MASTERARBEIT

Titel der Masterarbeit
„Drought-induced shifts in the greenhouse gas balance of an abandoned meadow in the Austrian Alps“

Verfasserin
Sandra Kienzl, BSc

angestrebter akademischer Grad
Master of Science (MSc)

Wien, 2013

Studienkennzahl lt. Studienblatt: A 066 833
Studienrichtung lt. Studienblatt: Masterstudium Ökologie
Betreuer: Univ.-Prof. Dr. Andreas Richter
## Contents

**General introduction** ................................................................................................................. 3
  - Climate change and land use change ................................................................................. 3
  - N$_2$O ......................................................................................................................................... 5
  - N-cycling ............................................................................................................................... 5
  - Controls over N$_2$O fluxes ................................................................................................. 10

**Study Aim** ............................................................................................................................. 12

**References** ............................................................................................................................. 13

**Manuscript** ............................................................................................................................... 18
  - Abstract ................................................................................................................................. 18
  - Introduction .......................................................................................................................... 19
  - Materials and Methods ........................................................................................................... 22
  - Results ................................................................................................................................... 30
    - Microclimate ..................................................................................................................... 30
    - Ecosystem respiration and green house gas net fluxes ......................................................... 31
    - Carbon/nitrogen pools ....................................................................................................... 34
    - Microbial community composition ..................................................................................... 36
    - Tables and contents ............................................................................................................ 37
  - Discussion .............................................................................................................................. 47
  - Acknowledgements ................................................................................................................ 53
  - References ............................................................................................................................. 54

**Zusammenfassung** ................................................................................................................... 60

**Curriculum vitae** ...................................................................................................................... 62
**General introduction**

**Climate change and land use change**

In Europe, many alpine meadows which were formerly used for livestock farming, i.e. by grazing and mowing, have been abandoned (Meeus 1993) and trees are re-growing. For example in the European Alps, between 20 and 70% of agricultural used meadows, were abandoned during 1980 and 2000 (Tappeiner, Tasser et al. 2008). Together with such changes in land use, climatic changes occurred (IPCC 2007) and these two phenomena are closely coupled, because climate change is one of the driving forces for land use change (Houghton, Hackler et al. 1999).

Climate models suggest that the frequency of extreme weather events, like drought or heavy rainfalls will increase and especially for mountain ecosystems a strong response is forecasted (IPCC 2012). Several hydrological model predict warmer and more humid winters and a strong increase in summer temperatures, combined with less precipitation (-10 to 40%). Winter temperatures are predicted to rise between 2 and 5.5°C and during the cold season precipitation will increase between 10 and 20%. Despite of the accession in precipitation, snow cover will decrease, because the effect of the rising temperatures is thought to overbalances the effect of precipitation increase (Laghari, Vanham et al. 2012). Little is known how abandoned meadows are affected by a changing climate.

Climate changes are closely linked to the amount of greenhouse gases (GHG) carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O), which are emitted to the atmosphere (IPCC 2007). Today’s concentrations of these GHG’s in the atmosphere are around 399.7 ppm for CO$_2$ (Dr. Pieter Tans, NOAA/ESRL, www.esrl.noaa.gov/gmd/ccgg/trends/; Status: 10.6.2013), around 1753.8 ppb for CH$_4$ (CSIRO Marine and Atmospheric Research and the Australian Bureau of Meteorology (Cape Grim Baseline Air Pollution Station), http://www.csiro.au/greenhouse-gases/GreenhouseGas/data/CapeGrim_CH4_data_download.txt; Status: 11.6.2013) and around 323 ppb for N$_2$O (Prinn, Weiss et al. 2000). Although N$_2$O contributes only in a small amount to the total GHG budget, it is the GHG with the highest potential for global warming (IPPC 2007).
The emission or absorption of a GHG is strongly controlled by the combination of environmental and climatic factors and changes in ecosystem functioning, due to alterations in climate, may lead to a positive or negative feedback in the GHG budget.

GHG fluxes from soils are closely linked to microbial community composition and activity, because microbially mediated processes, such as respiration (CO$_2$ production), methane oxidation (CH$_4$ consumption) or nitrification and denitrification (N$_2$O production and/or consumption) control the net GHG fluxes. Especially for N$_2$O, flux rates show a high spatial and temporal variety, leading to hot spots and hot moments of N$_2$O production (Groffman 2012).

For grazed and/or fertilized alpine meadows several studies deal with the impact of climate change on carbon and nitrogen cycling (including process rates) (Jiang, Yu et al. 2010; Seeber, Rief et al. 2012; Wang, Duan et al. 2012) and microbial community composition (Hirose, Shirouzu et al. 2009; Wang, Long et al. 2010; Yang, Wu et al. 2013). But little is known about abandoned meadows and their reactions due to climate change. Since abandoned meadows exhibit strong changes in N inputs, we were especially interested in N$_2$O flux patter.
**N\textsubscript{2}O**

N\textsubscript{2}O has 298 times the strength of CO\textsubscript{2} (IPCC 2007) and is the strongest ozone destroying component (Ravishankara, Daniel et al. 2009). In the atmosphere, it can be furthermore converted into nitrite (NO\textsubscript{2}\textsuperscript{-}) and washed back on the earth surface as acidic precipitation (NRC 1991). Around 62 % of total emitted N\textsubscript{2}O derives from natural and agricultural soils (Skiba and Smith 2000) and around 30 % arise from rivers and oceans (Bange 2010). Anthropogenic sources are mainly N fertilization, biomass burning, the use of fossil fuels (Vitousek, Aber et al. 1997).

**N-cycling**

The whole nitrogen (N) cycling, starting with nitrogen fixation and followed by the oxidation of ammonium (NH\textsubscript{4}\textsuperscript{+}) over several intermediates and conversions back to dihydrogen (N\textsubscript{2}), is driven by a variety of complex biochemical processes, which use N\textsubscript{2}O as intermediate and can alter the emission of N\textsubscript{2}O from soil into the atmosphere. Organisms of all three domains of life are involved in these processes, bacteria, archaea and eukaryotes (only fungi). Traditionally, bacterial nitrification (including ammonium oxidation) and anaerobic denitrification were thought to be the major sources and sinks for N\textsubscript{2}O (Conrad 2002), but newer studies suggest that under changing environmental conditions also other pathways like nitrifier denitrification may become more important (Kool, Wrage et al. 2010; Kool, Dolfing et al. 2011). Today a multitude of processes are known, which are involved in N cycling and directly or indirectly in N\textsubscript{2}O fluxes. Biotic processes include autotrophic and heterotrophic nitrification (bacterial as well as archaeal nitrification), aerobic and anaerobic denitrification, nitrifier denitrification, fungal denitrification, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA) and codenitrification.
Nitrification. Basically, nitrification can be divided into autotrophic and heterotrophic nitrification. Autotrophic nitrification is performed by chemolithoautotrophic ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). In a first step NH$_4^+$ is reduced to hydroxyl amine (NH$_2$OH) by the enzyme ammonium monooxygenase (amo) (Wood 1986) and then further reduced to NO$_2^-$ by the enzyme hydroxylamine oxidoreductase (hao) (Yamanaka and Sakano 1980). N$_2$O can be produced during the oxidation of NH$_4^+$ to NO$_2^-$, because when NH$_2$OH is reduced to NO$_2^-$ the instable intermediate HNO occurred, which can be spontaneously converted into N$_2$O (Hooper and Terry 1979).

Bacterial nitrification as well as archaeal nitrification has been found in a wide range of ecosystems, like in the ocean (Venter, Remington et al. 2004; Newell, Babbin et al. 2011), in sediments (Volant, Desoeuvre et al. 2012; Wang, Sheng et al. 2012) and in soils (De Boer and Kowalchuk 2001; Leininger, Urich et al. 2006; He, Hu et al. 2012). In the ocean archaeal nitrification dominates the production of N$_2$O (Santoro, Buchwald et al. 2011).
Heterotrophic nitrification is performed by a wide range of microorganisms, bacteria as well as fungi, with the ability to oxidize ammonia (NH$_3$), as well as reduce N containing compounds directly to NO$_2^-$ or nitrate (NO$_3^-$) (Focht 1977). Due to current knowledge the heterotrophic nitrification is not directly included in energy yielding metabolic processes and therefore is not involved in microbial cell growth (Hayatsu, Tago et al. 2008).

**Denitrification.** Denitrification is the process, in which NO$_3^-$ is stepwise reduced to N$_2$ via NO$_2^-$, nitric oxide (NO) and N$_2$O. Mostly facultative anaerobic bacteria are part of the “classic” denitrification and more than 60 genera of denitrifying microorganism are known (Philippot, Hallin et al. 2007). Additionally several archaeal denitrifiers have been found (Cabello, Roldan et al. 2004), but their contribution to total denitrification remains unclear.
Some fungal groups also show denitrifying activity, for example members of the ascomycota or basidiomycota (Shoun, Kim et al. 1992). It is important that in contrast to bacterial denitrification, the end product of fungal denitrification is always N₂O, because the enzyme, which converts N₂O to N₂, does not exist in fungi (Shoun, Fushinobu et al. 2012).

Enzymes, which are involved in denitrification are nitrate reductase (nar, transforming NO₃⁻ to NO₂⁻), dissimilatory nitrite reductase (nir, transforming NO₂⁻ to NO), nitrite oxide reductase (nor, transforming NO to N₂O) and nitrous oxide reductase (nos, transforming N₂O to N₂) (Hochstein and Tomlinson 1988). In bacterial denitrifier two different nir are found, the first is encoded by nirS genes and contains copper and the second is encoded by the nirK genes and contains heme c and heme d1 (Zumft 1997).

Archaeal denitrifying enzymes show differences in the structure and organization combined to bacterial denitrifier (Philippot 2002).

Overall the abundance of bacteria in soils, which are able to denitrify, is estimated to be less than 5% (Henry, Bru et al. 2006) and the amount of denitrifying fungi and archaea is unknown.

The term “coupled nitrification-denitrification” is used to describe the coupling between the two processes, when conditions are sufficient for both groups. It does not define a separate pathway.

**Codenitrification.** In contrast to the “classic” denitrification, in which two molecules of NO₂⁻ or NO₃⁻ are needed in the stepwise anaerobic reduction process to N₂O or N₂, in the codenitrification one of the compounds can be replaced by another N containing substrate, like NH₄⁺ or azide, and then they are directly transformed into N₂O or N₂ (Hayatsu, Tago et al. 2008). In grasslands fungal codenitrification seems to be an important source for N₂O emission (Laughlin and Stevens 2002).

**Nitrifier Denitrification.** During nitrifier denitrification NH₄⁺ is first reduced to NO₂⁻ and then further reduced to N₂ via NO and N₂O. The nitrifier denitrification seems to be less sensitive to higher oxygen (O₂) concentrations than the “classic” denitrification (Kool, Dolfing et al. 2011).

**Anammox.** Anammox or anaerobic ammonium oxidation is the process, in which NH₄⁺ and NO₂⁻ are directly transformed into N₂ under anaerobic conditions. Due to current knowledge anammox
bacteria belong to the phylum planctomycetes (Schmid, Maas et al. 2005). They strongly contribute to N cycling in marine environments (Dalsgaard, Canfield et al. 2003), for example in the Black Sea (Kuypers, Sliekers et al. 2003). In natural environments bacteria expressing anammox often coexists with AOB, because nitrification provides NO$_2^-$ (Ding, Zheng et al. 2013) and additionally, if urea is present, which cannot be converted into NH$_4^+$ by anammox bacteria themselves (Sliekers, Haaijer et al. 2004), also NH$_4^+$ can be provided by AOB.

Dissimilatory nitrate reduction to ammonium (DNRA). During DNAR NO$_3^-$ is reduced to NO$_2^-$ and NH$_4^+$; bacteria, as well as fungi are able to perform this pathway (Rutting, Boeckx et al. 2011). It occurs under anoxic conditions, but it is less sensitive to moderate O$_2$ concentrations than denitrification (Takaya 2002). A new study demonstrated that isolated soil strains of nitrate-ammonifying bacteria produce N$_2$O during DNRA and under certain conditions, such as high NO$_3^-$ concentrations, it may significantly contribute to the N$_2$O production (Streminska, Felgate et al. 2012) in soils.
Controls over $\text{N}_2\text{O}$ fluxes

$\text{N}_2\text{O}$ net fluxes are always a mixture of $\text{N}_2\text{O}$ consuming and producing processes and these processes are controlled by a number of different factors, including soil moisture, pH, nutrient availability (nitrogen as well as carbon) or soil temperature.

_Soil moisture and $\text{O}_2$ concentration._ Soil water content plays an important role, because it determines the amount of $\text{O}_2$ within the soil, i.e. low soil moisture leads to high $\text{O}_2$ concentrations and vice versa. Especially denitrifier activity is influenced by low soil moisture, because the enzyme nitric oxide reductase (NOR), which reduces NO to $\text{N}_2\text{O}$, is highly sensitive to $\text{O}_2$ (Boublikova, Kucera et al. 1985), and if soil water content (SWC) declines and less $\text{N}_2\text{O}$ is produced. In contrast to bacterial denitrification fungal denitrification shows less sensitivity to $\text{O}_2$ and also occurs at moderate $\text{O}_2$ levels (Zhou, Takaya et al. 2001). Due to its close relationship to the denitrifier also anammox microorganism are highly sensitive to increases in $\text{O}_2$ concentration (Egli, Fanger et al. 2001). Interestingly microorganism, which perform nitrifier denitrification, showed less sensitivity to moderate $\text{O}_2$ levels and therefore contribute stronger to $\text{N}_2\text{O}$ emissions from soils, if the $\text{O}_2$ concentration increases (Kool, Dolfing et al. 2011). Nitrifier, in contrast to denitrifier, need $\text{O}_2$ for the transformation of $\text{NH}_4^+$ to $\text{NH}_2\text{OH}$, and therefore are repressed at a high soil water content, due to a lack of $\text{O}_2$ (Rovita and Killorn 2009).

_Substrate availability._ For nitrifying bacteria (AOB, NOB) substrate availability is one of the strongest controlling factors, because they depend on $\text{NH}_4^+$ or $\text{NO}_2^-$ as energy source and the addition of fertilizers significantly increases their abundance in soil (Hayatsu, Tago et al. 2008). The availability of $\text{NH}_4^+$ for autotrophic nitrifier is closely linked to the amount of dissolved organic carbon (DOC); if enough DOC is present for growth of heterotrophis, autotrophic nitrifier are usually outcompeted, because of a slower metabolism (Taylor and Townsend 2010). During denitrification, if the availability of $\text{NO}_3^-$ is limited, atmospheric $\text{N}_2\text{O}$ can be used as electron acceptor (Rosenkranz, Bruggemann et al. 2006) and this alters the $\text{N}_2\text{O}$ absorption into soil. On the other hand, if $\text{NO}_3^-$ availability is high, for example because of fertilization, larger amounts of $\text{N}_2\text{O}$ are emitted into the atmosphere due to a stimulation of denitrification (Goossens,
De Visscher et al. 2001; Jager, Duffner et al. 2013). Also for denitrification a sufficient amount of DOC has to be present (Taylor and Townsend 2010). Anammox microorganism depends on the co-occurrence of $\text{NH}_4^+$ and $\text{NO}_2^-$ at the same time to perform their metabolic pathway (Egli, Fanger et al. 2001).

$p\text{H}$. Soil $p\text{H}$ influences the availability of $\text{NH}_4^+$, because it determines the amount at which $\text{NH}_4^+$ is formed out of $\text{NH}_3$. If $p\text{H}$ levels are low, more $\text{NH}_3$ is converted into $\text{NH}_4^+$ and vice versa. For pure cultures of nitrifier optimal $p\text{H}$ between 7-9 has been suggested (Allison and Prosser 1993) and for anammox a $p\text{H}$ of 7.5-8 has been determined to be optimal to favor growth and metabolic activity (Strous, VanGerven et al. 1997). Denitrifier in pure cultures showed the highest denitrification rates at nearly neutral $p\text{H}$ (Thomas, Lloyd et al. 1994) and at low $p\text{H}$ the activity of bacterial denitrifier is repressed (Herold, Baggs et al. 2012). Nevertheless, the involved enzymes in bacterial denitrification are not equally affected by a low $p\text{H}$. Although the nor enzyme of bacteria is sensitive to low $p\text{H}$ levels (Knowles 1982), nos, which transforms $\text{N}_2\text{O}$ to $\text{N}_2$, is even more repressed (Simek and Cooper 2002), possibly leading to a potential increase of $\text{N}_2\text{O}$ emission from soil. However, the specific microbial community composition determines the reaction due to changes in $p\text{H}$, because fungal denitrification showed no reactions to slight variations in $p\text{H}$ (Herold, Baggs et al. 2012).

Based on the different N transforming pathways, land use change from grazed and/or mowed meadows to abandoned meadows and simultaneous changes in soil moisture conditions, because of severe droughts, may strongly influence the contribution to the global $\text{N}_2\text{O}$ budget. If cattle or other grazing animals are no longer present on the meadows, there will be no input of fertilizing compounds, e.g. feces and urine, leading to a decrease in substrate availability for nitrifying and denitrifying microorganism and therefore maybe to a decrease of $\text{N}_2\text{O}$ production. Such abandoned meadows are also not fertilized. Likewise, drought affects the oxygen and soil moisture content in soils, thereby directly influencing $\text{N}_2\text{O}$ production and consumption.
**Study Aim**

The aim of the present study was to investigate how an extended summer drought affects net ecosystem CH$_4$ and N$_2$O fluxes and respiration of an abandoned meadow in the Austrian Alps. We focused on the greenhouse gas balance in order to assess a possible future behavior of such a system under a changing climate and to establish possible positive or negative feedbacks of drought and climate.

We hypothesized that prolonged summer droughts will decrease ecosystem respiration and N$_2$O production, but increase CH$_4$ uptake during growing season. To test these hypotheses rainout shelters were installed at the beginning of the season and net fluxes of CH$_4$ and N$_2$O and ecosystems respiration were measured in drought and control plots, at least once a month. In addition the associated soil processes, including N mineralization and nitrification, were determined at each sampling. After two month of drought a strong precipitation event (20mm) was simulated to test whether treatment differences still remain after drought.
References


Homepage

CSIRO Marine and Atmospheric Research and the Australian Bureau of Meteorology (Cape Grim Baseline Air Pollution Station),
Status: 11.6.2013

Dr. Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/) and Dr. Ralph Keeling, Scripps Institution of Oceanography (scrippsc2.ucsd.edu/). Status: 10.6.13
Manuscript

Drought-induced shifts in the green house gas balance of an abandoned meadow in the Austrian Alps.

With contribution of: Lucia Fuchslueger, Andreas Richter (University of Vienna)
Thomas Ladreiter-Knauss, Michael Bahn (University of Innsbruck)

Abstract

Climate models predict strong changes in hydrology for alpine ecosystems, such as an increased frequency of severe droughts (IPCC 2007) and in the last decades a high number of grazed and fertilized alpine meadows have been abandoned. The combination of land use change and climate change may strongly influence soil processes and plant community and therefore also the green house gas balance, but only a few studies deal so far with the effects of climate change on abandoned meadows.

In a climate manipulation experiment we simulated a severe summer drought on an abandoned meadow in the Austrian Alps, by installing rain out shelters during the vegetation season and measured ecosystem respiration ($R_{eco}$) and net fluxes of methane ($CH_4$) and nitrous oxide ($N_2O$) over the growing season.

Our results showed that drought significantly decreased $R_{eco}$ ($P<0.001$) and increased $CH_4$ net uptake ($P<0.001$), $N_2O$ net fluxes also increased, but were only weakly significant ($P=0.0655$). Soil water content strongly declined under drought ($P<0.0001$), but soil processes were relatively resistant to drought, neither the extractable organic carbon or nitrogen pool, nor soil microbial community composition and enzyme activity were significantly affected by the drought treatment. In summary a severe summer drought in an abandoned alpine meadow strengthened the $CH_4$ sink, but reduced ecosystem respiration and $N_2O$ consumption, which may overall lead to an improvement of GHG balance.
Introduction

In the Austrian Alps approximately 40 to 45% of total land area is covered by grasslands (Schiechtl 1974). In the last decades a huge area of these formerly agricultural used meadows (grazing and mowing) have been abandoned (Tappeiner, Tasser et al. 2008) and forest regrows. In addition to these land use changes, strong climatic changes are forecasted for mountain ecosystems (IPCC 2007). Models predict warmer and more humid winters and increased summer temperatures along with less precipitation and water deficits due to a lower snow cover in winter (Laghari, Vanham et al. 2012). Additionally, an increase in the number of extreme weather events, like droughts or storms, is forecasted (IPCC 2012). This increased frequency of drought may influence the contribution of mountain ecosystems to the global greenhouse gas (GHG) budget and therefore may potentially create a positive or negative feedback loop between land use change and climate change.

The most common GHG (beside water vapor) in the atmosphere is carbon dioxide (CO$_2$), which is produced by autotrophic and heterotrophic respiration and fossil fuel burning and consumed by photosynthesis or dissolution in the ocean. Over the last decades a high number of ecosystems, like forests (boreal, temperate, tropical) (Phillips, Malhi et al. 1998; Malhi, Baldocchi et al. 1999; White, Cannell et al. 2000) or peat lands (Hendriks, van Huissteden et al. 2007) were reported to be net sinks for CO$_2$ and also alpine meadows show a net consumption of CO$_2$ (Hirota, Zhang et al. 2009). Drought can reduce the consumption of CO$_2$, because of physiological (increased stomata closure) or structural (reduced leaf area and enzyme activity) changes in plants and vegetation (van der Molen, Dolman et al. 2011), therefore decreasing the terrestrial sink for CO$_2$.

On global scale methane (CH$_4$) is mainly produced in wetlands (Le Mer and Roger 2001) and the ocean (Grunwald, Dellwig et al. 2009), because under anaerobic conditions methanogenic archaea produce CH$_4$ as end product of the degradation of organic matter (Conrad 1996). In alpine grasslands CH$_4$ is usually net consumed, because of the oxidation of CH$_4$ by methanotrophic bacteria (Hiltbrunner, Zimmermann et al. 2012). Whereas land use change from agricultural to abandoned meadows hardly influences net CH$_4$ fluxes, climatic changes can have a greater impact, because methanotrophs in grasslands are mainly limited by the availability of CH$_4$ (Striegl 1993). Severe drought events may increase the molecular diffusion of CH$_4$ from the
atmosphere into soil, because of a lower soil water content (Striegl 1993) and drought potentially intensifies the sink for CH$_4$ in well aerated soils.

Nitrous oxide (N$_2$O), the green house gas (GHG) with the highest potential for global warming (IPCC 2007), is mostly net produced in wet soils via denitrification, as an intermediate of the reduction of NO$_3^-$ to N$_2$ (Flechard, Neftel et al. 2005). The amount of emitted N$_2$O is increased by the addition of fertilizer (Ellis, Yamulki et al. 1998) due to a better substrate availability. The abandonment of meadows therefore may reduce N$_2$O production. For managed grasslands also a net uptake of N$_2$O was reported (Chapuis-Lardy, Wrage et al. 2007) and these consumption rates are assumed to be linked to denitrification of N$_2$O to dinitrogen (N$_2$) (Yu, Chen et al. 2000). Under drought denitrifier activity may be reduced, because their enzymes are highly sensitive to oxygen (O$_2$) (Boublikova, Kucera et al. 1985) and dry soils contain higher amounts of O$_2$. But recent studies demonstrated that especially nitrifier denitrification is less sensitive to O$_2$ amounts (Kool, Dolfing et al. 2011) and may strongly contribute to net fluxes of N$_2$O under drought conditions.

Changes in land use practice coupled with more frequent drought events may modify the contribution of alpine meadows and wood lands to the global GHG budget. Whereas some studies performed climate manipulation experiments on agriculturally used meadows (Cantarel, Bloor et al. 2011; Signarbieux and Feller 2012; Wang, Duan et al. 2012), little is known about the reactions of meadows, which have been abandoned, to severe drought events. The goal of our experiment was to investigate the influence of drought on the GHG balance of this kind of grassland.

We hypothesize that drought will decrease ecosystem respiration (R$_{eco}$), mostly because of increased stomatal closures and reduced leaf area of plants. Also respiration rates may decrease, because of a reduced activity of soil microbes due to the lack of water and the combined reductions may totally lead to a decrease in R$_{eco}$. We further hypothesized that drought will increase the net CH$_4$ consumption, because of higher diffusion rates of atmospheric CH$_4$ into the soil.

Additionally, we hypothesized that drought will decrease denitrification and therefore also net N$_2$O production and due to the fact that alpine grasslands have been reported as net sinks for N$_2$O, we hypothesis that drought will increase net N$_2$O uptake.
Because drought periods are often terminated by heavy rain events (IPCC 2012) we simulated a heavy rainfall at the end of the drought period and hypothesized that it would increase $R_{eco}$ ("Birch effect", Birch and Friend 1956), decrease CH$_4$ uptake, due to lower diffusion rates from the atmosphere into the soil, and increase N$_2$O production, because of stronger denitrification activity in the wetter soil.

We conducted a climate manipulation experiment on an abandoned meadow in the Austrian Alps. A severe drought was simulated by installing rainout shelters during the growing season and CO$_2$, CH$_4$ and N$_2$O net fluxes were monitored over the season. In addition, we measured microclimate parameters, such as precipitation, soil and air temperature, and also several key soil processes, like C and N cycling and microbial community structure and activity, to determine if drought significantly influences these parameters.
Materials and Methods

Sampling Site. The sampling site is located on an alpine meadow (47°07´30.23´´N, 11°17´23.87´´E, 2001m a.s.l.) in the “Stubai”-valley, nearby Innsbruck, in the Austrian Alps (Fig.1). The meadow was abandoned in 1983 and since then it was only sporadically grazed by cattle and sheep in the late summer. Mean annual temperature and mean annual precipitation are around 3°C and 1097 mm, respectively. The meadow has an average inclination of 29 degree. The soil is classified as Dystric Cambisol with an pH of 5.4 (CaCl₂) (Schmitt, Bahn et al. 2010) and contains approximately 11.01% carbon (C) and 0.91% nitrogen (N). During our sampling period C:N content hardly varied. Plant community composition was classified as *Erico carnae - Pinetum prostratae* (Meyer, Leifeld et al. 2012).

Fig.1: Satellite pictures, © Google earth, of the Stubai Valley (A) and the “Kaserstatt-Alm” (B). Red rectangle directly marks the position of the sampling site.
**Experimental Design.** The experiment started in March 2011, including four control and four drought plots. Plots with highly similar plant communities were chosen to avoid bias due to different functional plant types and different microbial communities.

To simulate the conditions of an extreme summer drought rainout shelters (Fig. 2A) were built up on the 25th of May. Each tent covered an area of six square meters and the surrounding area was used as controls. Tents were fixed with ropes, to avoid damages due to storms and heavy wind.

At the end of the drought period on the 8th of August a heavy precipitation event with 20 mm was simulated, by pouring five liters of rainwater on each control and each drought plot. Watering was carefully carried out to prevent soil from mechanical destruction and together with the rewetting event all rainout shelters have been removed.

Ecosystem respiration ($R_{eco}$) and net soil gas fluxes of CH$_4$ and N$_2$O were measured at least once a month and with higher frequency before and after rewetting. Gas samples were taken monthly in May and June, twice in July, directly before the rewetting and one hour (August), one day (August), one week (August) and one month (September) after the rewetting.

We used grey, light tight (exclusion of photosynthesis) PVC chambers (0.5 x 0.5 x 0.5 m) for the gas sampling (Fig. 2B). The bottom edge, a plastic rectangle (0.5 x 0.5 m), was inserted 5 cm deep into the soil at the beginning of the growing season in May and stayed there during the whole summer. The chamber was placed for one hour on the rectangle and a little fan inside was responsible for a well mixture of the air. Chambers were fixed on the rectangle with ropes to avoid contamination by surrounding air. 24 ml air were taken at 0, 15, 30, 45 and 60 minutes with a 50 ml syringe and stored in a 12 ml exetainer® (Labco Limited), which was evacuated the day before.

At least once a month and together with gas sampling soil samples were collected. We took soil cores (5x10x10 cm) from the upper 15 cm (A-horizon), which was immediately sieved (2mm) in the field to remove stones, roots and plant tissue. During transport soil was stored below 10°C in the dark and directly used for further analyses in the lab.

In total 400 gas samples and 88 soil samples were taken in 2011.
Gas Analyses. Gas samples were measured with a TRACE GC Ultra Gas Chromatograph (Thermo Scientific, Wien) with a costume made injection system. We used a flame ionization detector (FID) with a methanizer for CO$_2$ and CH$_4$ detection and a pulsed discharge detector (PDD) (Model D-4-I-TQI-R, Valco Instruments Co. Inc.) for N$_2$O detection. Gas samples were injected into the sample loop at a pressure of 600 mbar. Peaks were analyzed with Chromeleon® 6.80 DU10a Build 2826 (171948) (Dionex). Net gas flux rates ($\mu$g CO$_2$-C/m$^2$/h, $\mu$g CH$_4$-C/m$^2$/h, $\mu$g N$_2$O-N/m$^2$/h) were calculated due to the linear increase or decrease of the concentration against time.

Environmental Parameter. Precipitation, air- and soil temperature were measured from January to December. In controls soil temperature was measured at a depth of 10 cm every 30 min with a thermocouple probe TCAV (Campbell Scientific, Logan, UT, USA) and soil water content was measured with a ML2x Delta-T Device (Cambridge, UK), both coupled with a logger CR10x (Campbell Scientific, Logan, UT, USA).

In drought plots soil temperature was measured with a thermocouple probe TCAV (Campbell Scientific, Logan, UT, USA) with a logger CR1000 (Campbell Scientific, Logan, UT, USA). Soil water content was measured with sensor ECH2O EC-5 and data logger Em50 (Decagon Devices Inc., Pullman, WA, USA).

Soil Analyses. The sieved soil was used to determine the extractable organic carbon (EOC) and extractable organic nitrogen (EON) pools, microbial carbon (C) and nitrogen (N), ammonium
(NH₄⁺) and nitrate (NO₃⁻) concentration, soil water content, mineralization and nitrification rates, actual and potential enzyme activity and microbial community composition (PLFA).

Soil water content was determined by drying 5 g sieved soil for at least 48 hours at 60°C. Water content was calculated due to the loss of weight.

EOC and total dissolved N (TDN) were measured in KCl extracts. We extracted 2 g sieved soil with 20 mL 2M KCl and stored the extracts at -20°C until they were analyzed with a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu).

Ammonium concentration. NH₄⁺ was measured calorimetrically (Hood-Nowotny, Hinko-Najera Umana et al. 2010). 2 g sieved soil were extracted with 0.5M K₂SO₄ and stored at -20°C. 150 µL of the sample was pipetted into a microtiter plate and then 75 µL of a colour reagent (a mixture 0.2 M NaOH, sodium salicylate solution and purified water 1:1:1; sodium salicylate solution was made by dissolving 8.5 g sodium salicylate and 63.9 mg sodium nitroprusside dihydrate in 50 mL purified water) and 30 µL oxidation solution (0.1g dichloroisocyanuric acid sodium salt dehydrate in 100 mL purified water) were added. Finally the absorption was measured at 660 nm (Tecan Infinite M200 fluorimeter) after 30 min incubation in the dark and the concentration was calculated due to the absorption of an ammonium standard series (500 µM to 1.95 µM).

Nitrate concentration. NO₃⁻ was measured in water extracts; therefore 2 g of sieved soil were extracted with 20 mL purified water and NO₃⁻ concentrations were determined via chemically suppressed ion-chromatography (DX500, Dionex, Vienna, Austria) with a AS11 column (Dionex, Vienna, Austria). DON was calculated by subtracting NO₃ and NH₄ from total dissolved N.

Microbial carbon and nitrogen. Microbial C (Cmic) and N (Nmic) were quantified by chloroform-fumigation-extraction method (Amato and Ladd 1988). We put 2 g of sieved soil for 48 hours in a desiccator (chloroform atmosphere) to destroy microbial cells. Finally the soil samples were extracted with 20 mL 2M KCl. In addition 2 g of fresh sieved soil were immediately extracted with 20 mL 2M KCl. Both extracts were analyzed on the TOC/TN analyzer (TOC-V CPH
E200V/TNM-1 220V, Shimadzu) and \( C_{\text{mic}} \) and \( N_{\text{mic}} \) were calculated due to the difference between fumigated and unfumigated soil.

*Mineralization/Nitrification.* Mineralization and nitrification were measured by pool dilution assays (Myrold and Tiedje 1986), modified by Kaiser et al (Kaiser, Fuchslueger et al. 2011). 2 g sieved soil (subsamples for each sampling point) were incubated (at room temperature) with 500 \( \mu \text{L} \) \( ^{15} \text{NH}_4\text{Cl} \) or 500 \( \mu \text{L} \) \( ^{15} \text{NO}_3 \) (250mM, 10 atom\% \( ^{15} \text{N} \)) for 4 or 24 hours. Then the subsamples were extracted with 20 mL 2M KCl and the extracts were stored at -20°C until further processing.

Gross N mineralization was determined by adding 100 mg MgO and an acid trap to each sample. Acids traps were made of Teflon tape, which contained two filter paper discs with 4 \( \mu \text{L} \) 2.5 M KHSO\(_4\) (Wanek, Mooshammer et al. 2010). \( \text{NH}_4^+ \) was absorbed into the acid traps and after five days the acid traps were removed and dried in a desiccator, which contained concentrated sulphuric acid. Finally the paper discs out of the acid traps were transferred into tin capsules and total N and at \( ^{15} \% \text{N} \) was measured with an elemental analyzer (EA 1110, CE Instruments) coupled with a mass spectrometer (Delta\(^{+}\)plus, Finnigan MAT, Thermo Fisher).

To measure gross nitrification we first added 100 mg MgO to each vial to remove \( \text{NH}_4^+ \), the vials were only loosely closed and put on a shaker for three days. Then 50 mg Devarda alloy was added to convert \( \text{NO}_3^- \) into \( \text{NH}_4^+ \) and the \( \text{NH}_4^+ \) was again trapped in acid traps. Once again after incubation (five days) acid traps were dried in a desiccator with sulphuric acid, transferred into tin capsules and analyzed with EA-IRMS.

*Extracellular enzyme activity.* Exoglucanase, endochitinase, phosphatase and protease activity was measured by microplate fluorimetric and photometric assays, as described by Koranda et al. (Koranda, Schnecker et al. 2011). 1 g sieved soil was extracted in 100 mL sodium acetate puffer (100 mM, pH 5.5) and homogenized with an ultrasonicator for 1 min at 10% power. 200 \( \mu \text{L} \) of suspension and 50 \( \mu \text{L} \) of substrate (MUF-celllobiosidase for exoglucanase, MUF-triacetylchitotrioside for endochitinase, MUF-phosphate for phosphatase, Leucin-aminomethylcoumarin for protease) were pipetted in black microtiter plates and incubated in the dark for approximately 140 min. Three analytical replicates for each sample were measured at 365 nm extinction and 450 nm emission with Tecan Infinite M200 fluorimeter.
Methylumbelliferone (MUF) was used as standard for exoglucanase, endochitinase and phosphatase and aminomethylcoumarin (AMC) for protease.

Phenoloxidase and peroxidase were measured by mixing 1 mL L-3,4-dihydroxyphenylalanin (20 mM) and 1 mL suspension. Supernatant was shaken for 10 min and centrifuged. 250 µl of supernatant were pipeted into a transparent microtiter plate and absorption was measured at 450 nm with Tecan Infinite M200 fluorimeter. For each samples six analytical replicates were pipeted and to determine peroxidase activity we additionally added 10 µL 0.3% H$_2$O$_2$ to three of the replicates. Absorption was measured at the starting point and after approximately 20 hour incubation in the dark at room temperature.

Enzyme activity was calculated due to the difference in absorption between the first and the second measurement.

**Actual protease and cellulase activity.** Actual protease and cellulase activity was determined after Kaiser et al. (Kaiser, Koranda et al. 2010). Soil suspensions were made with 4 g fresh soil and 100 ml purified water and 400 µl toluolene were added to each sample. After 30 and 240 min 2 mL suspension were transferred into a tube and centrifuged for 10 min. Then for cellulose activity 1mL solution was headed in a water bath (100°C) for 15 min and for protease activity 800 µL of the solution were mixed in equal parts with TCA solution (0.11 M trichloroacetic acid, 0.22 M natrium acetate and 0.33 M acetic acid).

Protease activity was determined by photometric measurement of amino acid production. 200 µL of sample were mixed with 200 µL ninhydrin reagent, cooked in a water bath for 10 min (100°C) and then 500 µL 50% ethanol were added. Finally 200 µL were pipeted into a transparent microtiter plate and absorption was measured at 570 nm. A leucine solution (7 µg leucine-N mL$^{-1}$ down to 0.02725 µg leucine-N mL$^{-1}$) was used as standard to calculate actual protease activity (µg AAS-N g$^{-1}$ h$^{-1}$).

Cellulose activity was determined by measuring the glucose concentration with HPLC (ICS-3000; CarboPac PA20 column; 20 mM NaOH; with pulsed amperiometric detection, DIONEX, Vienna, Austria).

**Phospholipid fatty acids.** PLFA’s were measured by using a modified version (Koranda, Schnecker et al. 2011) of a method first described by Frostegård (Frostegard, Tunlid et al. 1991).
2 g sieved soil were extracted with a mixture of chloroform: methanol: citrate buffer (1:2:0.8) over night and then the lower organic phase was transferred in a new vial and dried under N₂. Then samples were redissolved in chloroform and fractionated on a Supelclean™ LC-Si SPE column (Supelco). Chloroform was used to elute neutral fatty acids and acetone to purify the sample. Finally the phospholipid fraction was eluted with methanol and again dried under N₂.

Following 100 µL internal standard (methyl-nonadecanoate 200 µg mL⁻¹ in MeOH-Toluol 1:1 mixture) was added and alkaline methanolysis was used to convert phospholipids into fatty acid methyl ester.

At the end samples were transferred into GC vials with 100 µL 2,2,4-trimethylpentane and measured with a FID on a TRACE GC Ultra Gas Chromatograph (Thermo Scientific, Austria). A internal standard was used as reference peak for calculation.

We used Bacterial Acid Methyl Ester CP Mix (Supelco) as standard for bacterial PLFA`s and Supelco 37 Comp., FAME Mix 10 mg/mL in CH₂Cl₂ (Supelco) as standard for fungal PLFA`s.

The sum of fatty acids i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 was used as indicator for gram positive bacterial biomass, 16:1(7), 16:1(9)c, cy18:0(11,12), cy17:0(9,10), 18:1(11) and cy19:0(9,10) for gram negative bacterial biomass and 16:1(11), 10Me18:0, 18:1(9)c, 18:2(9,12)c and 18:3(6,9,12) as indicator for fungal biomass.

During the whole process only cleaned and muffled glassware were used to avoid contamination.

All measured parameter were reported per gram dry soil.

**Data preparation and statistical analyses.** Data calculation was done with excel (Microsoft Office, Version 2007) and graphs were created with Sigma Plot 12.0 (Systac Software). Statistical analyzes were performed with Statgraphic Centurion XVI (Version 16.1.11, 32-bit). Data were tested on normal distribution and variance homogeneity. If data didn’t fit into a normal distribution model, they were log or square transformed. Data were also searched for outliers which were excluded from further analysis.

Significant effects due to treatment and date were tested with a one-way analysis of variance (ONE-WAY ANOVA) and combined influence of sampling day x drought with TWO-WAY ANOVA. To evaluate the correlation coefficient between two variables we used a simple regression analysis and for multiple variables a Pearson correlation. The whole dataset was
divided into a drought period (25\textsuperscript{th} May-9\textsuperscript{th} August) and the post drought period (10\textsuperscript{th} August-27\textsuperscript{th} September) and the effect of rewetting was statistically analyzed with a ONE-WAY ANOVA one day (gas fluxes) and one week (all parameter) after the rewetting. Results were assessed to be significant, if $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***).
Results

Microclimate

Precipitation showed typical alpine pattern with dry winters and precipitation peaks in summer and one heavy rain event on the 10th of October (63 mm day−1) (Fig.3). Mean daily temperature (MDT) was strongly influenced by the current weather situation and varied highly (Fig.3). In July, August and September there were no MDT below 0°C. Soil temperature (controls) at a depth of 10 cm varied between 0.5-0.8°C from January to March, increased to 13.9°C in August and September and then slowly declined to 1.9°C in December (Fig.10). Soil water content (SWC) in the drought plots constantly decreased after the installation of the rainout shelters (P<0.0001), with the lowest amount (0.21 g H₂O g⁻¹ soil) directly before rewetting (Fig.4). SWC within the drought plots remained at a lower level than SWC in the controls (P<0.001), even after the removal of the rainout shelter.

Fig.3: Mean daily temperature (°C, red line) and precipitation (mm/day, grey bars) on the abandoned meadow during 2011.

Fig.4: Soil water content in gH₂O g⁻¹ soil in control (black circles) and drought (white circles) plots. Drought significantly reduced SWC (P<0.001) (multifactorial ANOVA) during drought and also in the post treatment period (P<0.001). Error bars are calculated with standard error (n=4).
**Ecosystem respiration and green house gas net fluxes**

Independent from our climate manipulation experiment we saw a significant intraannual dynamic of ecosystem respiration \((P<0.001)\) and \(\text{N}_2\text{O}\) net flux \((P<0.01)\), but not of \(\text{CH}_4\).

During the whole growing season the abandoned meadow was a sink for \(\text{CH}_4\) and net fluxes reached their minimum of absorption in June and August. Then absorption rates steadily rose until the end of the measurement period. Net flux rates were significantly increased by drought \((P<0.001)\) (Fig. 5A). On the contrary \(\text{CH}_4\) net fluxes revealed no significant reaction due to rewetting (Fig. 5B) and also in the post-drought period net fluxes of drought plots were lower than net fluxes of control plots \((P<0.01)\).

\(\text{N}_2\text{O}\) net flux rates in the drought plots constantly increased from the beginning of the growing season until rewetting (Fig.5C), but the lower consumption rates were only weakly significant \((P=0.0665)\). Nevertheless, \(\text{N}_2\text{O}\) net fluxes exhibited a crucial shift after rewetting, because the addition of water induced a switch from net absorption to net emission \((P=0.0722)\) (Fig.5D). At the end of the post treatment period \(\text{N}_2\text{O}\) net fluxes of drought and control plots reached the same level of net consumption.

Ecosystem respiration was highest in late summer and lowest in midsummer and it significantly decreased (27%-56%) under drought conditions \((P<0.001)\) (Fig.6A). One hour after rewetting CO\(_2\) emission rates strongly increased \((P<0.01)\) (“Birch effect”, Birch and Friend 1956) (Fig. 6B), but even after the removal of the rainout shelters net production of drought plots remained at a lower level than of control plots \((P<0.001)\).

All \(P\)-values, F-ratios and degrease of freedom for drought period, post treatment period and rewetting are listed in Table 2, Table 3 and Table 4.

To be able to compare \(\text{CH}_4\) and \(\text{N}_2\text{O}\) net fluxes we converted them into CO\(_2\) equivalents and calculated the cumulative flux during drought. From 25\(^{th}\) of May to 9\(^{th}\) of August both GHG’s were net consumed by the abandoned meadow, but although drought increased total \(\text{CH}_4\) consumption, it decreased \(\text{N}_2\text{O}\) consumption, leading in total to a decrease of the sink for GHG’s at around 14 % (Fig.7), even if CO\(_2\) emission decrease by drought is not taken into account.
Fig. 5: Graphs on the left hand show net fluxes of (A) CH$_4$ (µg CH$_4$-C m$^{-2}$h$^{-1}$) and (C) N$_2$O (µg N$_2$O-N m$^{-2}$h$^{-1}$) during the whole measurement period. Control plots are marked as black circles and drought plots as white circles. CH$_4$ net uptake significantly increased during drought (P<0.01) and also in the post treatment period (P<0.01) (multifactorial ANOVA). In the post treatment period N$_2$O net fluxes were significantly influenced by sampling day (P<0.01) and sampling day x drought (P<0.05) (multifactorial ANOVA), but the influence of drought was only weakly significant.

Graphs on the right hand show net fluxes immediately before and after rewetting (solid black line). (D) N$_2$O net fluxes increased due to rewetting (P=0.0722, one-way ANOVA), but (B) CH$_4$ net fluxes exhibited no changes. Error bars are calculated with standard error (n=4).
Fig. 6: (A) Graph shows the ecosystem respiration (mg CO$_2$-C m$^{-2}$ h$^{-1}$) during the measurement period. Control plots are marked as black circles and drought plots as white circles. It was significantly influenced by sampling day and treatment ($P<0.001$) during drought and in the post treatment period (multifactorial ANOVA). (B) Graph on the right hand shows net fluxes immediately before and after rewetting (solid black line). Ecosystem respiration ($P<0.01$) increased significantly due to rewetting (one-way ANOVA). Error bars are calculated with standard error (n=4).

Fig. 7: The graph shows the cumulative flux rates (25$^{th}$ May-9$^{th}$ August) of CH$_4$ and N$_2$O calculated in CO$_2$ equivalents (gCO$_2$e m$^{-2}$) during drought period. Net uptake of CH$_4$ was stronger in drought (white triangle) than in control (black triangle) plots, contrary to N$_2$O, because here the net uptake decreased in drought (white circle) compared to control (black circle) plots.
Carbon/nitrogen pools

One important controlling factor of N$_2$O production is the availability of reactive N. Total soil carbon and total soil nitrogen accounted for 11.01% and 0.91% (dry soil, control plots), respectively and both showed no seasonal pattern or changes due to drought. We measured extractable organic C (EOC) and extractable organic N (EON) pools and both showed no significant changes due to drought. However, intraannual dynamics highly influenced EOC pool ($P<0.001$). It peaked in May, decreased in June and July, peaked again in the early August and dropped to the lowest level in September (Fig. 8A). EON showed no significant changes due to sampling day and the pool peaked in August (Fig. 8B). Both pools strongly reacted to rewetting, by increasing EOC ($P<0.01$) and EON ($P<0.01$) pool. During the whole growing season EOC and EON were positively correlated ($R=0.41, P<0.05$).

Fig. 8: (A) Seasonal dynamics of extractable organic carbon (EOC, in $\mu$g C g$^{-1}$DM) in control (black circles) and drought (white circles) plots. The results suggested no significant influence of treatment, but of sampling date ($P<0.001$). (B) Seasonal dynamics of extractable organic nitrogen (EON, in $\mu$g N g$^{-1}$DM) in control and drought plots. Again there was no significant influence of treatment or sampling day (multifactorial ANOVA). The end of drought, the rewetting, is marked with a black line. Error bars are calculated with standard error (n=4). Note the different scales!
Microbial carbon ($C_{mic}$), but not microbial nitrogen ($N_{mic}$) highly varied seasonally ($P<0.5$). $C_{mic}$ ranged between 946 µgC g$^{-1}$DM and 7166 µgC g$^{-1}$DM with the highest amounts in June and August and the lowest amounts in mid June and October. $N_{mic}$ pool was highest at the beginning of May (around 400 µgN g$^{-1}$DM) and then steadily declined until August. There was a significant impact of drought on $C_{mic}$ ($P<0.01$), but not on $N_{mic}$. Both showed no changes one day and one week after rewetting.

$C_{mic}$ was positively correlated with water content ($R=0.42$, $P<0.05$), EOC ($R=0.44$, $P<0.05$), C:N$_{mic}$ ($R=0.90$, $P<0.001$) and $N_{mic}$ ($R=0.38$, $P<0.05$) and negatively correlated with C:N$_{soil}$ ($R=-0.40$, $P<0.05$). $N_{mic}$ was positively correlated with $C_{mic}$ ($R=0.38$, $P<0.05$) and N$_2$O net flux rates ($R=0.48$, $P<0.05$) and it was negatively correlated EON with ($R=-0.49$, $P<0.01$).

Especially N$_2$O net fluxes are highly influenced by belowground N cycling and we measured nitrification and mineralization rates, because we expected the availability of nitrate in combination with SWC to be an important controlling factor.

Gross nitrification rates (Fig. 9A) peaked in May and August and the rates were lowest in midsummer and in autumn. Gross nitrification varied significantly seasonally during drought ($P<0.01$), but there were no changes because of drought. Rewetting strongly reduced gross nitrification rates ($P<0.001$).

Gross nitrate immobilization rates showed similar dynamics than gross nitrification rates and were significantly influenced by drought ($P<0.05$) during the drought period, but only in the post treatment period there was a significant influence of the sampling day ($P<0.001$). Rewetting again decreased gross nitrate immobilization rates ($P<0.001$).

Both variables showed a strong positive correlation with N$_2$O net fluxes ($R^2=0.28-0.39$, $P<0.01$), indicating that higher gross nitrification rates led to higher N$_2$O net fluxes (Table 1).

Gross N mineralization (Fig. 9B) and immobilization rates peaked in May and August with the lowest rates in late June, but in contrast to gross nitrification and gross nitrate immobilization there was no significant influence of sampling day, drought or the combination of both factors. Even rewetting caused no significant changes.

Surprisingly, gross N mineralization was negative correlated with gross nitrification ($R= -0.36$, $P=0.0784$).
Microbial community composition

Microbial community composition was determined by phospholipid fatty acid analysis and PLFAs were divided into fungi PLFAs, gram+ PLFAs and gram- PLFAs (Fig 15). All three groups showed no significant reaction due to drought, rewetting, sampling day or the combination of sampling day and drought.

Fig.9: (A) Gross nitrification rates in µgN g⁻¹DMday⁻¹ in control (black circles) and drought (white circles) plots. Gross nitrification was significantly influenced by sampling day (P<0.001), but not by drought. (B) Gross N mineralization rates in µgN g⁻¹DMday⁻¹control and drought plots. There was no significant influence of sampling day or drought (multifactorial ANOVA). The black line indicates the end of drought. Error bars were calculated with standard error (n=4).
Tables and contents

Table 1: Pearson correlation of N$_2$O net flux, gross nitrification and gross NO$_3^-$ immobilization during drought (25$^{th}$ of May-9$^{th}$ of August). The first value is the correlation coefficients and the second value is the $P$-value.

<table>
<thead>
<tr>
<th></th>
<th>N$_2$O net flux</th>
<th>Gross nitrification</th>
<th>Gross NO$_3^-$ immobilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_2$O net flux</td>
<td>0.6380</td>
<td>0.5336</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0005</td>
<td>0.0050</td>
<td></td>
</tr>
<tr>
<td>Gross nitrification</td>
<td></td>
<td>0.7247</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0000</td>
<td></td>
</tr>
</tbody>
</table>

Correlation coefficients

$P$-Value

Fig.10: Soil temperature in a depth of 10 cm during one year on the abandoned meadow (control plot). The highest temperature were reached in September (13.9°C) and the lowest temperature from January to March (0.5-0.8°C).
Table 2: Multifactorial ANOVA of all measured parameters versus sampling day, treatment and the combination of sampling day x treatment with significance level ($P$), F ratio and degree of freedom (DF) during the drought period (25th May-9th August). Significant $P$ values are marked bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling day F-ratio (Df)</th>
<th>$P$-value</th>
<th>Treatment F-ratio (Df)</th>
<th>$P$-value</th>
<th>Sampling day x Treatment F-ratio (Df)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross nitrification</td>
<td>6.58 (3)</td>
<td><strong>0.0021</strong></td>
<td>3.58 (1)</td>
<td>0.0708</td>
<td>1.76 (3,24)</td>
<td>0.1825</td>
</tr>
<tr>
<td>Gross NO$_3^-$ immobilisation</td>
<td>2.44 (3)</td>
<td>0.0887</td>
<td>6.59 (1)</td>
<td><strong>0.0169</strong></td>
<td>0.28 (3,24)</td>
<td>0.8407</td>
</tr>
<tr>
<td>Gross NH$_4^+$ immobilisation</td>
<td>0.69 (2)</td>
<td>0.5156</td>
<td>1.11 (1)</td>
<td>0.3061</td>
<td>1.61 (2,18)</td>
<td>0.2279</td>
</tr>
<tr>
<td>Gross N mineralization</td>
<td>0.56 (2)</td>
<td>0.5825</td>
<td>0.70 (1)</td>
<td>0.4142</td>
<td>3.38 (2,18)</td>
<td>0.0568</td>
</tr>
<tr>
<td>Ecosystem respiration</td>
<td>14.06 (3)</td>
<td><strong>0.0000</strong></td>
<td>42.73 (1)</td>
<td><strong>0.0000</strong></td>
<td>1.40 (3,20)</td>
<td>0.2716</td>
</tr>
<tr>
<td>CH$_4$ net flux</td>
<td>1.38 (3)</td>
<td>0.2702</td>
<td>12.15 (1)</td>
<td><strong>0.0018</strong></td>
<td>0.91 (3,26)</td>
<td>0.4500</td>
</tr>
<tr>
<td>N$_2$O net flux</td>
<td>1.56 (3)</td>
<td>0.2292</td>
<td>3.80 (1)</td>
<td>0.0655</td>
<td>1.01 (3,20)</td>
<td>0.4106</td>
</tr>
<tr>
<td>Total C</td>
<td>4.41 (3)</td>
<td><strong>0.0132</strong></td>
<td>117.36 (1)</td>
<td><strong>0.0000</strong></td>
<td>1.52 (3,24)</td>
<td>0.2337</td>
</tr>
<tr>
<td>Total N</td>
<td>0.75 (3)</td>
<td>0.5347</td>
<td>2.94 (1)</td>
<td>0.0991</td>
<td>1.85 (2,42)</td>
<td>0.1625</td>
</tr>
<tr>
<td>C:N$_{total}$</td>
<td>1.37 (3)</td>
<td>0.2766</td>
<td>2.18 (1)</td>
<td>0.1530</td>
<td>1.59 (3,24)</td>
<td>0.2175</td>
</tr>
<tr>
<td>EOC</td>
<td>12.22 (3)</td>
<td><strong>0.0001</strong></td>
<td>1.69 (1)</td>
<td>0.2065</td>
<td>1.79 (3,23)</td>
<td>0.1770</td>
</tr>
<tr>
<td>EON</td>
<td>1.97 (3)</td>
<td>0.1489</td>
<td>1.72 (1)</td>
<td>0.2039</td>
<td>2.35 (3,21)</td>
<td>0.1011</td>
</tr>
<tr>
<td>C$_{mic}$</td>
<td>7.11 (3)</td>
<td><strong>0.0018</strong></td>
<td>14.06 (1)</td>
<td><strong>0.0012</strong></td>
<td>6.83 (3,21)</td>
<td><strong>0.0022</strong></td>
</tr>
<tr>
<td>N$_{mic}$</td>
<td>0.81 (3)</td>
<td>0.5040</td>
<td>0.17 (1)</td>
<td>0.6810</td>
<td>1.42 (3,21)</td>
<td>0.2641</td>
</tr>
<tr>
<td>C:N soil</td>
<td>13.22 (3)</td>
<td><strong>0.0000</strong></td>
<td>0.08 (1)</td>
<td>0.7826</td>
<td>5.32 (3,21)</td>
<td><strong>0.0069</strong></td>
</tr>
<tr>
<td>C:N$_{mic}$</td>
<td>22.45 (3)</td>
<td><strong>0.0000</strong></td>
<td>35.04 (1)</td>
<td><strong>0.0000</strong></td>
<td>17.65 (3,20)</td>
<td><strong>0.0000</strong></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.96 (3)</td>
<td>0.4264</td>
<td>0.15 (1)</td>
<td>0.7014</td>
<td>2.43 (3,24)</td>
<td>0.0896</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>1.35 (3)</td>
<td>0.2843</td>
<td>0.22 (1)</td>
<td>0.6435</td>
<td>0.68 (3,22)</td>
<td>0.5718</td>
</tr>
<tr>
<td>Cellulase (act)</td>
<td>7.09 (3)</td>
<td><strong>0.0014</strong></td>
<td>2.04 (1)</td>
<td>0.1665</td>
<td>6.41 (3,24)</td>
<td><strong>0.0024</strong></td>
</tr>
<tr>
<td>Protease (act)</td>
<td>3.01 (3)</td>
<td><strong>0.0497</strong></td>
<td>0.26 (1)</td>
<td>0.6176</td>
<td>1.03 (3,24)</td>
<td>0.3986</td>
</tr>
<tr>
<td>Exoglucanase (pot)</td>
<td>5.84 (3)</td>
<td><strong>0.0049</strong></td>
<td>1.12 (1)</td>
<td>0.3034</td>
<td>4.38 (3,20)</td>
<td><strong>0.0159</strong></td>
</tr>
<tr>
<td>Endochitinase (pot)</td>
<td>3.33 (3)</td>
<td><strong>0.0404</strong></td>
<td>0.01 (1)</td>
<td>0.9299</td>
<td>0.27 (3,20)</td>
<td>0.8478</td>
</tr>
<tr>
<td>Phosphatase (pot)</td>
<td>48.16 (3)</td>
<td><strong>0.0000</strong></td>
<td>3.07 (1)</td>
<td>0.0953</td>
<td>6.56 (3,20)</td>
<td><strong>0.0029</strong></td>
</tr>
<tr>
<td>Protease (pot)</td>
<td>24.12 (3)</td>
<td><strong>0.0000</strong></td>
<td>1.45 (1)</td>
<td>0.2425</td>
<td>5.83 (3,20)</td>
<td><strong>0.0049</strong></td>
</tr>
<tr>
<td>Phenoloxidase (Activity)</td>
<td>4.63 (3)</td>
<td><strong>0.0161</strong></td>
<td>1.55 (1)</td>
<td>0.2271</td>
<td>0.37 (3,20)</td>
<td>0.7732</td>
</tr>
<tr>
<td>Peroxidase (Activity)</td>
<td>2.42 (3)</td>
<td>0.0961</td>
<td>2.86 (1)</td>
<td>0.1063</td>
<td>0.03 (3,20)</td>
<td>0.9915</td>
</tr>
<tr>
<td>Fungi PLFA</td>
<td>1.72 (3)</td>
<td>0.1886</td>
<td>0.06 (1)</td>
<td>0.8059</td>
<td>0.99 (3,24)</td>
<td>0.4124</td>
</tr>
<tr>
<td>Gram+ PLFA</td>
<td>1.24 (3)</td>
<td>0.3183</td>
<td>0.02 (1)</td>
<td>0.8877</td>
<td>0.35 (3,24)</td>
<td>0.7912</td>
</tr>
<tr>
<td>Gram - PLFA</td>
<td>1.17 (3)</td>
<td>0.3413</td>
<td>0.04 (1)</td>
<td>0.8379</td>
<td>0.84 (3,24)</td>
<td>0.4847</td>
</tr>
</tbody>
</table>
Table 3: Multifactorial ANOVA of all measured parameters versus sampling day, treatment and the combination of sampling day x treatment with significance level ($P$), F ratio and degree of freedom (DF) of the post treatment period (10th of August-27th of September). Significant $P$ values are marked bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling day F-ratio (DF)</th>
<th>P-value</th>
<th>Treatment F-ratio (DF)</th>
<th>P-value</th>
<th>Sampling day x Treatment F-ratio (DF)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross nitrification</td>
<td>2.58 (1)</td>
<td>0.1344</td>
<td>2.48 (1)</td>
<td>0.1411</td>
<td>1.96 (1,12)</td>
<td>0.1868</td>
</tr>
<tr>
<td>Gross NO$_3$ immobilisation</td>
<td>20.54 (1)</td>
<td>0.0007</td>
<td>0.37 (1)</td>
<td>0.5525</td>
<td>2.56 (1,12)</td>
<td>0.1294</td>
</tr>
<tr>
<td>Gross NH$_4$$^+$ immobilisation</td>
<td>0.26 (2)</td>
<td>0.7757</td>
<td>0.14 (1)</td>
<td>0.7163</td>
<td>0.25 (2,18)</td>
<td>0.7813</td>
</tr>
<tr>
<td>Gross N mineralization</td>
<td>0.04 (2)</td>
<td>0.9606</td>
<td>0.12 (1)</td>
<td>0.7299</td>
<td>0.46 (2,18)</td>
<td>0.6376</td>
</tr>
<tr>
<td>Ecosystem respiration</td>
<td>48.87 (2)</td>
<td>0.0000</td>
<td>22.46 (1)</td>
<td>0.0001</td>
<td>1.15 (2,19)</td>
<td>0.3390</td>
</tr>
<tr>
<td>CH$_4$ net flux</td>
<td>1.68 (2)</td>
<td>0.2180</td>
<td>14.80 (1)</td>
<td>0.0014</td>
<td>3.00 (2,16)</td>
<td>0.0785</td>
</tr>
<tr>
<td>N$_2$O net flux</td>
<td>7.99 (2)</td>
<td>0.0037</td>
<td>0.04 (1)</td>
<td>0.8395</td>
<td>5.17 (2,17)</td>
<td>0.0176</td>
</tr>
<tr>
<td>WC</td>
<td>2.16 (2)</td>
<td>0.1441</td>
<td>22.03 (1)</td>
<td>0.0002</td>
<td>4.39 (2,18)</td>
<td>0.0279</td>
</tr>
<tr>
<td>Total C</td>
<td>1.71 (1)</td>
<td>0.2172</td>
<td>0.89 (1)</td>
<td>0.3662</td>
<td>0.94 (1,11)</td>
<td>0.3535</td>
</tr>
<tr>
<td>Total N</td>
<td>1.26 (1)</td>
<td>0.2854</td>
<td>1.40 (1)</td>
<td>0.2613</td>
<td>0.34 (1,11)</td>
<td>0.5699</td>
</tr>
<tr>
<td>C:N$_{total}$</td>
<td>0.88 (1)</td>
<td>0.3678</td>
<td>0.86 (1)</td>
<td>0.3750</td>
<td>1.65 (1,11)</td>
<td>0.2249</td>
</tr>
<tr>
<td>EOC</td>
<td>15.31 (2)</td>
<td>0.0001</td>
<td>1.43 (1)</td>
<td>0.2468</td>
<td>0.95 (2,18)</td>
<td>0.4066</td>
</tr>
<tr>
<td>EON</td>
<td>18.10 (2)</td>
<td>0.0000</td>
<td>0.98 (1)</td>
<td>0.3358</td>
<td>0.98 (2,18)</td>
<td>0.3949</td>
</tr>
<tr>
<td>C$_{mic}$</td>
<td>4.72 (2)</td>
<td>0.0225</td>
<td>0.36 (1)</td>
<td>0.5536</td>
<td>0.28 (2,18)</td>
<td>0.7582</td>
</tr>
<tr>
<td>N$_{mic}$</td>
<td>0.59 (2)</td>
<td>0.5672</td>
<td>0.12 (1)</td>
<td>0.7281</td>
<td>0.48 (2,18)</td>
<td>0.6288</td>
</tr>
<tr>
<td>C:N$_{soil}$</td>
<td>0.28 (2)</td>
<td>0.7555</td>
<td>0.45 (1)</td>
<td>0.5093</td>
<td>1.72 (2,18)</td>
<td>0.2077</td>
</tr>
<tr>
<td>C:N$_{mic}$</td>
<td>21.85 (2)</td>
<td>0.0000</td>
<td>0.22 (1)</td>
<td>0.6465</td>
<td>0.88 (1,18)</td>
<td>0.4337</td>
</tr>
<tr>
<td>NH$_4$$^+$</td>
<td>20.89 (2)</td>
<td>0.0000</td>
<td>0.01 (1)</td>
<td>0.9185</td>
<td>0.16 (2,18)</td>
<td>0.8515</td>
</tr>
<tr>
<td>NO$_3$$^-$</td>
<td>2.89 (2)</td>
<td>0.0845</td>
<td>0.15 (1)</td>
<td>0.7053</td>
<td>0.57 (2,16)</td>
<td>0.5747</td>
</tr>
<tr>
<td>Cellulase (act)</td>
<td>4.35 (2)</td>
<td>0.0287</td>
<td>1.19 (1)</td>
<td>0.2897</td>
<td>1.85 (2,18)</td>
<td>0.1859</td>
</tr>
<tr>
<td>Protease (act)</td>
<td>1.19 (2)</td>
<td>0.3269</td>
<td>5.03 (1)</td>
<td>0.0377</td>
<td>2.89 (2,18)</td>
<td>0.0815</td>
</tr>
<tr>
<td>Exoglucanase (pot)</td>
<td>4.87 (2)</td>
<td>0.0204</td>
<td>0.26 (1)</td>
<td>0.6157</td>
<td>0.46 (2,18)</td>
<td>0.6397</td>
</tr>
<tr>
<td>Endochitinase (pot)</td>
<td>6.26 (2)</td>
<td>0.0086</td>
<td>3.44 (1)</td>
<td>0.0800</td>
<td>0.17 (2,18)</td>
<td>0.8457</td>
</tr>
<tr>
<td>Phosphatase (pot)</td>
<td>32.13 (2)</td>
<td>0.0000</td>
<td>0.90 (1)</td>
<td>0.3549</td>
<td>1.77 (2,18)</td>
<td>0.1989</td>
</tr>
<tr>
<td>Protease (pot)</td>
<td>28.18 (2)</td>
<td>0.0000</td>
<td>1.38 (1)</td>
<td>0.2553</td>
<td>0.26 (2,18)</td>
<td>0.7727</td>
</tr>
<tr>
<td>Phenoloxidase (Activity)</td>
<td>7.77 (2)</td>
<td>0.0037</td>
<td>1.64 (1)</td>
<td>0.2164</td>
<td>0.01 (2,18)</td>
<td>0.9930</td>
</tr>
<tr>
<td>Peroxidase (Activity)</td>
<td>40.34 (2)</td>
<td>0.0000</td>
<td>5.40 (1)</td>
<td>0.0320</td>
<td>0.04 (2,18)</td>
<td>0.9633</td>
</tr>
<tr>
<td>Fungi PLFA</td>
<td>0.68 (2)</td>
<td>0.5215</td>
<td>0.73 (1)</td>
<td>0.4026</td>
<td>1.08 (2,18)</td>
<td>0.3619</td>
</tr>
<tr>
<td>Gram+ PLFA</td>
<td>0.20 (2)</td>
<td>0.8192</td>
<td>0.34 (1)</td>
<td>0.5649</td>
<td>0.59 (2,18)</td>
<td>0.5659</td>
</tr>
<tr>
<td>Gram - PLFA</td>
<td>0.05 (2)</td>
<td>0.9517</td>
<td>0.00 (1)</td>
<td>0.9695</td>
<td>0.87 (2,18)</td>
<td>0.4357</td>
</tr>
</tbody>
</table>
Table 4: One-way ANOVA for net fluxes of CH$_4$ and N$_2$O and ecosystem respiration (only in drought plots) one hour after rewetting (left column) and for gas fluxes and the other parameters one day after rewetting (right column) with F-ratio, degree of freedom (Df) and significance value $P$. One hour after rewetting only gas fluxes were measured and the first soil samples were taken one day after rewetting. Bold marked values are significant.

<table>
<thead>
<tr>
<th></th>
<th>Rewetting +1 hour</th>
<th>Rewetting +1 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-ratio (Df)</td>
<td>P-value</td>
</tr>
<tr>
<td>Gross nitrification</td>
<td>36.08 (1)</td>
<td><strong>0.0010</strong></td>
</tr>
<tr>
<td>Gross NO$_3^-$ immobilisation</td>
<td>69.65 (1)</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>Gross NH$_4^+$ immobilisation</td>
<td>0.00 (1)</td>
<td>0.9932</td>
</tr>
<tr>
<td>Gross N mineralization</td>
<td>0.12 (1)</td>
<td>0.7436</td>
</tr>
<tr>
<td>Ecosystem respiration</td>
<td>21.94 (1)</td>
<td><strong>0.0034</strong></td>
</tr>
<tr>
<td>CH$_4$ net flux</td>
<td>0.61 (1)</td>
<td>0.4656</td>
</tr>
<tr>
<td>N$_2$O net flux</td>
<td>5.89 (1)</td>
<td>0.0722</td>
</tr>
<tr>
<td>WC</td>
<td>0.62 (1)</td>
<td>0.4625</td>
</tr>
<tr>
<td>Total C</td>
<td>1.50 (1)</td>
<td>0.2745</td>
</tr>
<tr>
<td>Total N</td>
<td>0.81 (1)</td>
<td>0.4103</td>
</tr>
<tr>
<td>C:N$_{total}$</td>
<td>1.92 (1)</td>
<td>0.2243</td>
</tr>
<tr>
<td>EOC</td>
<td>15.84 (1)</td>
<td><strong>0.0073</strong></td>
</tr>
<tr>
<td>EON</td>
<td>21.06 (1)</td>
<td><strong>0.0059</strong></td>
</tr>
<tr>
<td>C$_{mic}$</td>
<td>3.92 (1)</td>
<td>0.1047</td>
</tr>
<tr>
<td>N$_{mic}$</td>
<td>3.76 (1)</td>
<td>0.1100</td>
</tr>
<tr>
<td>C:N$_{soil}$</td>
<td>12.06 (1)</td>
<td><strong>0.0178</strong></td>
</tr>
<tr>
<td>C:N$_{mic}$</td>
<td>2.09 (1)</td>
<td>0.2080</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.08 (1)</td>
<td>0.7915</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>7.13 (1)</td>
<td><strong>0.0370</strong></td>
</tr>
<tr>
<td>Cellulase (act)</td>
<td>1.83 (1)</td>
<td>0.2244</td>
</tr>
<tr>
<td>Protease (act)</td>
<td>1.97 (1)</td>
<td>0.2098</td>
</tr>
<tr>
<td>Exoglucanase (pot)</td>
<td>0.15 (1)</td>
<td>0.7124</td>
</tr>
<tr>
<td>Endochitinase (pot)</td>
<td>0.21 (1)</td>
<td>0.6664</td>
</tr>
<tr>
<td>Phosphatase (pot)</td>
<td>0.15 (1)</td>
<td>0.7082</td>
</tr>
<tr>
<td>Protease (pot)</td>
<td>4.75 (1)</td>
<td>0.0725</td>
</tr>
<tr>
<td>Phenoloxidase (Activity)</td>
<td>2.19 (1)</td>
<td>0.1897</td>
</tr>
<tr>
<td>Peroxidase (Activity)</td>
<td>3.99 (1)</td>
<td>0.0926</td>
</tr>
<tr>
<td>Fungi PLFA</td>
<td>0.08 (1)</td>
<td>0.7908</td>
</tr>
<tr>
<td>Gram+ PLFA</td>
<td>0.03 (1)</td>
<td>0.8747</td>
</tr>
<tr>
<td>Gram - PLFA</td>
<td>0.01 (1)</td>
<td>0.9228</td>
</tr>
</tbody>
</table>
Table 5: Pearson Correlation of all measured soil parameters vs. net gas fluxes of \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) and ecosystem respiration during the drought period (25\textsuperscript{th} of May - 9\textsuperscript{th} of August). Only significant results are noted in the table. The first value is the correlation coefficients (r) and the second value the significance level (P-value).

<table>
<thead>
<tr>
<th></th>
<th>Ecosystem respiration</th>
<th>( \text{CH}_4 )</th>
<th>( \text{N}_2\text{O} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>-0.4080 0.0429</td>
<td>-0.5355 0.0019</td>
<td>0.3546 0.0820</td>
</tr>
<tr>
<td>Ecosystem respiration</td>
<td>0.4418 0.0210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{CH}_4 )</td>
<td>0.4418 0.0210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total C</td>
<td>-0.3703 0.0404</td>
<td>0.4874 0.0115</td>
<td></td>
</tr>
<tr>
<td>Total ( \text{N} )</td>
<td></td>
<td></td>
<td>0.5191 0.0066</td>
</tr>
<tr>
<td>( \text{N}_{\text{mic}} )</td>
<td></td>
<td></td>
<td>0.4782 0.0244</td>
</tr>
<tr>
<td>C: ( \text{N}_{\text{mic}} )</td>
<td>-0.4270 0.0475</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EON</td>
<td>-0.5610 0.0066</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease (act)</td>
<td>0.5673 0.0025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatase (pot)</td>
<td>0.5746 0.0033</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease (pot)</td>
<td>0.4721 0.0307</td>
<td>0.5908 0.0024</td>
<td></td>
</tr>
<tr>
<td>Exoglucanase (pot)</td>
<td></td>
<td></td>
<td>0.5554 0.0048</td>
</tr>
<tr>
<td>Endochitinase (pot)</td>
<td></td>
<td></td>
<td>0.5335 0.0073</td>
</tr>
<tr>
<td>Phenoloxidase</td>
<td>-0.3981 0.0359</td>
<td>0.5434 0.0061</td>
<td></td>
</tr>
<tr>
<td>(activity)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peroxidase</td>
<td>-0.4268 0.0235</td>
<td>0.5101 0.0109</td>
<td></td>
</tr>
<tr>
<td>(activity)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross nitrification</td>
<td></td>
<td></td>
<td>0.6380 0.0005</td>
</tr>
<tr>
<td>Gross ( \text{NO}_3^- ) immobilization</td>
<td></td>
<td>0.5336 0.0050</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 11: (A) NH$_4$-N pool in control (black circle) and drought (white circle) plots in µg NH$_4$-N g$^{-1}$DM. The pool peaks in July and late August, but there were no significant changes due to drought. Only in the post treatment period the pool size varied significantly due to sampling day ($P<0.001$). (B) NO$_3^-$ pool in µg NO$_3^-$ g$^{-1}$DM in drought (white circles) compared to control (black circles) plots. Sampling day dynamics were similar to NH$_4$-N and again there was no significant response to drought, sampling day, sampling day x drought or rewetting. The black line in both graphs marks the end of drought with the rewetting event. Statistical tests were performed with multifactorial ANOVA and error bar are calculated with standard error (n=4).
Fig. 12: (A) Actual cellulase activity in µg Glucose g⁻¹DM h⁻¹, which was significantly influenced by sampling day (P<0.01) and the combination of sampling day x treatment (P<0.01). (B) Actual Protease activity in µg AAs-N g⁻¹DM h⁻¹ was neither influenced by sampling day, drought, rewetting or the combination of sampling day x drought. In both graphs drought plots are symbolized by white circles and controls by black circles. The end of drought is marked with a black line. Multifactorial ANOVA was used for statistical analysis and error bars were calculated with standard error (n=4).
Fig. 13: (A) Potential endochitinase, (B) exoglucanase, (C) phosphatase and (D) protease activity in nmol g$^{-1}$ DMh$^{-1}$ in control (black circles) and drought (white circles) plots. All four enzymes showed significant changes due to sampling day (exoglucanase, phosphatase, protease $P<0.0001$; endochitinase $P<0.05$), but no changes due to drought or rewetting. Nevertheless, exoglucanase, phosphatase and protease were significantly influenced by the combined factor of sampling day x drought ($P<0.05$). Error bars are standard error (n=4) and we used multifactorial ANOVA for statistical analysis. The solid black line indicates the end of drought with the rewetting event.
Fig. 14: (A) Phenoloxidase activity and (B) peroxidase activity in µmol g⁻¹DM h⁻¹. Phenoloxidase was highly influenced by sampling day \((P<0.01)\) and in the post treatment period peroxidase activity was significantly lower in drought plots \((P<0.01)\). Both enzymes showed no changes after rewetting and the rewetting event is marked with the solid black line. Error bars were calculated with standard error \((n=4)\) and statistical analysis were performed with a multifactorial ANOVA.
Fig 15: Graphs on the left side show the total amount of (A) fungi-PLFAs (µg C-PLFA g^{-1}DM), (C) gram + PLFAs (µg C-PLFA g^{-1}DM) and (E) gram – PLFAs (µg C-PLFA g^{-1}DM). Drought plots are marked with white circles and control plots with black circles. Neither fungi nor bacteria showed any significant reactions due to drought or rewetting (multifactorial ANOVA). Graphs on the right side show the relative abundance of (B) fungi PLFAs, (D) gram+ PLFAs and (F) gram – PLFAs. Again there were no significant changes due to drought or rewetting (multifactorial ANOVA). The black line marks the end of drought. Error bars are calculated with standard error (n=4).
Discussion

On global scale mountain ecosystems play a crucial role, as they provide a wide range of ecosystem services like the purification of water or erosion protection to the lowlands (Gret-Regamey, Brunner et al. 2012). Mountain ecosystems are forecasted to be strongly affected by global climate change (Koerner 2000) and land management changes. During the last decades a huge areas of agricultural used meadows in alpine ecosystems have been abandoned (Tappeiner, Tasser et al. 2008). Thus, we conducted a drought simulation experiment, followed by a heavy rain event on an abandoned meadow in the Austrian Alps.

Our results showed that net GHG fluxes significantly changed during the two month experimental drought, while the soil processes overall proved to be relatively resistant. Soil water content was highly influenced by drought and showed a slow resilience, as even after the rainshelters were removed, soil moisture in the drought plots remained at a lower level than in controls. One explanation for the weaker wettability in drought plots is increased soil repellency. In moist soils the polar ends of soil organic matter compounds align towards the soil solution and cover the hydrophobic parts (Kleber, Sollins et al. 2007). Under drought these polar compounds possibly bind to each other, by forming hydrogen bonds, and parts of the inner hydrophobic layer can be exposed (Horne and McIntosh 2000). This exposition can lead to higher soil repellency and a minor wettability after drought.

Ecosystem respiration

Ecosystem respiration (R\textsubscript{eco}) strongly decreased under drought, presumably because of morphological changes of plants. We did not asses changes in plant biomass, but such changes were clearly visible and are well documented for alpine meadows. Cantarel (Cantarel, Bloor et al. 2013) showed that a drought period significantly reduced above ground biomass production and, in combination with warming, changed plant traits and functional diversity. Additionally, some studies reported that drought reduced the leaf area index (LAI) of different grass species (Dermody, Weltzin et al. 2007) and that the negative effect of drought was stronger on aboveground biomass of grasses, than on forbs or legumes (Gilgen and Buchmann 2009).
Ecosystem respiration consists of the respiration of autotrophic and heterotrophic organism, therefore being closely linked to the extractable organic carbon (EOC) pool in soil. We expected a strong influence of drought on EOC pool size, because under drought plants should exhibit a lower C fixation rate and reduced root exudations. Our results however suggested only a weak influence of drought on the size of the EOC pool. Sanaullah (Sanaullah, Chabbi et al. 2012) reported, that under drought plants allocated a relatively large amount of assimilates to their root system, where it was incorporated into root biomass and increased root exudations. In our study microbial community structure (Fig.15) and enzyme activity (Fig.12, Fig.13, Fig.14) exhibited no alterations, even though other studies demonstrated that drought, in combination with warming, significantly reduced soil microbial diversity and abundance (Sheik, Beasley et al. 2011). 

$R_{eco}$, as well as EOC pool, significantly increased after rewetting. This pulse of soil respiration is well known as “Birch effect” (Birch and Friend 1956), which can be explained by two contrary hypotheses. The “physical hypothesis” (Utomo and Dexter 1982) claims that the changes between drying and rewetting cycles physically break the soil microstructure and therefore provide new substrate for microorganism. The “metabolic hypothesis” (Halverson, Jones et al. 2000) suggests that solutes, which were build-up by microbes during drought to protect them from dehydration (compatible solutes), are released after rewetting and directly consumed by microbes. Both hypotheses may be an explanation for our observations. The “physical hypothesis” seems to be more likely, as it was shown by Navarro-García (Navarro-Garcia, Casermeiro et al. 2012).

Land use change, especially from cropland to grassland, has a great impact on ecosystem respiration and net CO$_2$ fluxes, because due to the lack of disturbance by tilling and the change to perennial plants with a lower shoot-root ratio, plants can transfer higher amounts of C into their root system, causing a larger root systems and soil organic carbon pool (Frank, Liebig et al. 2006). The abandonment of meadows also lead to changes in aggregate size and formation and a lower amount of carbon is converted into a stable fraction (Meyer, Leifeld et al. 2012). Similar changes may also have taken place in our system.

Our study showed that drought significantly reduced ecosystem respiration, but due to the fact that we excluded photosynthesis with our method, we are not able to give a clear statement about
the influence of drought on the net flux of CO₂. Most likely drought also reduced net uptake rates, because of reduced water availability and structural and functional changes in plants (reduced leaf area and enhanced stomata closure), and therefore may in total reduce the net consumption of CO₂ in the abandoned meadow.

CH₄ net fluxes
CH₄ net consumption significantly increased during drought, intensifying the sink for CH₄. This increase in CH₄ uptake rates is likely due to a higher diffusion rate of atmospheric CH₄ into the drier soil. The molecular diffusion from atmospheric CH₄ is faster in well aerated soils than in wet soils (Striegl 1993), because soil moisture inhibits CH₄ diffusion by blocking micro- and macro-pores (Hartmann, Buchmann et al. 2011). In addition, at high soil moisture, methanotrophs are covered by a thick water film, which forms a diffusion barrier between the surrounding air and the cell (Koschorreck and Conrad 1993) limiting substrate availability. Only at very low soil water concentrations, like in deserts, methanotrophic activity can be limited due to the lack of water (Striegl, McConnaughey et al. 1992). This was not the case in our study. An increase of CH₄ consumption during drought was also shown for other ecosystems, such as forests (Borken, Davidson et al. 2006). In forests the atmospheric CH₄ was consumed by methanotrophs in the A-horizon. Similarly, in mountain grassland the highest methanotrophic activity was reported between 12 and 25 cm (Torn and Harte 1996).

In the literature various responses of CH₄ fluxes to rewetting are reported (Kim, Vargas et al. 2012), but the underlying mechanism are still not well understood. Net uptake rates could decrease, due to an increased activity of methanogenic microbes (Blankinship, Brown et al. 2010) or due to a limitation of the diffusion rate, because of higher soil moisture. Shortly, after rewetting residual CH₄ should be push out of soil, because of the infiltration of pores by water (Yavitt 2013). In our study, however, CH₄ net flux rates exhibited no significant change after rewetting.

The influence of land use change on net CH₄ fluxes is closely coupled with the use of N fertilizer. Methanotrophic activity was shown to decrease with the addition of fertilizer (Alam and Jia 2012), because they microbes switch from CH₄ to ammonia (NH₃) oxidation (Dunfield and Knowles 1995), if a sufficient amount of NH₃ is present. The sensitivity of CH₄ consumption to
fertilizer application strongly depends on the history of use, e.g. managed or formally managed sites showed a stronger response of the methanotrophic community to fertilizer addition than natural sites (Aronson and Helliker 2010).

In summary drought was shown to increase the net consumption of atmospheric CH$_4$.

$N_2O$ net flux

The abandoned meadow net consumed N$_2$O with uptake maxima at around 30 µg N$_2$O-N m$^{-2}$h$^{-1}$ at the end of the growing season, but compared to other ecosystems, such as croplands (wheat, Hidalgo, Mexico, uptake maxima: 484 µg N$_2$O-N m$^{-2}$h$^{-1}$ (Longoria-Ramirez, Carbajal-Benitez et al. 2003)) or some temperate forests (Belgium, uptake maxima: 16 g N$_2$O-N m$^{-2}$day$^{-1}$, (Goossens, De Visscher et al. 2001)), our system consumed only a small amount of atmospheric N$_2$O.

Contrary to our hypothesis that drought will decrease denitrification rates and therefore strengthen the sink, as it was shown for forests (Davidson, Ishida et al. 2004; van Haren, Handley et al. 2005), we detected a decrease in net consumption rates during drought. We found that drought initially decreased ammonium (NH$_4^+$) and nitrate (NO$_3^-$) pools (Fig.11) thereby reducing the substrate availability for N processing groups (i.e. nitrifier, denitrifier, co-denitrifyer and nitrifier denitrification). The initial decrease of NH$_4^+$ and NO$_3^-$ was also accompanied by lower mineralization and nitrification rates.

N$_2$O is either produced during nitrification, when NH$_2$OH is reduced to NO$_2^-$ (Hooper and Terry 1979) or it is produced and consumed during denitrification or by DNRA. A decreased soil water content leads to higher O$_2$ concentrations in soil, which could repress the activity of denitrifier (Boublikova, Kucera et al. 1985), but not that of nitrifier. In the drought plots, compared to controls, denitrification activity was decreased and less N$_2$O was consumed, but it was still produced during nitrification, leading in total to a reduced uptake of N$_2$O.

Another possible explanation for our results can be based on the competition between heterotrophic and autotrophic nitrifying microbes, as it was shown for a mixed culture of Thiosphaera pantotropha (heterotrophic nitrifier) and Nitrosomonas europaea (autotrophic nitrifier) (Vanniel, Arts et al. 1993). Heterotrophic nitrifier can use organic N (Barraclough and Puri 1995) as well as NH$_4^+$ (Focht 1977) and oxidize them to NO$_2^-$ or nitrate (NO$_3^-$). Additionally they also require organic C, such as DOC or plant exudates (Brierley and Wood 2001). Although heterotrophic nitrification probably contributes only to a small extend to total N$_2$O production in
soils (Tortoso and Hutchinson 1990), under drought there competitive strength may decrease compared to autotrophic nitrifier. Under drought some studies showed that less C is transferred into soil via roots (Sanaullah, Chabbi et al. 2012) and heterotrophic activity may be repressed, because they require a 1:1 ratio of N-substrate and dissolved organic carbon (DOC) (Taylor and Townsend 2010). Now autotrophic nitrifier are no longer limited by substrate availability, which can finally lead to an increased N_2O production during nitrification and in total to a decreased uptake in drought plots. Although our results suggested only a trend towards a smaller EOC pool in drought plots, nitrification rates strongly increased at the end of the drought period, indicating a higher activity of autotrophic, as well as heterotrophic nitrifier and therefore a higher production of N_2O.

At the end of the drought period NH_4^+ and NO_3^- pools reached the highest level and were significantly larger in drought plots compared to controls. The high amounts of available substrate were also accompanied by the lowest N_2O consumption rates and the highest nitrification rates, indicating an increased N_2O production, because of nitrifier activity.

Directly after rewetting (1 hour) soil N_2O net fluxes showed a shift from net consumption to net production of N_2O. This change can be explained by several factors: Microbes which died during drought (Cregger, Schadt et al. 2012; Tian, Zhu et al. 2012), provide easily decomposable organic matter (Saetre and Stark 2005). Additionally, rewetting can also disrupt soil aggregates and provide new substrate (Navarro-Garcia, Casermeiro et al. 2012). Together with lower O_2 amounts, caused by higher soil moisture, this increase in available substrates can activate denitrifier and the higher amounts of available nutrients can also stimulate nitrification, leading in total to an increased N_2O production.

N_2O flux rates are also closely linked to land use changes, because N_2O emissions are stimulated by the addition of fertilizer (Kim, Mishurov et al. 2010), and so the change from a grazed to an abandoned meadow can decrease N_2O production and an even switch the meadow from a source into a sink for N_2O.

It is important to note that especially for N_2O fluxes high spatial and temporal variations are reported (Groffman 2012), which may explain the large standard error and weaker significance compared to the other measured gas fluxes. Nevertheless our results suggest that the
abandonment of alpine meadows in combination with prolonged drought periods, can decrease the net consumption of N$_2$O and after a strong rain event it can even switch into a net production.

In conclusion our drought simulation experiment suggests that severe drought events in the future, accompanied by changes in management practice, can have a strong influence on the production and consumption of GHG’s on abandoned meadows in temperate regions. Negative feedbacks may occur between land use change, drought and CH$_4$ net fluxes, leading to an intensification of the potential sink. However, under drought the consumption of atmospheric N$_2$O decreased and if drought periods end with heavy rain events, N$_2$O fluxes may even switch from net consumption to net production.

The underlying soil processes still seem to have a stronger resistance against drought and altered precipitation in winter may be able to buffer some of the effects of summer drought in alpine ecosystems.
Acknowledgements

I want to thank my supervisor Andreas Richter for providing me the opportunity of this master thesis, the critical discussions and his help and support during the work. Special thanks go to Lucia Fuchslueger for her patience with my endless questions, her help with the calculation and interpretation of the data and many hours of shared field and lab work.
I wish to acknowledge the useful discussions about my data and the help with the statistical analysis provided by my colleges Birgit, Daniel, Maria, Flo and Jörg and the technical support by Margarete.

Finally, I wish to thank my parents for their endless encouragement and I gladly dedicate this work to them!
References


Zusammenfassung


Eine treibende Kraft hinter diesen globalen Veränderungen sind klimarelevante Gase, wie Kohlendioxid (CO\(_2\)), Methan (CH\(_4\)), Lachgas (N\(_2\)O) oder Wasserstoff (H\(_2\)). Sie führen, durch die großen Mengen an antrophogen induzierten Treibhausgasen, in den letzten Jahrzehnten zu einer kontinuierlichen Erwärmung der Atmosphäre.

Über den Zusammenhang und mögliche positive oder negative Rückkopplungen zwischen den Änderungen der Landnutzungsform und den Veränderungen im Klima gibt es aktuell kaum Studien.

Zusätzlich zur Dürre wurde noch ein starker Regenfall (20mm) am Ende der Trockenperiode simuliert, um festzustellen, wie groß die Auswirkungen dieses Regens auf die gemessenen Parameter sind.

Zusammenfassend lässt sich für das Treibhausgas-Budget der Brache folgendes Fazit ziehen: Auf Grund der von uns gewählten Methode (lichtundurchlässige PVC-Kammern, die die Photosyntheseaktivität der Pflanzen während der Messung verhinderten) konnten wir nur die ökosystemare Respiration ermitteln. Diese ist unter Dürre signifikant geringer ($P<0.001$) als in den Kontrollflächen, hauptsächlich aufgrund struktureller und funktioneller Änderungen in der Pflanzengemeinschaft, die in den trockengestressten Arealen erheblich weniger oberirdische Biomasse aufgebaut hat. Das Wiederbefeuchten am Ende der Dürre führte zu einem kurzfristigen, sehr starken Anstieg der Respiration („Birch Effect“) ($P<0.001$).

Die Nettoaufnahme von CH$_4$ wurde durch die Trockenheit noch zusätzlich verstärkt ($P<0.001$), bedingt durch eine erhöhte Diffusionrate von atmosphärischem CH$_4$ in den Boden, während durch das Wiederbefeuchten keine signifikanten Änderungen auftraten.

In den trockengestressten Flächen wurde weniger N$_2$O konsumiert, allerdings mit geringer Signifikanz. Dies war möglicherweise durch die hohe Heterogenität der N$_2$O-Aufnahme und – Abgabe bedingt. Durch das Wiederbefeuchten am Ende der Dürre, wurde kurzfristig sogar netto N$_2$O produziert und die Brache wurde von einer Senke von N$_2$O zu einer Quelle von N$_2$O.

Im Gegensatz zu den Gasflüssen, zeigten die zugrundeliegenden Bodenprozesse kaum Veränderungen auf Grund der Dürre und die Brache scheint eine sehr hohe Resistenz gegenüber einer längerer, einmalig auftretende Dürre zu haben. Nur der Wassergehalt des Bodens verringerte sich erheblich ($P<0.001$) während der Trockenperiode und blieb auch nach Ende des Versuches (nach dem Abbau der Zelte) in den vormals trockengestressten Flächen niedriger als in den Kontrollflächen. Die Zusammensetzung der mikrobiellen Gemeinschaft zeigte keine starke Reaktion auf die Dürre, weder im Verhältnis der Pilze zu Bakterien, noch im Verhältnis der gram+ zu den gram- Bakterien.
Curriculum vitae

Personal Information

Name: Sandra Kienzl
Date of Birth: 17.12.1988
Address: Strindbergweg 4
          4040 Linz
Citizen: Austria

Education

1995-1999: Primary School Linz, VS 25
1999-2007: Europagymnasium Auhof, Linz
June 2007: Matura (university entrance diploma)
2007-2010: Bachelor of Biology, University of Vienna
July 2010: Bachelor thesis: “Limitation of heterotrophic respiration in an alpine ecosystem”
           (Department of Terrestrial Ecology)
2010-2013: Master of “Microbial, Molecular and Chemical ecology”,
           University of Vienna
           (Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research)

Working experience

                              Department of Terrestrial Ecology, University of Vienna
Winter term 2012: Tutor of the course “Chemische Methoden der Ökologie”
                  Department of Terrestrial Ecology, University of Vienna