Publizierbarer Endbericht

Gilt für Studien aus der Programmlinie Forschung

A) Projektdaten

Allgemeines zum Projekt	
Kurztitel:	ExtremeGrass
Langtitel:	Interactive effects of warming, elevated CO_2 and weather extremes on nitrogen gas fluxes in a managed grassland
Zitiervorschlag:	
Programm inkl. Jahr:	ACRP 8th Call for Proposals
Dauer:	01.04.2016 bis 31.10.2016
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Schlagwörter:	Klimawandel, Stickstoffflüsse, Bodenmikroorganismen
Projektgesamtkosten:	327.173 €
Fördersumme:	296.447 €

Allgemeines zum Projekt	
Klimafonds-Nr:	B566960, KR15AC8K12624
Erstellt am:	31.01.2020

B) Projektübersicht

1 Kurzfassung

Für den Alpenraum ist ein Anstieg der atmosphärischen CO_2 Konzentration um 300 ppm, sowie ein Anstieg der Oberflächentemperatur um 3 °C bis zum Ende des Jahrhunderts prognostiziert. Dies erhöht die Häufigkeit und Intensität starker Wetterextreme, wie z.B. Dürreperioden, gefolgt von kurzen, starken Regenfällen. Während die Auswirkungen einzelner Umweltfaktoren auf die mikrobielle Gemeinschaft (Struktur und Funktion), sowie auf biogeochemischen Zyklen von Ökosystemen, relativ gut untersucht sind, gibt es nur wenige Studien, die die Auswirkung kombinierter Faktoren wie z.B. Erwärmung, erhöhter Kohlendioxidgehalt und Dürreereignisse untersucht haben. Unseres Wissens ist dies das erste Experiment, das sich gleichzeitig mit einzelnen und kombinierten Klimawandelfaktoren mit unterschiedlichem Stufen an Erwärmung und CO_2-Begasung, sowie mit Wetterextremen in Bezug auf biogeochemische Kreisläufe und die mikrobielle Struktur und Funktion von Ökosystemen befasst.

Um die Komplexität eines multifaktoriellen Klimamanipulationsversuches zu umzusetzen wurde ein „Second-order Response Regression" Ansatz entwickelt. Bisher haben nur wenige Ökosystem-Experimente die Notwendigkeit erkannt, nicht-lineare Reaktionen zu untersuchen, und wenn beschränkten sich diese Experimente weitgehend auf einzelne Faktoren.
Dies stellt eine erhebliche Wissenslücke dar, zumal Metaanalysen darauf hindeuten, dass die Reaktionen von Ökosystemen auf Umweltveränderungen, insbesondere Klimaveränderungen aufgrund der kombinierten Ergebnisse von Einzelfaktorexperimenten nicht vorhersehbar sind. Das Ziel des Projekts war es, mikrobielle Prozesse im Zusammenhang mit dem terrestrischen N-Kreislauf, der aus Sicht der Pflanzenproduktion, der Ökosystemfunktionen und der Treibhausgasemissionen (THG) einer der wichtigsten biologischen Kreisläufe ist, zu untersuchen.

Um diese Ziele zu erreichen, (1) wurde in 3 aufeinander folgende Jahren, während der Vegetationsperiode die N_2O- und CH_4-Emissionen im Freiland gemessen. (2) Im zweiten Projektjahr wurde eine Dürreperiode mit anschließendem Regenereignis simuliert, und der Einfluss auf N_2O- und CH_4-Konzentrationen in verschiedenen Bodentiefen (0, 3, 9, 18 und 36) mit traditionellen Kammermessungen kombiniert. (3) Im ersten Projektjahr wurden intakte Bodenbohrkerne im Feld geworben, und im Labor inkubiert, um die Emissionen von N_2O, NO_x, CO_2 und NH_3 zu erfassen. Anschließend wurden die intakten

Bohrkerne bei verschiedenen Temperaturen inkubiert, um die Temperaturempfindlichkeit der N_2O- und NO_x-Flüsse im Boden zu bewerten. (4) Ein zweites Laborexperiment wurde mit intakten Bodenkernen durchgeführt, um die Auswirkung von Trockenheit und verschiedenen Bodenwassergehalten auf N_2O-Emissionen des Bodens zu bestimmen. (5) Bodenproben wurden 2016 nach der Ernte entnommen. Im zweiten Projektjahr wurde der Boden dreimal während des „Dürreexperiments mit Starkregensimulation" entnommen. Die Bodenproben wurden im Labor analysiert, um verschiedene N-Pools (gelöster organischer Stickstoff, mirkobieller Kohlenstoff und Stickstoff, Ammonium und Nitrat), die mikrobielle Funktion (extrazelluläre Enzymaktivität) zu bestimmen. (6) Pilz- und Bakteriengemeinschaften wurden mittels Hochdurchsatzsequenzierung analysiert. (7) Zur Bewertung der mineralischen und organischen Stickstoffflüsse/-konzentrationen während des „Dürreexperiments mit Starkregensimulation" wurde ein Mikrodialysesystem implementiert. Dieser kombinierte Ansatz der N-Pool-Bestimmung im Boden, der Messung des Spurengasflusses und der Profilierung der mikrobiellen Gemeinschaft im Boden ermöglichte es uns, Prozesse und Akteure im biogeochemischen N-Zyklus zu verknüpfen.

Feldversuche sind von entscheidender Bedeutung, um multifaktorielle Treiber des Klimawandels zu identifizieren. Die Durchführung von Feldexperimenten ist jedoch sehr arbeits- und kostenintensiv. Zusätzlich erschwert die große Heterogenität des Bodens im Feldexperiment und viele weitere Faktoren, wie z.B. Wassergehalt, verschiedenste Pflanzenarten etc. die Dateninterpretation. In unseren Studien haben wir herausgefunden, dass die unterschiedlichen Ergebnisse vor allem auf indirekte Effekte, wie z.B. Bodenfeuchtigkeit, Pflanzenzusammensetzung, Bodenfauna zurückzuführen ist. Ausgefeilte statistische Ansätze, wie der „Response Surface Regression" Ansatz sind entscheidend signifikante Ergebnisse zu erhalten

Zusammenfassung: Die geplanten Experimente wurden erfolgreich durchgeführt und die Datenauswertung abgeschlossen. Erste Ergebnisse wurden bereits in international anerkannten Journalen (z.B.: Applied Soil Ecology und Soil Biology and Biochemistry) publiziert, und die Ergebnisse auf nationalen und internationalen Tagungen einem breiten Publikum präsentiert.

2 Executive Summary

The most likely future climate scenario that is predicted for the Alpine region is an increase in atmospheric CO_2 by 300 ppm along with an increase in surface temperature by 3 °C by the end of this century. Thus, will increase the frequency and severity of extreme weather events, such as severe droughts followed by short and heavy rainfall events. While the impact of individual environmental factors on microbial structure and functions, such as nitrification and denitrification and biogeochemical cycles of ecosystems is studied relatively well, there are few studies that have investigated the combined effects of several factors such as warming, elevated carbon dioxide (CO_2) and drought events.

This is the first experiment, to our knowledge, that addresses simultaneously individual and combined climate change factors with varying levels of warming and CO_2 fumigation as well as weather extremes on biogeochemical cycles and microbial structure and function of ecosystems. In order to master the complexity of the experimental setup, a second-order response surface regression approach was used to disentangle quantitative multifactorial manipulation effects. Response surface regression is an attractive approach to optimize statistical power also in ecology. Up to now, only few ecosystem experiments have recognized the need to account for non-linear responses and these experiments were largely limited to single factors. This represents a significant gap in knowledge, especially as meta-analyses indicate that the responses of ecosystems to environmental change based on the combined results of single factor experiments are unpredictable.

The objectives of this project focused on microbial processes related to the terrestrial N cycle, which is one of the most important biological cycles, from the perspective of crop production, ecosystem functions and greenhouse gas (GHG) emissions. Furthermore, a second aim of proposed work is to examine the microbial drivers of N-pools and fluxes from montane grassland soil under factorial climate change manipulation treatments, including an extreme event such as drought.

In order to achieve our objectives, (1) we determined N_2O and CH_4 emissions in the field over the growing season for three years. (2) In the second project year, during the summer-drought experiment we combined “traditional” static chamber greenhouse gas (GHG) measurements with online monitoring of N_2O and CH_4 concentrations in different soil depth (0, 3, 9, 18 and 36 cm) on 6 plots (2 of them with rain-out shelters) with water-tight, gas-permeable tubes connected to a laser-based gas monitor. (3) In the first year (autumn 2016) intact soil cores were sampled to conduct a lab incubation experiment, where N_2O, NO_x, CO_2 and NH_3 were monitored at a water filled pore space according to field conditions. Subsequently, soil cores were incubated at different temperatures to evaluate the temperature sensitivity of soil N_2O and NO_x fluxes. (4) A second lab experiment was conducted with intact soil cores, to determine the effect of soil moisture and drought-re-wetting on soil N_2O emissions. (5) Soil samples were taken 2016 during the vegetation season after grass harvesting, while in the second project year the soil sampling was conducted thrice during the summer drought experiment (before and after rewetting as well as recovery in 3 months later). Soil samples were analyzed in the laboratory to examine different N pools (DON, N_{mic}, NH_4^+ and NO_3^-), and microbial function via extracellular enzyme activities. (6) Fungal and bacterial communities were analyzed via high-throughput sequencing. (7) To assess mineral and organic nitrogen fluxes during drought-rewetting event a microdialysis system was implemented in the field. This combined approach of N-pool determination in soil, trace gas flux measurements, gene quantification and soil microbial community profiling allows us to link processes and players in the biogeochemical N-cycle.

Field experiments are of crucial importance to identify multifactorial drivers of climate change. However, conducting field experiments is highly labor and cost intensive, and the large heterogeneity and factors that vary in the field are not simple to consider for data interpretation. Results of N trace gases and microbial community structure seem to be more driven by indirect effects, especially on soil moisture but also on plant composition, soil fauna, that is different among the treatments. Sophisticated statistical approaches, such as the surface response regression approach coupled with the anisotropic power model is crucial to identify significant difference due to possible spatial variability batch effects occurring in the lab and field.

We conclude that size/intensity and interactions in climate change determine ecological outcomes, and we seriously have to take them into account in future projections. Further, field experiments are of crucial importance to identify multifactorial drivers of climate change. Essentially all experiments in the ExtremeGrass projected pointed to the overarching importance of water. Soil water balance is influenced by temperature, atmospheric CO_2 and through changes in precipitation. In turn, soil water influences plant physiology and vegetation, microbial community composition and function and consequently soil microbial processes and GHG emissions. It became obvious that interactions are highly complex, and indirect effects tend to be stronger than direct effects. This makes it necessary to collect as many additional data as possible. Similar to microbial communities, it is expected that prolonged treatment will not only influence plant physiology but also vegetation. This will add an additional factor, which must be taken into consideration.

3 Hintergrund und Zielsetzung

Initial situation / motivation for the project

Climate projections for the next decades predict a significant increase in air temperature, atmospheric CO_2 concentrations and the frequency and intensity of extreme weather events. Weather extremes are of particular importance because they have a disproportionately strong impact on the ecosystem feedbacks to the climate system. While the impact of individual environmental factors on microbial structure and functions, such as nitrification and denitrification and biogeochemical cycles of ecosystems is studied relatively well, there are few studies that have investigated the combined effects of several factors such as warming, elevated carbon dioxide (CO_2) and drought events.
The quantification of the impact of these combined environmental changes is still fraught with uncertainty, both, in terms of magnitude and the interactions. This represents a significant gap in knowledge, especially as meta-analyses indicate that the responses of ecosystems to environmental change based on the combined results of single-factor experiments are unpredictable.

Objectives of the project

ExtremeGrass investigated the response of warming, elevated CO_2 concentrations, their combined effect as well as extreme weather events on N-gas emissions, microbial community structure and function in a managed grassland site.
Workpackage 1 ensured the efficient management of the project in line with the work plan
Workpackage 2 studied changes among N related trace gas emissions (N_2O, NO_x, NH_3) together with available mineral N forms (NH_4 and NO_3) and organic N (DON and N_{mic}), and how these are related to factorial increases in temperature and CO_2 levels as well as drought conditions.
Workpackage 3 investigated the connections between climate change scenarios (increases in CO_2 and temperature) and soil microbial community structure and function in managed grassland.
Workpackage 4 studied episodic measurement of trace gas concentrations across the soil profile (4 depths) and at the soil surface. Furthermore, it provides soil microclimate (temperature and moisture) across the soil profile. It combined trace gas concentrations across the soil profile and microclimate data in a gradient flux model to determine soil trace gas fluxes and source-sink relations in the soil and at the soil-atmosphere interface. Furthermore, combined trace gas concentrations with microbial processes as analyzed by WP2 and WP3.
Workpackage 5 provided the experimental basics for testing the effects of warming, elevated CO_2, and weather extremes in permanent grassland.

4 Projektinhalt und Ergebnis(se)

Workpackage 1: Project management

Project management and coordination within the working groups was the first milestone of WP 1. The main objective was to ensure the efficient management of the project in line with the work plan and to ensure sufficient support for each individual task. Project meetings were organized by WP 1, the Kick-off meeting was conducted at the field site (Figure 1) so that all researchers got to know each other and to get an impression of the field site and the infrastructure available, or to be implemented during the project period. Further project meetings were held at BOKU and in Gumpenstein. The ongoing fieldwork was coordinated among the work packages, while common larger decisions were made during the meetings, smaller adoptions were discussed and decided by regular phone calls. In addition, the project meetings served to present the results of the previous work conducted within the project to be discussed within the consortium. WP1 ensured the timely submission of the 1st, the 2nd and the final report. Further all collected data were implemented in the "Climgrass" database provided by the WP5. These data will serve for the ongoing field experiment and for future research. These data provide the basis for the synthesis paper as soon as the manuscripts currently in preparation are published.

Figure 1 Kick-off meeting at the experimental site

Workpackage 2: Sampling, analytical pipeline, and data collection

Milestone 1 & 2 of the WP2 were fully achieved. The gas measurement campaigns, sampling for molecular biology, and sampling for NO_x, NH_3 and N_2 incubations were finished as expected. The sampling campaigns for gaseous measurement in the field were prolonged by one year, as we observed difficulties with the methodology and data evaluation of first-year's data (see section 5 Description of difficulties, if any, encountered in the achievement of project targets). One publication on major results (Experiment 1-2a, incubation Experiment) was published in Applied Soil Ecology in January 2019 (Deltedesco et al., 2019). The results of the paper which was currently published in Soil Biology and Biochemistry were elaborated together with WP 3 Deltedesco et al. (2020). Further, a manuscript on N trace gas emission is currently in preparation, with the working "Interactive effects of warming, elevated CO_2 and extreme weather events on the production and consumption of

N_2O and CH_4 in a managed grassland soil", by Eugenio Diaz-Pines et al.; for more information, please see below in Annex e/a Master thesis Alexandre Fahringer.

Results Experiment 1

N_2O emission showed generally low soil emission rates in the second and the third project year at several time points during the growing season (Figure 2). Elevated temperature plots (eT; +3 °C) showed lowest fluxes, while the highest mean emission rates could be observed during the second campaign after harvest on C2T2. Generally, no visible harvest effects could be detected over the growing season. C0T2 showed the lowest N_2O fluxes over the whole growing season, while C2T2 showed the highest (Diaz-Pines et al. in preparation).

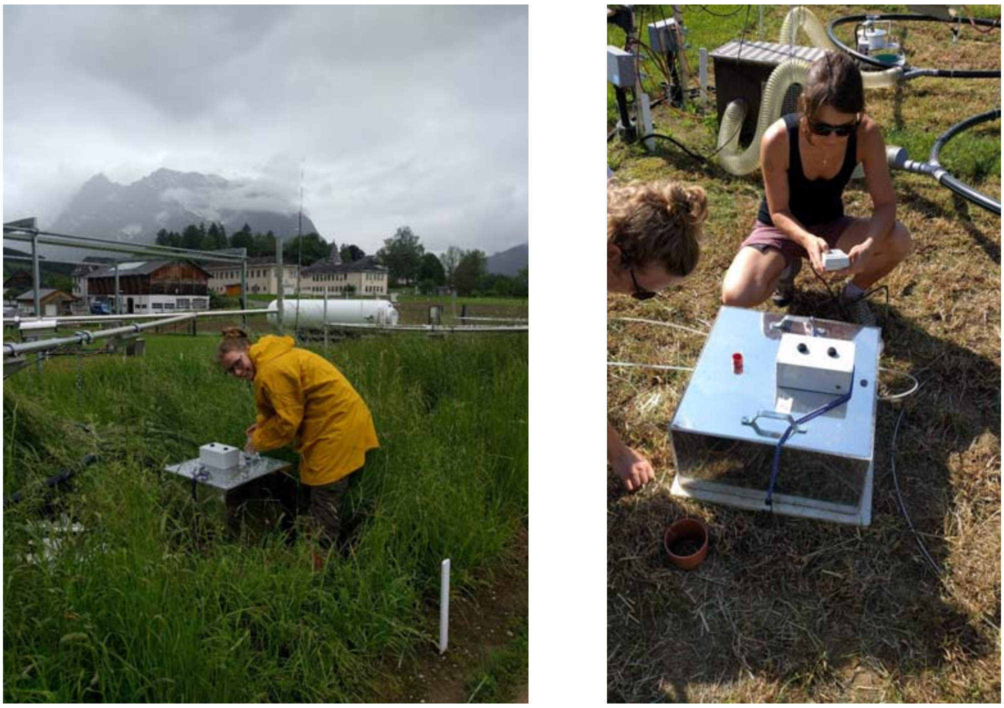
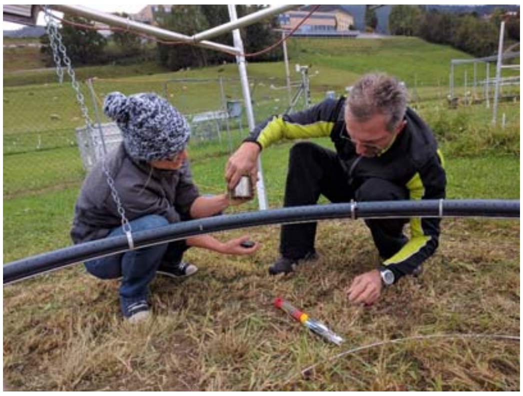


Figure 2 Gas sampling with high vegetation and large chambers using the syringe method (left side); trace gas measurements after harvest, with small chambers with Laser method (right side) Photo credit: Evi Deltedesco.

Results Experiment 1a

The results of the incubation study are well described in the already published manuscript, Deltedesco et al. (2019).



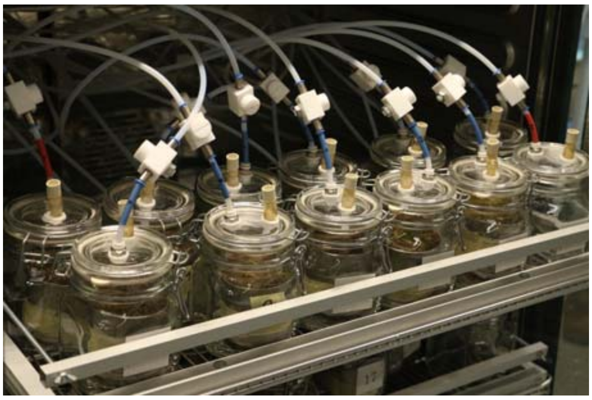
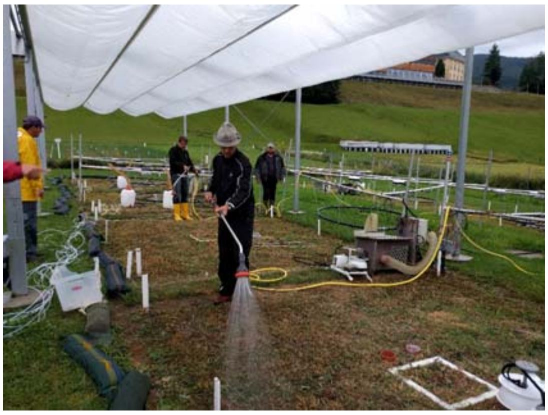


Figure 3 Sampling of intact soil cores (left side) and incubation in Kilnar jars (right side) for experiment 1a Photo credit: Evi Deltedsco.

Results Experiment 2 (drought-rewetting)

Results Experiment 2a1

Over the 11-day measuring period, fluxes ranged from the highest N_2O uptake on C2T2R at the start of the experiment (-5.3 µg N_2O-N m^{-2} h^{-1}) to the highest emission after fertilization on C0T0R (76.3 µg N_2O-N m^{-2} h^{-1}). Harvesting did not show discernible effects on N flux behavior, while rewetting led to modest fluxes (C0T0R and C2T2R). In contrast, the highest N_2O fluxes were observed one and two days after the fertilization in all treatments, also in non-rewetted plots (Master thesis Alexandre Fahringer).



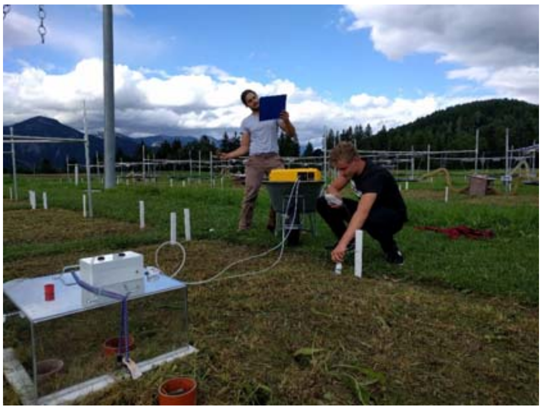


Figure 4 Rewetting of the plots under the rain-out shelter roofs, to simulate a heavy rain event after a severe drought period (left side) and trace gas measurements (right side) Photo credit: Evi Deltedesco.

Experiment E2a2 (Microdialysis)

Harvesting caused an immediate increase of NH_4^+ fluxes in drought-stressed plots, rewetting amplified the short-lived flush and further led to an immediate but short-lived increase of NO_3^- in drought-stressed plots determined by microdialysis. These effects could not be seen in water extracts. Interestingly, no response of rewetting to amino acid fluxes could be seen, since they reached their maximum 1 hour after the rewetting event in all treatments, also in those who were not rewetted. The major part of diffusive N fluxes was amino acids, while water extracts were dominated by the mineral N in the form of NH_4^+ and NO_3^- (Deltedesco et al In Prep).

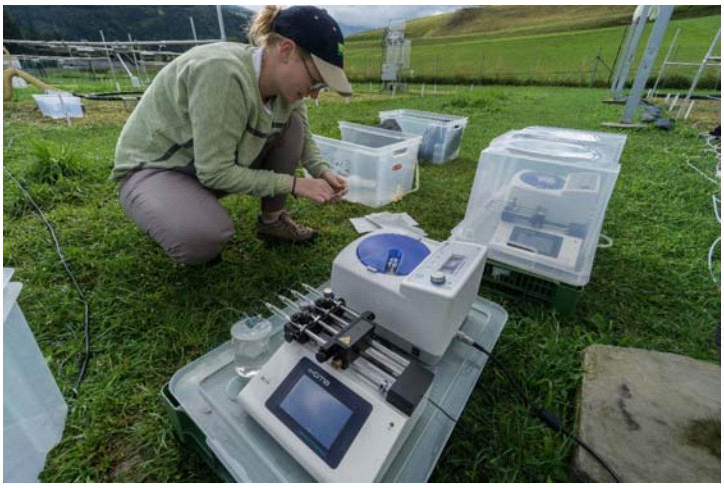


Figure 5 Usage of the microdialysis device in the field (Photo credit: Erich Pötsch)

Results Experiment 2a3 (incubation experiment)

Lowest fluxes were determined before rewetting at all WHC. Highest nitrous oxide fluxes, with high error-bars, were measured one day after rain treatment with 75 % and 100 % WHC. Even N_2O emissions at 50 % WHC were low one day after rewetting, and reached a maximum at 75% WHC with values similar to 100% saturation one day after treatment. One week after rewetting N2O emissions stabilized with a peak for 100 % saturated Gumpenstein samples (Ecker et al., in Prep).

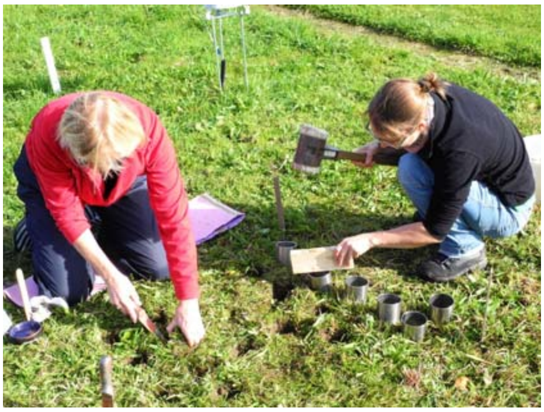


Figure 6 left side -sampling of intact soil cores for the incubation experiment 2a3 (Photo credit: Erich Pötsch), right side – intact soil cores for incubation (Photocredit: Evi Deltedesco)

Workpackage 3: Sequencing, Data Collection & Bioinformatics

The WP3 achieved both Milestones, data on soil microbial communities were available at the right time. Marker genes were close to or below the limit of detection, therefore no reasonable conclusion can be drawn from this data.

Therefore, as a proxy for microbial function extracellular enzyme activity was measured.

Results

A description of results from the first sampling year (2016) is provided in the recently published manuscript (Deltedesco et al., 2020).

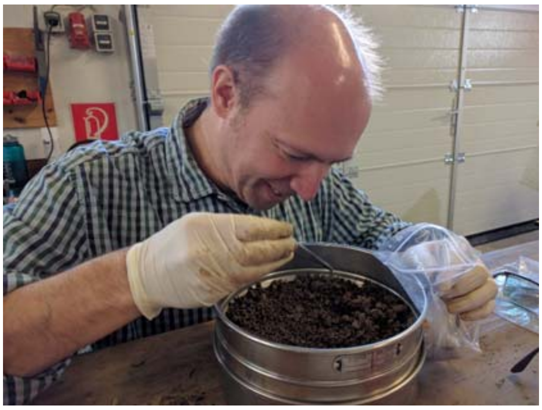



Figure 7 Soil sampling and homogenization.

Workpackage 4: Soil trace gas fluxes & Modelling

WP4 achieved all milestones, the in-situ gas measurements and gas analyses were completed after the second growing season. The modeling of soil trace gas fluxes was finished and the linkage between N pools and fluxes with microbial processes established. The major results were implemented in publications, already published, and currently in preparation.

Results Microclimate

Treatment effects on microclimate were detected in both, soil temperature [Ts] and soil water content [SWC], resulting in higher Ts and lower SWC in heated plots and higher SWC in CO_2 fumigated plots. Effects of the combined treatments were to some degree additive, though effects of warming on SWC were more pronounced than those of elevated CO_2. Effects of the drought treatment on soil moisture were observed across the soil profile, with highly consistent effects across the whole main rooting horizon.
Effects of the different treatments were detected across all investigated layers of the soil profile, but were not consistent throughout the observation period, synoptic changes in weather conditions dampening the effects of the climate manipulations.

Results for soil profile diffusivity for trace gas transport

Via flux gradient modelling of CO_2 fluxes, effective diffusivity (D_{eff}) could be calculated. D_{eff} was highly dynamic especially in the uppermost soil layer. Soil moisture had the biggest influence on the of dynamics D_{eff}, followed by soil temperature. Future climate, i.e. a combination of warming and elevated CO_2, increased effective diffusivity. Treatment effects on D_{eff} increased with soil depth. Under future climate (C2T2), especially in combination with drought (C2T2D corresponds to the C2T2R treatment), higher diffusivity could be observed, which was related to an increase of air-filled pore space resulting from lower soil moisture.

Results on modelling soil trace gases

Highest mean N_2O concentrations could be observed in C0T0, while lowest were measured in treatments under rainout sheltering. At 0 cm mean concentration were similar in all treatments, while concentration increases with increasing depth in all treatments. At 36 cm depth mean concentrations ranged from 1396 ppb (C2T2R) to 2191 ppb (C0T0). No general trend could be observed in changes over time, since they varied between treatments. Effects of rewetting and fertilisation did generally increase with depth (Diaz-Pines et al In Prep).

Figure 8 Belowground control-unit of the depth profile trace gas measurements

Workpackage 5: Experimental setup & Coordination

Markus Herndl, Erich Pötsch, Andreas Schaumberger

Milestone 1 – 4 were fully achieved. The main objective was to ensure to ensure sufficient support/coordination of the experiments at the experimental site. General field work (harvest, fertilization etc.) and the setting up the adaptions for the extreme weather event treatment were organized by WP5. Further, a big data warehouse was created to collect all data within the ExtremeGrass consortium as well as data from other projects dealing on the same experimental field site. Management recommendations for farmers were frequently realized during excursions and field days at the ClimGrass-site.

During the trial period (2016/04-2019/10) the heating of the field plots, realized with infrared heaters as described by Kimball (2011), started/stopped when the snow depth fell below or exceeded 10 cm. The CO_2 fumigation, based on an adapted miniFACE system according to Miglietta et al. (2001), started/stopped at the beginning and at the end of the vegetation period. The three intended cuts were carried out at intervals of approximately 8 weeks, which, depending on the year, means end of May for the first, end of July for the second and beginning of October for the third cut.

The performance quality of the ClimGrass-experiment is expressed by the target achievement ratio (TAR). This index gives evidence how well the application of temperature and CO_2 approximates the respective target value. The target value represents the averaged base value added with the respective factor level that is 1.5 and 3.0°C for temperature and 150 and 300 ppm CO_2 for fumigation. More than 90% of all heating treatments are within the stringent target achievement range, thus providing evidence of high performance.

4 Schlussfolgerungen und Empfehlungen

H1a) A rise in temperature is likely to increase **N gas emissions** because of multiplying effects of a series of underlying processes, which are all temperature sensitive. **Elevated CO_2 could counter this effect,** since elevated CO_2 positively influences denitrification and nitrification.

Supported

H1b) Drought slows down the N-cycle and **rewetting leads to pulses of N-release**, which result in enhanced N-trace gas emission rates; during the NO/N_2O ratio and the N_2O/N_2 ratio will increase.

Supported

H2a) Elevated CO_2 and warming lead primarily to physiological adaptions of microbial communities and **cause slow shifts in microbial community structure**.

Partially supported

H2b) whereas **drought results in rapid and pronounced shifts** in microbial community structure and function, in changes of the soil food-web and reduction of trace gas emissions.

Rejected

H3a) Ammonia oxidizing bacterial (AOB) populations negatively affect the available **inorganic N pool** that is supposed to increase under global warming conditions, thus counteracting increased growth of other copiotrophic populations.

Still to be judged

H3b) Changes in NO_x, N_2O, N_2 and N_2O emissions are reflected in changes of the respective functional guilds within the microbial community, especially the activity of **genes from nitrifiers and denitrifiers** which will be determined through qPCR of N target genes (nirK, NirS, amoA and nosZ).

Still to be judged

Q1: What is the impact of combined environmental changes on GHG emissions?

Our results highlight the importance of considering different steps of enrichment and warming as well as combined manipulation treatments (eCO_2 and eT) to evaluate synergetic, antagonistic or additive effects. Maximum CO_2 emissions occurred at the moderate eCO_2 level while extreme treatments showed similar CO_2 emissions as the control. On the contrary, the linear model in the response surface approach suggests the highest NO_x emissions occur with eT, whereas warmed and dry soils tend to lead to the lowest N_2O emissions. The latter indicates that N_2O and NO_x emissions were mainly influenced by the indirect effects of warming on soil moisture, which had immediate effects and effective diffusivity. No significant legacy effects affecting temperature sensitivity of microbial trace gas emissions were found after two years of future climate change scenarios. Clearer long-term trends are to be expected with sustained climate change manipulation treatments. Based on currently available data it is assumed that secondary effects through alterations in plant performance (e.g., stomatal closure and evapotranspiration) and abiotic soil characteristics (e.g., water content) are primary drivers for soil microbial processes, which in turn determine trace gas emissions. It is thus of the utmost importance to continue disentangling these complex interactions to improve predictions of climate change scenarios on biogeochemical soil processes.

Q2: How does warming and elevated CO_2 concentration affect microbial community structure and function in a managed grassland, and how does community structure respond to drought?

We conclude that at the ClimGrass site the detection of spatial effects through microbial indicators is of high importance for the interpretation of already existing and newly generated data. In several experiments carried out as part of ongoing other projects, site imbalance has therefore already been included into the statistical models. A reevaluation of plant parameters collected since the experimental site has been installed indicated that spatial effects are not restricted to microbial parameters. Current data do not show strong modulation of soil microbial community composition, function or processes by warming and/or fumigation. Indirect effects of future climate change scenarios prevail over direct effects on soil microbial community composition and function. Soil water content, nutrient pools, atmospheric CO_2 and plant root identity were identified as drivers of the observed changes after removal of unintended spatial effects. More pronounced effects of elevated atmospheric CO_2 concentration and surface warming on soil microbial community structure and function are expected on the longer-term, but indirect effects will most likely remain the dominant drivers.

Q3: How does warming and elevated CO_2 concentration affect the combined response of microbial community structure and function with N gas emissions in a managed grassland?

We showed that rewetting caused an immediate but short-term increase of diffusive NH_4^+ and NO_3^- in a Pre-alpine managed grassland, which is was likely caused by the release of mineralizable compounds following stress-induced microbial cell lysis. Furthermore, we suggest that the flush of amino acids after rewetting event in all treatments was caused by a delayed effect of harvest. Likely through a direct release of amino acids as root exudates and/or amino acid accumulation in soil through a short-term plant nutrient absorption. Besides, we showed that N availability was dominated by amino acid and not by mineral N when using microdialysis. Our study suggests, that extreme events, such as prolonged drought followed by heavy rainfall had caused faster and more distinct "visible" effects than an increase in atmospheric CO_2 and warming. It is likely that prolonged climate change (elevated atmospheric CO_2 and warming) will cause more pronounced effects on plant and microbial N dynamics, but extreme events will most likely remain the stronger drivers.

We have to understand interactive and additive effects of climate change factors as well as damage thresholds in order to adequately respond to climate change. In our study we see several important aspects of interacting effects, e.g. opposite effects of eCO_2 and warming on N_2O, maximum respiration at moderate intensities, indirect climate change effects on microbial communities such as stimulation of coprophilous fungi. We conclude that size/intensity and interactions in climate change determine ecological outcomes, and we seriously have to take them into account in future projections. Further, field experiments are of crucial importance to identify multifactorial drivers of climate change. However, conducting field experiments is highly labor and cost intensive, and the large heterogeneity and factors that vary in the field are not simple to consider for data interpretation. Sophisticated statistical approaches, such as the surface response regression approach coupled with the anisotropic power model is crucial to identify significant difference due to possible spatial variability batch effects occurring in the lab and field.

Essentially all experiments in the ExtremeGrass projected pointed to the overarching importance of water. Soil water balance is influenced by temperature, atmospheric CO_2 and through changes in precipitation. In turn, soil water influences plant physiology and vegetation, microbial community composition and function and consequently soil microbial processes and GHG emissions. Future research must, therefore, put a strong focus on soil water balance.

The current data set provides a good and comprehensive baseline for future studies. It became obvious that interactions are highly complex, and indirect effects tend to be stronger than direct effects. This makes it necessary to collect as many additional data as possible. Similar to microbial communities, it is expected that prolonged treatment will not only influence plant physiology but also the vegetation. This will add an additional factor, which must be taken into consideration

C) Projektdetails

6 Methodik

Workpackage 1: Project management

<u>Sophie Zechmeister-Boltenstern</u>

WP 1 organized the Kick-off meeting as well as regular project meetings to ensure efficient project management, coordination of the workgroups. In this WP the supervision of compliance with the project plan and the financial mentoring was conducted. Further, WP 1 integrated and synthesized results, wrote interim and the final report.

Workpackage 2: Sampling, analytical pipeline and data collection
Katharina Keiblinger, Evi Deltedesco

Experiment 1 (factorial climate change manipulation – surface response model approach)

The first experiment conducted during the growing season 2016 tested combined effects of warming and elevated CO_2 on N_2O and CH_4 emissions (see interim report 01). This experiment was repeated also during the growing season 2017 and 2018 with minor modifications. Instead of the use of syringe, static chambers were connected with a Los Gatos Research Ultraportable Greenhouse Gas Analyzer (Laser) to examine CH_4 and N_2O emissions. To test combined effects of warming and elevated CO_2 on N_2O and CH_4 emissions intensive sampling campaigns were conducted during harvests. GHG were measured before and after harvests and immediately after the fertilization. In-situ GHG measurements (Experiment 1 and 2) has been carried out in collaboration with WP4.

Soil samples were taken on 27 plots (see Table 1) in October 2016 after harvest. For more details about soil sampling procedure see Deltedesco et al. (2020). The soil samples were analyzed for N pools and some basic soil characteristics to link them with N-gas emissions as well as microbial community structure and function, and to identify the effects of factorial climate manipulation on those.

Table 1 A 3 x 3 response surface design with treatment-codes (C = atmospheric CO_2, T = Temperature) for a total of 27 plots, the number of replicates are given in parenthesis (Deltedesco et al., 2019).

	eCO_2 (ppm)		
eT (C°)	0	150	300
0	C0T0 (7)	C1T0 (3)	C2T0 (3)
1.5	C0T1 (3)	C1T1 (2)	
3	C0T2 (3)		C2T2 (6)

Experiment 1a (incubation study, for more details about methods, see Deltedesco et al. (2019))

Simultaneously with soil sampling for "Experiment 1" in October 2016 two intact soil cores from each of the 27 plots were taken to determined fluxes of nitrogen oxide (NO) and nitrogen dioxide NO_2 (NO + NO_2 = nitric oxide NO_x), ammonia

(NH_3) as well as nitrous oxide (N_2O) and CO_2. For more details about methods and additional parameters that were analysed and linked with the dataset (Annex a) Deltedesco et al. (2019) of this report)

Experiment 2 - climate manipulation with extreme drought event

The experiment 2 consisted of 12 experimental plots (see Table 2), half of which were roofed for two months to create experimental summer drought conditions (soil moisture < 4 %). Only plots under the roof were subject to rewetting after harvest by adding 40 mm collected rainwater. Plots without roof served as control for the drought treatment (for more details see WP 5).

Further soil samples were taken before and after rewetting as well as in October (recovery) from 12 plots from the upper 2 cm using a 1.5 cm diameter corer, 24 hours prior to wetting and 24 h post-wetting and again 60 days post-wetting. Soil samples have been subject to same analyses for N-pools and fluxes as described above in Experiment 1.

Table 2 Treatment code of the drought-rewetting experiment

Treatment-Code	Treatment
C0T0	ambient
C0T0R	ambient, drought
C2T2	+ 300 ppm, + 3°C
C2T2R	+ 300 ppm, + 3°C, drought

Experiment 2a1 – drought rewetting effects on trace gases along a soil profile

An intense sampling campaign to measure N_2O and CH_4 emissions with a Los Gatos Research UltraporTab. Greenhouse Gas Analyzer was conducted during the drought-rewetting experiment. N_2O were measured once a day for a week after the re-wetting experiment, since plots were fertilized.

Additionally, we combined the traditional static chamber GHG measurements with online monitoring of N_2O and CH_4 concentrations in different soil depth (0, 3, 9, 18 and 36 cm) on 6 plots (2 of them with rain-out shelters) with water-tight, gas-permeable tubes connected to a laser-based gas monitoring corporation with WP5.

Experiment 2a2 - drought rewetting effects on short-term N-fluxes

Further, to assess nitrogen fluxes throughout the drought-rewetting experiment, microdialysis systems were deployed on 4 plots (within the 6 plots were N-trace gases are monitored with soil depth – on C0T0, C0T0R, C2T2 and C2T2R) to determine short-term *in situ* N diffusion during a drought-rewetting experiment. Membranes of microdialysis were installed to a soil depth of 1.5 cm and dialysates were collected at 1 h intervals. Initial samples were taken 48 h before rewetting (24 h before harvest) and immediately after harvest (24 h before rewetting) of extreme-treatments, for both soil sampling and dialysis. Further, samples were taken immediately after rewetting the drought-stressed plots 1, 2, 3, 4, 5, 6 and 20 h from all treatments with dialysis, while only after 20 h for soil sampling. Samples were analysed for inorganic N (NH_4 and NO_3) as well as organic N (amino acid) pools and fluxes (Deltedesco et al. In Prep)

Experiment 2a3 – Lab incubation of intact soil cores to study the effects of moisture and drought-rewetting on N trace gas fluxes

Intact soil cores were taken in Autumn right beside the "Climgrass" experimental field. The grassland was established at the same time and treated equally as the "Climgrass" experimental field. Four spatial clusters were used as spatial replicates and comprised an area of one square meter (24 soil cores from each replicate). To estimate soil-headspace trace gas emissions a dynamic chamber approach was used. To determine soil CO_2, NO, NO_x, N_2O and CH_4 flux intact soil cores were incubated in gas tight Kilnar jars and measured simultaneously in a fully automated system with open flow technique as already described in Deltedesco et al. (2019). The incubation was five days at 22°C, followed by four different re-wetting treatments (25 %, 50 %, 75 % and 100 % water holding capacity [WHC]) which lasted for eight days also at 22°C to assess the response of soil-atmosphere GHG fluxes. As precipitation liquid A standard rain mixture was used as precipitation liquid ($CaCl_2$ = 73,5 mg, KCl = 122 mg, Na_2SO_4 93 mg for five litres) (Ecker et al. In Prep).

Workpackage 3: Sequencing, Data Collection & Bioinformatics

<u>Markus Gorfer, Evi Deltedesco</u>

For Experiment 1 soil samples were taken in collaboration with the WP 2 to conduct analysis on the same sample aliquot. Soil DNA-based analyses were conducted from a well homogenized aliquot. While root biomass was separated from each soil core during sampling, washed, lyophilised and ground to a fine powder for nucleic acid. For soil microbial community analyses bacterial (SSU; Illumina Nextera protocol) and fungal (ITS2; Tedersoo et al., 2015) barcodes were amplified and indexed according to manufacturer's instructions and followed by Illumina MiSeq sequencing. Additionally, the plant ITS2-region was amplified from root DNA for Illumina MiSeq sequencing (Chen et al., 2010).

For the experiment 1 and 2 potential hydrolytic enzyme activity of cellobiohydrolase (CBH), acid phosphatase (PHO), β-1,4-glucosidase (BGL), ß-*N*-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) as well as oxidative enzyme activity of phenoloxidase activity (PHE) and peroxidase activity (PER) were measured on a spectrophotometer according to Ameur et al. (2018).

For more details on used methods and the bioinformatic see interim report 02 and Annex b) Deltedesco et al. (2020).

Workpackage 4: Soil trace gas fluxes & Modelling

Michael Bahn, Eugenio Diaz-Pines, David Reinthaler

An automated membrane-based system for monitoring trace gas concentrations across the soil profile was developed, constructed and tested, and was installed at five soil depths (0, 3, 9, 18 and 36 cm) on six plots representing the major climate treatments. The setup was initially designed for monitoring soil CO_2 concentrations and was extended in May 2017 for the analysis of further trace gases. Soil microclimate (temperature and moisture) is continuously logged by logger stations. The measurement depths of the sensors installed is analogous to the depth of the membrane tubes. N_2O and concentrations were measured with a Los Gatos Research UltraporTab in different soil depth (0, 3, 9, 18 and 36 cm) on 6 plots (2 of them with rain-out shelters) as pointed out above (Experiment 2a1 – WP2). Microclimate data served for modelling the effects of temperature and moisture on trace gas concentrations among the soil profile and to identify potential sink and sources of these microbial processes, among the profile to relate it with atmospheric emissions.

Workpackage 5: Experimental setup & Coordination

Markus Herndl, Erich Pötsch, Andreas Schaumberger

The grassland vegetation (nutrient-rich meadow) was established in the year 2007. The soil of the experimental site was ploughed up to a depth of 0.25 m, totally levelled with a curry-comb and sown with a seed mixture for permanent grassland using a seed density of 27 kg ha^{-1}. In the year 2010 the east part of the experimental site (east, plots no. 1–24) and in 2012 the west part (west, plots no. 25–54) was equipped with vertically adjustable metal constructions to carry the warming and fumigation devices on the plot scale, 16 m^2 each (Pötsch et al., 2019) (for more details see Deltedesco et al. (2020) and Pötsch et al. (2019). The experiment was designed by a response surface regression approach (Piepho et

al., 2017) and treatments were completely randomized. The all-out operation (warming and fumigation) of the ClimGrass site started in May 2014 after the first harvest. For more details about the treatment realization and experimental set-up see Deltedesco et al. (2019) and Deltedesco et al. (2020). The grassland is harvested three times during the growing season from April to the end of October, and mineral fertilizer is applied in three batches (total load of 90 kg N ha^{-1} y^{-1}, 65 kg P ha^{-1} y^{-1}, 170 kg K ha^{-1} y^{-1}).
At the study site, a drought-rewetting experiment has been set up in 2017, including four treatments: a) ambient conditions (C0T0), handled as a ambient control and apply to all of the following treatments, b) ambient conditions with simulated drought-rewetting event (C0T0R, extreme-control), c) combination of increase in temperature by 3 °C and increase in CO_2 by 300 ppm (C2T2), and d) combination of increase in temperature by 3 °C and increase in CO_2 by 300 ppm with simulated drought-rewetting event (C2T2R, extreme future climate change). Further, C0T0 and C2T2 are handled as control treatments to C0T0R and C2T2R (extreme treatments), respectively. The treatments with increased temperature and CO_2-fumigation started in May 2014 after the first harvest. For the drought experiment, C0T0R and C2T2R were covered with rainout-shelters positioned at 3.3 m above the ground surface to exclude rainfall for the period of the second grassland growth from 23rd of May to 27th of July 2017. During this period the plots outside the rainout-shelters received 251 mm of rainfall whereas the plots under the shelters only got 40 mm. Immediately after the harvest, the rainout-shelters were opened again, and the extreme-treatments were rewetted with 40 mm of rainwater that was collected at the site during spring 2017. To avoid a surface run-off, the rewetting procedure was done gently by splitting the total amount into four doses of 10 mm each (Deltedesco et al in Prep).

7 Arbeits- und Zeitplan

Gas measurements were successfully conducted in all three project years during the vegetation season (2016-2018). The initial plan, bi-weekly measurement of GHG in the field was reduced to three intensive measurement campaigns in the vegetation season, for the years 2017 and 2018, as the travel costs already exceeded the planned expenditure. GHGs measurement were conducted during the harvest (before and after harvest, as well as after fertilization). Further, for the drought-re-wetting event we`ve planned an additional recovery soil sampling in October 2017, to determine possible legacy effects of the extreme event. We did not experience any substantial delay from sample reception in the lab until analysis of GHGs, nutrients, microbial biomass and microbial community analyses.

8 Publikationen und Disseminierungsaktivitäten

Project meeting

5th – 6th April 2016	Kick-Off meeting at Gumpenstein
23rd – 24th March 2017	at AREC
20th – 21st February 2018	at BOKU
11th – 12th November	at BOKU

Work shop

Statistical Analysis: Insights into the Response Surface Regression Model with Professor Hans-Peter Piepho from the University of Hohenheim, Institute of Crop Science, Biostatistics Unit, 70599, Stuttgart, Germany at the University of Natural and Life Sciences, Vienna (BOKU), 19th – 20th October 2016.

Peer-reviewed articles

Deltedesco E, Keiblinger KM, Piepho HP, Antonielli L, Pötsch EM, Zechmeister-Boltenstern S, Gorfer M. (2020) Soil microbial community structure and function mainly respond to indirect effects in a multifactorial climate manipulation experiment Soil Biology and Biochemistry (142) https://doi.org/10.1016/j.soilbio.2020.107704

Deltedesco E, Keiblinger K.M, Naynar M, Piepho H-P, Gorfer M, Herndl M, Bahn M, Pötsch E.M, Zechmeister-Boltenstern S. (2019) Trace gas fluxes from managed grassland soil subject to multifactorial climate change manipulation Applied Soil Ecology (137) 1-11 https://doi.org/10.1016/j.apsoil.2018.12.023

In preparation

Deltedesco E, Inselsbacher E, Pötsch EM, Gorfer M, Zechmeister-Boltenstern S, Keiblinger Short-term soil mineral and organic N dynamics during a drying-rewetting event Soil Biology and Biochemistry (to be submitted in February 2020)

Ecker et al. Re-wetting effects on the production of trace gases from forest and grassland sites

Gorfer M, Deltedesco E, Keiblinger K, Pötsch EM, Zechmeister-Boltenstern S Change in soil microbial community structure and function during a drying-rewetting event

Díaz-Pinés E et al. Interactive effects of warming, elevated CO_2 and extreme weather events on the production and consumption of N_2O and CH_4 in a managed grassland soil

Non peer-reviewed publications

Deltedesco E, Keiblinger K.M, Naynar M, Piepho H-P, Gorfer M, Pötsch E.M, Zechmeister-Boltenstern S Einfluss des Klimawandels auf Stickstoffflüsse im Grünlandökosystem. 21. Alpenländisches Expertenforum 26-27 März 2019. Höhere Bundeslehr- und Forschungsanstalt ISBN 13: 978-3-902849-69-4

Deltedesco E, Keiblinger K.M, Piepho H-P, Antonielli L, Zechmeister-Boltenstern S, Pötsch E.M, Gorfer M Wie reagiert die Bodenmikrobiologie im Grünland auf den Klimawandel? 21. Alpenländisches Expertenforum 26-27 März 2019. Höhere Bundeslehr- und Forschungsanstalt ISBN 13: 978-3-902849-69-6

Zechmeister-Boltenstern S, Keiblinger K.M, Deltedesco E, Pötsch EM, Herndl M, Schaumberger A, Gorfer M EXTREMEGRASS Interaktive Auswirkungen von Erwärmung, erhöhten CO_2- und Wetterextremen auf Stickstoffgasflüsse im Grünland, In: Austrian Climate Research Programme in Essence, Berichte zur Klimafolgenforschung 2019

Zechmeister-Boltenstern S, Keiblinger KM, Deltedesco E, Herndl M, Schaumberger A, Pötsch EM, Gorfer M, EXTREMEGRASS Interaktive Auswirkungen von Erwärmung, erhöhten CO_2- und Wetterextremen auf Stickstoffgasflüsse im Grünland, Austrian Climate Research Programme in Essence, Bericht zur Klimafolgenforschung

Presentations

Deltedesco E, Keiblinger K.M, Naynar M, Piepho H-P, Gorfer M, Pötsch E.M, Zechmeister-Boltenstern S Einfluss des Klimawandels auf Stickstoffflüsse im Grünlandökosystem. 21. Alpenländisches Expertenforum 26th-27th März 2019. Höhere Bundeslehr- und Forschungsanstalt ISBN 13: 978-3-902849-69-4

Deltedesco E, Keiblinger K, Piepho H-P, Antonielli L, Zechmeister-Boltenstern S, Pötsch E.M, Gorfer M Wie reagiert die Bodenmikrobiologie im Grünland auf den Klimawandel? 21. Alpenländisches Expertenforum 26th-27th März 2019. Höhere Bundeslehr- und Forschungsanstalt ISBN 13: 978-3-902849-69-6

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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.

Die Fördernehmerin/der Fördernehmer erklärt mit Übermittlung der Projektbeschreibung ausdrücklich über die Rechte am bereitgestellten Bildmaterial frei zu verfügen und dem Klima- und Energiefonds das unentgeltliche, nicht exklusive, zeitlich und örtlich unbeschränkte sowie unwiderrufliche Recht einräumen zu können, das Bildmaterial auf jede bekannte und zukünftig bekanntwerdende Verwertungsart zu nutzen. Für den Fall einer Inanspruchnahme des Klima- und Energiefonds durch Dritte, die die Rechtinhaberschaft am Bildmaterial behaupten, verpflichtet sich die Fördernehmerin/der Fördernehmer den Klima- und Energiefonds vollumfänglich schad- und klaglos zu halten.