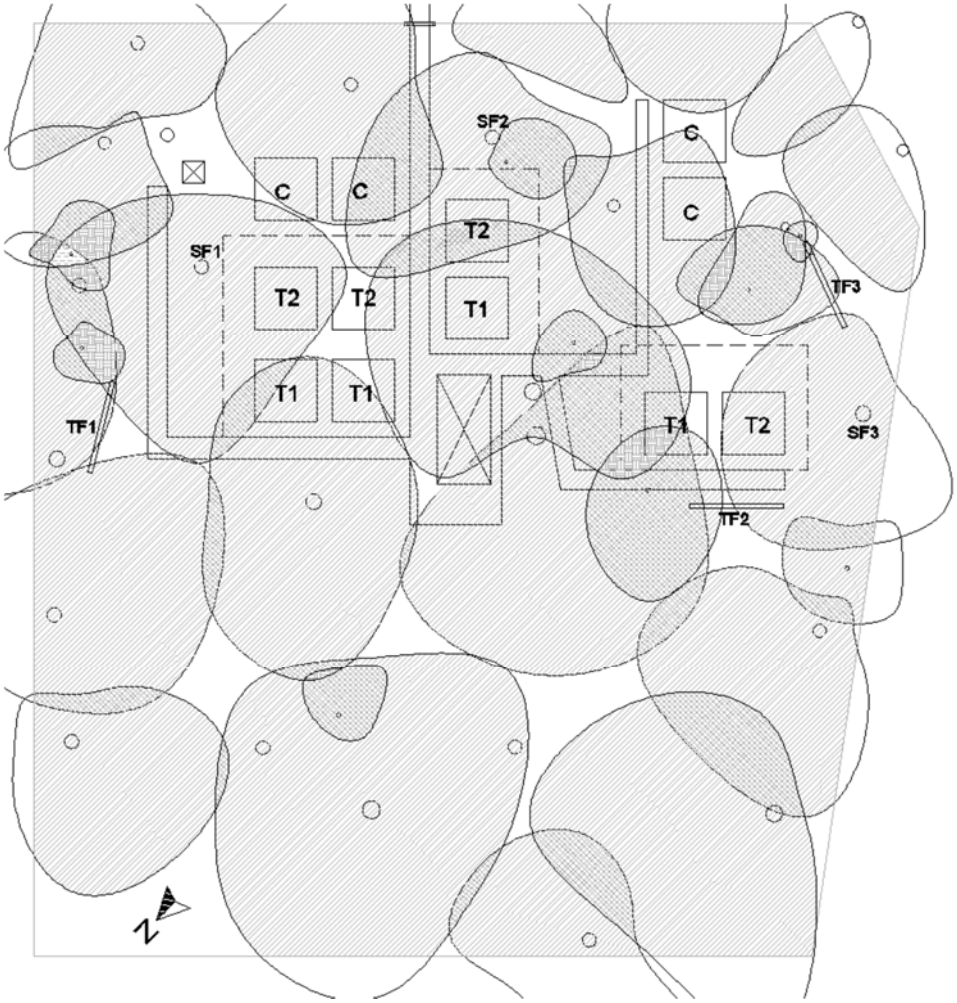

Figure 6.2. Spatial arrangement of experimental plots (C: Control, T1: Moderate stress treatment; T2: Severe stress treatment) and canopy cover distribution of the Rosalia test site. ST and TF show the location of stemflow and throughfall measurements, respectively. Credit: Bockholt, Jan.

6.3. *Soil-atmosphere GHG exchange monitoring*

We investigated the soil-atmosphere exchange rates of CO_2, CH_4, and N_2O with an automated gas flux measuring system, which consists of a set of 12 transparent chambers, a controlling unit, a CO_2 measurement device (Li-Cor 840A, Li-Cor Inc, Lincoln, NE, USA) and a gas chromatograph (SRI 8610, SRI Instruments, Torrance, CA, USA). The controlling unit governs the opening and closing of the chambers and allows the gas sampling from them into the gas detectors. The gas chromatograph is equipped with a flame ionization detector and a ^{63}Ni electron capture detector, for the determination of CH_4 and N_2O concentrations, respectively The system allows for continuous, high temporal resolution of the measurements.

6.4. *Soil sampling*

Soil samples of the top mineral soil layer (0-10 cm) were taken regularly over the course of the experiment. One day before the onset of the rain-exclusion roofs (2014 and 2015), soil samples were taken for all the plots, in order to assess differences by the beginning of the vegetation period, before the drying-wetting manipulation begun. Over the vegetation period soil samples were taken every eight weeks (three times each year), coinciding with the artificial precipitation of the severe-stress treatment (roughly 25th of June, 20th of August, 15th of October) an additional soil sampling campaign was carried out. In this case, samples were collected one day before, one day after and 3-7 days after the rainfall simulations. Collected samples from all twelve plots were immediately transported to the lab for analyses of different parameters.

Soil nutrients: Chemical composition of mineral soil samples was analyzed with an elemental analyzer (EA) for determination of total C and N contents. Extractable C and N were determined after extraction with KCl. While total dissolved organic C and total dissolved N were determined DOC/TN analyzer, estimation of NO_3^- and NH_4^+ was done photometrically (for NO_3^-, via reduction with vanadium, and development of color at 540 nm using sulfanilic acid and naphtylethylendiamin; for NH_4^+, the green indophenol method at 660 nm was applied).

Microbial biomass C and N were measured following the fumigation-extraction technique. Briefly, soil samples were fumigated with chloroform to break down microbial cell membranes, so that the microbial cellular content (both membrane tissues and intra-cellular storage compounds) is released into the soil solution. After this, soil was extracted in a similar way as for determination of dissolved C and N. Microbial biomass C and N estimates were obtained by subtracting the amounts of dissolved C and N in fumigated vs. control, un-fumigated samples after applying an extraction factor (Brookes XX).

Further, and in order to characterize microbial community structure, phospholipid fatty acids (PLFAs) were investigated. Phospholipid fatty acids were extracted after an adapted protocol of the Bligh and Dyer method. The method allows for differentiation of main microbial community groups: Gram + and Gram – bacteria and Fungi. Further, some physiological and stress indicators can be derived (e.g the ratio between cyclopropyl PLFA and their monounsatured precursors).

The samples revealing the most pronounced changes as caused by the drought/rewetting experiment were further analyzed for metaproteomics. Metaproteomics approach involves the study of microbial community changes by culture-independent methods, which allow to capture the entire proteome (proteins) that can be found in the soil samples. The proteomic analysis comprised three steps: (i) sample preparation including protein extraction, purification and precipitation, (ii) enzymatic digestion, protein or peptide separation and analysis, and (iii) assigning of peptide/protein sequences (bioinformatics). Potential activities of extracellular enzymes were determined by MUF/DOPA-assays.

Extraction of soil proteins with SDS-Phenol

Five grams of soil were mixed with 10% (w/w) polyvinylpolypyrrolidone (PVPP) in mortar and grounded with pestle in liquid nitrogen. The mixture was then transferred into 50 ml sterile centrifuge tube (Greiner Bio-One, Germany) and extraction buffer (1% SDS-Phenol-Tris HCl, 1:1 (v: v)) in ratio 1:3 was added. The suspension was sonicated with an ultrasonic homogenizer (SONOPULS, Bandelin, Berlin, Germany) on ice at 90% pulsing and 20% energy twice for 1 min and incubated on a horizontal shaker at 150 rpm and room temperature for 1 h. Centrifugation of suspension at 3220 g 20 min at 4°C followed.

Protein precipitation

The intermediate phenol phase was precipitated over night at −20°C with 5-fold amount of 0.1 M ammonium acetate in methanol (p. a). Precipitated protein pellets collected by centrifugation at 10640 g for 20 min at 4°C were washed with 100% pre-chilled acetone by gentle vortexing (Genie 1 Touch Mixer, USA) and afterwards once again centrifuged under the same conditions. Pellets were then further re-suspended with Tris-EDTA (TE) buffer by gentle mixing at 4°C overnight. On the next day, the protein extract (supernatant) was collected by centrifugation at 17,960 g for 3 min at 4 °C and stored at -20°C for further analysis.

Ninhydrin-Assay: quantitative evaluation of protein extracts

Prior to further processing, the effectiveness of the extraction procedure was checked by quantification of proteins in the samples. This was done by Ninhydrin Assay (calibration curve and straining of amino acids with ninhydrin) on a spectrophotometer at 575 nm.

SDS-PAGE – separation and straining/destraining of proteins

Extracted proteins were separated by SDS-PAGE in a 12% polyacrylamide gel. Three gels in total were prepared for 27 samples and protein extracts were applied carefully on a separate slot, thus each band on gel represent proteins extracted from one soil sample. The gel bands were strained overnight with blue silver after fixation with 40% ethanol and 10% acetic acid and afterwards washed until no acetic acid would be smelled. Each band was sliced in 10 pieces (size of the pieces was same for all 27 samples) resulting in 270 gel lines to be subjected to protein digestion to peptides. Those were transferred in 2 ml Eppendorf tubes and discolored by incubation with 0,2M acetonitrile: purified water solution for 15 min at 37°C. If necessary, this step was repeated until their complete discoloration. Washing solution was discarded and gel lines dried on concentrator for 30 min at 30°C.

Protein digestion and Ziptip purification

Dried gel lines were covered with trypsin (10-20µl) and digestion took place overnight at 37°C. On the next day, gel pieces were covered with sterile deionized water and placed in an ultrasonic bath (SONOREX, Bandelin, Berlin, Germany) for 15 min. Liquid was then transferred into new 2 ml Eppendorf tubes for the final purification of the extracts that was performed by C18 Ziptips (Merck Millipore). For this step four solutions were needed: wetting (70% ACN in MilliQ)-, equilibration (3% ACN, 0.1% acetic acid in MilliQ)-, washing (0.1% acetic acid in MilliQ)-, and elution solution (60% ACN 0.1% acetic acid in MilliQ). Bounded peptides were than eluted, transferred into GC glass vials with conical inserts, concentrated in a speed-vacuum centrifuge (Eppendorf Vacuum Concentrator plus, Hamburg, Germany) at 30°C (to remove ACN), and stored in 0.1% acetic acid in MilliQ solution at -70°C.

Samples were further analyzed by liquid chromatography and electrospray ionization mass spectrometry detection. Mass spectra of at least two hits on the same protein were assigned using stringent filters and multiple search engines SEQUEST and MASCOT. Application of the established workflow named PROPANE finally combines data on microbial taxonomy at different levels with functional categorization of the determined proteins.

6.5. Nutrient leaching and water fluxes

Throughfall, stemflow, litter percolate and soil water were measured at least biweekly for a vegetation period for estimating nutrient leaching and water fluxes in the experimental site.

Stainless steel throughfall collectors (3 m x 0.1 m x 0.1 m , n=3) were placed in spots representing the total average canopy over ration for the test area (88%) and connected to a 100 liter plastic container. The total amount of water collected for each sampling period was determined volumetrically. Stemflow (SF) was measured on three selected trees with a representative diameter at breast height (43-50 cm) with L-shaped collars fitted spirally around the trees. The collars encircled the trunk about 1.5 times and were connected to a 200 liter buffer tank.

This tank was further connected to a tipping bucket rain gauge (200 liter), and the tips recorded on a datalogger. After each sampling all buffer and collector bins were purged and cleaned. Tension-free lysimeters (n=12), connected via tubing to collection bottles were installed on the plots for hydrological and chemical analyses. Gross precipitation was measured using a tipping bucket rain gauge (ARG100, UMS GmbH, Munich, Germany), connected to a datalogger. It was mounted at a height of one meter at a clear-cut area 110 m away from the study site. Soil water was sampled via porous ceramic suction cups (10 cm long, 40 mm diameter, SK20, UMS GmbH n = 12) placed in a depth of 10 cm beneath the ground surface. An automatic vacuum system was installed to provide the soil water sampling. Suction cups were connected to evacuated 500 ml collector bottles made of borosilicate (SF-500, UMS GmbH).

6.6. Short-term nutrient dynamics following rewetting

To determine in-situ N diffusing before and during the first 20 h after artificial precipitation, we deployed two microdialysis systems, each consisting of a syringe infusion precision pump (CMA 400, CMA Microdialysis AB, Kista, Sweden) equipped with four gas-tight microsyringes (5 ml, Hamilton, Bonaduz, Switzerland). Each syringe was connected to a microdialysis probe. Membranes were carefully installed 2 h prior to the irrigation to a soil depth of 1.5 cm. Membranes were then perfused with MilliQ water and samples were collected continuously in 300 µl vials. Collected samples were analysed collorimetrically for NO_3^- and NH4+ similar to the determination of soil nutrients. Further, concentrations of 19 individual amino acids were measured on an Agilent 1200 HPLC system. Nitrogen diffusion rates over the membrane surface were calculated based on the membrane surface, the N concentration in the sample and the sampling time needed to obtain a specific volume of sample.

7 Arbeits- und Zeitplan / Work and Time Plan

Activity	2014	2015	2016	2017
WP1				
Preparation of plots				
Drying-wetting cycles				
GHG monitoring				
Data Evaluation				
Manuscript publication				
PhD Defense				
WP 2				
Soil sampling				
Laboratory analysis				
Data evaluation				
Manuscript publication				
WP3				
Soil sampling				
Laboratory analysis BOKU				
Metaproteomics analyses				
Manuscript publication				
WP 4				
Coordination				
Data synthesis				
Reporting				
Manuscript publication				

8 Publikationen und Disseminierungsaktivitäten / Publications and Dissemination Activities

So far, the project led to the publication of six articles in high-ranked scientific journals, one book chapter and three more manuscripts currently in preparation will be submitted in the following months. I addition, we contributed to a large number of international conferences and supervised several Master Thesis

Published scientific articles (**highlighted,** members of the DRAIN project)*:*

- **Leitner S**, Sae-Tun O, Kranzinger L, **Zechmeister-Boltenstern S, Zimmermann M**. 2016. *Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest*. Plant and Soil 403: 455-469.

- **Leitner S**, Homyak PM, Blankinship JC, Eberwein J, Jenerette GD, **Zechmeister-Boltenstern S**, Schimel JP. 2017. *Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils*. Soil Biology and Biochemistry 115: 461-466.

- Schwen A, **Zimmermann M, Leitner S**, Woche SK. 2015. *Soil Water Repellency and its Impact on Hydraulic Characteristics in a Beech Forest under Simulated Climate Change*. Vadose Zone Journal. doi:10.2136/vzj2015.06.0089.

- **Leitner S**, Minixhofer P, Inselsbacher E, **Keiblinger KM, Zimmermann M, Zechmeister-Boltenstern S**. *Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying-wetting events*. Applied Soil Ecology 114: 28-33.

- Filipovic V, Weninger T, Filipovic L, Schwen A, Bristow KL, **Zechmeister-Boltenstern S, Leitner S**. 2018 *Inverse estimation of soil hydraulic properties and water repellency following artificially induced drought stress*. Journal of Hydrology and Hydromechanics 66, 2, 170-180.

- **Keiblinger KM**, Fuchs S, **Zechmeister-Boltenstern S**, Riedel K. 2016. *Soil and leaf litter metaproteomics – a brief guideline from sampling to understanding*. FEMS Microbiology Ecology, 92, fiw180

Book chapter (**highlighted,** members of the DRAIN project):

- **Zechmeister-Boltenstern S, Díaz-Pinés E**, Spann C, Hofmann K, Schnecker J, Reinsch S. Soil – *The hidden part of climate: Microbial processes regulating soil-atmosphere exchange of greenhouse gases*. In: Lal, R (ed). *Advances in Soil Science*. Taylor & Francis Group. In press.

Manuscripts in preparation (**highlighted,** members of the DRAIN project)*:*

- **Leitner S**, Kobler J, **Díaz-Pinés E, Saronjic N, Zechmeister-Boltenstern S, Zimmermann M**. *Repeated extreme drought and rainfall events reduce soil respiration and affect its temperature and moisture sensitivity*.

- **Díaz-Pinés E, Leitner S, Zimmermann M, Zechmeister-Boltenstern S**. *Impact of repeated drying-wetting cycles on soil-atmosphere exchange of N_2O and CH_4 in a temperate beech forest*.

- Liu D, **Keiblinger KM, Leitner S**, Wegener U, **Zimmermann M**, Fuchs S, Lassek C, Riedel K, **Zechmeister-Boltenstern S**. *Response of microbial communities and their metabolic functions to drought-rewetting stress in a temperate forest soil*.

Contribution to conferences (**highlighted,** members of the DRAIN project)**:**

- **Leitner S**, **Zimmermann M**, Bockholt J, Schartner M, Brugner P, Holtermann C, **Zechmeister-Boltenstern S**. *Impact of repeated dry-wet cycles on soil greenhouse gas emissions, extracellular enzyme activity and nutrient cycling in a temperate forest*. EGU General Assembly Conference Abstracts, 2014

- **Zechmeister-Boltenstern S, Leitner S, Zimmermann M**: *Impact of droughts and heavy rain on greenhouse gas emissions and soil microbial activities in a beech forest*. In: Holzheu S & Thies B (eds.), BIOGEOMON 2014, Book of Abstracts, Band 119/2014, ISSN 0944 – 4122. BIOGEOMON, 8th International Symposium on Ecosystem Behaviour, Bayreuth, GERMANY, JUL 13-17, 2014.

- **Zimmermann M**, Orracha ST, Kranzinger L, **Leitner S, Zechmeister-Boltenstern S**: *Impact of litter removal on greenhouse gas fluxes, soil nutrients and microbial communities*. In: Holzheu S & Thies B (eds.), BIOGEOMON 2014, Book of Abstracts, Band 119/2014, ISSN 0944 – 4122. BIOGEOMON, 8th International Symposium on Ecosystem Behaviour, Bayreuth, GERMANY, JUL 13-17, 2014.

- **Leitner S, Zimmermann M**, Holtermann C, **Keiblinger K, Saronjic N, Zechmeister-Boltenstern S**: *Impact of repeated dry-wet cycles on soil CO_2 efflux and extracellular enzyme activities in a beech forest*. Annual Conference of the Austrian Soil Science Society, Vienna, Sep 22, 2014.

- Minixhofer P, **Leitner S, Zimmermann M**: *Impact of extreme weather events on plant-available nitrogen and amino acids using microdialysis*. Annual Conference of the Austrian Soil Science Society, Vienna, Sep 22, 2014.

- **Saronjic N, Leitner S, Keiblinger K, Zechmeister-Boltenstern S, Zimmermann M**: *Impact of droughts and heavy rainfall events on soil microbial communities in a beech forest*. Annual Conference of the Austrian Soil Science Society, Vienna, Sep 22, 2014.

- **Leitner S, Zimmermann M**, Kobler J, Holtermann C, **Keiblinger K, Zechmeister-Boltenstern S**: *Impact of repeated dry-wet cycles on soil CO_2 efflux*

and extracellular enzyme activities in a temperate beech forest. In: EU COST Action – ES1308, Integrating climate change experiments, data syntheses and modelling. 1st ClimMani COST action workshop, Aveiro, Nov 12-14, 2014.

- **Zimmermann M, Leitner S, Saronjic N, Zechmeister-Boltenstern S**: *Impact of manipulation experiments on soil greenhouse gas fluxes at the LTER site Rosalia Lehrforst.* Joint Conference of LTER Europe and EXPEER Related Sites Group , Vienna, Feb 17-19, 2015.

- **Leitner S**, Homyak PM, Blankinship JC, Eberwein J, Jenerette D, Z**echmeister-Boltenstern S**, Schimel JP: *Linking NO, N_2O and CO_2 emission peaks to the mobilization of mineral N upon rewetting of dry soil*. ClimMani/INTERFACE Workshop "After the extreme: Measuring and modeling impacts on terrestrial ecosystems when thresholds are exceeded". Florence, Italy, 12-15 April, 2016.

- **Saronjic N, Leitner S, Keiblinger K, Zechmeister-Boltenstern S, Zimmermann M**. *How do soil microbial communities react on droughts and heavy rainfall events?* EGU General Assembly Conference Abstracts, 2015

- **Leitner S, Saronjic N**, Kobler J, Holtermann C, **Zechmeister-Boltenstern S**, Zimmermann M. *Impact of repeated dry-wet cycles on soil CO_2 efflux in a beech forest*. EGU General Assembly Conference Abstracts, 2015.

- **Leitner S**, Kobler J, Holtermann C, **Zechmeister-Boltenstern S, Saronjic N, Zimmermann M**. *Response of Soil Respiration to Repeated Extreme Events in a Temperate Beech Forest in Austria*. AGU Fall Meeting Abstracts, 2015.

- **Zimmermann, M; Leitner, S; Saronjic, N; Zechmeister-Boltenstern, S**. *Extreme weather events and soil greenhouse gas fluxes: what do we see by automatic chambers?* NORA ICOS workshop 2015, Götheburg , Germany, May 10-13, 2015.

- **Saronjic N, Leitner S, Keiblinger K, Zechmeister-Boltenstern S, Zimmermann M**. *Response of soil microbial communities to droughts and heavy rainfall events*. 16h International Symposium on Microbial Ecology of the International Society for Microbial Ecology, Montreal, August 2016.

- **Saronjic N, Leitner S, Keiblinger K, Zechmeister-Boltenstern S, Zimmermann M.** *Response of soil microbial communities to droughts and heavy rainfall events*. Rosalia Workshop, Vienna, Austria, 20.10. 2016,

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- **Leitner, S**; Kobler, J; **Zimmermann, M; Zechmeister-Boltenstern, S**. *Moisture sensitivity of soil respiration in the context of extreme dry-wet events.* SOMmic International Workshop on Microbial Contribution and Impact on Soil Organic Matter, Structure and Genesis, Leipzig, Germany, November 2016.

- **Leitner S**, Minixhofer P, Inselsbacher E, **Saronjic N, Zechmeister-Boltenstern S, Zimmermann M, Díaz-Pinés E**: *DRAIN – Impact of Droughts and heavy RAIN on greenhouse gas emissions and soil nitrogen cycling.* Climate day of the Climate Change Centre Austria, Vienna, May 24, 2017.

- **Leitner S**, Inselsbacher E, Homyak PM, Schimel JP, **Zechmeister-Boltenstern S**: *Mobilization of mineral and organic N upon rewetting of dry soils.* BIOGEOMON 2017, 9th International Symposium on Ecosystem Behavior, Litomyšl, Czech Republic, Aug 20-24, 2017.

- **Díaz-Pinés E, Leitner S**, Kitzler B, **Zechmeister-Boltenstern S**. *Estimations of N_2O emissions from natural and managed ecosystems in the LTER-Austria Network*. In: International Workshop on N_2O emissions in various ecosystems. Taichung, Taiwan, 28-nov-1 dec 2017.

Finalized dissertations in the frame of DRAIN:

- **Leitner S**. *Impact of Extreme Weather Events on Soil Nitrogen Cycling and Greenhouse Gas Emissions*. University of Natural Resources and Life Sciences Vienna, Department of Forest and Soil Sciences, Institute of Soil Research. Supervisor: Prof. Dr. Sophie Zechmeister-Boltenstern. Defence: December 2017

Master Theses supervised in the frame of DRAIN

- Bockholt, Jan. 2014. Rainfall partitioning and solute fluxes in a pure beech stand and the effect of droughts and heavy rain events at plot scale. University of Natural Resources and Life Science (BOKU). Supervisors: Sophie Zechmeister-Boltenstern, Michael Zimmermann and Andreas Schwen.

- Brugner, Paul. 2014. Influence of weather extremes on forest-soil nutrient cycling. University of Natural Resources and Life Science (BOKU). Supervisor: Sophie Zechmeister-Boltenstern

- González Escolano, Flavia. 2017. Recovery of forest soil microbial activity after multiyear drought and heavy rainfall event simulations. University of Natural Resources and Life Science (BOKU) and University of Hohenheim. Supervisors: Sophie Zechmeister-Boltenstern and Ellen Kandeler.

- Haller, Helmut. 2015. Ammonia emissions from beech litter as source of reactive nitrogen gas in deciduous forests. University of Vienna. Supervisor: Sophie Zechmeister-Boltenstern

- Kranzinger, Lukas. 2014. Impact of litter removal and seasonality on soil greenhouse gas fluxes and nutrient cycling in an Austrian beech forest. University of Natural Resources and Life Science (BOKU). Supervisors: Sophie Zechmeister-Boltenstern and Michael Zimmermann

- Minixhofer, Pia. 2015. Impact of extreme weather events on plant available nitrogen and amino acids using microdialysis. University of Natural Resources and Life Science (BOKU). Supervisor: Sophie Zechmeister-Boltenstern

- Sae-Tun, Orracha. 2014. Impact of litter-removal and seasonality on soil microbial community composition in a beech forest. University of Natural

Resources and Life Science (BOKU). Supervisors: Sophie Zechmeister-Boltenstern & Michael Zimmermann.

- Schartner, Markus. 2015. Impact of repeated dry-wet cycles on macro-aggregate stability and organic carbon stabilization of a beech forest soil. University of Natural Resources and Life Science (BOKU). Supervisors: Sophie Zechmeister-Boltenstern & Michael Zimmermann.

- Yang, Li. 2015. Influence of nitrogen nutrition form and drought cycles on the fine root respiration of two tree species. University of Natural Resources and Life Science (BOKU) & University of Eastern Finland. Supervisors: Boris Rewald and Hans Göransson.

Diese Projektbeschreibung wurde von der Fördernehmerin/dem Fördernehmer erstellt. Für die Richtigkeit, Vollständigkeit und Aktualität der Inhalte sowie die barrierefreie Gestaltung der Projektbeschreibung, übernimmt der Klima- und Energiefonds keine Haftung.

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