Publizierbarer Endbericht

Gilt für Studien aus der Programmlinie Forschung

A) Projektdaten

Allgemeines zum Projekt	
Kurztitel:	EXAFOR
Langtitel:	Extreme weather events and soil greenhouse gas fluxes in Austrian Forests. Evaluating the feedbacks under global change
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Projekt- und KooperationspartnerIn (inkl. Bundesland):	Bundesforschungszentrum für Wald, Naturgefahren und Landschaft (BFW), Wien. Austrian Institute of Technology GmbH (AIT), Wien
Schlagwörter:	Soil greenhouse gas fluxes, extreme events, forest soils
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Klimafonds-Nr:	KR19AC0K17557
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B) Projektübersicht

1 Kurzfassung

Ausgangssituation - Motivation des Projekts

Waldböden sind für das Klimasystem von entscheidender Bedeutung aufgrund der großen Mengen an Treibhausgasen (THG), die sie mit der Atmosphäre austauschen. Österreichische Wälder wirken derzeit als THG-Senke, doch der globale Wandel könnte die Senkenwirkung reduzieren. Extremwetterereignisse wie Dürren und Starkregen verändern die Umweltbedingungen erheblich und führen zu Veränderungen in den Bodenmikroben und der Nährstoffverfügbarkeit, was wiederum die zeitlichen Dynamiken der Boden-THG-Flüsse und des C- und N-Kreislaufs beeinflusst. Das Verständnis für das Feedback zwischen höherer Niederschlagsvariabilität, N-Verfügbarkeit und Boden-THG-Flüssen ist weitgehend unbekannt und schwer zu erfassen, und es fehlen evidenzbasierte Quantifizierungen.

Ziele: Projektziele

Das EXAFOR-Projekt zielt darauf ab, die interaktiven Auswirkungen von wiederholten Bodentrocknungs-Wiederbefeuchtungszyklen und atmosphärischer N-Ablagerung auf die Bodenbiogeochemie österreichischer Waldökosysteme zu untersuchen. Zu diesem Zweck werden Boden-THG-Flüsse (CO_2, CH_4 und N_2O) in hoher zeitlicher Auflösung bestimmt, Bodenmethanotrophen-Gemeinschaften analysiert und Boden THG-Flüsse unter verschiedenen Klimawandelszenarien modelliert. Hypothesen untersuchen kurz- und langfristige Auswirkungen der Niederschlagsänderungen sowie den Effekt von N auf THG-Flüsse unter erhöhten Trocken-Wiederbefeuchtungszyklen.

Methodik und Aktivitäten

Manipulationsexperimente (Trockenheit und extreme Niederschläge sowie erhörter N-Eintrag) wurden mit natürlichen Klima- und N-Depositionsgradienten kombiniert. Die Untersuchungen fokussierten auf den Boden-Atmosphäre-THG-Austausch und umfassen Beobachtungen der Bodenmikroben. Das Projekt führte u.a. frühere Aktivitäten eines ACRP-Projektes fort. Das Projekt gliederte sich in drei Arbeitspakete: 1) Boden-Atmosphäre-Treibhausgas-Austausch; 2) Bodenmikrobielle Gemeinschaften und Nährstoffkreislauf; 3) Harmonisierung und Feedback im globalen Wandel

Wir simulierten meteorologische Trockenheit und Niederschlagsereignisse in Kombination mit erhöhtem atmosphärischen N-Eintrag an drei österreichischen Waldstandorten (Lehrforst Rosalia, Klausen-Leopoldsdorf und Zöbelboden). Zu diesem Zweck wurden Dächer installiert, die verhindern, dass Niederschlag während der Vegetationsperiode den Boden erreicht. Darüber hinaus wurde ein Bewässerungssystem installiert, um alle acht Wochen während der Vegetationsperiode ein episodisches Niederschlagsereignis zu simulieren. Unser

Ziel war es, die zeitliche Verteilung des Niederschlags zu verändern, jedoch nicht die Gesamtmenge. Ein erhöhter atmosphärischer N-Eintrag (+ 50 kg N ha^{-1} a^{-1}) wurde durch das Versprühen von in Wasser gelöstem Stickstoff simuliert. Insgesamt betrachten wir vier Behandlungen: Umweltkontrolle (C); Trocken-Wiederbefeuchtung (DRW); erhöhter N-Eintrag (C+N); und Trocken-Wiederbefeuchtung und erhöhter N-Eintrag (DRW+N).

Kontinuierliche Messungen von Boden-Atmosphäre-Treibhausgasflüssen wurden mit automatisierten Kammer-Systemen durchgeführt, die mit laserbasierten Gasanalysatoren verbunden waren. Bodenproben wurden an allen Standorten und Behandlungen wiederholt entnommen, um die Saisonalität spezifischer Bodenparameter zu überwachen. Bodenproben wurden zu Beginn der Vegetationsperiode, zum Höhepunkt der Trockenperiode und nach jedem Wiederbefeuchtungsereignis geworben und im Labor auf Nährstoffgehalt, mikrobielle Biomasse und methanotrophe Gemeinschaften untersucht.

Ergebnisse und Schlussfolgerungen des Projekts

Trocken-Wiederbefeuchtungszyklen und eine Erhöhung der atmosphärischen N-Eintrag wurden erfolgreich an unseren Versuchsstandorten simuliert. Die Zunahme der Häufigkeit und Intensität von Trocken-Wiederbefeuchtungszyklen führte zu einer Reduzierung von 30 bis 40 % der Bodenatmung, einer Verringerung der Boden-N_2O-Flüsse und einem Trend zu höheren Boden-CH_4-Oxidationsraten. Die meisten Effekte waren der Trockenperiode zuzuschreiben; die beobachteten Emissionspulse nach dem Wiederbefeuchten trockener Böden waren geringfügig und kurzlebig. Der Effekt des erhöhten N-Eintrages war marginal und unabhängig vom anfänglichen Stickstoffstatus des Systems. Das deutet darauf hin, dass Veränderungen in den Niederschlagsmustern in Zukunft wichtiger sind als die Dynamik des N-Eintrages. Dennoch legen unsere Ergebnisse nahe, dass eine erhöhte N-Deposition sowohl die Fähigkeit des Bodens zur CH_4-Senke als auch die Vielfalt der methanotrophen Gemeinschaften und die Reduzierung des mikrobiellen Biomasse-C im Boden verringern kann. Am Standort Rosalia nahmen die Auswirkungen des Bodens auf veränderte Niederschlagsmuster langfristig ab, wobei die Bodenatmung weniger betroffen war und die CH_4-Aufnahme sieben Jahre nach Beginn der Manipulation nicht mehr betroffen war.

Ausblick und Zusammenfassung

Die Häufigkeit und Intensität von Trocken-Wiederbefeuchtungszyklen hat einen starken Einfluss auf Bodenprozesse. Während die beobachtete Verminderung des Boden-CO_2- und N_2O-Ausstoßes und ein Trend zu erhöhter CH_4-Aufnahme aufgrund unserer Niederschlagsmanipulation als negative Feedbacks auf den Klimawandel betrachtet werden können, sind langfristige und in-situ-Untersuchungen auf Ökosystem-Ebene (samt Bäumen) erforderlich, um den zu erwartenden Ökosystemstatus in einer sich verändernden Welt genau vorherzusagen und zu verstehen.

2 Executive Summary

Initial situation - Motivation of the project

Forest soils are critical for the climate system, due to the large amounts of greenhouse gases (GHG) they exchange with the atmosphere. Austrian forests are currently a net GHG sink but this may change in the frame of global change, which comes hand in hand with an increase in the frequency and the intensity of extreme weather events, such as drought periods and heavy episodic rainfalls. Such disturbances strongly change the environmental conditions and induce changes in soil microbial communities and nutrient availability, thereby affecting the overall balance and the temporal dynamics of soil GHG fluxes and C and N cycling. Our understanding of the overall feedback between higher precipitation variability or excess N availability and soil GHG fluxes is still largely unknown and challenging to address, and evidence-based quantifications are missing.

Targets: Objectives of the project

The overall aim of the EXAFOR project is to investigate the interactive impact of extreme weather events (i.e., repeated soil drying-wetting cycles caused by increased precipitation variability) and atmospheric N deposition on the soil biogeochemistry of representative Austrian forest ecosystems. For this purpose, we will specifically quantify and evaluate soil GHG (CO2, CH4 and N2O) fluxes in high temporal resolution, explore changes in size, structure and functioning of soil methanotrophic communities and model GHG emissions of selected Austrian forests as affected by different climate change scenarios. We developed hypotheses looking at short-term vs. long-term effects of precipitation manipulation; and effect of N on CO2, N2O and CH4 fluxes under increased drying-rewetting cycles.

Methodology and activities

We combined active manipulation experiments (drought and extreme precipitation and addition of N to soil) with natural climate and N deposition gradients. Observations focus on the soil-atmosphere GHG exchange, complemented with in-detail observations of soil microbial communities. The project strongly relied on activities initiated in a previous ACRP project but expands in terms of both the spatial coverage of the observations and the number of issues addressed. The project is structured in three work packages:

- WP1: Soil-Atmosphere Greenhouse Gas Exchange
- WP2: Soil Microbial Communities and Nutrient Cycling
- WP3: Harmonization and Feedback under Global Change

We performed a simulation of meteorological drought and episodic rainfall events in combination with increased atmospheric N deposition in three Austrian forest sites (Lehrforst Rosalia, Klausen-Leopoldsdorf and Zöbelboden). For this purpose, rain-out-shelters were installed, preventing that throughfall reaches the soil during the vegetation period. Furthermore, an irrigation system was installed, so that an episodic rainfall event was simulated every eight weeks during the vegetation period. In all sites, we aimed at altering the temporal distribution of the precipitation, but not the total amount. Increased atmospheric N deposition (+ 50 kg N ha-1 a-1) was simulated by spraying N dissolved in water. In total, we consider four treatments: environmental control (C), drying-rewetting (DRW), increased N deposition (C+N) and drying-rewetting and increased N deposition (DRW+N)

Continuous measurements of soil-atmosphere greenhouse gas fluxes were implemented, using automated chamber systems connected to laser-based gas analysers. Soils were sampled in all sites and treatments repeated times to monitor the seasonality of specific soil parameters. Thus, soil samples were collected by the beginning of the vegetation period, by the peak of the drought period and after each rewetting event. Soil samples were investigated in the lab for nutrient content, microbial biomass and methanotrophic communities.

Results and conclusions of the project

We were able to successfully mimic drying-rewetting cycles and enhancement of atmospheric N deposition rates across our experimental sites in a harmonized manner. The increase in frequency and intensity of drying-rewetting cycles resulted in a reduction of 30 to 40 % in soil respiration rates, a virtual suppression of soil N_2O fluxes and a trend of higher soil CH_4 oxidation rates. Most of the effects where attributable to the drought period, since the emission pulses observed after rewetting of dry soils were modest and short lived. Likewise, the effect of increased N deposition was marginal, regardless of the initial nitrogen status of the system, suggesting that changes in precipitation patterns are likely to be more important in the future than dynamics of N deposition rates. Still, our results suggest that increased N deposition may decrease both the soil CH_4 sink capacity and diversity of methanotrophic communities and reduce soil microbial biomass C. In Rosalia, soil responses to altered precipitation patterns decreased in the long run; seven years after the onset of the manipulation, soil respiration was less affected, and CH_4 uptake not being affected anymore as compared to the environmental control.

Outlook and summary

Increased frequency and intensity of drying-rewetting cycles has a strong influence on soil processes. While the observed reduction in soil CO_2 and N_2O efflux and a trend toward increased CH_4 uptake due to our manipulation of the precipitation can be seen as negative feedback to climate change, long-term, in situ investigations involving alteration of the trees are still needed to accurately forest response to anticipate ecosystem status in a changing world.

3 Hintergrund und Zielsetzung

Initial situation / motivation for the project

Forest soils are critical for the climate system, due to the large amounts of greenhouse gases (GHG) they exchange with the atmosphere. In Austria, forests are currently a net GHG sink, but this may change in the frame of global change and the anticipated increase in frequency and intensity of extreme weather events, such as drought spells and heavy episodic rainfalls. Such disturbances modify the soil environmental conditions, and induce changes in microbial communities and nutrient availability, thereby affecting the GHG production and consumption patterns in the soil. Our current understanding of the overall feedback between extreme weather events and soil GHG fluxes is still largely unknown and challenging to address. Precipitation is uneven by nature, with a global pattern of heavy rains contributing disproportionately to total precipitation and relatively large periods of time with little or none precipitation. Multiple sources of data indicate that it is highly likely that the frequency and intensity of drying-wetting cycles will continue to increase in the coming years, with strong implications for the soil biogeochemical processes and the losses of C and N.

Soils play a crucial role for climate regulation, since they are involved in the production of roughly 50 % of the GHG emissions globally. Further, the temporal dynamics of GHG turnover rates in the soil are governed to a large extent by both the availability of substrate and environmental conditions. Usually, drying-wetting cycles lead to two contrasting environmental situations separated by time: the amount of GHG released from the soils into the atmosphere is strongly limited during drought and following the rewetting event, a pulse of gases has been usually observed).

Anthropogenic activities have dramatically increased the release of reactive N, potentially amplifying the response of ecosystems to global change, but multiple interaction of GHG emissions with soil, climate and vegetation obscure our understanding of the underlying mechanisms behind the impact of N input.

Objectives of the project

The overall aim of the EXAFOR project was to understand and quantify the interactive impact of extreme weather events (i.e., repeated soil drying-wetting cycles caused by increased precipitation variability) and atmospheric nitrogen deposition on the soil greenhouse gas balance of representative Austrian forest ecosystems. For this purpose, we specifically: Quantified and evaluated soil greenhouse gas (CO_2, CH_4 and N_2O) fluxes in high temporal resolution, elucidated the dominant processes producing N_2O; explore changes in size, composition, structure and functioning of soil methanotrophic communities and modelled the greenhouse gas emissions of representative Austrian forests as affected by different climate change scenarios. In order to fulfil the aims of the project, the following hypotheses were tested:

- Hypothesis 1: *Increased frequency and intensity of drying-wetting cycles will affect the soil GHG fluxes in the long-term at a lower intensity than in the short-term. Thus, while we hypothesize that continuation of precipitation manipulation will further decrease* CO_2 *and* N_2O *fluxes and increase* CH_4 *uptake rates, the magnitude of the effect will diminish with time*.

Our previous ACRP project, DRAIN, showed a clear effect of increasing intensity and frequency of drying-wetting cycles on the soil-atmosphere GHG fluxes. During 2013-2015 (main observation period) we observed a consistent reduction of CO_2 fluxes and N_2O fluxes of about 30 and 45 %, respectively, while CH_4 uptake increased by 20 % on average. The differences attributed to the increased intensity of drying-wetting cycles were lower in the third year of observation, suggesting some degree of acclimation and/or adaptation to the new environmental conditions. We therefore hypothesized that the changes in GHG fluxes due to simulated extreme events are still persistent but the magnitude of the differences are lower.

- Hypothesis 2: *Alleviation of microbial nutrient limitation in the soil will amplify the GHG pulses after rewetting of dry soils. Thus, cumulative* N_2O *and* CO_2 *fluxes under increased drying-wetting cycles will be higher than under control plots if enough nitrogen is available for soil microorganisms*.

The observed responses upon rewetting in the CO_2 and N_2O fluxes during the DRAIN project were moderate and short-living. As a consequence, the reduction of fluxes during drought periods clearly outweighed pulses after rewetting. The modest response of soil N_2O and CO_2 fluxes following rewetting was somehow unexpected and related to the low N availability at our Rosalia site, featuring low soil N contents, high soil C:N ratio and low N deposition rates. These conditions may have limited the response of microbes at this site; however, areas subjected to larger N deposition rates may react differently. We hypothesized that the release of CO_2 and N_2O from soil after rewetting is exacerbated if N is not a limiting factor for soil microbial activity. To test this hypothesis, we used a natural N deposition gradient (by including the forest sites Klausen-Leopoldsdorf and Zöbelboden) in combination with artificial N fertilization.

- Hypothesis 3: *High nitrogen deposition rates will decrease the soil methanotrophic bacteria while altered precipitation patterns will enhance them*.

Nitrogen additions may reduce the soil CH_4 sink strength, as it has been observed several forests, including one of our sites (Klausen-Leopoldsdorf), attributably driven by N-induced changes in the methanotrophs and its activity. We therefore anticipated a negative relationship between N deposition rates and methanotrophic communities. We further expected an enhancement in soil methanotrophic bacterial abundance upon increased intensity of drying-wetting cycles, in line with our observations in Rosalia (higher soil CH_4 uptake rate in severe stress plots).

4 Projektinhalt und Ergebnis(se) /Project content and results

Results Work package 1: Soil-Atmosphere Greenhouse Gas Exchange

This work package involves the execution of measurements of greenhouse gas fluxes between the soil and the atmosphere at high temporal resolution by using automated chamber systems.

Rosalia:

Soil Greenhouse gas flux data analysis in Rosalia focused in the time period from 2019 to 2020 and considered the DRAIN plots. In these plots, manipulation of the precipitation had started already in 2013 (Schwen et al., 2015) and a throughout analysis of the period 2013-2015 was already available (Díaz-Pinés et al., 2018). Thus, investigation and comparison of both datasets allowed for shedding light on our first working hypothesis ("*the effect of drying-rewetting cycles diminishes with time since the onset of the manipulation")*. In the period 2013-2015, drying rewetting stress reduced CO_2 fluxes by 30 %, while CH_4 uptake was enhanced by roughly 20 %. Seven years after onsetting the manipulation, the effect on soil respiration was roughly half of the one during the first three years, and effect on CH_4 uptake virtually disappeared. Reduction in soil N_2O fluxes due to drying-rewetting was maintained or even enhanced, but these large percentage reduction (45 % in 2013-2015, 55 % in 2019-2020) indeed correspond to modest absolute differences, due to the low magnitude of the natural N_2O fluxes in Rosalia. Thus, our first hypothesis was confirmed, which suggests that in the long run, acclimation and/or adaptation to the new environmental conditions by the microbial community drives the soil GHG balance towards levels before the onsetting of the manipulation

Vegetation period	CO_2 efflux	CH_4 uptake	N_2O efflux
2013-2015	-30%	+20%	-45%
2019-2020	-17%	+1%	-55%

Table 1. Relative change in mean soil GHG fluxes in the vegetation period (May to October) between severe drying-wetting stress and control plots in Rosalia during the 2013-2015 and 2019-2020 observation periods. (Thoma, 2023)

To assess the success of the newly stablished manipulation experiment, soil moisture data was processed. Soil moisture peaks after rewetting were clearly identified, followed by a dry-out phase with continuously declining soil moisture levels. When considering the whole time period under investigation, soil moisture levels were lower in DRW plots than in controls, but these differences were less pronounced in Rosalia than in the other sites (Table 1). In 2021 DRW plots had 3.4% lower soil moisture than controls (χ2(1)=4490, p<0.001), whereas due to a summer drought in Rosalia in 2022 (Figure 1), DRW reduced soil moisture by only 1.5% (χ2(1)=9852, p<0.001). Seasonal drought under natural conditions in 2022 was the likely driver responsible for the similar soil moisture contents for Control and DRW plots.

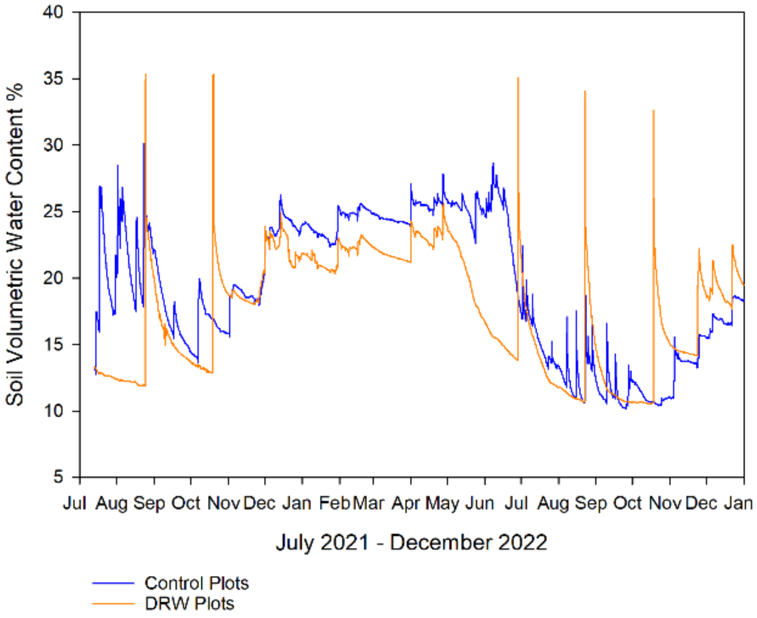

Figure 1 Volumetric soil moisture content for Rosalia in 2021 and 2022. Sensors were installed July 2021. Seasonal drought conditions in 2022 resulted in similar soil moisture contents for Control and DRW plots.

Klausen-Leopoldsdorf:

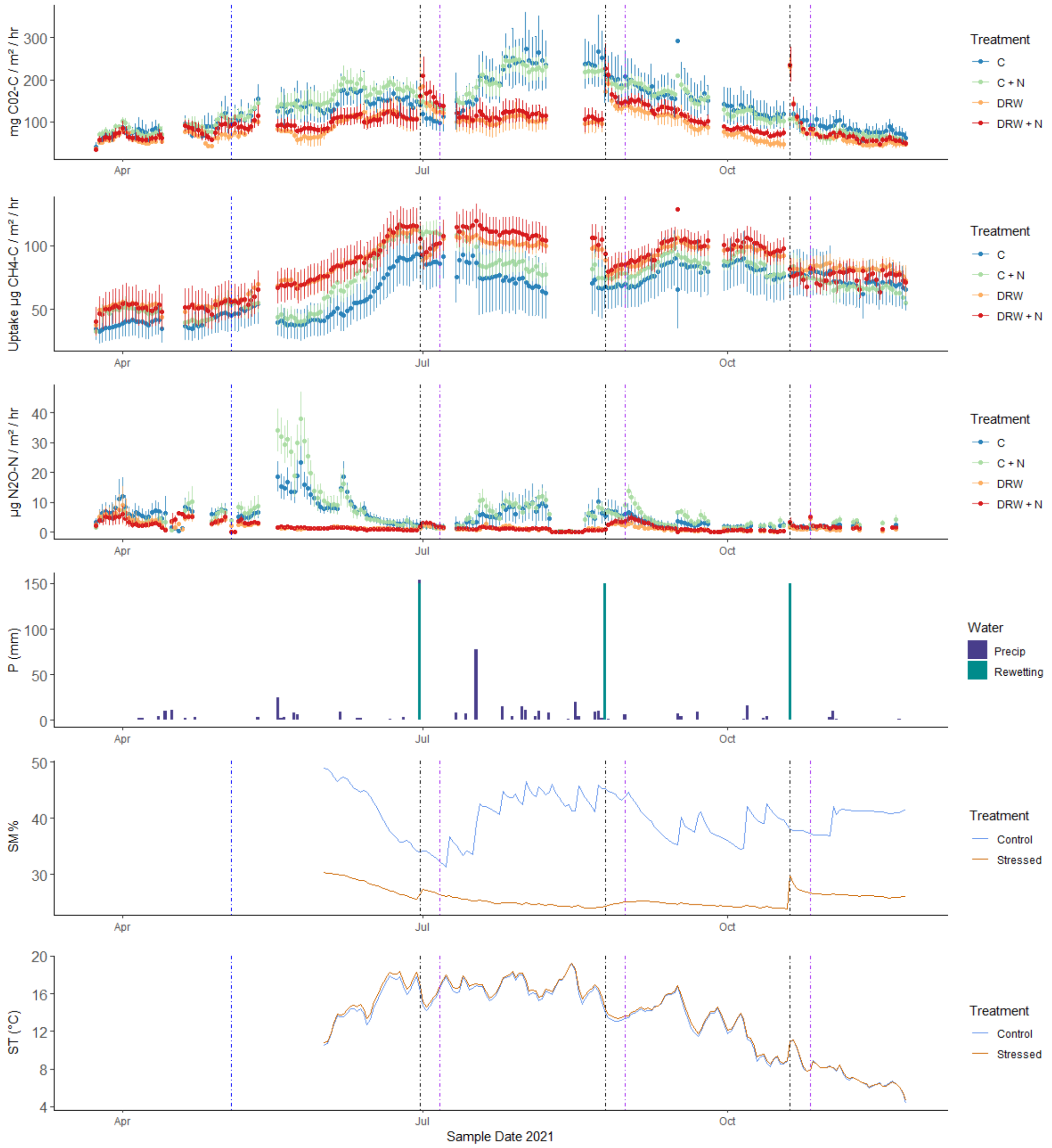

Figure 2. Soil CO_2, CH_4 and N_2O fluxes, precipitation, soil moisture and soil temperature (5 cm depth) in Klausen-Leopoldsdorf 2021. Blue dashed line indicates beginning of rainfall manipulation, black dashed lines indicate rewetting events and purple dashed lines indicate fertilization.

In Klausen Leopoldsdorf, the rain-out-shelters in combination with the episodic artificial rainfall events had an effect on soil moisture in interaction with depth (Table 2). DRW treatment caused a reduction of 13.6% volumetric water content at 5cm soil depth ($\chi2(1)=58267$, $p<0.001$), whereas DRW plots had 3.47% higher soil moisture than control plots at 15cm ($\chi2(1)=4332$, $p<0.001$). At 30cm soil depth, DRW plots had almost 5% lower soil moisture than control plots ($\chi2(1)=19377$, $p<0.001$).

Site	Depth	Mean Soil Moisture % Control	Mean Soil Moisture % DRW
Klausen Leopoldsdorf 2021	5cm	39.9 ± 0.04	26.3 ± 0.02
	15cm	28.1 ± 0.07	31.6 ± 0.02
	30cm	32.3 ± 0.04	27.4 ± 0.02
Rosalia 2021	10cm	18.9 ± 0.04	15.4 ± 0.05
Rosalia 2022	10cm	16.3 ± 0.06	14.9 ± 0.04
Zöbelboden 2022	10cm	27.8 ± 0.04	18.9 ± 0.03

Table 2. Mean (± 1 standard error) soil moisture in the vegetation period according to site and sensor depth for Control and Drying-Rewetting (DRW) treatments.

To determine the effect of drying-rewetting and fertilization on fluxes, linear mixed effects models were run to test for these effects individually and as interactive effects. Control and Control + Nitrogen treatments both emitted about 150 mg CO_2-C m^{-2} h^{-1} whereas both rainfall exclusion treatments had lower fluxes (100 - 110 mg CO_2-C m^{-2} h^{-1}), (Table 1). Drying-rewetting lowered CO_2 fluxes by 45 mg CO_2-C m^{-2} h^{-1} ($\chi 2(1)=4.224$, $p=0.04$). All treatments had a soil CH_4 uptake rate of about 100 µg CH_4-C m^{-2} h^{-1} except for the Control + Nitrogen plots, which absorbed on average 77 µg CH_4-C m^{-2} h^{-1}; Control and Control + Nitrogen plots had N_2O emissions of around 5 µg N_2O-N m^{-2} h^{-1}, while DRW and DRW + Nitrogen plots were over three times lower (1.3 – 1.6 µg N_2O-N m^{-2} h^{-1}). Drying-rewetting lowered N_2O fluxes by 3.58 µg N_2O-N m^{-2} h^{-1} ($\chi 2(1)=8.309$, $p=0.004$). Fertilization had no effect on fluxes for any of the three gases, and there were no interactive effects between fertilization and drought.

				Klausen-Leopoldsdorf 2021				
	C	se ±	**C + N**	se ±	**DRW**	se ±	**DRW + N**	se ±
mg CO2-C m^{-2} h^{-1}	153.12	4.36	154	3.31	99.79	2.7	112.7	2.32
µg CH4-C m^{-2} h^{-1}	-95.53	1.16	-77.26	0.78	-100.5	1.01	-96.92	1.05
µg N2O-N m^{-2} h^{-1}	4.89^{a}	0.3	5.25^{a}	0.23	1.25^{b}	0.07	1.64^{b}	0.07
				Zöbelboden 2022				
mg CO2-C m^{-2} h^{-1}	169.14^{a}	1.73	162.02^{a}	1.94	117.21^{b}	1.18	95.96^{b}	1.41
µg CH4-C m^{-2} h^{-1}	-49.3	0.59	-35.59	0.48	-58.79	0.93	-53.28	0.79

Table 3. Mean (± 1 standard error) soil-atmosphere fluxes of CO_2, CH_4 and N_2O in Klausen-Leopoldsdorf and Zöbelboden for Control, (C), Control + Nitrogen (C+N), Drying-Rewetting (DRW) and Drying-Rewetting + Nitrogen (DRW+N) treatments.

Zöbelboden

Similar to Klausen-Leopoldsdorf, soil moisture in Zöbelboden was strongly impacted by rainfall exclusion and irrigation in the drying-rewetting plots (Table 1). Drying-rewetting treatment lowered soil moisture by 8.9% lower moisture compared to controls ($\chi 2(1)=59974$, $p<0.001$).

Figure 3. Soil CO_2 and CH_4 fluxes, precipitation, soil moisture and soil temperature (5 cm depth) in Zöbelboden 2022. Blue dashed line indicates beginning of rainfall manipulation, black dashed lines indicate rewetting events and purple dashed lines indicate fertilization.

Control and Control + Nitrogen treatments emitted on average between 160 and 170 mg CO_2-C m^{-2} h^{-1}, DRW and DRW + Nitrogen treatments had much lower emissions (between 95 and 117 mg CO_2-C m^{-2} h^{-1}) (Table 1). Drying-rewetting resulted in a reduction in CO_2 emissions of 65 mg CO_2-C m^{-2} h^{-1} ($\chi 2(1)=18.786$, $p < 0.001$). As was the case in Klausen-Leopoldsdorf, average CH_4 uptake was slightly lower in Control + Nitrogen plots (36 µg CH_4-C m^{-2} h^{-1}) than the other treatments (49 – 59 µg CH_4-C m^{-2} h^{-1}). According to linear mixed models, drying-rewetting had no effect on CH_4 uptake in Zöbelboden and there were no interactive effects of drying-rewetting and fertilization on either CO_2 or CH_4 fluxes.

Results Work package 2: Soil Microbial Communities and Nutrient Cycling

Precipitation patterns and N deposition are expected to affect soil microbes and nutrient cycling. Distinct effects of drying-rewetting on soil CH_4 consumption have been observed in Rosalia in previous projects (DRAIN) and N fertilization has been shown to reduce soil CH_4 uptake in Klausen-Leopoldsdorf (Gundersen et al 2012). It is assumed that at least part of the observed changes in CH_4 consumption are mediated through alterations in the size and the composition of soil methanotrophic bacteria. Newly generated data can be correlated with a wealth of information from previous and ongoing measurement campaigns for soil characteristics and gas fluxes in the three sites.

ddPCR, soil microbial biomass and nutrient data

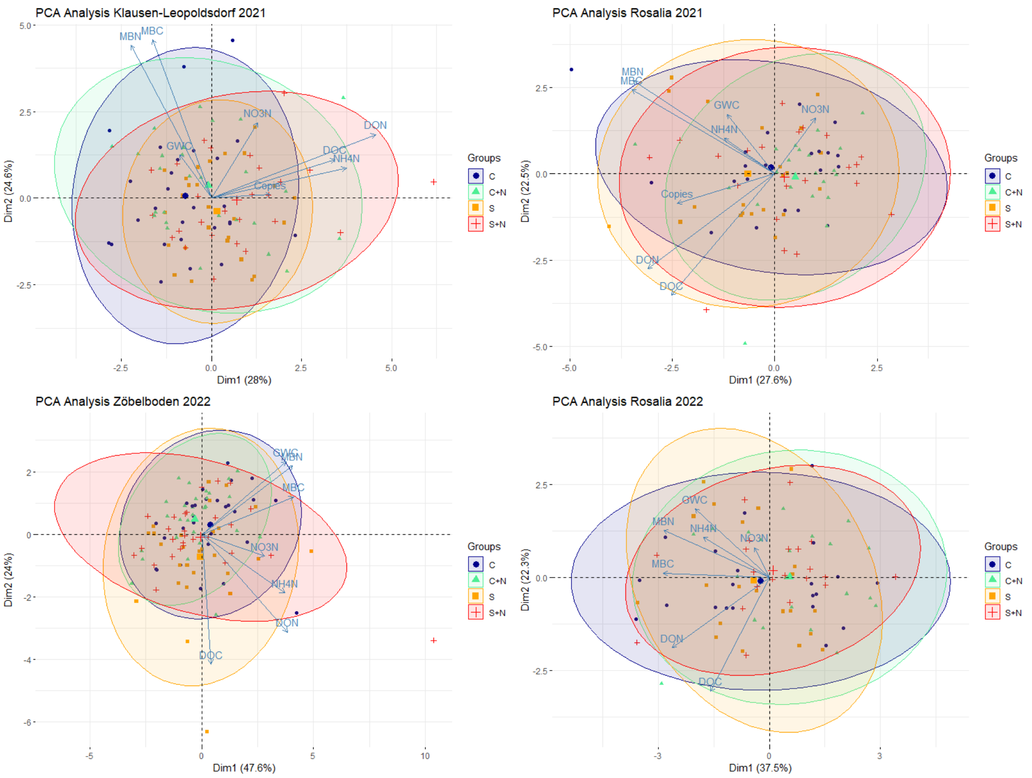

Figure 4. PCA analysis of each EXAFOR site in both years of treatment. GWC = gravimetric water content %, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen, DOC = dissolved organic carbon, DON = dissolved organic nitrogen, NH4N = nitrogen from ammonium, NO3N = nitrogen from nitrate, Copies = concentration of methanotrophic bacteria from upland soil cluster alpha. S and S + N treatments are synonymous with DRW and DRW + N.

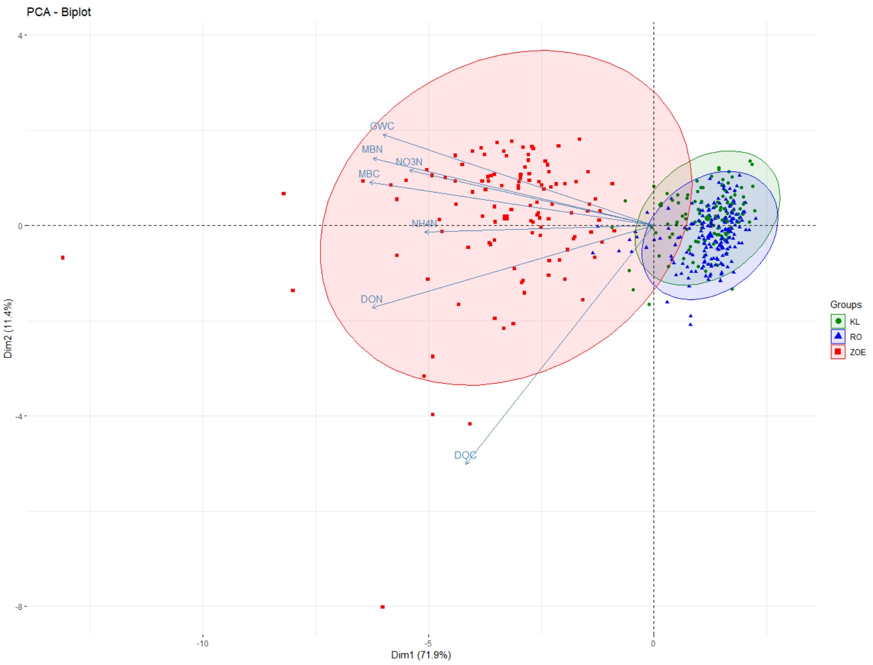

Figure 5. PCA analysis of all soil parameters across all sites (KL: Klausenleopolsdorf, RO: Rosalia, ZOE: Zöbelboden).

Soil samples from the measurement campaigns were analyzed for NO_3, NH_4, DOC, DON after extraction with K_2SO_4, and microbial biomass carbon and nitrogen were analyzed using the fumigation extraction method. At a site level, all soil sample measurements were higher in Zöbelboden than in Rosalia and Klausen-Leopoldsdorf. A comprehensive set of tables with the soil parameters data can be found in the annexes.

In our preliminary results from the samples before the onset of the treatments, we found a correlation between USCα and soil CH_4 uptake rates (see first period report). Once the manipulation was operative, the USCα – CH_4 uptake relationship was further observed (Figure 9), indicating that USCα play a relevant role for the CH_4 budget of these soils. The relationship seems to be similar regardless of the treatment, as suggested by the similar slope of the correlation. Furthermore for, Klausen – Leopoldsdorf, we found that the abundance of USCα was highest during the first drought period compared to the last rewetting event and that the effect of treatment was not significant ($p = 0.82$). The resistance index was calculated to assess the effect of the disturbance (drying and rewetting) on the abundance of USCα and soil CH_4 uptake. Overall, both S and S+N treatments were resistant to the stress events, as the values obtained at each cycle were above zero. No significant effect of treatments was found. The resistance was lower after the second cycle compared to the first or the third cycle. Our results also show a high resistance index with regard to the soil CH_4 uptake (values 0.52-0.88 depending on treatment and cycle).

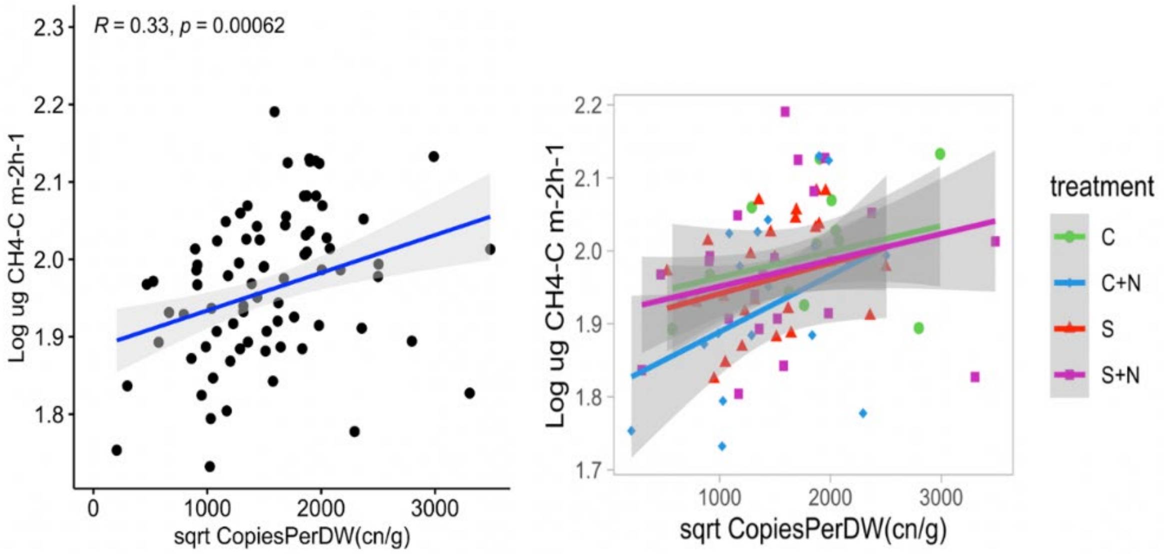

Figure 6. Pearson's correlation between USCα abundance and soil CH_4 uptake with the average of the treatments (left) and for each treatment (right) in Klausenleopoldsdorf.

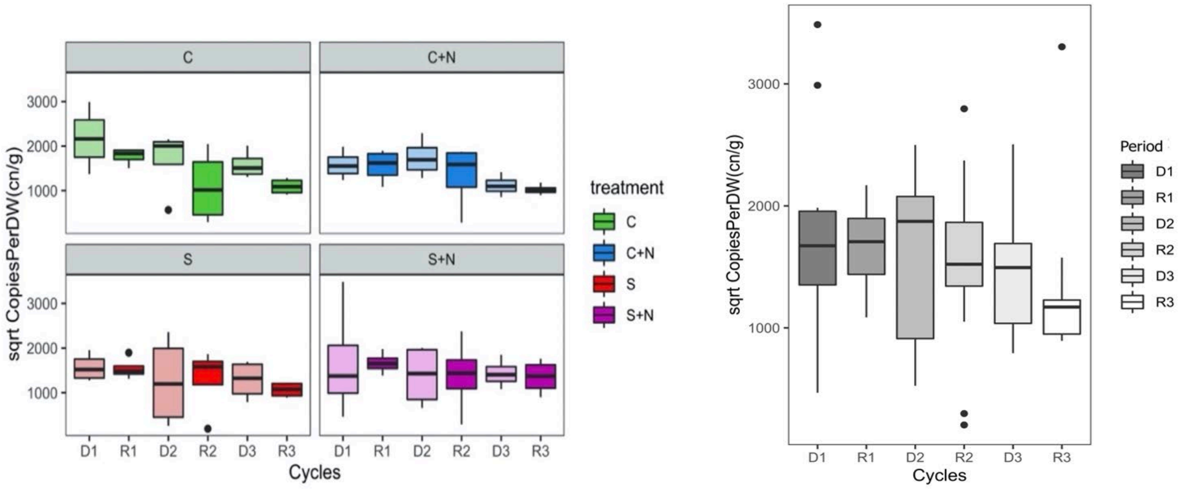

Figure 7. Left: USCα abundance per treatment in Klausenleopoldsdorf in 2021. The values are square root transformed. Treatments: C= Control; C+N= Control + Nitrogen addition; S= Stress (drying & rewetting); S+N = Stress + Nitrogen addition. Right: USCα abundance at each drying period and rewetting events; all treatments were used for this graph. (D = drought; R = Rewetting).

Structure of methanotrophic communities (M2.4)

Initial testing of available assays for molecular detection and quantification of methanotrophic communities in soil samples revealed that at all three investigated sites only the Upland Soil Cluster alpha (USCα, (Kolb et al., 2003; Pratscher et al., 2018)) was present at detectable amounts. Methanotrophs from this cluster belong to the family Beijerinckiaceae in the Alphaproteobacteria and contain a high-affinity methane uptake system especially suited for (facultative) methanotrophy at low methane concentrations. USCα is commonly detected in forest soils including acidic sites. Therefore, all further work was done with primer pairs specifically targeting USCα.

Composition of USCα communities at the three sites in Klausen-Leopoldsdorf, Rosalia and Zöbelboden was assessed by high-throughput amplicon sequencing on the MiSeq platform. A reduction of α-diversity (Shannon-Index) was seen in the C+N treatment at Klausen-Leopoldsdorf and Rosalia but not at Zöbelboden (Figure 11). Differences between sampling depth, seasons and years were statistically not significant.

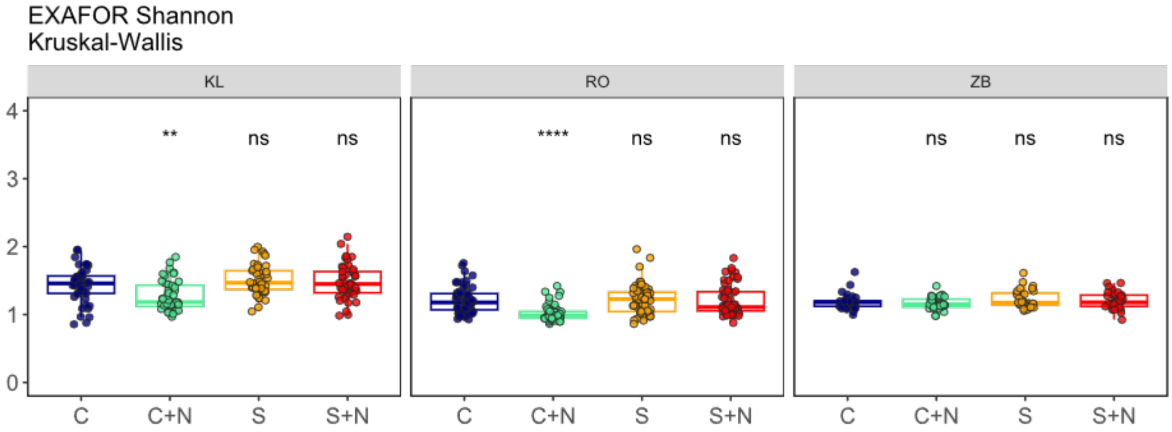

Figure 8: Alpha-Diversity (Shannon-Index) of soil methanotrophic communities at the three sites Klausen-Leopoldsdorf (KL), Rosalia (RO) and Zöbelboden (ZB)**.** Data from all sampling time points (seasons) from both years (2021 and 2022) were collapsed by treatment. For statistical analysis (Kruskal-Wallis test), values were compared to the control for each site. Significance levels are indicated.

A comparison of the community composition (Figure 12) revealed pronounced differences between the three sites (PERMANOVA, $R^2 = 0.43$, $p = 0.001$) and marginal differences for seasons ($R^2 = 0.047$, $p = 0.001$). Although still significant, the treatment effect was very weak ($R^2 = 0.017$, $p = 0.001$), even when interaction with site was taken into account ($R^2 = 0.03$, $p = 0.001$).

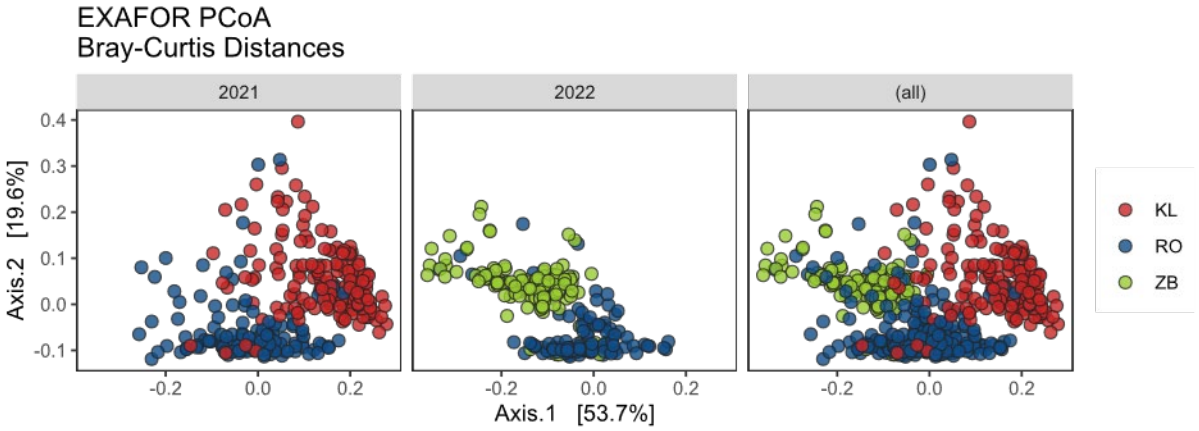

Figure 9: Principal Coordinate Analysis (Bray-Curtis distances) of soil methanotrophic communities at the three sites Klausen-Leopoldsdorf (KL), Rosalia (RO) and Zöbelboden (ZB). Data from all treatments, sampling depths and sampling time points were collapsed. Sites are shown in different colours and sampling years in different panels.

To disentangle more subtle effects of the treatments, the dataset was split into the three different sites and analysed separately. For the site-specific subsets of the full data set, the factor treatment was slightly more pronounced with an R² of ca. 0.05 at each site. The richest data set was available from Rosalia, where sampling and methanotroph community analysis was done in both years – 2021 and 2022. Although the difference between the two years was significant, the effect was very small (R² = 0.019; p = 0.001). Data for the Rosalia set split into the sampling months and sampling depths are shown in Figure 13.

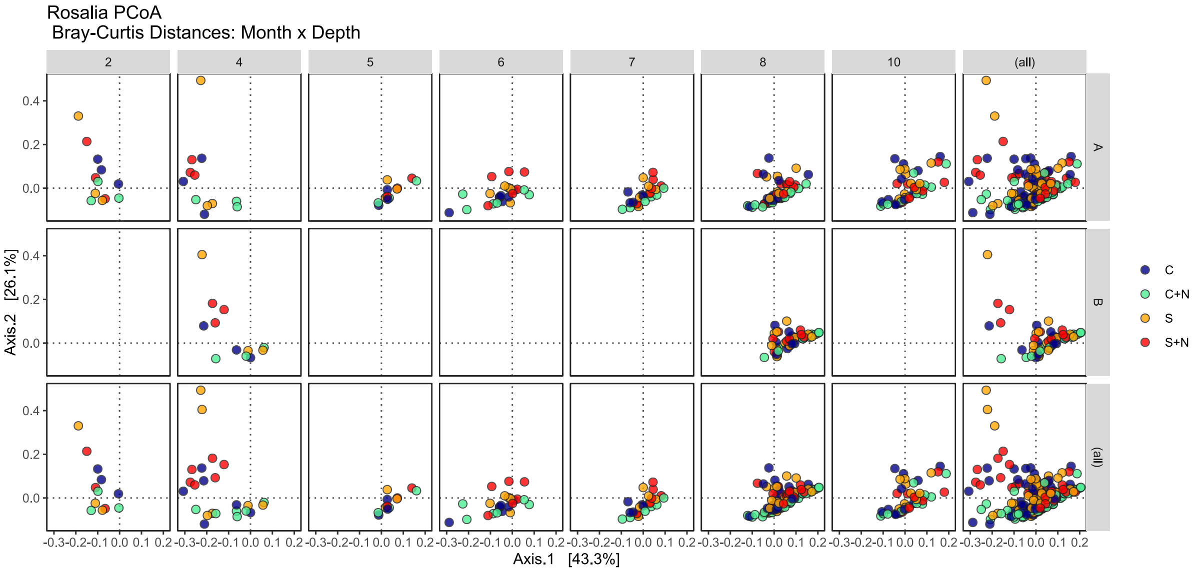

Figure 10: Principal Coordinate Analysis (Bray-Curtis distances) of soil methanotrophic communities at Rosalia. Data from the two sampling years were collapsed and split by months (2-10) and depths (A, B). Treatments are shown in different colours.

A closer look at the phylogenetic composition of the methanotrophic communities at the three sites revealed a dominance of members of the Beijerinckiaceae in the Alphaproteobacteria as would be expected from the use of USCɑ specific primers for molecular analyes. There was, however, at the Zöbelboden site a substantial fraction of members in the Methylococcales, an order in the Gammaproteobacteria, which lies outside of the intended range of the USCɑ assay (Figure 14).

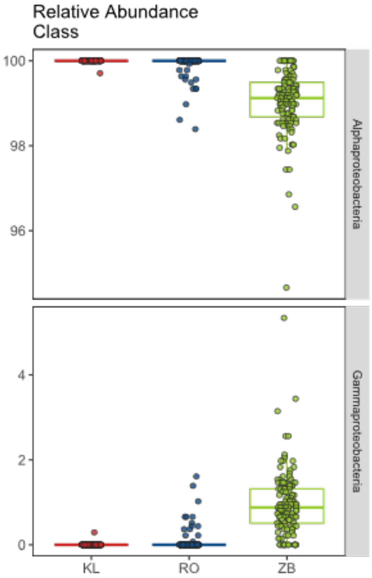

Figure 11: Relative abundance (%) of pmoA from the classes Alpha- and Gammaprotebacteria at the three sites Klausen-Leopoldsdorf (KL), Rosalia (RO) and Zöbelboden (ZB).

Work package 3: Harmonization and feedbacks under global change

This work package was responsible for harmonization between activities of the other work packages, installation of central infrastructure, modelling and integration of activities with other projects and initiatives.

In the course of the project, experimental activities of EXAFOR were linked to those of the eLTER-PLUS, which investigated biogeochemical data and the effect of extreme events. Furthermore, in this frame, access to the EXAFOR experimental set up was provided and international collaborators visited our plots for conducting related experiments, in the frame of provision of transnational access. Thus, colleagues from the University of Coimbra made use of our plots to set up a litter decomposition experiment under global change. Litterbags were installed in the field in autumn 2021 and are being collected at different time intervals to assess the degree of decomposition according to the different environmental conditions. The last sample collection is foreseen for autumn 2024. Colleagues from Ben Gurion University in Israel, visited the EXAFOR plots in Rosalia in August 2023 and performed measurements of nitric oxide emissions in the frame of the RETRACE experiment.

Activities with regard to the installation of the rain-out-shelters, the irrigation infrastructure and the GasFluxTrailer are reported in the section C (project details)

Soil Moisture and Temperature Models

As CO_2 fluxes were more affected by the drying-rewetting regime than by fertilization, the relationship between CO_2 flux and soil moisture and temperature was explored using the least squares method, first with CO_2 flux as a function of soil temperature, then as a function of soil moisture. CO_2 values generated by Gaussian temperature models fit the measured CO_2 fluxes relatively well. On the other hand, the Quadratic soil moisture models generated CO_2 flux data which only partially reflected measured CO_2 values. Finally, both models were combined, considering the CO_2 flux data as a function of soil temperature and moisture, which provided the best fit for modelled vs observed values:

$$\mathbf{R_S = a * \exp(b * T_{soil} + c * T_{soil}^2) * (d * WFPS + e * WFPS^2)}$$

The model was run for each treatment and site separately. In Klausen-Leopoldsdorf, the model fit was better in the Control and Control + Nitrogen plots when soil temperature and moisture data from 5cm depth were used as explanatory variables ($R^2 = 0.70 – 0.93$). In contrast, modelled data fit measured data from DRW and DRW + Nitrogen plots better with soil temperature and moisture data from 15cm ($R^2 = 0.74 – 0.79$) whereas the goodness of fit for Control and Control + Nitrogen plots were lower than at 5cm depth (Figure 12, for fitting with 15cm soil depth data, see annex, Figure 25). Soil moisture below 10cm in Klausen-Leopoldsdorf therefore plays an important role in CO_2 flux when soils are subjected to drought.

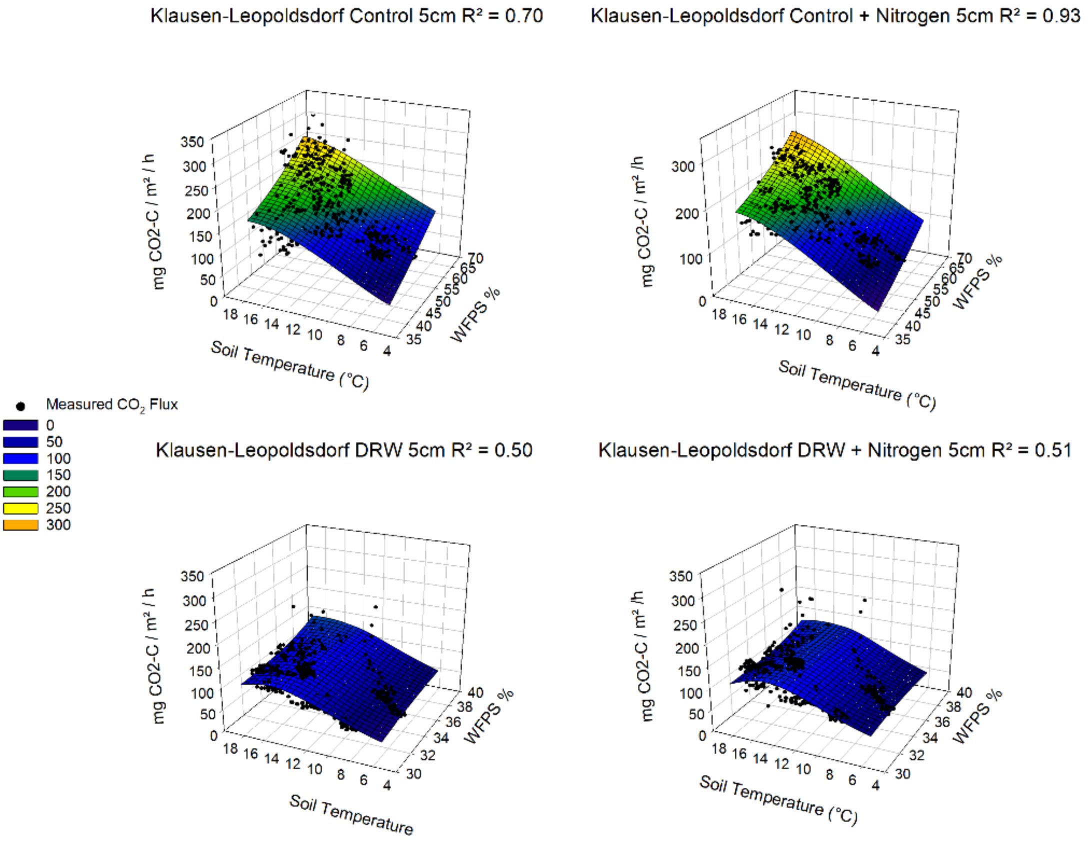

Figure 12 Measured CO_2 fluxes (black points) against Gaussian*Quadratic models (mesh graphs) for Klausen-Leopoldsdorf 2021. Soil temperature and WFPS% from 5cm soil depth were used for the models.

In Zöbelboden, model fit was better in the Control and Control + Nitrogen plots (Figure 13). Compared to Klausen Leopoldsdorf the goodness of fit for the temperature-moisture model was only slightly better than that of the soil-temperature model. This indicates that temperature rather than moisture has the larger influence on CO_2 fluxes in Zöbelboden.

To assess temperature sensitivity, Gaussian * Quadratic models were used to calculate Q_{10} values for Klausen-Leopoldsdorf and Zöbelboden (Figure 14). Q_{10}

values in Klausen-Leopoldsdorf were higher in Control and Control + Nitrogen plots than in DRW and DRW + Nitrogen plots at both 5cm and 15cm soil depths. In Zöbelboden DRW plots had the highest Q_{10} values, suggesting that these plots were less sensitive to changes in temperature.

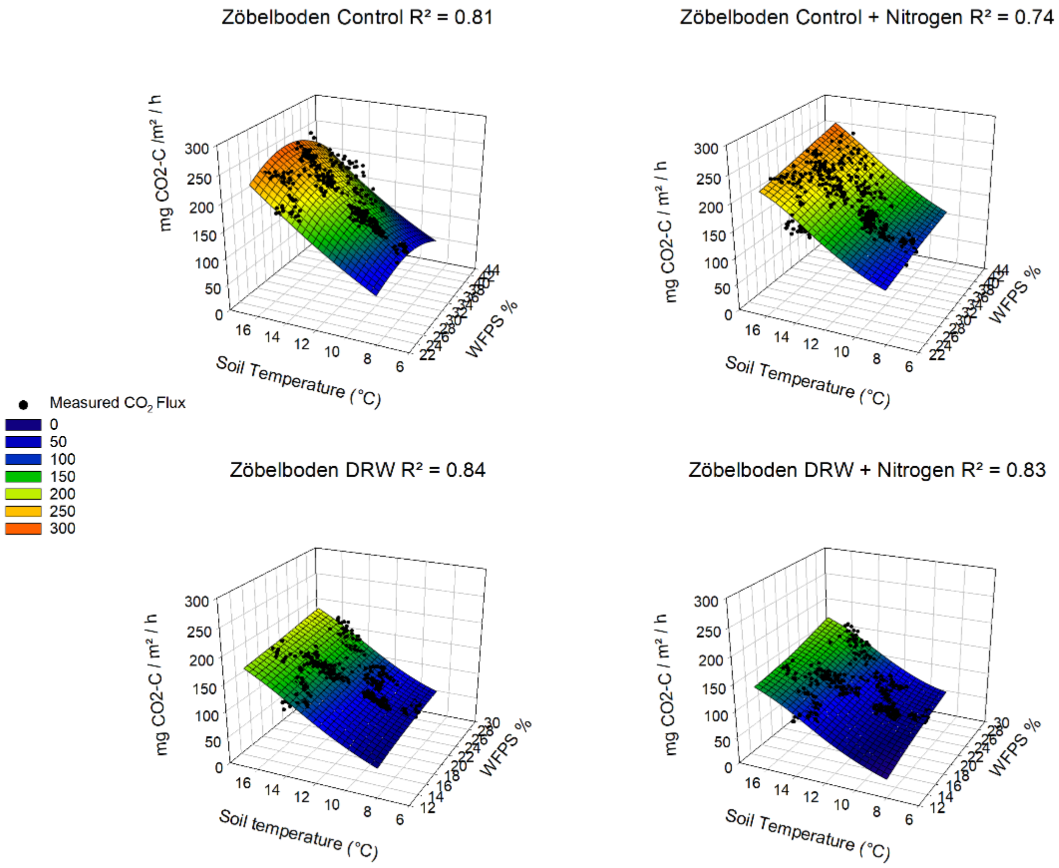

Figure 13. Measured CO_2 fluxes (black points) against Gaussian*Quadratic models (mesh graphs) for Zöbelboden 2022, using soil temperature and WFPS% from 10cm soil depth.

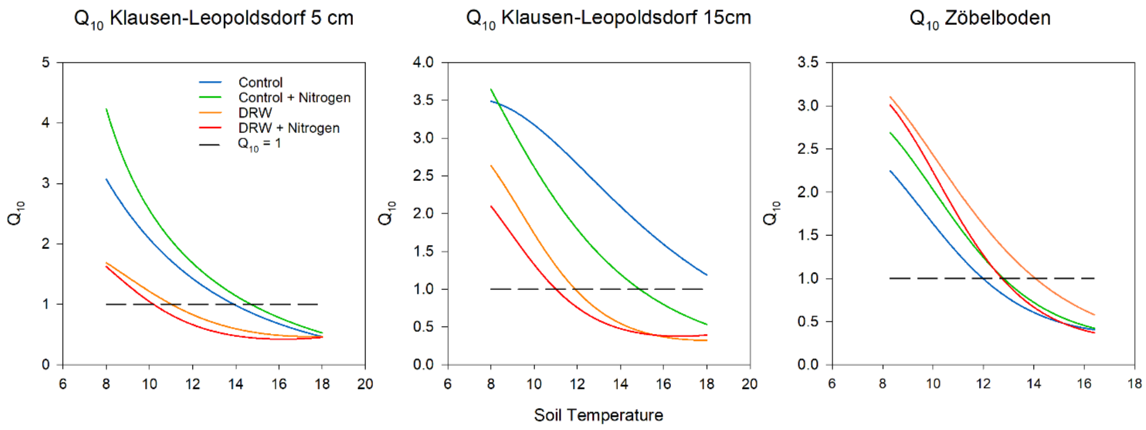

Figure 14. Q_{10} values for Klausen-Leopoldsdorf and Zöbelboden, calculated with Gaussian*Quadratic models.

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5 Schlussfolgerungen und Empfehlungen / Conclusions and recommendations

Answers to the research hypotheses developed for the EXAFOR project

- **Hypothesis 1:** *Increased frequency and intensity of drying-wetting cycles will affect the soil GHG fluxes in the long-term at a lower intensity than in the short-term. Thus, while we hypothesize that continuation of precipitation manipulation will further decrease* CO_2 *and* N_2O *fluxes and increase* CH_4 *uptake rates, the magnitude of the effect will diminish with time.*

The first hypothesis was based on the likely decrease of the manipulation effect in the long-term, partially based on our observations towards the end of our previous ACRP project. The hypothesis was clearly confirmed for CO_2 fluxes, which were reduced in 2019-2020 by 17 % only instead of 30 % as initially. In the case of CH_4 fluxes, the manipulation did not seem to affect uptake rates anymore, suggesting a strong adaptation of the microbial communities to the new environmental conditions. Finally, for N_2O a strong relative reduction was still appreciated (from 45 % reduction in 2013-2015 to 55 % in 2019-2020). However, due to the extremely low N_2O emissions in this forest ecosystem, the absolute difference, as well as the implications for the climate system, are not critical.

- **Hypothesis 2:** *Alleviation of microbial nutrient limitation in the soil will amplify the GHG pulses after rewetting of dry soils. Thus, cumulative* N_2O *and* CO_2 *fluxes under increased drying-wetting cycles will be higher than under control plots if enough nitrogen is available for soil microorganisms.*

In order to test these hypotheses, we had a natural nitrogen deposition gradient (low deposition rates in Rosalia, medium deposition rates in Klausen-Leopoldsdorf and high deposition rates in Zöbelboden) in combination with artificial increase of atmospheric N deposition rates via fertilization. Unfortunately, soil N_2O fluxes could not be assessed in Zöbelboden, where atmospheric N deposition rates are highest, but we could still make of natural and artificially increased N deposition rates in Klausenleopoldsdorft, already significantly higher than in Rosalia. For soil CO_2 fluxes, we could still check the hypothesis with both Klausenleopoldsdorf and Zöbelboden. Our results showed that the effect of drought clearly overweighs the pulse effect, in both N_2O and CO_2, for all plots. Table 2 summarizes the obtained results: on average, drying-rewetting reduced soil respiration by 45 and 55 mg CO_2-C m^{-2} h^{-1} in Klausen-Leopoldsdorf and Zöbelboden, respectively, and regardless of the N effect. We only observed modest, non-significant differences in CO_2 fluxes due to the addition of N, with an interaction with site: in Klausen-Leopoldsdorf, DRW-N CO_2 fluxes were slightly larger than DRW ones (113 vs. 100 mg CO_2-C m^{-2} h^{-1}). In Zöbelboden, site known to be N saturated, the addition of N likely induced a reduction in soil respiration of 7 mg CO_2-C m^{-2} h^{-1} under natural precipitation regime and 23 mg CO_2-C m^{-2} h^{-1} under drying-rewetting cycles. To us, these are indicators that a further increase in the N deposition in Zöbelboden may induce a shift in belowground processes; differences found are small and non-significant. We therefore conclude that N may modulate to some extent the response of greenhouse gas fluxes to drying-rewetting cycles, but the dominant driver for the production and consumption of greenhouse gases in the soil is the exposure to drought.

- **Hypothesis 3:** *High nitrogen deposition rates will decrease the soil methanotrophic bacteria while altered precipitation patterns will enhance them*.

Our previous experience gained in the DRAIN project showed that increased frequency and intensity of drying-rewetting cycles do enhance the CH_4 sink capacity of forest soils, and CH_4 uptake rates react different to changes in soil moisture and soil temperature (Díaz-Pinés et al., 2018), suggesting an influence on the soil methanotropic communities. Having this in mind, we elaborated the part of the hypothesis related to the altered precipitation patterns. Following literature on the effects of deposition rate on soil methanotrophic bacteria (e.g. (Fang et al., 2014), we hypothesized that N deposition will have a negative impact on methanotrophic microbes. We did observe a seasonality effect in Klausen-Leopoldsdorf, with a non-significant trend of reduction from spring towards the end of the summer. When looking at the diversity of the USCα communities, there was indeed a reduction in Klausen-Leopoldsdorf and Rosalia but not at Zöbelboden. Treatment effects observed were actually quite modest, suggesting that the short-term effects of either high nitrogen deposition rates or altered precipitation patterns only had a very minor effect on the investigated USCα communities. Further investigations on long-term effects, as well as different lag times after rewetting may help to disentangle more about USCα communities and their environmental drivers.

Summary of effects of drying rewetting cycles and increased atmospheric N deposition on selected soil parameters

Our set up allow to derive conclusions of soil responses to changes in the distribution of the precipitation and to enhanced nitrogen deposition rates. While acknowledging that our results are valid to assess the short-term responses, our multi-site in-situ approach is able to anticipate soil response for a large area of broadleaf-forests in Austria.

Parameter	**Effect of drying-rewetting**	**Effect of increased N deposition**
Soil Moisture	Usually decrease in soil moisture	No effect
Soil CO_2 emissions	Decrease 30-40 %	Site dependent (modest effect)
Soil N_2O emissions	Decrease 75 %	No effect
Soil CH_4 uptake	Increase (non-significant)	Decrease (non-significant)
Soil Microbial biomass	No evident change (highly variable)	Usually, reduction in C
Soil Nutrients	No clear pattern (highly variable)	No clear pattern (highly variable)
Methanotrophic microbes	No evident change	Reduced diversity

Table 4. Summary Table of main effects in the project. Summary of flux data is only relevant to Klausen-Leopoldsdorf and Zöbelboden.

Expected patterns were often obscured by high temporal and spatial variability, as in the case of nutrients. We further need to clearly point out that our environmental controls undergo episode of natural drought and episodic rainfall events. What we designed as severe stress scenarios beyond observed situations, turn out that it is already occurring due to global change. This is clearly shown in Figure 15; soil moisture levels in the environmental controls experienced several times over the vegetation period strong dry-out phases and short-term increases in soil moisture.

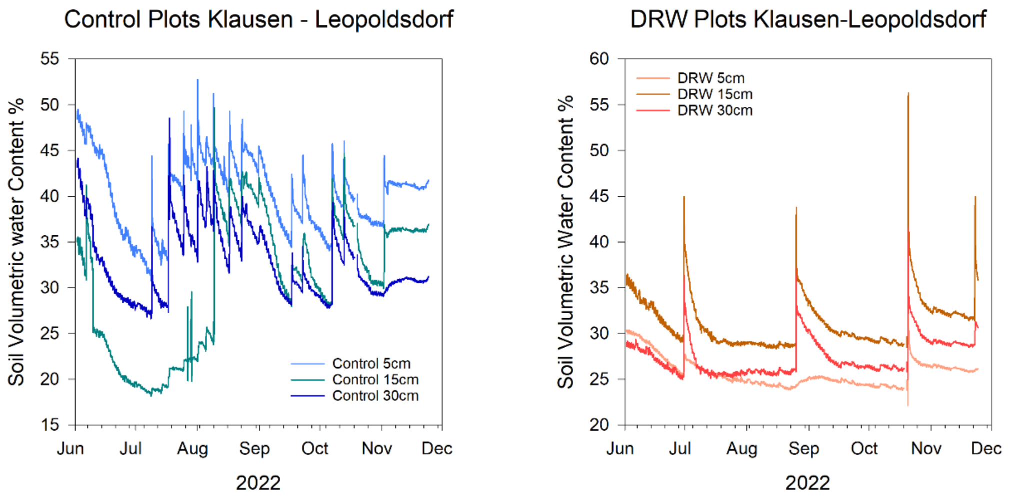


Figure 15. Volumetric moisture content % from Klausen-Leopoldsdorf 2021. Soil moisture was better retained after rewetting in the 15cm soil depth of DRW and DRW + Nitrogen plots.

The behaviour is not exclusive to Klausen-Leopoldsdorf. In Rosalia, a summer drought in 2022 resulted in similar soil moisture conditions in control and DRW plots for much of the vegetation season (Figure 1). As conditions in the DRW plots in our project are designed to simulate extreme drought events under future climate scenarios, it seems likely that at least periodical occurrences of such extreme droughts are already a reality for forests in Central Europe.

Our project tested two effects on soil GHG fluxes: drying-rewetting and fertilisation. We analysed the impact of these effects on fluxes both in isolation and as interactive effects. Fertilisation did not have a statistically significant effect on GHG fluxes in Klausen-Leopoldsdorf or in Zöbelboden, either in Control or DRW plots. In contrast, drying-rewetting reduced CO_2 fluxes in both Klausen-Leopoldsdorf and Zöbelboden by 30 – 40%. There was no interactive DRW-fertilisation effect in either site for CO_2. CH_4 fluxes did not respond to either treatment, however there was a significant DRW effect on N_2O fluxes in Klausen-Leopoldsdorf, with fluxes almost zero during peak drought. Fertilisation did not influence either CH_4 or N_2O fluxes in either site. The lack of differences in plots subjected to fertilisation by either CO_2 or N_2O fluxes under DRW conditions rejects our hypothesis that CO_2 and N_2O fluxes are higher in these plots when nitrogen is not limited.

We modeled CO_2 flux as a function of soil temperature, and obtained a good fit to measured flux data in Zöbelboden (R^2 0.71 – 0.82). This fit only improved slightly by adding soil moisture as an explanatory variable to the model (see Annex). Soil temperature therefore seems to have the largest effect on CO_2 fluxes in Zöbelboden. Water regime rather than soil temperature affected CO_2 fluxes in Klausen–Leopoldsdorf. Model fits for the DRW and DRW + Nitrogen plots improved greatly when soil temperature and moisture from 15cm soil depth was used, contrasting with a slight reduction in R^2 values for the Control and Control + Nitrogen plots. This is likely to be due to soil below 10cm having a greater capacity to retain soil moisture under drought conditions at this site. Therefore, it is important to investigate soil conditions below 10cm soil depth when analysing the effect of drying-rewetting treatments on forest soils.

Analysis of soil microbial biomass and nutrients did not reveal any clear response to treatments at any site. High variation occurred within treatments in the sample set. In general, all soil microbial and nutritional values measured during soil sampling were greater in Zöbelboden than in Klausen-Leopoldsdorf or Rosalia. However, this was not reflected in greater GHG fluxes in Zöbelboden, perhaps due to temperature limitation on microbial activity at these high-altitude soils. PCA analysis also did not reveal any clear relationships between microbial biomass and other soil parameters within sites.

Methanotrophic bacteria belonging to upland soil cluster alpha (USCɑ) were detected after failure to detect Type 1 and Type 2 methanotrophs. As with other soil parameters, there was high variation within treatments, and differences were greater between sites than between treatments. This reflects CH_4 fluxes, which were not affected by either DRW or fertilisation treatments in Klausen-Leopoldsdorf or in Zöbelboden. Shannon diversity of methanotrophic bacteria in Control + Nitrogen plots in Rosalia and Klausen-Leopoldsdorf was lower than in Controls. This may be due to a combination of lower methane availability due to reduced diffusion combined with nitrogen addition, which can be a limiting factor for methanotrophic bacteria.

C) Projektdetails

6 Methodik

Activities performed within the framework of the project, including methods employed

Brief description of the experimental design

Four different treatments were considered for our experimental set up:

Environmental control (C): This treatment was not exposed to any manipulation, and thus received natural throughfall and background rates of N deposition.

Drying-rewetting (DRW): In this treatment, the soil was exposed to meteorological drought by the use of rain-out-shelters that were installed by the beginning of May. Every eight weeks, a rainfall event of 150 mm (for Rosalia and Klausen-Leopoldsdorf) or 300 mm (Zöbelboden) took place. Thus, a total of three episodic rainfall events were simulated (June, August, October) with no incoming rainfall in the periods in between.

Increased N deposition (C+N): This treatment had no manipulation of rain, but was subjected to an increased N deposition rate of 50 kg N ha^{-1} a^{-1} above the background N deposition rates. N was applied to the soil surface in a water solution every 2 months.

Drying-rewetting and increased N deposition (DRW+N): In this treatment, plots were subjected to alteration of the precipitation in the same way as the DRW treatment, and also received an increased N deposition rate of 50 kg N ha^{-1} a^{-1} as the C+N treatment.

Installations in Rosalia and Klausenleopoldsdorf

Installation of rain-out-shelters and irrigation system in Rosalia and Klausenleopoldsdorf started in October 2020 and were finished in May 2021, coinciding with the start of the vegetation period. At Klausenleopoldsdorf four rain-out shelters with an area of 24 m^2 each were installed. In order to place the GasFluxTrailer in a horizontal position, a wooden platform had to be built. Additionally, soil moisture and soil temperature sensors were installed at two control and two drought plots. Sensors were placed in three different soil depths (5, 15 and 30 cm) in Klausen -Leopoldsdorf, and at 10cm soil depth in Rosalia. Data is measured in a 15-minute interval and stored in a Delta-T Logger where data is retrieved manually. Pictures and sketches of the installations and manipulation are found below

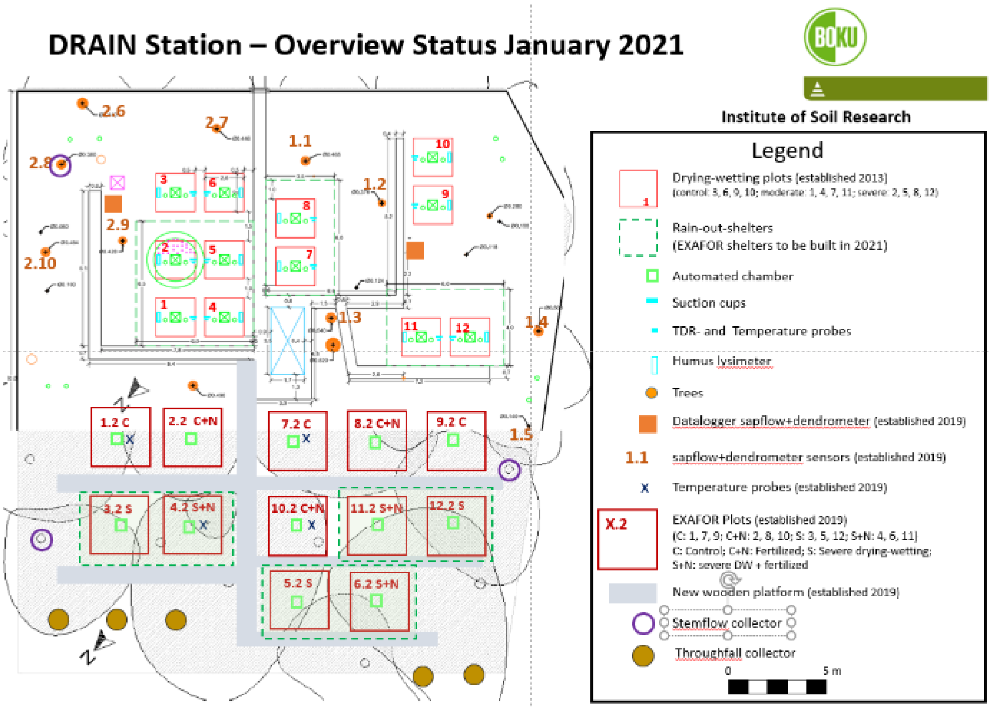


Figure 16. Spatial arrangement of plots and devices in Rosalia

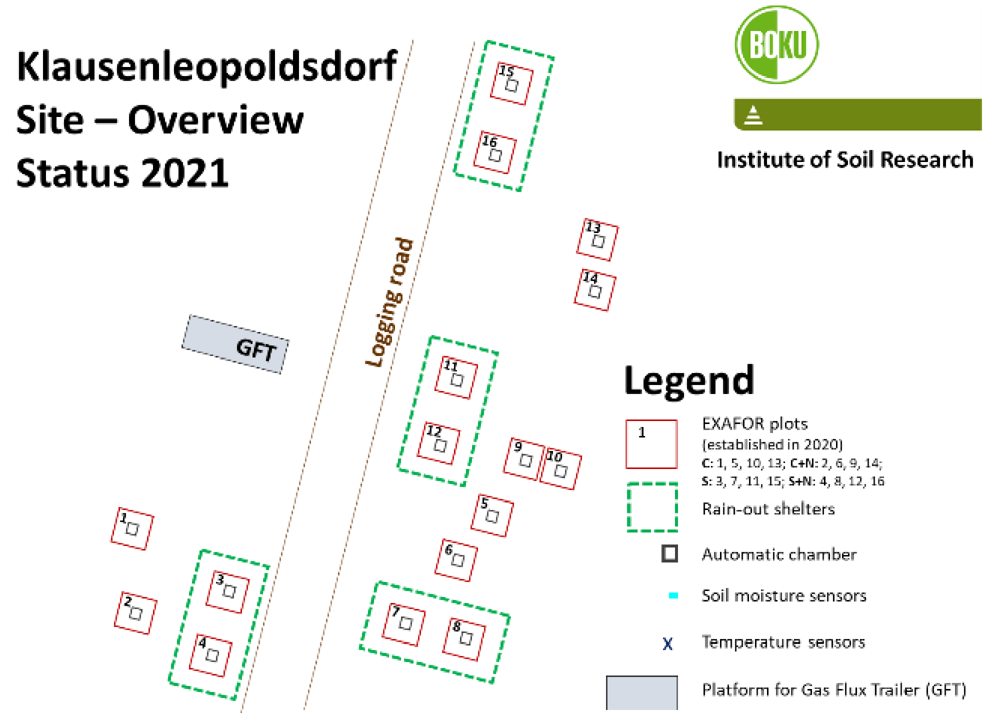


Figure 17 Spatial arrangement of plots and devices and Klausenleopoldsdorf.

Figure 18. Transport of the GasFluxTrailer to the plots (a) GasFluxTrailer on site (b) automated gas chambers and rain-out shelter (c), and water reservoir for the rewetting experiment (d) at Klausenleopoldsdorf. Source: B. Kitzler, 2021

Installations in Zöbelboden

Installation of rain-out-shelters and irrigation system in Zöbelboden took place in Spring 2022, and works were finished by the start of the vegetation period (end of April). four rain-out shelters with an area of 8 m^2 each were installed. In order to place the GasFluxTrailer in a horizontal position, a wooden platform had to be built. Additionally, soil moisture and soil temperature sensors were installed at two control and two drought plots. Sensors were placed at 10cm soil depth. Data is measured in a 15-minute interval and stored in a Delta-T Logger where data is retrieved manually. In Figure 23 some pictures of the installations and manipulation can be seen; Figure 24 shows the plots and spatial arrangement of the infrastructure.

Figure 19. Installation works at Zöbelboden B. (c): Djukic, 2022

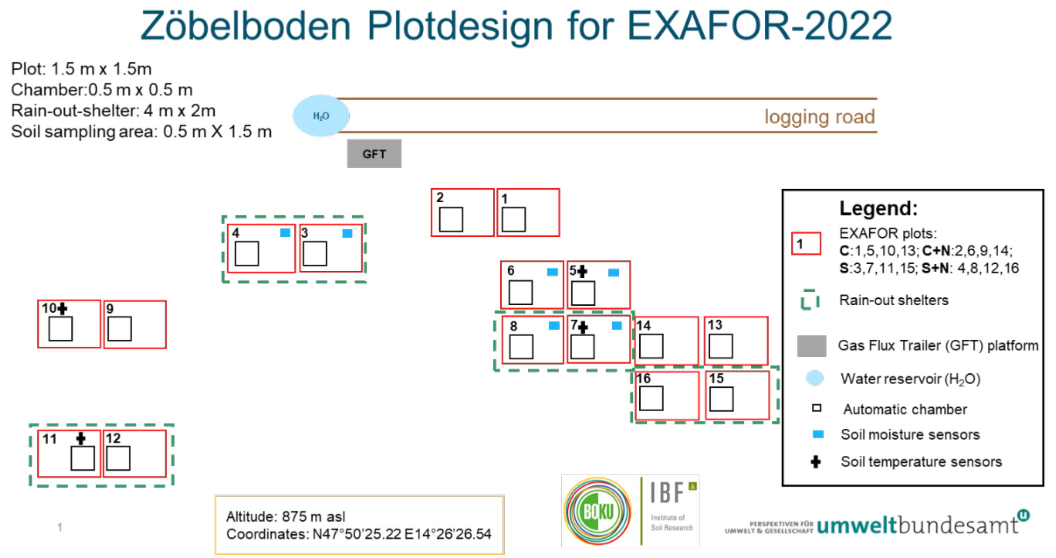


Figure 20. Spatial arrangement of plots and devices in Zöbelboden.

Soil sampling

We were interested in investigating several soil parameters that are known for having a strong temporal dynamic. Further, environmental conditions in the soil are contrastingly different depending on the onset of the drought or the irrigation events. Soils were sampled at each site before the installation of rain out shelters as a pre-treatment control, then sampled before each irrigation event and one-week post-irrigation. We avoided repeated sampling on the same area of the plots by implementing a grid system, whereby samples were taken from a different square of the grid at each sampling event. Soils were sampled at 10cm soil depth for all samples, with additional samples at 10 – 20cm soil depth in the pre-treatment sampling, and before and after the second irrigation event each year.

Microbial Biomass, DOC, DON, NO_3^- and NH_4^+.

Microbial biomass was analysed using the fumigation extraction method. 5g of soil per plot and soil depth was placed in glass petri dishes in a desiccator and fumigated with ethanol-free chloroform for 24 hours. A parallel set of samples was left unfumigated. Both sets of samples were extracted with 50ml 0.5M potassium sulphate (K2SO4), shaken for 1 hour and filtered through N-free filters (Sartorius AG, Qual. Grade 3hW, Gottingen, Germany). A TOC/TN analyser (TOC-L, Shimadzu, Kyoto, Japan) was used to measure dissolved organic C (DOC) total dissolved N (TDN), NO3- and NH4+. Dissolved Organic N was determined as the difference between TDN measurement and NO3- and NH4+ combined. Microbial biomass C was calculated as the difference in DOC in the fumigated and non-fumigated samples, whereas microbial biomass N was the difference in TDN. These differences were corrected by kEC for microbial C and by kEN for microbial N to account for the extractable part of the biomass in the sample.

Methanotrophic Bacteria

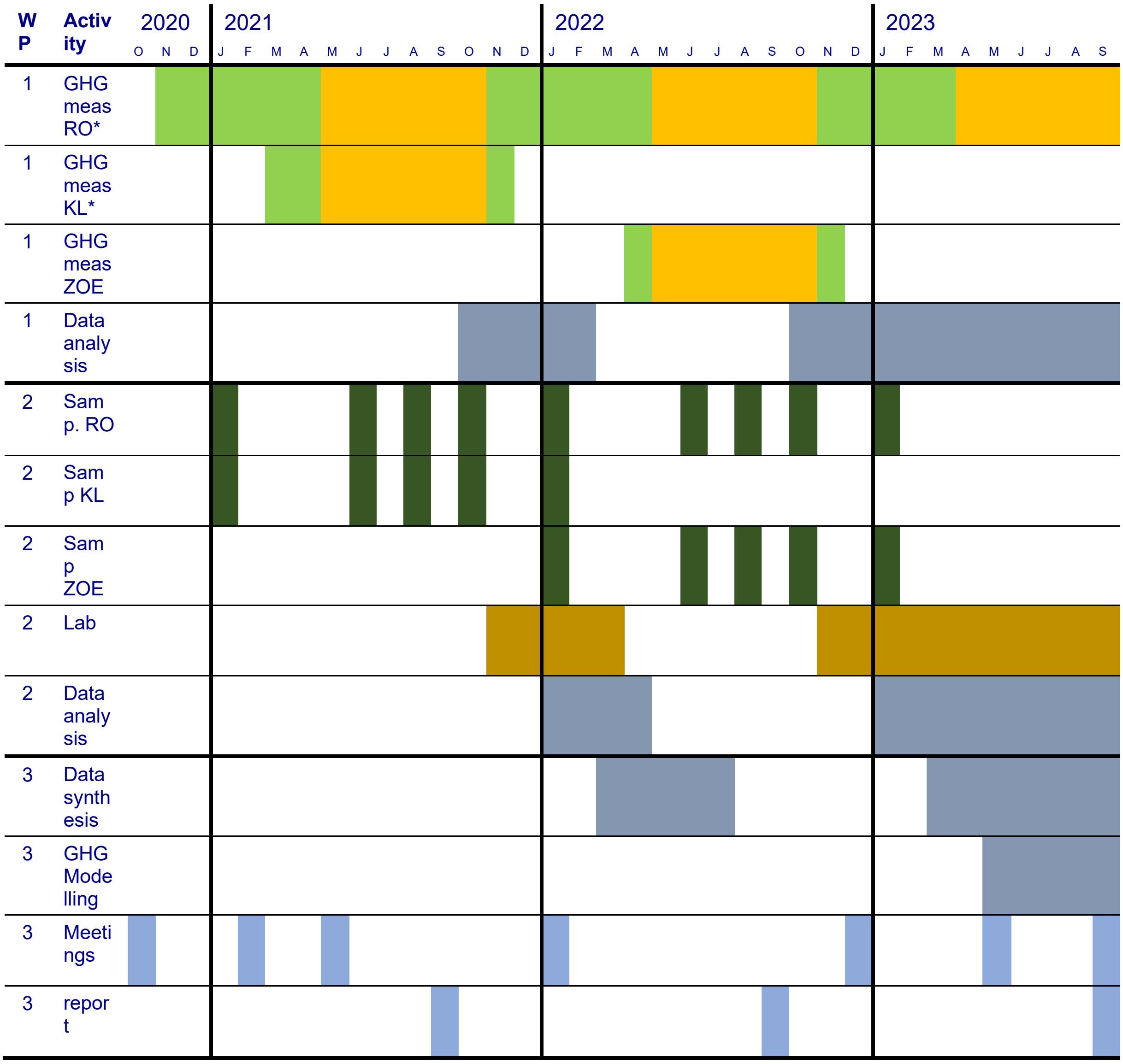
Methanotrophic Bacteria were analysed using the digital droplet Polymerase Chain Reaction technique (ddPCR). DNA was isolated from 0.25g of soil from each plot. using the MagAttractTM Power Soil EP Kit (Quiagen, Hamburg, Germany). The DNA extraction preparation consisted of centrifugation, the addition of IR solution followed by incubation, further centrifugation and then the transfer of the supernatant to a new collection plate. This was then analysed by colleagues in AIT to confirm that DNA had been properly isolated.

Samples were then analysed for the presence of Type I and Type II methanotrophic bacteria, and subsequently for USCɑ using standard PCR analysis. Microplates were filled with DNA from the samples in replicate, primers for each type of bacteria, GoTaq DNA polymerase and water. This mixture was run in a thermocycler for initial denaturation at 95ºC for 2:30 min, 35 cycles of 20s at 94ºC, annealing (temperature gradient from 60ºC to 65ºC, 30s), extension (72ºC, 30 sec) and final extension at 72ºC for 5 min. Following this, Gel electrophoresis was performed at 120v for 50 minutes in a 2% agarose gel. Based on this analysis, it was determined that methanotrophs of USCɑ were the most abundant, and only these bacteria were targeted during ddPCR analysis, using the primers A189f and Forest 675r.

ddPCR analysis allows absolute quantification of DNA from samples and is highly accurate. For this analysis, each well is filled with DNA, A189f forward and Forest 675r reverse primers, bovine serum albumin (BSA), and QX200™ ddPCR™ EvaGreen Supermix (Bio-Rad Laboratories, Inc.). Samples are then mixed with QX200™ Droplet Generation Oil and emulsified. The mixture is subsequently run in a thermocycler for the following sequence: Initial denaturation at 95ºC for 5 min; 40 cycles consisting of 30s at 95ºC, an annealing phase with temperature of 57.5ºC for 1 min and 72ºC for 90 s; these 40 cycles are followed by 5 minutes at 4ºC, and finally a temperature extension for 90ºC for 5 min.

After thermocycling, samples are put in a QX200TM droplet reader and analysed using QuantaSoftTM Analysis Pro software (Bio-Rad Laboratories). The abundance of methanotrophic soil bacteria was considered by the number of gene copies of USCɑ per g of dry soil

7 Arbeits- und Zeitplan



WP	**Activity**	2020			2021												2022												2023								
		O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
1	GHG meas RO*		🟩	🟩	🟩	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨
1	GHG meas KL*						🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟩																						
1	GHG meas ZOE																			🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟩										
1	Data analysis													▨	▨	▨	▨	▨								▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
2	Samp. RO				■					■		■		■			■					■		■		■			■								
2	Samp KL				■					■		■		■			■																				
2	Samp ZOE																■					■		■		■			■								
2	Lab														🟫	🟫	🟫	🟫	🟫								🟫	🟫	🟫	🟫	🟫	🟫	🟫	🟫	🟫	🟫	🟫
2	Data analysis																▨	▨	▨	▨									▨	▨	▨	▨	▨	▨	▨	▨	▨
3	Data synthesis																		▨	▨	▨	▨									▨	▨	▨	▨	▨	▨	▨
3	GHG Modelling																																▨	▨	▨	▨	▨
3	Meetings	🟦				🟦			🟦								🟦											🟦					🟦				🟦
3	report												🟦												🟦												🟦

8 Publikationen und Disseminierungsaktivitäten

Tabellarische Angabe von wissenschaftlichen Publikationen, die aus dem Projekt entstanden sind, sowie sonstiger relevanter Disseminierungsaktivitäten.

Scientific publications:

Type of item	Description	Link
Scientific article	Fernández-Alonso MJ, Díaz-Pinés E, Ortiz C, Rubio A. *Drivers of soil respiration in response to nitrogen addition in Mediterranean mountain forests.* Biogeochemistry, 155 (3), 305-321.	https://10.1007/s10533-021-00827-2
Scientific article	Gillespie LM, Triches NY, Abalos D, Finke P, Zechmeister-Boltenstern S, Glatzel S, Díaz-Pinés E. 2023. *Land inclination controls CO_2 and N_2O fluxes, but not CH_4 uptake, in a temperate upland forest soil*. SOIL, 9, 517-531.	https://doi.org/10.5194/soil-9-517-2023
Scientific pre-print	Triches NY, Gillespie LM, Abalos D, Finke P, Zechmeister-Boltenstern S, Glatzel S, Díaz-Pinés E *Inclination controls CO_2 and N_2O fluxes, but not CH_4 uptake, from a temperate upland forest soil*	https://doi.org/10.21203/rs.3.rs-1803105/v1
Scientific article	Gillespie LM, Kolari P, Kulmala L, Leitner S, Pihlatie M, Zechmeister-Boltenstern S, Díaz-Pinés E. 2024. *Drought effects on soil greenhouse gas fluxes in a boreal and a temperate forest*. Biogeochemistry 167(2), 155-175	https://doi.org/10.1007/s10533-024-01126-2
Scientific pre-print	Dirnböck T, Bahn M, Diaz-Pines E, Djukic I, Englisch M, Gartner K, Gollobich G, Hofbauer A, Ingrisch J, Kitzler B, Knaebel K, Kobler J, Maier M, Wohner C, Offenthaler I, Peterseil J, Pröll P, Venier S, Zechmeister-Boltenstern S, Zolles A, Glatzel S. *High-resolution Carbon cycling data from 2019 to 2021 measured at six Austrian LTER sites*. Earth System Science Data Discussion	https://doi.org/10.5194/essd-2024-110

Presentations at scientific congresses and meetings:

Authors	Title	Event	Date
Díaz-Pinés E	*Effects of repeated soil drying-wetting cycles in a beech forest. A long-term manipulation study*	Invited seminar (online), The French Associates Institute for Agriculture and Biotechnology of Drylands (FAAB), Ben-Gurion University, Israel	December 2020
Díaz-Pinés E	*Experimental networks and eLTER*	eLTER VENUS Meeting (virtual)	April 2021
Gillespie L	WAILS: *Biogeochemical controls of ecosystem functions*	eLTER VENUS Meeting (virtual)	April 2021
Díaz-Pinés E; Goff D, Gorfer M, Kitzler B	*EXtreme weather events and soil greenhouse gas fluxes in Austrian FORests. Evaluating the feedbacks under global change*	22. Klimatag (Climate Change Centre Austria), Vienna	April 2022
Gonzalez, F; Goff, D; Kitzler, B; Knohl, A; Zechmeister-Boltenstern, S; Díaz-Pinés, E	*Soil carbon dioxide fluxes from two Austrian forests under drying rewetting stress*	7. WABO Student Conference 2022: Featuring future in forest and soil sciences, Vienna, AUSTRIA	May 2022
Lopez-Montoya, I; Goff, D; Kitzler, B; Gorfer, M; Zechmeister-Boltenstern, S; Díaz-Pinés, E	*Response of methanotrophic bacteria to soil drying and rewetting in Austrian forests*	7. WABO Student Conference 2022: Featuring future in forest and soil sciences, Vienna, AUSTRIA	May 2022
Gillespie L, Díaz-Pinés E	*Plot scale ecosystem process understanding / Biogeochemical variables*	eLTER PLUS Meeting, Palma de Mallorca, Spain	May 2022
Goff D, Gorfer M, Kitzler B, Díaz-Pinés E	EXtreme weather events and soil greenhouse gas fluxes in Austrian FORests. Evaluating the	In LTER-Austria Conference, Vienna	November 2022

	feedbacks under global change		
Gillespie L, Díaz-Pinés E	*Plot scale ecosystem process understanding / eLTER Biogeochemistry legacy data*	eLTER PLUS Meeting, Frankfurt, Germany	April 2023
Díaz-Pinés E	Measuring soil greenhouse gas fluxes between terrestrial ecosystems and the atmosphere	Seminar Institute of Meteorology and Climatology BOKU, Vienna, Austria	May 2023
Goff D, Kitzler B, Gorfer M, Djukic I, Díaz-Pinés E	Impact of Drought and Rewetting Cycles on Soil Greenhouse Gas Fluxes across an N Deposition Gradient in Austrian Forests	In: IUFRO World congress, Forests and Society towards 2025. Session: Response of forest ecosystems to global change: Learning from experimental manipulations and natural gradient studies	June 2024

One ongoing PhD-Thesis:

Goff D. Impact of drought and rewetting events on greenhouse gas fluxes in Austrian forest soils. Expected finalization: 2024/2025

Six Master Theses:

Author	Title	University	Date
Gabriela Fernanda Villalba Ayala	*Spatial and temporal variability of Methanotrophic Bacteria in two Austrian Forest Soils*	BOKU	2021
Nathalie Triches	*Climate signals on biogeochemistry in Europe: CO_2, CH_4 and N_2O emissions in a temperate forest in eastern Austria*	Uni Ghent	2021
Itzel Lopez-Montoya	*Response of methanotrophic bacteria to soil drying and rewetting in Austrian forests*	BOKU	2022
Franco Alexis González	*Soil Carbon Dioxide Fluxes from Two Austrian Forests under Drying Rewetting Stress*	BOKU	2022
Miriam Dunzendorfer	*Beech transpiration dynamics under drought stress in the Rosalia Demonstration Forest*	BOKU	2022
Christopher Thoma	*Effects of extreme weather events on soil GHG fluxes in an Austrian Forest*	BOKU	2023

Seven Datasets:

Authors	Title	Link
Gillespie LM, Triches NY, Abalos D, Finke P, Zechmeister-Boltenstern S, Glatzel S, Díaz-Pinés E	Soil CO_2, CH_4 and N_2O fluxes, soil and litter parameters and meteorological data from a temperate upland forest along a land inclination gradient	https://doi.org/10.23728/b2share.3900981a45684bfea5d648eb744622d3
Leitner S, Díaz-Pinés E, Zimmermann M, Holtermann C, Zechmeister-Boltenstern S.	Rosalia Lehrforst Austria - Soil Greenhouse Gas Flux Data DRAIN Experiment 2013-2016	https://doi.org/10.23728/b2share.3983fd5a8c574e12af21c4a8682bac88
Díaz-Pinés E, Leitner S, Holtermann C, Zechmeister-Boltenstern S	Rosalia Lehrforst Austria - Soil Greenhouse Gas flux data DRAIN Experiment 2017	https://doi.org/10.23728/b2share.36363cccc85c448cbb00214628acb561
Diaz-Pines E, Holtermann C, Zechmeister-Boltenstern S.	Lehrforst Rosalia Austria - Soil greenhouse gas flux data DRAIN experiment 2019-2020	https://doi.org/10.23728/b2share.04e1295b523d495eaefc2877bd8b1bbe
Leitner S, Zimmermann M, Holtermann C, Zechmeister-Boltenstern S, Díaz-Pinés E	Rosalia Lehrforst AUSTRIA - Soil Moisture and soil temperature data DRAIN Experiment 2013-2020	https://doi.org/10.23728/b2share.d4f34aa2b2584323ae18d699a40695f7
Díaz-Pinés E, Gasch J	Rosalia Lehrforst AUSTRIA - Discharge data Grasriegelgraben 2001-2020	https://doi.org/10.23728/b2share.206dc52077a64150855c706d6a5b70d4
Díaz-Pinés E, Gasch J	Rosalia Lehrforst AUSTRIA - Meteorological Data 2001-2020	https://doi.org/10.23728/b2share.681966be29a34f3ebc6015ac255ab143

Press releases:

https://short.boku.ac.at/exrx34

https://www.umweltbundesamt.at/news220830

https://www.bfw.gv.at/auswirkungen-wetter-boeden-exafor/

https://blickinsland.at/extremwetter-veraendert-wald-mikrobiom/

https://www.bauernnetzwerk.at/extremwetter-veraendert-wald-mikrobiom/

https://www.biologischevielfalt.at/service/chmnews/2022/exafor

https://epaper.heute.at/titles/heutewien/13000/publications/361/pages/6

https://www.diepresse.com/17770013/waldboeden-mischen-rege-im-globalen-klimasystem-mit