UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA
DEPARTMENT OF FOREST AND SOIL SCIENCES
INSTITUTE OF SOIL RESEARCH

Doctoral thesis

Impact of Extreme Weather Events on Soil Nitrogen Cycling and Greenhouse Gas Emissions

submitted by

Mag. Sonja Leitner

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Supervisor: Prof. Dr. Sophie Zechmeister-Boltenstern

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“Climate change does not respect border; it does not respect who you are - rich and poor, small and big. Therefore, this is what we call 'global challenges,' which require global solidarity.”

— Ban Ki-moon

TABLE OF CONTENTS

I. Abstract 5

II. Zusammenfassung 7

1. General introduction 9

1.1. Climate change and extreme weather events 9
1.2. The role of soils for the global greenhouse gas budget 11
1.3. Influence of drying and rewetting on soil greenhouse gas fluxes 15
1.4. Scientific and practical relevance of the study 18

2. Aims & outline of the thesis 20

2.1. Research questions & hypotheses 20
2.2. Thesis outline 22

3. Results 25

3.1. Paper #1: Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest 25
3.2. Paper #2: Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying-rewetting events 41
3.3. Paper #3: Repeated extreme drought and rainfall events reduce soil respiration and affect its temperature and moisture sensitivity 51
3.4. Paper #4: Linking NO and N2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils 83

4. Conclusions and remark 95

5. References 98

6. Acknowledgements 106

7. Curriculum vitae 107

I. ABSTRACT

Climate change research anticipates a shift in global precipitation patterns and an increase in the frequency and intensity of extreme weather events like severe droughts and heavy rainstorms. Changes in precipitation affect soil moisture, which is one of the main determinants of soil nutrient cycling and greenhouse gas (GHG) emissions. However, recent studies suggest that current soil moisture responses of GHG emissions cannot be extrapolated to future climate scenarios. This PhD thesis targeted this knowledge gap and focused on the influence of extreme events on soil GHG emissions and nitrogen (N) cycling.

First, the contribution of litter layer to total soil GHG emissions was assessed in a litter-removal experiment in an Austrian beech forest. The litter layer contributed 30 % to total soil respiration (R_s) and influenced its temperature sensitivity. Furthermore, litter removal increased soil CH_4 uptake by 16 %. In soil with intact litter layer, N_2O emissions peaked after rainfall events, but these peaks were absent when the litter layer was removed. These findings improve our understanding of forest soil biogeochemistry and should be accounted for in C models, given that litter represents an important component of C input to soils.

To examine the impact of repeated severe drying-rewetting cycles on R_s and soil N cycling, a precipitation manipulation experiment was conducted in an Austrian beech forest in 2013 and 2014 during the vegetation period (May-October). Drought generally inhibits the activity of soil microorganisms and plant roots, reducing the production of CO_2 in soil, whereas rewetting often leads to disproportionately high CO_2 efflux rates. In the present study, the drought-induced reduction in R_s was not compensated by rewetting CO_2 pulses, leading to a reduction in total R_s in soil subjected to repeated severe drying-rewetting cycles compared to controls receiving natural rainfall. Furthermore, repeated severe drying-rewetting cycles shifted the climate sensitivity of R_s from temperature-dependence to moisture-dependence. During the last irrigation in 2014, soil microdialysis was used to follow the response of soil N movement to rewetting. Results showed that upon rewetting, NO_3^- and amino acids diffused through the soil, and the rewetting N flush was larger if the preceding drought period had been longer. This indicates that N accumulates in dry soil and can be mobilized upon rewetting, which bears the potential of N leaching loss when a severe drought is followed by heavy rainfall.

The last part of this thesis describes a rewetting experiment that was conducted in a semi-arid grassland in California, USA, simulating a rain shower at the transition from dry to wet season. Soil microdialysis was used in combination with high-resolution GHG flux measurements to follow the dynamics of soil N diffusion and emissions of NO and N_2O. Immediately upon rewetting, N diffused through the soil, with NO_3^- contributing ~80 % to total N diffusion, and NO and N_2O flux increased. This

soil rewetting N flush was short-lived and disappeared after 2 h, probably indicating high immobilization rates of the resuscitated microbial community. 27 h after rewetting, NH_4^+ diffusion increased, coinciding with peak N-gas emissions, which indicated that at this time point microbial nitrification was the primary NO and N_2O production pathway. This is the first study that combined microdialysis measurements with the determination of GHG emissions to link aboveground and belowground N cycling processes. Our results corroborate the theory that N compounds accumulate in dry soil, and, upon rewetting, can be mobilized and fuel GHG emissions from soil.

In conclusion, this PhD thesis showed that although natural ecosystems are buffered against weather fluctuations, extreme events like severe drought and heavy rainfalls can affect soil GHG emissions and N cycling. Extreme weather events can trigger rapid and disproportionate responses that are short in duration but can contribute substantially to ecosystem C and N cycling. Models that predict ecosystem C balance under a changing climate need to account for the duration and frequency of drought periods and heavy rainfall events.

Keywords: extreme events, drought, CO_2, CH_4, N_2O, NO, microdialysis

II. ZUSAMMENFASSUNG

Durch den Klimawandel verschiebt sich die globale Verteilung des Niederschlags, wodurch Extremwetterereignisse wie Dürre und Starkregen häufiger werden und an Intensität zunehmen. Eine Veränderung des Niederschlagsmusters beeinflusst die Bodenfeuchte, welche wiederum den Nährstoffkreislauf im Boden und die Emission von Treibhausgasen (THG) aus dem Boden kontrolliert. Neueste Studien warnen jedoch davor die Zusammenhänge zwischen Bodenfeuchte und THG Emissionen, die unter gegenwärtigen Bedingungen gemessen werden, auf zukünftige Klimaszenarien zu extrapolieren. Die vorliegende Dissertation versucht, diese Wissenslücke ein stückweit zu schließen und untersucht den Einfluss von Extremwetterereignissen auf den Stickstoffumsatz im und THG Emissionen aus dem Boden unter kontrollierten Bedingungen.

Zunächst wurde untersucht, wie viel die Streuschicht zu den gesamten Bodentreibhausgasemissionen beiträgt. Hierzu wurde die Streuschicht in einem österreichischen Buchenwald entfernt und THG Emissionen mit jenen aus dem intakten Bodenprofil verglichen. Es zeigte sich, dass die Streuschicht 30 % zur Bodenatmung beitrug, und auch die Temperatursensitivität der Bodenrespiration beeinflusste. Außerdem stieg durch das Entfernen der Streuschicht die CH_4 Aufnahme durch Bodenmikroorganismen um 16 %. In Versuchsflächen mit intakter Streuschicht kam es nach Regenfällen zu N_2O Emissionen, in Boden ohne Streuschicht wurden diese jedoch nicht beobachtet. Die Ergebnisse dieser Studie verbessern unser Verständnis der Biogeochemie des Waldbodens und sollten außerdem in Kohlenstoffmodellen berücksichtigt werden, da die Laubstreu einen wichtigen Eintrag von Kohlenstoff in den Boden darstellt.

Um den Einfluss von wiederholten extremen Dürre-Niederschlags-Zyklen auf die Bodenrespiration und den Stickstoffkreislauf zu untersuchen wurde der Niederschlag mit Dächern und einer Bewässerungs-anlage in einem österreichischen Buchenwald in den Jahren 2013 und 2014 während der Vegetationsperiode (Mai-Oktober) manipuliert. Trockenheit reduzierte die Aktivität von Mikroorganismen und Wurzeln im Boden, wodurch die Bodenatmung sank, während Wiederbefeuchtung oft zu überproportionaler CO_2 Ausgasung führte. In der vorliegenden Studie war die Verringerung der Bodenatmung während der Trockenheit stärker als die Ausgasung während der Bewässerung, was in Folge zu einer verringerten Gesamtatmung des Bodens durch wiederholte extreme Dürre-Niederschlags-Zyklen führte. Außerdem verschob sich die Klimasensitivität der Bodenatmung von trockensensitiv zu feuchtesensitiv. Während der letzten Bewässerung im Jahr 2014 wurde die Reaktion des mobilen Bodenstickstoffs auf die Wiederbefeuchtung mit Hilfe der Bodenmikrodialyse-technik untersucht. Kurz nach der Bewässerung diffundierten Nitrat und Aminosäuren durch den Boden, wobei mehr Stickstoff freigesetzt wurde, wenn die vorangegangene Trockenperiode länger gewesen war. Das bedeutet, dass sich Stickstoff in trockenem Boden anreichert

und durch Wiederbefeuchtung freigesetzt werden kann, was die Gefahr einer Stickstoffauswaschung birgt, wenn ein Starkregenereignis auf eine lange Trockenperiode folgt.

Der letzte Teil der Arbeit beschreibt ein Beregnungsexperiment in einem semiariden Grasland in Kalifornien, USA, wo ein Regenguss am Übergang von Trocken- zu Regenzeit simuliert wurde. Die Dynamik der Stickstoffdiffusion im Boden und der Emissionen von NO und N_2O wurden mittels Bodenmikrodialyse in Kombination mit zeitlich hochaufgelösten THG-Messungen untersucht. Unmittelbar nach der Bewässerung war die Stickstoffdiffusion im Boden erhöht, wobei ~80 % des Stickstoffs in Form von Nitrat vorlag. Gleichzeitig stieg die Ausgasung von NO und N_2O stark an. Die Stickstoff-freisetzung im Boden war nur von kurzer Dauer und verschwand nach 2 h, was auf eine erhöhte Stickstoffaufnahme durch Bodenmikroorganismen hindeutet. 27 h nach der Bewässerung stieg die Diffusion von Ammonium stark an, und zeitgleich wurden die höchsten NO und N_2O Emissionen gemessen, was darauf hindeutet, dass diese beiden Gase hauptsächlich durch Nitrifikation gebildet wurden. Diese Studie ist die erste, die Bodenmikrodialyse mit THG-Messungen kombiniert, um die Zusammenhänge zwischen über- und unterirdischen Stickstoffprozessen zu untersuchen. Des Weiteren untermauern diese Ergebnisse die Theorie, dass sich Stickstoff in trockenem Boden anreichert und bei Wiederbefeuchtung freigesetzt werden kann, was die THG Produktion im Boden antreibt.

Obwohl Ökosysteme Wetterschwankungen ausgleichen können, zeigt die vorliegende Dissertation, dass Extremwetterereignisse wie Dürre und Starkregen Bodentreibhausgasemissionen und den Stickstoffkreislauf stark beeinflussen. Extremereignisse lösen abrupte und überproportionale Reaktionen aus, die zwar oft nur von kurzer Dauer sind aber dennoch erhebliche Auswirkung für den Kohlenstoff- und Stickstoffkreislauf eines Ökosystems haben können. Modelle zur Vorhersage von Kohlenstoffbilanzen unter veränderten Klimaszenarien sollten daher sowohl Länge als auch Häufigkeit von Trockenperioden und Starkregenereignissen berücksichtigen.

Schlüsselworte: Extremereignisse, Dürre, CO_2, CH_4, N_2O, NO, Mikrodialyse

1. GENERAL INTRODUCTION

1.1. CLIMATE CHANGE AND EXTREME WEATHER EVENTS

Climate change is caused by the anthropogenic disturbance of the global carbon (C) and nitrogen (N) cycle (Bindoff *et al.*, 2013). Human activities have led to an increase in atmospheric concentrations of the greenhouse gases (GHG) carbon dioxide (CO_2, +40 %), methane (CH_4, +150 %) and nitrous oxide (N_2O, +20 %) compared to pre-industrial times (Ciais *et al.*, 2013). After water vapor, these gases are the driving force behind the "greenhouse effect" that makes our planet inhabitable. However, anthropogenic actions like the use of fossil fuels, land-use change, forest conversion, or agricultural practices add additional GHGs to the atmosphere, which disturbs the fine-balanced global C and N cycle and enhances the greenhouse effect. The human-induced increase in atmospheric GHG concentrations is causing a rise in global surface temperature, which has tremendous impacts on the biosphere. Climate projections estimate that mean global surface temperature will increase between 2.6 °C and 4.8 °C compared to the 1981-2005 average by the end of the 21st century if no measures are taken to reduce GHG emissions (CMIP5-RCP8.5 model, Collins *et al.*, 2013, p.1055). Some regions including northern mid latitudes and the Arctic will warm more rapidly than the global mean, with temperature increases up to 11 °C by the end of the 21st century. Scientists agree that globally all social, biological and geophysical systems will be massively disturbed by a global mean temperature increase above 4 °C (IPCC, 2014). To combat climate change, policymakers have recently adopted the ambitious goal to hold the rise in global average temperature below 2 °C, and to pursue efforts to keep it even below 1.5 °C compared to pre-industrial levels, which was laid down in the Paris Agreement in 2015 (COP21, 2015). Among strategies for the mitigation of climate change, Intended Nationally Determined Contributions (INDCs) include annual reports of national GHG budgets, which require precise and robust estimations of natural and anthropogenic GHG fluxes. Furthermore, to develop reasonable mitigation and adaptation strategies, reliable projections of future GHG emissions are needed. However, it has recently been stressed that (i) results from studies investigating GHG fluxes under current climatic windows cannot be extrapolated to future climate scenarios (Vicca *et al.*, 2014), and (ii) that there is a knowledge gap concerning ecosystem responses to extreme changes in climate (De Boeck *et al.*, 2015). It was recommended to explicitly include extreme events in future climate change experiments, considering timing and intensity of global change factors, and evaluating potential thresholds or ecosystem "tipping points" (Rustad, 2008).

Apart from an increase in global mean temperature, climate change will lead to a shift in precipitation patterns. There is convincing evidence that within the 21st century, extreme weather events (*i.e.* weather events like dry spells and heavy rainstorms that are rarer than the 10th or 90th percentile of the observed probability function) will become more frequent in most regions of the globe (Kirtman *et*

al., 2013; Fischer & Knutti, 2014). In this context, the precipitation regime in Central Europe is expected to shift, which will lead to an increase in precipitation and a higher flood risk in the winter months, and a decrease in precipitation in the summer months with more exceptional summer droughts and heat waves (Kromp-Kolb *et al.*, 2014). In addition, the days of heavy rain events (>30 mm day^{-1}) between May and September will increase. Given that moisture is one of the most important controls of biological processes, this has tremendous implications for terrestrial ecosystems, which hold the largest global organic C pool (Xu & Shang, 2016). Furthermore, it has been widely agreed that feedback effects between altered precipitation and changed GHG emissions from natural and anthropogenic ecosystems could further intensify climate change and might annihilate all human effort to reduce CO_2 emissions (Reichstein *et al.*, 2013). Hence it is apparent that understanding the influence of extreme events and shifting precipitation regimes on the terrestrial C and N cycle is urgently needed.

There are many natural and anthropogenic sources and sinks of GHGs, which are ultimately linked to the global C and N cycle. The annual C fluxes between the atmosphere and the terrestrial biosphere are enormous, with an uptake of 123 Pg C yr^{-1} fixed by terrestrial gross primary production (GPP), and emissions of 119 Pg C yr^{-1} originating from respiration and fire (Ciais *et al.*, 2013). At the moment, terrestrial systems are net C sinks, fixing a total of 4.3 Pg C yr^{-1} (Le Quere *et al.*, 2009). Considering the global C balance, the land C sink alleviates some of the anthropogenic CO_2 emissions that originate from fossil fuel combustion and cement production (+7.8 Pg C yr^{-1}) and net land-use change (+1.1 Pg C yr^{-1}) (Houghton *et al.*, 2012). However, there is evidence that climate change could decrease the strength of the land C sink if CO_2 emissions from respiration increase faster with rising temperatures than CO_2 uptake by net primary production (Meehl *et al.*, 2007). As a result, a larger fraction of anthropogenic CO_2 will remain in the atmosphere. Furthermore, it was shown recently that a few extreme weather events dominate global interannual variability of GPP, with most of the negative impacts being attributed to a decrease in photosynthetic activity due to water scarcity (Zscheischler *et al.*, 2014). If extreme events like droughts and storms exceed thresholds of ecosystem coping capacity (Figure 1), this can result in severe disturbances and lead to irreversible shifts in C and N cycling processes (Frank *et al.*, 2015), which can increase GHG emissions from natural ecosystems (Kurz *et al.*, 2008). While previous studies have examined how processes respond to gradual changes

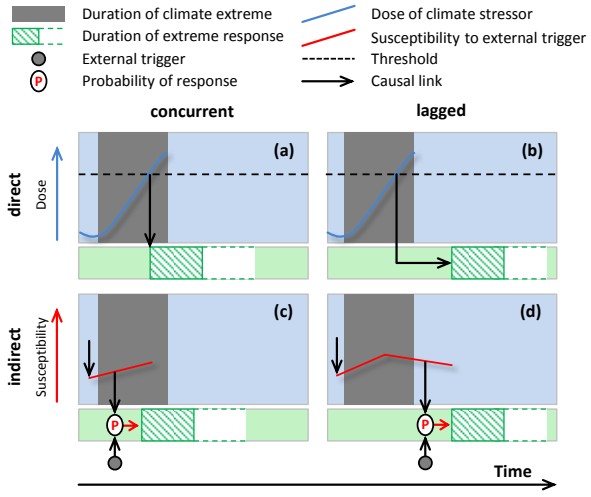

Figure 1: Direct concurrent and lagged (a, b) and indirect concurrent and lagged (c, d) impacts of climate extremes and corresponding extreme ecosystem responses. In the direct case, the extreme impact occurs if a threshold is reached, that is a critical dose (blue line) is passed. In the indirect case, the climate extreme increases the susceptibility (red line) to an external trigger. Concurrent responses start during the climate extreme, but may last longer for indefinite time (dashed extensions of green boxes). Lagged responses only happen after the climate extreme. Figure redrawn after Frank *et al.* (2015), CC BY 4.0 © 2015.

in climate parameters within current climatic windows, there are many uncertainties about the vulnerability of ecosystems to extreme events, and about the thresholds of ecosystem resistance and resilience beyond which system functioning is irreversibly disrupted (Beier *et al.*, 2012).

1.2. THE ROLE OF SOILS FOR THE GLOBAL GREENHOUSE GAS BUDGET

Soil C cycle

Soils contain more C than plant biomass and the atmosphere combined (Jobbágy & Jackson, 2000), with current estimates of the terrestrial soil C stock ranging from 1,500-2,400 Pg C, with another 1,700 Pg C in permafrost soils (Ciais *et al.*, 2013). The largest fraction of soil C is stored as soil organic matter (SOM), which accumulates when C inputs to the soil via plant litter and microbial residues exceed SOM decomposition. Soil organic matter is considered to be relatively stable over long time spans (centuries to millennia) because it is either stabilized via association with clay minerals, protected in soil aggregates and spatially inaccessible, or biochemically hard to decompose (Six *et al.*, 2002; Mikutta *et al.*, 2006). However, even small shifts in the balance between uptake and loss of soil C due to changes in temperature or moisture could trigger rapid and enormous increases in CO_2 and CH_4 emissions to the atmosphere (Christensen *et al.*, 2004; Davidson & Janssens, 2006). Therefore, recent research recognizes the need to manage C flows rather than C stocks to enhance C sequestration and maintain SOM storage (Lehmann & Kleber, 2015). Given that decomposition of SOM is tightly controlled by temperature and moisture (Curiel Yuste *et al.*, 2007; Conant *et al.*, 2011), there is urgent need to quantify the impact of shifts in precipitation patterns on SOM mineralization.

Soil respiration is the release of CO_2 from soil, which is formed during the oxidation of C-containing compounds (*e.g.* sugars, amino acids, lipids) for the production of energy to support life (catabolism). Depending on the source of C that is being oxidized, soil respiration is divided into (i) autotrophic respiration, which uses freshly assimilated C from photosynthesis and includes root respiration and also respiration of root-associated microorganisms (mycorrhiza and rhizospheric microorganisms), and (ii) heterotrophic respiration of saprotrophic organisms that mineralize litter and SOM. Root and microbial respiration respond differently to temperature and moisture (Epron *et al.*, 2001; Högberg *et al.*, 2001), which is important because the balance between SOM mineralization and CO_2 fertilization effects on photosynthesis drive future changes in the global C cycle. Decomposition or formation of SOM strongly depends on decomposer carbon-use efficiency (CUE), which is defined as the ratio of growth over total C uptake. High CUE promotes growth and C stabilization in soils, while low CUE increases respiration and C loss. Research shows that CUE in terrestrial ecosystems is temperature and moisture sensitive: there is evidence that warmer conditions lead to lower CUE and decreases in soil C storage (Manzoni *et al.*, 2012), and it was also shown that CUE can decrease with increasing drought duration (Tiemann & Billings, 2011). A negative effect of dry conditions on CUE seems logical because (i) substrate diffusion is strongly determined by soil water content (Or *et al.*, 2007), and (ii) water limitation requires increased C allocation for stress protection (Fierer & Schimel, 2003), decreasing the

amount of C available for growth. Given the importance CUE for C sequestration, understanding the moisture dependency of soil respiration is highly relevant for climate change research.

On the other end of the soil moisture curve, water-saturated conditions lead to a switch in microbial metabolism from aerobic to anaerobic pathways with different end products, including acetate and CH_4 (Burgin *et al.*, 2011). Due to the lack of O_2 these end products are not completely oxidized, which leads to a decrease in CUE (Šantrůčková *et al.*, 2004). However, water saturation decreases total C mineralization, leading to an accumulation of organic matter in water-logged soils. Methane fluxes from soils are the net balance of two antagonistic but related processes: (i) methanogenesis by strictly anaerobic archaea in water-saturated soil layers, and (ii) CH_4 oxidation by methanotrophic bacteria in aerated soil layers, who can consume up to 90 % of the CH_4 produced by methanogens in the same soil (Le Mer & Roger, 2001). Furthermore, recent studies report an anaerobic CH_4-oxidizing pathway that uses NO_3^- or sulfate (SO_4^{2-}) as electron-acceptor and was described in marine bacteria (Raghoebarsing *et al.*, 2006) and archaea (Wang *et al.*, 2014), but its relevance for upland forest soils is unclear. In terms of atmospheric CH_4 concentrations, soils are both sources and sinks of CH_4: ~30 % of global CH_4 emissions originate from natural wetlands (177-284 Tg CH_4 yr^{-1}) and rice fields (33-40 Tg CH_4 yr^{-1}), whereas CH_4 oxidation in well-aerated upland soils consumes 9-47 Tg CH_4 yr^{-1} (Ciais *et al.*, 2013). However, the large variability in these numbers displays the level of uncertainty associated with the assessment of CH_4 source and sink strengths of soils. For example, it was estimated that ~29 Tg of the annually emitted CH_4 is re-oxidized to CO_2 within soils, but the calculated uncertainty range was very wide, ranging from 7 up to 100 Tg CH_4 yr^{-1} (Smith *et al.*, 2000). Given the fact that CH_4 is a very potent GHG, and bearing in mind that within the 21st century thawing of permafrost soils due to rapid climate warming in northern regions could dramatically increase soil CH_4 emissions (Koven *et al.*, 2011), there is urgent need for reliable CH_4 flux data from soils. Furthermore, soil CH_4 emissions are highly susceptible to climate change and have been shown to coincide with temperature and precipitation anomalies (Dlugokencky *et al.*, 2009). This complicates the projection of future CH_4 budgets and underlines the need for CH_4 flux measurements under future climate scenarios.

Soil N cycle

The C cycle is tightly linked to the N cycle, because plants require N to fix C and produce biomass, and soil microorganisms degrade plant litter and SOM to acquire N for growth and enzyme production (Elser *et al.*, 2000). In many ecosystems primary productivity is N limited (Vitousek & Howarth, 1991), and plants and soil microorganisms are thought to compete for N (Rennenberg & Dannenmann, 2015). Therefore, SOM stabilization and mineralization are key drivers of soil N cycling. However, some intermediates of the N cycle are highly reactive and, when present in excess, can have negative impacts including eutrophication, groundwater and air pollution, toxicity, acidification, biodiversity loss, and global warming. Since the beginning of industrialization, humans are transforming the global N cycle at an ever accelerating rate, which has massive consequences for the C cycle and the radiative forcing of the atmosphere (Ciais *et al.*, 2013). After introducing the Haber-Bosch process that allows fixation of

atmospheric dinitrogen (N_2), the human creation of reactive nitrogen (N_r, including but not limited to ammonium [NH_4^+], ammonia [NH_3], nitrate [NO_3^-], nitrite [NO_2^-], nitric oxide [NO], nitrogen dioxide [NO_2], nitrous oxide [N_2O]) has rapidly increased, exceeding natural production since the 1970s (Galloway *et al.*, 2008). In 2010, N fixation through Haber-Bosch (120 Tg N yr^{-1}) was double the natural terrestrial sources of N_r (63 Tg N yr^{-1}) (Fowler *et al.*, 2013). Essentially all of the anthropogenic N_r is spread into the environment, either immediately after creation (fossil fuel combustion) or after its use in food production (fertilizer) and industry. Use of nitrogenous fertilizers is responsible for N_2O emissions of 1.7-4.8 Tg N yr^{-1}, originating mostly from enhanced rates of nitrification and denitrification in soils. Burning of fossil fuels and biomass emits an additional 0.4-2.8 Tg N yr^{-1}. In comparison, natural N_2O emissions from soils, oceans and the atmosphere together account for 5.4-19.6 Tg N yr^{-1} (Ciais *et al.*, 2013). Furthermore, it was shown that soil processes and burning of fossil fuels contribute equally to global fluxes of NO (Davidson & Kingerlee, 1997), which is an air pollutant and a secondary GHG that can lead to the formation of tropospheric ozone (O_3) (Lammel & Grassl, 1995). Once anthropogenic N_r is introduced into the environment, it also affects natural ecosystems via atmospheric N deposition, which is responsible for additional N_2O emissions in the range of 0.4-1.3 Tg N yr^{-1} (Ciais *et al.*, 2013). Some studies suggest that globally, most ecosystems are already affected to some degree by N deposition, including remote and uninhabited regions (Bobbink *et al.*, 2010; Holtgrieve *et al.*, 2011). While the manifold consequences of increasing anthropogenic N_r loads have been in the focus of many national and international research programs, less emphasis has been placed on interactions between the global N and C cycle, especially in the context of climate change (Gruber & Galloway, 2008).

Processing of total N_r accounts for 100 Tg N yr^{-1} in the atmosphere and 240 Tg N yr^{-1} in terrestrial ecosystems (Fowler *et al.*, 2013). In natural soils, plant litter represents the most important N input. After senescence or death, plant biomass is mineralized by decomposer organisms, and during decomposition N undergoes various transformation processes. Nitrogen can be removed from the active N_r pool when it is incorporated into microbial biomass and stabilized into SOM via microbial residues. However, N_r can also be lost from soil via leaching (aqueous loss) or emission (gaseous loss). There is a multitude of N-transforming processes that can lead to the formation or consumption of N_2O and NO, as reviewed by Butterbach-Bahl *et al.* (2013) for N_2O and Pilegaard (2013) for NO. Briefly, during mineralization of plant litter or SOM, organic N compounds (*e.g.* amino acids) are decomposed into NH_4^+ (ammonification). In the presence of O_2, NH_4^+ can be oxidized to NO_2^- (ammonia oxidation) and further to NO_3^- (nitrite oxidation) during autotrophic or heterotrophic nitrification. Under O_2-limiting conditions, microorganisms can use nitrogen oxides as alternative electron acceptors, and as a result NO_3^- or NO_2^- are consecutively reduced to NO, N_2O, and N_2 (denitrification). Nitrification and denitrification can also co-occur, either during coupled nitrification-denitrification by distinct microorganisms that coexist in adjacent aerobic (nitrifier) and anaerobic (denitrifier) microsites (Wrage *et al.*, 2001), or during nitrifier denitrification within the same nitrifier organism under aerobic conditions (Colliver & Stephenson, 2000). Aerobic nitrifier denitrification might be especially relevant for NO and N_2O formation in forest soils (Pilegaard, 2013). Organic N compounds can also be co-

denitrified with NO or N_2O to N_2. In addition to biological transformations, there are also abiotic processes that can contribute to NO and N_2O formation, like (i) chemo-denitrification of NO_2^-, which has been shown to produce substantial amounts of NO in semi-arid drylands (Homyak *et al.*, 2016), (ii) chemical decomposition of hydroxylamine (NH_2OH), or (iii) surface decomposition of ammonium nitrate (NH_4NO_3).

Taken together, microbial nitrification and denitrification in soils contribute ~70 % to global N_2O emissions (Syakila & Kroeze, 2011). Generally, both NO and N_2O are produced by the same processes, but the exact NO:N_2O ratios are unknown (Pilegaard, 2013). Following the conceptual "hole-in-the-pipe" model (Firestone & Davidson, 1989), the amount of N-gas production depends on (i) the rates of N transformation processes (the amount of "fluid" running through the pipe, as metaphor for the amount of N that is transformed), and (ii) on the control of relative proportion of end products (the "holes" in the pipes that represent the fractions of NO and N_2O that "leak out" before complete reduction to N_2). Nitrification rates are regulated primarily by O_2 and availability of reduced N (NH_4^+ or organic N compounds), and denitrification rates are regulated by O_2, oxidized N (NO_2^-, NO_3^-, organic N) and the availability of organic C. All of these controlling factors (substrate diffusion and aeration) are strongly dependent on soil water content. Furthermore, soil moisture also controls the ratio of NO:N_2O emissions. When NO and N_2O diffusion is restricted by high soil moisture, emissions of these gases are reduced and complete denitrification to N_2 increases. Therefore, total N-gas emissions are dominated by NO at low water contents, because it is the more oxidized of the two gases. In moist to wet soils, N_2O is the most important end product, and in very wet to water-saturated soils, complete denitrification to N_2 prevails (Davidson & Verchot, 2000). Because of the importance of soil moisture for N transformation processes, NO and N_2O formation and consumption processes will very likely be affected by global warming and changes in precipitation patterns. However, there is lack of knowledge about possible climate feedback effects and the impact of extreme weather events on N transformation processes.

Contribution of litter layer to total soil greenhouse gas emissions

While total GHG emissions from soils of different ecosystems have been extensively studied in the last decades, the influence of the litter layer on GHG emissions from mineral soil and the contribution of emissions originating from the litter layer have received less attention. However, there is evidence that the litter layer can contribute substantially to total soil GHG emissions. For example, respiration of decomposing plant litter can contribute between 8-30 % to total soil CO_2 emissions in temperate forests (Bowden *et al.*, 1993; Ngao *et al.*, 2005; Sulzman *et al.*, 2005). Plant litter substantially affects soil nutrient cycling, and these effects are mediated largely by the microbial community (Grayston & Prescott, 2005). The litter-inhabiting microbial community in beech forests is dominated by fungi that can decompose cellulose and lignin (Schneider *et al.*, 2007), and it seems plausible that the litter layer also affects the fungi:bacteria (F:B) ratio in the mineral soil. Because most fungi have a higher C use efficiency (CUE) than bacteria (Keiblinger *et al.*, 2010), a shift in the F:B ratio is likely to affect soil CO_2

emissions. Furthermore, Dong *et al.* (1998) showed that up to 50 % of N_2O emissions originated from the leaf litter/humus layer in a German beech forest, and Regina (1998) reported that the litter layer produced as much as 75 % of NO emitted from a boreal peatland. Furthermore, litter represents an important source of dissolved organic C (DOC) that can be transported to the mineral soil via water percolation (Michel & Matzner, 1999), and it is well established that the availability of labile C is an important determinant of denitrification. In line with this, it was shown that labile C leaching from litter layer to mineral soil promoted N_2O emissions in tropical ecosystems, which was attributed to either a direct result of the stimulation of heterotrophic denitrifiers or an indirect effect of increased heterotrophic O_2 consumption and formation of anaerobic microsites (Wieder *et al.*, 2011). Furthermore, N_2O fluxes were shown to be negatively related to litter C:N in tropical dry forests (Erickson *et al.*, 2002) and temperate grasslands (Hungate *et al.*, 1997). However, the contribution of the litter layer itself to total soil emissions of N oxides from temperate forests as well as the importance of DOC leaching from litter to mineral soil is not well-studied. Similarly, the importance of the litter layer for soil CH_4 emissions is still poorly understood. Soils of upland forests act mostly as CH_4 sinks through the consumption of CH_4 by methanotrophic bacteria. Litter itself does most likely not produce or consume CH_4 (Smith *et al.*, 2000; Gritsch *et al.*, 2016), but the litter layer can act as diffusion barrier and decrease soil CH_4 oxidation capacity, which is particularly relevant for broad leaf tree species like beech (*Fagus sylvatica* L.) (Brumme & Borken, 1999). However, if the litter layer also influences CH_4 emissions via nutrient leaching to the mineral soil has not been studied. Partitioning the contribution of litter and mineral soil to total soil GHG fluxes as well as improving our understanding on how the litter layer influences soil processes and microbial communities will help to reduce uncertainties in biogeochemical models and improve our forecasts of future GHG budgets for terrestrial ecosystems.

1.3. INFLUENCE OF DRYING AND REWETTING ON SOIL GREENHOUSE GAS FLUXES

Soil C and N cycling processes are influenced by soil temperature and soil moisture (Davidson *et al.*, 2000; Borken & Matzner, 2009). After soil temperature, soil moisture has been reported to be the second-most important driver of CO_2 emissions (Skopp *et al.*, 1990), especially when above or below the optimum moisture range. For CH_4, soil moisture is of special relevance given that soil-atmosphere CH_4 flux is the net balance of anaerobic methanogenesis in water-saturated soil and aerobic CH_4 oxidation in well-aerated soils, with the latter dominating net CH_4 fluxes in upland forests (Hanson & Hanson, 1996; Le Mer & Roger, 2001). However, heavy rainfall and the resulting water saturation of soil can disturb CH_4 oxidation and turn upland forests into CH_4 sources (Megonigal & Guenther, 2008). Furthermore, the CH_4-oxidizing capacity of soils is highest at intermediate water-saturation levels and can be reduced by drought (Xu & Luo, 2012). As for N-bearing gases, drought is thought to drive a shift from N_2O emissions, which are primarily driven by denitrification under sub-oxic conditions, towards increased NO emissions from nitrification. Furthermore, rewetting of dry soil was shown to increase NO emissions within minutes due to abiotic chemo-denitrification of nitrite (Galbally *et al.*, 2008; Homyak *et al.*, 2016). Recent research recognizes the complexity of N cycling and the multitude of

biological processes that can lead to NO and N_2O emissions and that might respond differently to changes in soil moisture (Butterbach-Bahl *et al.*, 2013; Pilegaard, 2013; Medinets *et al.*, 2015). Because soil moisture is one of the primary controls of NO and N_2O emissions, changes in precipitation patterns will most likely lead to shifts in NO and N_2O emission budgets.

While arid, semi-arid, Mediterranean, and some tropical regions are characterized by a distinct dry season and episodic rewetting, even temperate and boreal climate regions, where rainfall is erratic and distributed over the entire year, can experience days or weeks without precipitation. The responses of soil processes to drying and rewetting are complex and include changes in microbial physiology (Placella & Firestone, 2013) and community composition (Landesman & Dighton, 2011) as well as chemical and physical processes (Homyak *et al.*, 2016). Because microorganisms cannot escape changing environmental conditions, they have developed mechanisms of resistance and resilience to water stress, which affects microbial resource allocation (Figure 2). This includes the formation of thick peptidoglycan cell walls in gram-positive bacteria (Wallenstein & Hall, 2012), and the formation of osmo-regulatory substances or "osmolytes" (Csonka, 1989; Warren, 2014) to resist loss of cytoplasmatic water. Filamentous fungi that can access deeper and moister soil regions or ground water with their mycelium are more tolerant against water stress than bacteria (Harris, 1981). Some microorganisms might form endospores or enter a state of dormancy to survive dry spells (Chen & Alexander, 1973). Taxa that are resilient to water stress might be more susceptible to cell death, but can compensate losses with rapid population regrowth from surviving cells (Lennon & Jones, 2011).

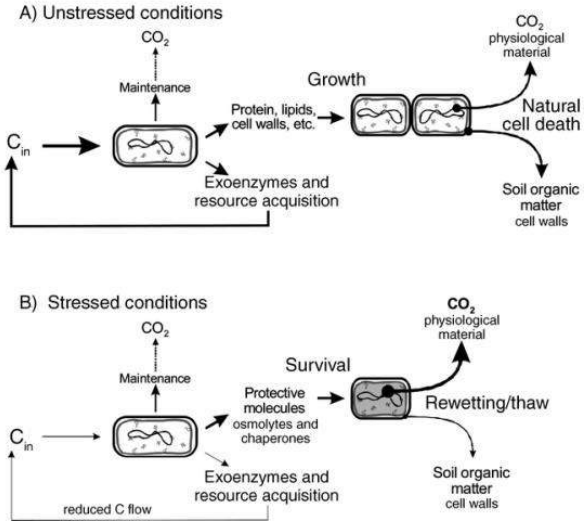

Figure 2: Resource allocation patterns in microorganisms under (A) unstressed and (B) stressed conditions. Under stress there is a reallocation of resources from growth pathways to the production of protective molecules. This makes C and N vulnerable to loss during rewetting events. Figure from Schimel *et al.* (2007) with permission from the Ecological Society of America © 2007.

Apart from direct water stress, soil drying leads to a decrease in C and N supply to microorganisms and plants. When soils dry and diffusion of water-soluble substances is reduced, soil microorganisms become physically separated from their substrates and microbial growth and metabolism slows down (Figure 3), leading to a decrease in soil respiration during dry periods (Moyano *et al.*, 2013). At the same time, labile C and N accumulates in dry soil because enzymatic decomposition of organic matter can continue even at low soil moisture levels while microbial activity and nutrient uptake are low (Manzoni *et al.*, 2014). Furthermore, in response to water shortage trees close their stomata to reduce

transpiration, which constrains photosynthesis and belowground C allocation (Grayston *et al.*, 1997), thus decreasing respiration from roots, mycorrhiza and rhizospheric microorganisms (Bréda *et al.*, 2006). Extended drought periods can lead to reduced tree C storage, heat damage and premature senescence of leaves, which increases C and N input from falling litter to the soil, while at the same time reducing plant growth and N uptake in the following years (Hartl-Meier *et al.*, 2015). In 2003, an extreme drought and heat wave over Europe reduced GPP by 30 % and turned European ecosystems into strong anomalous C sources, reversing the effect of four years of net ecosystem C sequestration (Ciais *et al.*, 2005). However, although the mechanistic responses of plants and microorganisms to drought stress are more or less understood, the effects of drought on *in situ* process rates under future climate scenarios still contain large uncertainties (Borken & Matzner, 2009).

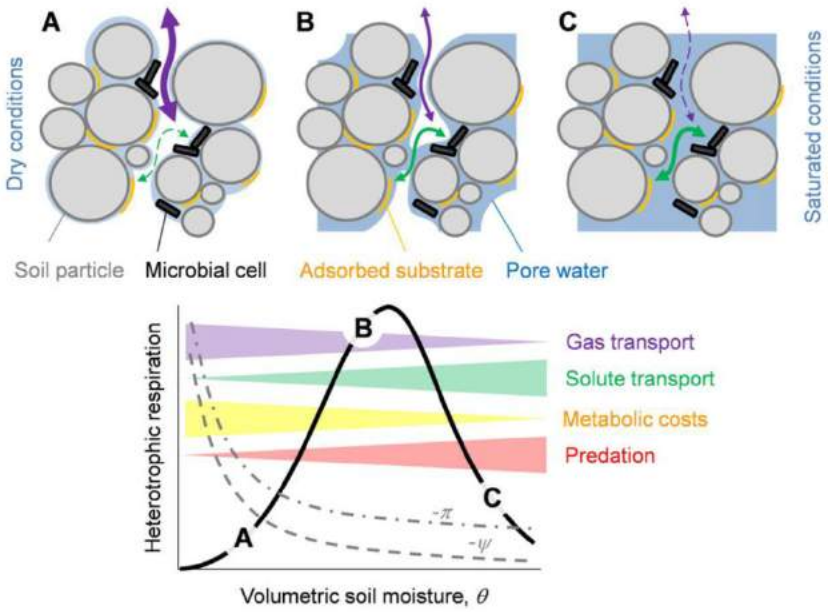

Figure 3: Soil moisture effects on microbial activity. The relationship between heterotrophic respiration and water availability is the result of a number of interacting effects, ranging from diffusion limitations to physiological, biochemical, and ecological processes. Because these effects often act in different directions (*e.g.*, substrate transport decreases with decreasing soil moisture, whereas O_2 transport increases), a peak in respiration occurs at intermediate values of soil moisture. In the lower panel, Ψ indicates the soil water potential, and π is the cell osmotic potential that would allow maintaining a stable turgor pressure as Ψ declines. Figure from Moyano *et al.* (2013) with permission from Elsevier Ltd © 2013.

Rewetting of dry soil reconnects microorganisms with their substrates by restoring disrupted water films around soil particles. It can further generate additional substrate from discarded microbial osmolytes (Fierer & Schimel, 2003; Warren, 2014), from cell lysis of microorganisms that could not cope with the sudden change in water potential (Kieft *et al.*, 1987; Evans & Wallenstein, 2012; Blazewicz *et al.*, 2014), or from breaking of soil aggregates and the exposure of protected SOM (Van Gestel *et al.*, 1991; Fierer & Schimel, 2003). The latter is especially critical in the context of climate change because it could expose occluded C that had been protected from microbial attack and, thus, withdrawn from biogeochemical cycling (Schimel *et al.*, 2011; Navarro-García *et al.*, 2012), which represents an additional input to the global C cycle. It was suggested that the flush of labile substrate at least partially drives "hot moments" of disproportionately high biogeochemical process rates after rewetting of dry soil, a phenomenon that has been termed "Birch effect" after its discoverer (Birch,

1958). This effect includes an immediate peak in soil-atmosphere flux rates of CO_2 (Kim *et al.*, 2012), NO (Homyak *et al.*, 2016) and N_2O (Whiteley *et al.*, 2006; Colman *et al.*, 2007; Harms & Grimm, 2012) together with increased N transformation rates in the first hours post-wetting followed by elevated rates over the next days (Borken & Matzner, 2009; Reich *et al.*, 2010). Furthermore, there are also physico-chemical rewetting effects that contribute to rewetting gas pulses: for example, soil degassing during rewetting drives soil CO_2 that is stored in the pore space out of the soil and can release large quantities of CO_2 within minutes as the water front passes through the soil (Xu *et al.*, 2004). Furthermore, chemo-denitrification of NO_2^- or NH_2OH can lead to NO and N_2O emissions within minutes after a rewetting event (Heil *et al.*, 2016; Homyak *et al.*, 2016).

This strong rewetting effect on C and N cycling can have tremendous impacts on annual GHG budgets: for example, Lee et al. (2004) found a soil CO_2 flush equivalent to 5-10 % of net ecosystem CO_2 exchange caused by a single storm event. Similarly, ~80 % of annual forest soil N_2O fluxes are emitted after extreme events like drying-rewetting or freeze-thaw cycles (Wolf *et al.*, 2010). Furthermore, fewer but heavier precipitation events can lead to decreased N mineralization-immobilization turnover during dry periods, leading to a retarded stabilization of N in organo-mineral associations and a greater risk of N leaching losses during extreme rainfall events (Bimuller *et al.*, 2014). Unger et al. (2010) reported that the magnitude of the response of soil CO_2 efflux to rewetting depends on both duration and intensity of the preceding drought, with longer and more intense drought periods leading to larger emission pulses. Laboratory studies have provided some insight into the mechanisms underlying the response of soil processes (Franzluebbers *et al.*, 2000; Fierer & Schimel, 2002; Göransson *et al.*, 2013), and soil microbial communities (Fierer *et al.*, 2003; Iovieno & Baath, 2008; Evans & Wallenstein, 2012; Blazewicz *et al.*, 2014; Aanderud *et al.*, 2015) to drying and rewetting. However, it remains uncertain how much rewetting GHG pulses contribute to annual soil GHG budgets, and how changes in precipitation patterns will affect GHG emissions and soil nutrient cycling processes. Moreover, if repeated drying-rewetting cycles lead to soil aggregate destruction releasing physically protected SOM, rewetting respiration bursts could overcompensate drought-induced reductions in R_s, increasing total R_s over time.

1.4. SCIENTIFIC AND PRACTICAL RELEVANCE OF THE STUDY

Scientists have been investigating soil-atmosphere GHG fluxes for more than 150 years. The first studies about soil respiration were already published in the 19th century and tried to characterize “soil metabolism” (Wollny, 1831; Boussingault & Levy, 1853; Möller, 1879). In the early 1900s, soil respiration was increasingly used as a measure for soil fertility and microbial activity in the context of fertilizer application in agricultural soils (Russell & Appleyard, 1915), and soil microorganisms were acknowledged as primary producers of GHGs (Sewell, 1914). Shortly after that, the importance of soil moisture for biological processes was reported (Greaves & Carter, 1920), and the first *in situ* measurements of soil CO_2 efflux in the field were conducted using a removable chamber to cover the soil for a period of time and to draw gas samples for analysis in the laboratory (Lundegårdh, 1927). In

the 1950s, after WWII, the infrared gas analyzer (IRGA) was developed, which simplified field measurements of soil respiration (Haber, 1958; Golley *et al.*, 1962) and moved the general research interest from lab incubations to field observations of ecological processes. At the same time, Birch demonstrated that rewetting of dried soil enhanced decomposition of SOM, leading to a flush of CO_2 and increased N mineralization (Birch, 1958). In the 1970s, the "greenhouse effect due to man-made perturbations of trace gases" was described (Wang *et al.*, 1976) and marked a milestone for GHG research, leading to the foundation of the Intergovernmental Panel on Climate Change (IPCC) by the World Meteorological Organization (WMO) and the United Nations Environment Program (UNEP) in 1988. Reports of ecosystem-level responses to increasing atmospheric CO_2 levels (Tans *et al.*, 1990; Mooney *et al.*, 1991) have attracted researchers' attention to the land biosphere. The awareness of the contribution of GHGs to the greenhouse effect and their relevance for global warming ignited a massive research interest, and since the 1990s the number of studies investigating GHG fluxes in the context of climate change has increased rapidly each year. However, despite a combined research effort, central questions like the short- and long-term responses of C and N cycling to changing climatic conditions, the susceptibility of ecosystem processes to extreme weather events, and the magnitude of the feedback between climate change and the C cycle remain unsolved (Marotzke *et al.*, 2017).

Given that climate change is one of the main challenges facing humanity, GHG research is not only important for purely academic reasons, but it is also crucial for political, societal and commercial sectors. There is urgent need to quantify ecosystem GHG balances under present and future climatic conditions in order to reliably predict the development of the atmospheric composition under future climates and under different mitigation scenarios. Furthermore, global C emission trading is one possible way to promote reductions of GHG emissions, which could make soil preservation and SOM management for C sequestration more attractive to farmers, foresters, and government officials. This could mean that if soil GHG emissions can be effectively reduced via adequate management strategies, farmers and foresters can earn cash awards in global C trading markets while simultaneously contributing to climate change mitigation. In addition to monetary incentives, increasing soil C stocks has been shown to improve soil fertility and agricultural productivity: recently, France launched an international initiative under the Global Climate Action Agenda (GCAA) in order to increase global soil C storage by 4 ‰ annually (four per mil, "4 per 1000") using appropriate soil management practices in agriculture. This could halt the annual increase in atmospheric CO_2 over the next 10-20 years while simultaneously ensuring food security especially in developing countries (Minasny *et al.*, 2017). Finally, simulations and models predicting future atmospheric conditions, climate scenarios, and biosphere-atmosphere feedbacks are only as good as the underlying data, and upscaling from local to regional and global scales is only reliable if spatial and temporal variability are adequately represented, which underlines the need for robust data of soil processes *in situ*.

2. AIMS & OUTLINE OF THE THESIS

2.1. RESEARCH QUESTIONS & HYPOTHESES

The aim of my thesis was to investigate the impact of extreme weather events on soil GHG emissions and nutrient cycling processes. First, I assessed the contribution of litter layer to total forest soil GHG emissions, because litter is a major source of C and nutrients and influences forest soil biogeochemistry. Then I focused on the impact of rewetting on N mobilization in soil, how mineral and organic N forms contributed to the rewetting N flush, and how N mobilization during rewetting affected soil GHG emissions. Furthermore, I investigated how repeated extreme drying-rewetting events influenced total soil respiration and its temperature and moisture sensitivity. In addition, I wanted to know how the length of the drought period and the intensity of the precipitation event affected soil GHG emissions and nutrient cycling, *i.e.* if there was a difference between more frequent short drought periods compared to less frequent but longer drought periods followed by heavy rain.

Research questions

Q1: How much of forest soil greenhouse gas flux is litter-induced, *i.e.* is either produced in or influenced by the litter layer? How does removal of the litter layer affect soil greenhouse gas emissions and soil microbial community composition?

Q2: How do frequency and intensity of drought periods and rain events affect soil N availability? What are the contributions of mineral and organic compounds to total N diffusion after rewetting in a temperate forest?

Q3: How much are soil greenhouse gas emissions reduced by extended drought periods? Can potential pulses during and after rewetting compensate or even outweigh the drought-induced reduction, thereby leading to increased overall greenhouse gas fluxes?

Q4: Can soil nutrient cycling dynamics be linked to pulses of greenhouse gas emissions after a rewetting event? What are the contributions of mineral and organic N forms to total N diffusion after rewetting in a semi-arid grassland?

Hypotheses

H1: (i) Plant litter is a source of C and N, which can be transported to the mineral soil via fungal hyphae and leaching. Therefore, litter removal reduces soil concentrations of mobile C and N compounds.

(ii) Respiration of decomposing plant litter produces CO_2; therefore, removal of the litter layer reduces soil CO_2 flux.

(iii) Because the litter layer acts as physical barrier that affects gas diffusion, litter removal enhances soil CH_4 uptake.

(iv) Leaching of C and N to the mineral soil can enhance soil nitrification and denitrification; therefore, litter removal reduces soil N_2O efflux.

(v) Because litter-inhabiting microbial communities in temperate forests are dominated by fungi, litter removal will decrease the fungi:bacteria ratio in the soil.

H2: (i) In dry soil, diffusion is inhibited and N accumulates during drought. Upon rewetting, this accumulated N is mobilized and can be lost via leaching if it is not immobilized fast enough. Therefore, repeated long drought periods followed by heavy rainfall increase losses of mobile N forms like nitrate.

(ii) The amount of N that accumulates and is prone to mobilization increases with the length of the drought; therefore, longer drought periods will lead to larger N losses during subsequent rewetting events.

(iii) Because temperate tree species like Beech are able to take up amino acids, organic N contributes to plant nutrition in temperate forests.

H3: (i) Drought inhibits the activity of soil microorganisms and plant roots, which decreases soil respiration during dry periods.

(ii) Rewetting triggers a disproportionate increase in CO_2 efflux that originates (a) from C that accumulated during drought, and (b) from C that is exposed upon rewetting. Repeated extreme drying-rewetting cycles lead to faster breakdown of soil aggregate structures, which enhances the decomposability soil organic matter and leads to increased overall soil respiration.

H4: (i) Emissions of N-bearing gases depend on the availability of N compounds in soil; therefore, N mobilization in soil fuels pulses of NO and N_2O emissions upon rewetting.

(ii) In semi-arid grasslands nitrification is considered to be the most important N-transforming process; therefore, N diffusion is dominated by nitrate.

2.2. THESIS OUTLINE

The thesis is structured into four papers. The first three papers describe work that was conducted in the BOKU University Forest "Rosalia" in Lower Austria. Paper #4 emerged from a short-term scientific exchange at the University of California, Santa Barbara, US. The four papers are summarized below. For a detailed description of the applied methods, results and discussion please refer to the individual chapters.

Paper #1: Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest

In the first paper I investigated how much of total soil GHG emissions originate from the litter layer and how the litter layer influences soil nutrient concentration and microbial decomposer communities in the mineral soil. To this end, a litter removal experiment in the BOKU University Forest "Rosalia" was conducted and soil-atmosphere fluxes of CO_2, CH_4 and N_2O were measured on 22 occasions between July 2012 and February 2013. Furthermore, samples from the mineral soil (0-10 cm) were collected on 8 occasions to measure soil nutrient concentrations (NH_4^+, NO_3^-, PO_4^{3-}, DOC, water-soluble sugars) and phospholipid fatty acids (PLFAs) as markers for the soil microbial community composition. Results showed that respiration from the litter layer contributed 30 % to total soil CO_2 flux, and removal of the litter layer increased the temperature sensitivity of soil respiration (Q_{10}). Furthermore, litter removal facilitated diffusion of CH_4 into the soil and increased CH_4 uptake by 16 %. Nitrous oxide emissions from soil with an intact litter layer peaked after rain events in summer and autumn, but no N_2O peaks were observed when the litter layer was removed. In contrast, litter removal turned soils from net N_2O sources to slight N_2O sinks. Mineral soil nutrient concentrations were not affected by litter removal. Microbial communities showed no response to litter removal but were strongly influenced by seasonality. Fungal and bacterial PLFA markers increased from summer to winter and were controlled primarily by soil pH, temperature, and water content. In conclusion, this study shows that the litter layer (i) contributes directly to soil GHG fluxes, and (ii) influences GHG emissions originating from mineral soil. This should be taken into account when assessing forest GHG budgets. Furthermore, it was demonstrated that the litter layer affects the temperature sensitivity of soil CO_2 fluxes, which should be accounted for in C models, given that litter represents a major component of total C input to soils. Finally, the outcome of this study suggests that in the short term, the litter layer controls soil GHG fluxes primarily via physical processes and not via nutrient leaching to or impacts on microbial composition in the mineral soil.

Paper #2: Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying-rewetting events

In the second paper I focused on the temporal dynamics of soil N availability before, during and after a rewetting event. This work was carried out at the same study site in the BOKU University Forest as the previous paper and was part of a precipitation-manipulation experiment that was conducted from May

2013 until October 2015. During the vegetation period (May-October) of each year, rainfall was excluded with roofs to simulate drought, and soils were irrigated monthly to simulate moderate stress (6 drying-rewetting cycles per vegetation period), and every two months to simulate severe stress (3 drying-rewetting cycles per vegetation period). In October 2014, at the end of the vegetation period and after two years of precipitation manipulation, soil N availability before, during and after soil rewetting was measured using two different approaches: (a) conventional soil extraction with high-purity water in the laboratory to determine N concentrations, and (b) *in situ* soil microdialysis to determine diffusive N flux. While soil water extraction revealed mainly mineral N forms (NH_4^+ and NO_3^-), diffusive N flux was dominated by organic N (amino acids). This outcome was surprising given that traditionally, mineralization of organic N compounds to mineral N forms is considered to be the rate-limiting step of N supply in temperate forests. However, there is growing evidence that temperate tree species can take up intact amino acids and that organic N could contribute to plant N nutrition. In support of this, results from the present study indicated that organic N could constitute a more important plant N source than previously thought. Furthermore, microdialysis results showed that rewetting of dry soil led to a fast but short-lived mobilization of mobile N forms, primarily NO_3^- and some neutral amino acids (lysine, glutamine, cysteine, glycine). The rewetting N flush was larger when the preceding drought period had been longer, indicating that more N had accumulated during the longer drought. In contrast, the rewetting N flush was not detected by laboratory water extraction, highlighting the importance of *in situ* measurements that minimize soil disturbance to detect short-term soil processes. This study shows that soil microdialysis is well suited to monitor changes in soil N dynamics during drying–rewetting cycles and is therefore of particular interest for future studies examining the effects of climate change on plant and microbial N nutrition.

Paper #3: Repeated extreme drought and rainfall events reduce soil respiration and affect its temperature and moisture sensitivity

The third paper of this thesis examines how soil temperature and moisture interactively control soil respiration, and how these controls are affected by extreme weather events. This work was part of the same precipitation-manipulation experiment in the BOKU University Forest as the previous paper and summarizes two years of repeated moderate and severe drying-rewetting stress. From April 2013 to March 2015, soil-atmosphere CO_2 flux was measured in 3-hourly resolution with automated flux chambers in moderately stressed plots (6 cycles of one-month drought followed by 75 mm irrigation per vegetation period), severely stressed plots (3 cycles of two-months drought followed by 150 mm irrigation per vegetation period), and control plots that received natural precipitation (n = 4). This resulted in a data set of approx. 42,000 soil respiration measurements, which was used to test different temperature and moisture response functions. Results showed that repeated severe drying-rewetting cycles reduced total soil respiration compared to control plots in both 2013 and 2014, indicating that rewetting CO_2 pulses did not outweigh drought-induced decreases in soil respiration. Respiration from the moderate drying-rewetting stress treatment was not different from controls. At soil moisture levels >30 % WFPS, respiration from all three treatments was best described by a Gauss model that used soil temperature as predictor. At WFPS <30 % moisture became limiting and decreased the explanatory

power of the temperature model. This indicates that at low soil moisture levels, moisture limitation could override the temperature control of soil respiration. Therefore, a combined temperature-moisture model, which used a Gauss function to express temperature dependence and a quadratic function to express moisture dependence, was used to predict soil respiration. This model could well describe soil respiration even at low soil moisture levels. Comparison of stress treatments and controls showed that severe drying-rewetting stress increased the temperature and moisture sensitivity of soil respiration compared to controls, whereas moderate drying-rewetting stress had no effect. This underlines the need for experiments that apply manipulations that are outside current climatic windows in order to detect process responses to extreme weather events. The main outcome from this study is that repeated severe drying-rewetting cycles decrease soil respiration, and that both temperature and moisture sensitivity have to be accounted for in predictions of future GHG emissions.

<u>Paper #4</u>: Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils

This paper summarizes work that I did during a four-month scientific exchange at the University of California, Santa Barbara, US. There I conducted a rewetting experiment in a semi-arid Oak savanna at the end of the dry season in November 2015, after approx. six months of drought. Soil was irrigated with 15 mm water to simulate a rain shower at the transition from dry to wet season, and soil N dynamics were monitored over 32 h following rewetting. Soil-atmosphere fluxes of NO and N_2O were measured every 1-4 h using a portable chamber connected to a N_2O laser detector and a NO chemi-luminescence detector. Diffusive soil fluxes of mineral and organic N were measured hourly using microdialysis. Results show that rewetting led to a pulse in NO and N_2O emissions from soil that coincided with rapid mobilization of mineral and organic N in soil. Fluxes of NO and N_2O increased rapidly after rewetting and remained elevated compared to pre-wetting rates for the duration of our measurements, with NO accounting for ⅔ of N-gas flux. In soil, NO_3^- contributed ~80 % to total N diffusion after rewetting, but this NO_3^- flush disappeared 2 h after rewetting. 27 h post-wetting, NH_4^+ diffusion increased and dominated total N diffusion, coinciding with peak N-gas emissions. This indicates that at this time point, nitrification was the most important process of NO and N_2O formation. Rewetting also led to a mobilization of amino acids, which contributed ~10 % to total N diffusion immediately after rewetting, but this flush was only short-lived, indicating that organic N did not contribute much to NO and N_2O emissions at this site. In line with my previous results from the temperate forest precipitation-manipulation experiment, this study shows that N-compounds accumulate in dry soil and are rapidly mobilized during rewetting. This mobilization of N-bearing substrates upon rewetting can fuel pulses of NO and N_2O emissions when they are transformed by microorganisms.

3. RESULTS

3.1. PAPER #1: CONTRIBUTION OF LITTER LAYER TO SOIL GREENHOUSE GAS EMISSIONS IN A TEMPERATE BEECH FOREST

Sonja Leitner[1], Orracha Sae-Tun[1,2], Lukas Kranzinger[1,3], Sophie Zechmeister-Boltenstern[1], Michael Zimmermann[1]

[1] Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

[2] Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand

[3] Department of Environmental and Energy Process Engineering, Montanuniversitaet Leoben, Leoben, Austria

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Contributions:

SL selected the sampling site and designed the study, supervised gas sampling and laboratory analysis, calculated statistics, wrote the manuscript

OST performed gas sampling and PFLA analysis

LK performed gas sampling and nutrient analysis, calculated gas fluxes

SZB supervised the study, wrote the manuscript

MZ selected the sampling site and designed the study, wrote the manuscript

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REGULAR ARTICLE

Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest

Sonja Leitner · Orracha Sae-Tun · Lukas Kranzinger · Sophie Zechmeister-Boltenstern · Michael Zimmermann

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Abstract
Background and aims The litter layer is a major source of CO_2, and it also influences soil-atmosphere exchange of N_2O and CH_4. So far, it is not clear how much of soil greenhouse gas (GHG) emission derives from the litter layer itself or is litter-induced. The present study investigates how the litter layer controls soil GHG fluxes and microbial decomposer communities in a temperate beech forest.
Methods We removed the litter layer in an Austrian beech forest and studied responses of soil CO_2, CH_4 and N_2O fluxes and the microbial community via phospholipid fatty acids (PLFA). Soil GHG fluxes were determined with static chambers on 22 occasions from July 2012 to February 2013, and soil samples collected at 8 sampling events.
Results Litter removal reduced CO_2 emissions by 30 % and increased temperature sensitivity (Q_{10}) of CO_2 fluxes. Diffusion of CH_4 into soil was facilitated by litter removal and CH_4 uptake increased by 16 %. This effect was strongest in autumn and winter when soil moisture was high. Soils without litter turned from net N_2O sources to slight N_2O sinks because N_2O emissions peaked after rain events in summer and autumn, which was not the case in litter-removal plots. Microbial composition was only transiently affected by litter removal but strongly influenced by seasonality.
Conclusions Litter layers must be considered in calculating forest GHG budgets, and their influence on temperature sensitivity of soil GHG fluxes taken into account for future climate scenarios.

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S. Leitner (✉) · O. Sae-Tun · L. Kranzinger · S. Zechmeister-Boltenstern · M. Zimmermann
Institute of Soil Research, University of Natural Resources and Life Sciences Vienna (BOKU), Peter Jordan-Str. 82, 1190 Vienna, Austria
e-mail: sonja.leitner@boku.ac.at

O. Sae-Tun
Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, 50 Ngamwongwan Rd., Ladyao Chatuchak, Bangkok 10900, Thailand

L. Kranzinger
Department of Environmental and Energy Process Engineering, Montanuniversitaet Leoben, Franz-Josef-Str. 18, 8700 Leoben, Austria

Keywords Litter removal · Seasonality · CO_2 · CH_4 · N_2O · PLFA

Abbreviations

LR	Litter removal
CO_2	Carbon dioxide
CH_4	Methane
N_2O	Nitrous oxide
GHG	Greenhouse gases
SOC	Soil organic carbon
TN	Total nitrogen
C_{mic}	Microbial carbon
N_{mic}	Microbial nitrogen
NH_4^+	Ammonium

NO_3^-	Nitrate
PO_4^{3-}	Phosphate
WSS	Water-soluble sugars
VWC	Soil volumetric water content
T_{soil}	Soil temperature
DaLR	Day after litter removal
PLFA	Phospholipid fatty acids
gram-	Gram-negative bacteria
gram+	Gram-positive bacteria
Q_{10}	Temperature sensitivity
CCA	Canonical correspondence analysis

Introduction

Forest soils play an important role in controlling global greenhouse gas (GHG) budgets because they act mostly as carbon dioxide (CO_2) sources, methane (CH_4) sinks and nitrous oxide (N_2O) sources (IPCC 2013). Soil microbial communities strongly influence soil GHG fluxes (Conrad 1996; Schimel and Gulledge 1998), and are typically adapted to the type of plant litter in a certain environment (Ayres et al. 2009; Madritch and Lindroth 2011). Although plant litter contributes the largest input of C and nutrients to forest soils (FAO 2010), there is a lack of knowledge on the explicit impact of the litter layer on forest soil GHG fluxes. Atmospheric CO_2 is the major driver of global warming, and CH_4 and N_2O are potent GHGs with 100-year global warming potentials of 28 and 265, respectively (IPCC 2013). Partitioning the contribution of litter and mineral soil to total soil GHG fluxes as well as improving our understanding on how the litter layer influences soil processes and microbial communities will help to reduce uncertainties in biogeochemical models and improve our forecasts of future GHG budgets for terrestrial ecosystems. Because ecosystem GHG sinks can be used to a limited extend to compensate for emission reductions stipulated in the Kyoto protocol (IPCC 2014), a precise quantification of ecosystem C and N budgets is of utmost importance for climate change mitigation.

Forests cover 31 % of land area and contain 652 GtC, 45 % in soils and 11 % in dead wood and litter (FAO 2010). Respiration from plant litter decomposition contributes between 5 and 45 % to total soil CO_2 emissions in temperate forests (Borken and Beese 2005; Bowden et al. 1993; Vose and Bolstad 2007). The litter-inhabiting microbial community in beech forests is dominated by fungi that can decompose litter cellulose and lignin (Schneider et al. 2012). Removing the litter might decrease the fungi:bacteria (F:B) ratio in the soil. Because most fungi have a higher C use efficiency (CUE) than bacteria (Keiblinger et al. 2010), a shift in the F:B ratio is likely to affect soil CO_2 emissions.

Furthermore, temperate forests are considered to be important CH_4 sinks through the consumption of CH_4 by methanotrophic bacteria in well-aerated forest soils (Dalal and Allen 2008; Le Mer and Roger 2001). Litter itself does apparently not produce or consume CH_4 (Dong et al. 1998; Reith et al. 2002; Smith et al. 2000). However, the litter layer has been reported to influence soil CH_4 uptake by controlling gas diffusion into the soil (Peichl et al. 2010; Wang et al. 2013), which can be particularly important in broad-leaved forests like beech (Brumme and Borken 1999). Furthermore, soils that receive high N loads due to N fertilization or atmospheric N deposition often consume less CH_4 than undisturbed soils (Butterbach-Bahl et al. 1998; Macdonald et al. 1997; Steudler et al. 1989) because NH_4^+ inhibits oxidation of CH_4 to CO_2 by methanotrophic bacteria (Bodelier and Laanbroek 2004). However, whether litter N content influences soil CH_4 fluxes, for example via leaching of N to the mineral soil, remains to be demonstrated.

Soils under natural vegetation are mostly regarded as N_2O sources and account for 6.6 Tg N_2O-N yr^{-1} to the global terrestrial N_2O input to the atmosphere (IPCC 2013). How the litter layer affects soil N_2O flux is not clear. Dong et al. (1998) reported that removal of leaf litter/humus layer significantly decreased N_2O emissions in a German deciduous forest, which they attributed primarily to emissions of the humus layer itself. Wieder et al. (2011) found a priming effect of labile C leaching from plant litter on soil N_2O emissions for tropical ecosystems, which can either be a direct result of stimulation of heterotrophic denitrifiers or occur indirectly by increased heterotrophic O_2 consumption and formation of anaerobic microsites in the soil. However, contribution of litter itself to total soil N_2O emissions in temperate forests as well as the importance of dissolved organic carbon (DOC) leaching from litter to mineral soils is not well studied.

The purpose of the present study was to quantify how much of forest soil GHG flux is litter-induced, as well as to investigate how removal of the aboveground litter layer (henceforth referred to as 'litter removal') influences the soil processes and microbial community

composition in the short term. We hypothesized that litter removal (i) reduces soil concentrations of mobile C, N and P, (ii) reduces soil CO_2 efflux, (iii) enhances soil CH_4 uptake, (iv) reduces soil N_2O efflux, and (v) reduces the proportion of fungi in the soil microbial community.

Materials and methods

Study site

The study was conducted in a pure mature beech forest (*Fagus sylvatica* L.) at the 'Rosalia Lehrforst' site, which is part of the 'long-term ecological research' network (LTER-Austria) and is located in the Rosalien Mountains, Austria (47° 42′ 26″ N /16° 17′ 59″ E). The soil at the study site was a pseudo-gleyic Cambisol over metamorphic crystalline bedrock. Mean annual temperature and mean annual precipitation were 6.5 °C and 796 mm, respectively. The study site was at an elevation of 600 m asl and exposed to the west.

Experimental design

Twelve pairs of experimental plots were randomly positioned along a 20 m horizontal line, each consisting of one control and one litter-removal (LR) plot. The litter layer was removed carefully by hand in an area of 0.5 m × 0.5 m from the LR plots in June 2012. Total removed litter accounted for 1.39 kg dw m^{-2}, which contained 0.55 kg C m^{-2}. The bare mineral soil was covered with a black water-permeable textile mat to prevent excessive soil-drying due to litter removal, which allowed us to focus on the influence of nutrient leaching from the litter rather than changes in soil microclimate. A metal mesh cage (25 cm height) was placed over the LR plots to prevent new litter input. On all 24 plots, PVC collars of 20 cm diameter and 10 cm height were inserted carefully 2–3 cm into the ground to be used as closed headspace chambers to collect air samples. Between July 2012 and February 2013 air samples were collected 22 times and soil samples 8 times. Microbial community composition was determined via phospholipid fatty acid (PLFA) analysis at 5 time points.

Soil analysis

Soil samples were collected from all 24 plots with metal cylinders of 4 cm diameter and 5 cm height. At each sampling, 5 soil cores from each plot were taken and pooled together. Before soil cores were taken from control plots, the litter layer was carefully moved aside locally and only mineral soil was sampled to make soil samples from control plots comparable to those from LR plots. At the same time, soil temperature in 5 cm depth was determined with a penetration thermometer (Voltcraft DET3R, Switzerland), and volumetric water content (VWC) was measured with a TDR probe (SM300, Delta-T, UK). Soil samples were transported to the laboratory in Vienna, sieved (<2 mm) and stored at 4 °C for nutrient and microbial biomass analysis, and at −18 °C for PLFA analysis. All soil samples were analyzed for pH, NO_3^-, NH_4^+, PO_4^{3-}, water-soluble sugars (WSS), microbial biomass, and soil organic C (SOC) and total nitrogen (TN) contents. Soil pH was determined with a calibrated pH-meter (WTW 537, Germany) in a suspension of 2 g fresh soil in 25 ml 0.01 *M* $CaCl_2$ (Schinner et al. 1996). Nitrate, NH_4^+ and PO_4^{3-} concentrations were measured in suspensions of 5 g fresh soil in 50 ml 1 *M* KCl with a photometer (Perkin Elmer 2300 EnSpire, USA) as described elsewhere (Hood-Nowotny et al. 2010; Schinner et al. 1996). Hot-water soluble reducing sugars (WSS) were detected with the Prussian-blue method (Schinner and Von Mersi 1990; Slaughter et al. 2001). Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) of the samples was calculated as difference of DOC and total dissolved nitrogen (TDN), respectively, before and after chloroform fumigation (Schinner et al. 1996). Soil organic C and TN were quantified on oven-dried (105 °C) soil with an elemental analyzer (NA-1500 Carlo Erba, Italy). Additionally, the textile mat was tested for leaching of C, N and P, and no leaching was detected.

Soil greenhouse gas fluxes

To collect gas samples, the 24 dark chambers (total volume 2.51 L) were closed with air-tight lids and gas samples were collected with a syringe through a rubber septum in the lid 0, 10, 20 and 60 min after chamber closure. 30 ml gas samples were injected into 20 ml pre-evacuated glass vials (clear flat-bottom headspace vials with aluminum crimp caps and grey butyl septa, all from Agilent Technologies, Austria) and transported to the

lab. Gas samples were stored at air temperature and analyzed within 1 week. Concentrations of CO_2, CH_4 and N_2O of all gas samples were determined with an Agilent GC-system (Agilent Technologies). Detector 1 was an electron capture detector (ECD) for N_2O measurements, and detector 2 was a flame ionization detector (FID) with Ni-methanizer to quantify CO_2 and CH_4 (all Agilent Technologies, Austria). For calibration, gas mixes of CO_2, CH_4 and N_2O in N_2-gas in 3 different concentrations (CO_2 250, 500, 1000 ppm; CH_4 1, 2, 4 ppm; N_2O 0.5, 2.5, 5 ppm, respectively) were used (Linde Gas, Austria). Limit of detection (LoD) of the chamber measurements was 3.6 mg CO_2-C m^{-2} h^{-1}, 9.2 μg CH_4-C m^{-2} h^{-1} and 10.1 μg N_2O-N m^{-2} h^{-1}, respectively (Parkin et al. 2012). Because N_2O fluxes from temperate forest soils are known to be highly variable in time and space, with high fluxes during "hot moments" such as drying-rewetting or freeze-thaw events, and low fluxes during the rest of the year (Groffman et al. 2009). Therefore, in the present study fluxes below the LoD were not excluded from the calculation of average fluxes over the study period, because this would have caused a bias towards higher emissions. Nevertheless, the reader should be aware that values below LoD bear a high analytical uncertainty.

Hourly GHG flux rates for each chamber were calculated based on Eq. (1) as described by Metcalfe et al. (2007),

$$\text{GHG flux} = \Delta C/\Delta t * 273.15/(T_{air} + 273.15) * p/1000 * M/22.41 * V/A \quad (1)$$

Where *GHG flux* is the flux of the respective greenhouse gas, $\Delta C/\Delta t$ is the concentration change (ppm for CO_2, ppb for CH_4 and N_2O) over time (h), T_{air} is air temperature (°C), p is atmospheric pressure (Pa), M is molecular weight (g), 22.41 is the molar volume of an ideal gas at Standard Temperature and Pressure (1 mol^{-1}), V is the chamber volume (m^3) and A the chamber area (m^2). The term (T_{air} + 273.15) is used to convert air temperature from degree Celsius to Kelvin. For calculation of CO_2 and CH_4 fluxes, M is 12.01 g (the molecular weight of C) and units are mg CO_2-C h^{-1} m^{-2} and μg CH_4-C h^{-1} m^{-2}, respectively. For calculation of N_2O flux, M is 28.02 g (the molecular weight of 2 N atoms) and units are μg N_2O-N h^{-1} m^{-2}. Concentration changes over time were determined with quadratic best-fit equations for CO_2 and N_2O, and an exponential best-fit equation for CH_4. Greenhouse gas fluxes were discharged if regression coefficients (r^2) were below 0.70 for CH_4 and N_2O, and below 0.90 for CO_2 (Barton et al. 2008; Chadwick et al. 2014; Unteregelsbacher et al. 2013). Positive fluxes represent net GHG emissions, negative fluxes represent net GHG uptake. Greenhouse gas fluxes of control and LR plots were averaged for each sampling event and are given together with standard errors (n = 12 per treatment). Litter-induced GHG flux was calculated as the difference between GHG flux from control plots (soil & litter) and LR plots (soil only):

$$\text{Litter-induced GHG flux} = \text{GHG flux}_{control} - \text{GHG flux}_{LR} \quad (2)$$

Temperature sensitivity values (Q_{10}) were calculated for soil GHG fluxes that were significantly correlated with soil temperature after a Lloyd & Taylor function (Eq. 3) according to Tuomi et al. (2008):

$$\text{GHG flux} = a * \exp((E/(283.15*8.314))*(1-283.15/(T_{soil} + 273.15))) \quad (3)$$

with a and E as fitted parameters, T_{soil} the soil temperature (°C), which is converted to Kelvin by adding 273.15, 8.314 is the universal gas constant (J mol^{-1} K^{-1}), and 283.15 is some reference temperature (10 °C, see also Lloyd and Taylor 1994).

Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acids were analyzed in pooled soil samples for reasons of feasibility (from the 12 soil samples per treatment, 4 were combined to one composite sample, which resulted in 3 composite samples per treatment and time point). Phospholipid fatty acids were extracted after an adapted protocol of the Bligh and Dyer method (Frostegård et al. 1991) as described elsewhere (Brandstätter et al. 2013; Djukic et al. 2010). Briefly, 2 g field-moist soil were extracted overnight in the dark with chloroform:methanol:citrate buffer (1:2:0.8) and chloroform:methanol (1:2), fractionated by sequential elution with chloroform, acetone and methanol on silica solid-phase columns (Isolute SI 500 mg 3 ml^{-1}, Biotage, Sweden) to separate phospholipids from neutral lipid fatty acids and glycolipids. Samples were methylated with methanol:toluol (1:1), 0.2 *M* methanolic KOH and 1 *M* acetic acid. Phospholipids where re-dissolved in 200 μl iso-octane and analyzed with an HP 6980 series GC-system and 7683 series injector and auto-sampler on an HP-5 50 m

capillary column (all Hewlett Packard, USA) using a flame ionization (FID) detector. A mix of bacterial acid methyl esters (Supelco BAME CP Mix # 47080-U, Sigma-Aldrich, USA) was used as qualitative standard to identify PLFAs. Concentrations of individual PLFAs were quantified relative to the internal standard nonadecanoate fatty acid (19:0, 20 mg l^{-1}).

Absolute amounts of PLFAs are given in μmol PLFA g^{-1} SOC. The PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and 10Me18:0 were used as markers for gram+ bacteria, cy17:0, cy19:0, 16:1ω5c, 16:1ω7c, 14:0, 15:0, 17:0 for gram- bacteria, 10Me16:0 and 10Me17:0 for unspecific bacteria, and 18:2ω6,9 for fungi (Baath 2003; Djukic et al. 2010, 2013; Zelles 1999). Total bacterial PLFAs were calculated from the sum of gram+, gram- and unspecific bacterial markers. Bacteria:fungi ratio was calculated as the sum of bacterial PLFAs divided by the fungal PLFA 18:2ω6,9.

Statistical analysis

To identify effects of time and litter removal, first a two-way ANOVA was used to check for interactions between factors. If interactions were found, the dataset was split into control and LR subsets, and one-way ANOVA followed by Tukey's *post hoc* test was employed to identify differences between time points. Differences between treatments were analyzed by separate *t*-tests for each time point. Homogeneity of variance was tested with Levene's test, and data were log-transformed if necessary. If transformation did not ensure homogeneity of variance, robust ANOVA as described by Wilcox (2005) was employed. Microbial community composition was analyzed by canonical correspondence analysis (CCA), using the mole percentage of PLFAs as community matrix and soil parameters and time as constraining factors. Interactions between soil GHG fluxes and soil temperature and moisture were analyzed on data from 22 gas samplings by Spearman's rank correlation with Benjamini & Hochberg correction to test for false positives (type I error) in multiple comparisons (Benjamini and Hochberg 1995). Statistical analysis was conducted with Statgraphics (StatPoint Technologies, United States), SigmaPlot (Systat Software, USA), and R 3.0.2 using packages "vegan" for CCA (Oksanen et al. 2014) and "WRS" for robust ANOVA (Wilcox 2005).

Results

Soil properties

Average soil temperatures were 11.2 ± 1.4 °C and 11.1 ± 1.4 °C between July 2012 and February 2013 for control and LR plots, respectively, and were not significantly altered by litter removal (Fig. 1a). Soil temperature changed according to seasons and decreased from 18 °C during July and August to 2–5 °C in December to February. Volumetric soil water content (Fig. 1a), which averaged 22.4 ± 1.8 % and 23.5 ± 2.1 % for control and LR plots, respectively, was also not significantly affected by litter removal and increased from July to February, with a large peak in the first 2 weeks of August 2012 due to strong rainfall events. Soils at our site were strongly acidic with a mean soil pH of 3.9 ± 0.1, which was not affected by litter removal. Bulk density was 0.595 ± 0.143 g m^{-3}.

Soil nutrients were only affected by litter removal at the start of the experiment (Table 1). One week after removing the litter layer, NH_4^+ increased by 134 % from 302 ± 26 mg N m^{-2} in controls to 710 ± 20 mg N m^{-2} in LR plots. Stocks of SOC (2.1 ± 0.24 kg C m^{-2}) and TN (0.11 ± 0.01 kg N m^{-2}) in the uppermost 5 cm were not influenced by litter removal. At the consecutive samplings, concentrations of NO_3^-, NH_4^+, PO_4^{3-} and WSS did not differ between treatments (Table 2).

Soil greenhouse gas fluxes

Litter removal significantly decreased CO_2 fluxes from soil by 29.9 % (Fig. 1b, Table 2). On average, control plots emitted 128 ± 13 mg CO_2-C h^{-1} m^{-2}, whereas LR plots respired 90 ± 10 mg CO_2-C h^{-1} m^{-2}. Soil CO_2 fluxes of both control and LR plots followed the seasonal trend of soil temperature and decreased from July to February. Absolute litter-induced CO_2 fluxes (difference between control and LR plots) decreased from July 2012 to February 2013, and relative contribution of litter-induced to total soil CO_2 efflux ranged from 15.6 to 46.1 %.

The forest soil acted as atmospheric CH_4 sink during the entire study period (Fig. 1c). Litter removal significantly increased soil CH_4 uptake by 16.0 % (i.e., CH_4 fluxes were 16.0 % more negative) with average CH_4 uptakes of 40.0 ± 2.3 μg CH_4-C h^{-1} m^{-2} in control plots and 46.4 ± 2.6 μg CH_4-C h^{-1} m^{-2} in LR plots. Differences between control and LR plots where large

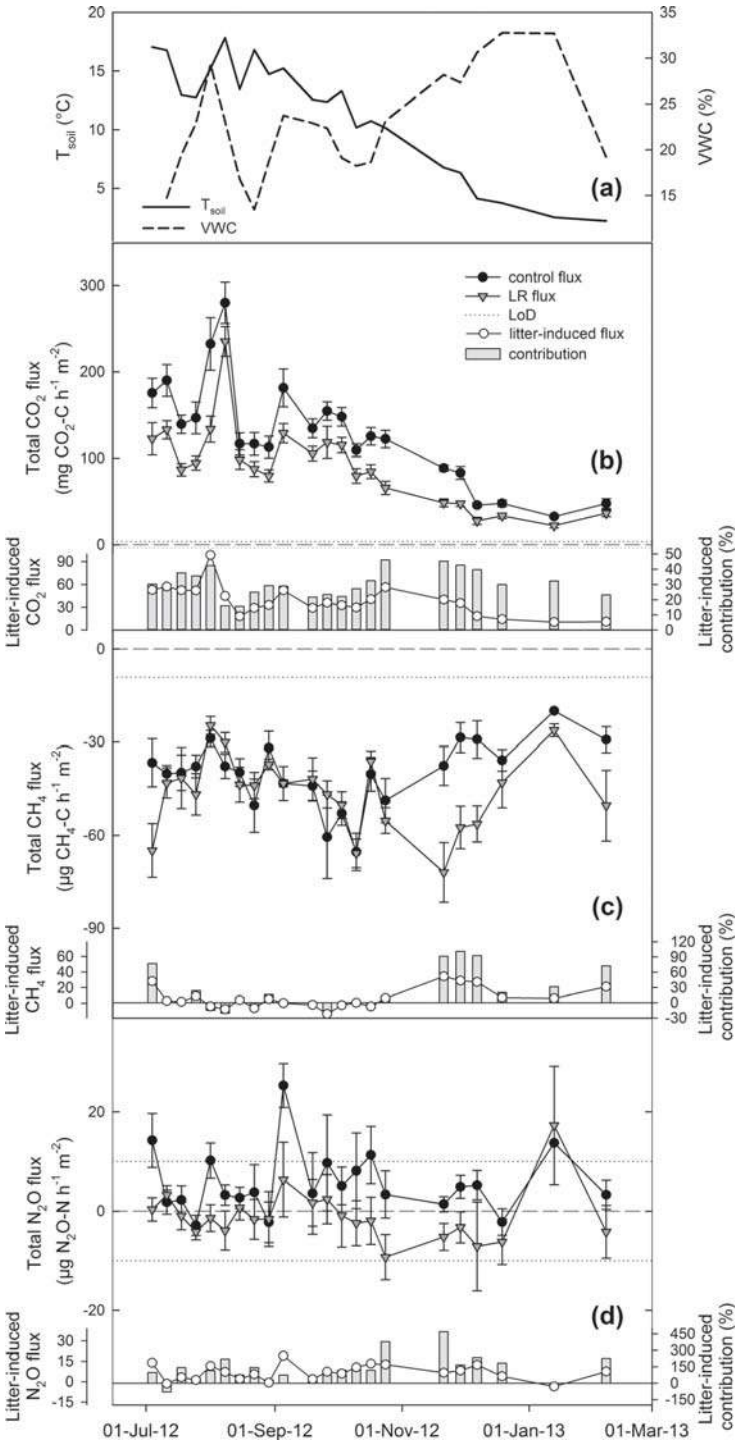

Fig. 1 **a**, soil temperature (T_{soil}, *solid line*) and soil volumetric water content (VWC, *dashed line*) in the experimental plots; **b**–**d**, total greenhouse gas (GHG) flux from control (●) and litter-removal (▽) plots as well as litter-induced GHG flux (○) and contribution of litter-induced to total GHG flux (*grey bars*) (mean ± SE, n = 12): **b**, CO_2; **c**, CH_4; **d**, N_2O. Limit of Detection (LoD, dotted line) of the used GC system was 3.6 mg CO_2-C m^{-2} h^{-1}, 9.2 µg CH_4-C m^{-2} h^{-1} and 10.1 µg N_2O-N m^{-2} h^{-1}, respectively. Positive fluxes (CO_2 and N_2O) indicate soil GHG emissions, negative fluxes (CH_4 and N_2O) indicate soil GHG uptake. Litter-induced flux was calculated as difference between average control and litter-removal GHG fluxes, therefore no standard errors are given

at the beginning of the experiment and in the time from November 2012 to February 2013, with highest absolute litter-induced CH_4 fluxes of 28.2 µg CH_4-C h^{-1} m^{-2} in July 2012 and 34.3 µg CH_4-C h^{-1} m^{-2} in November 2012, which correspond to a 76.8 and 100.9 % increase in CH_4 uptake, respectively, if the litter layer was

Table 1 Soil chemical properties of the uppermost 5 cm from July 2012 until February 2013 in control and litter-removal (LR) plots

Date		C_{mic} (g C m^{-2})		N_{mic} (g N m^{-2})		NO_3^-(mg N m^{-2})		NH_4^+ (mg N m^{-2})		PO_4^{3-} (mg P m^{-2})		WSS (mg Glc-equ m^{-2})	
02-Jul-12	control	**27.0**[a]	±2.3	4.3	±0.4	387	±111	**302**[a]	±25	42.5	±11.2	29.3	±3.7
	LR	**46.6**[b]	±8.2	8.0	±1.8	470	±128	**710**[b]	±20	53.2	±10.8	33.8	±3.9
15-Jul-12	control	28.5	±4.0	8.2	±0.8	124	±40	225	±28	59.6	±12.5	43.0	±3.6
	LR	24.7	±4.7	7.1	±0.8	126	±42	284	±21	55.6	±8.9	36.6	±3.4
30-Jul-12	control	39.8	±6.1	5.8	±1.1	628	±230	449	±49	59.7	±8.2	42.7	±3.9
	LR	30.5	±3.1	4.2	±0.5	284	±97	393	±45	46.8	±3.5	39.8	±3.6
20-Aug-12	control	39.2	±3.7	5.8	±0.7	489	±140	336	±26	44.9	±5.7	19.8	±1.8
	LR	39.6	±5.4	6.2	±1.0	332	±86	402	±42	49.0	±8.4	19.7	±2.0
24-Sep-12	control	43.3	±4.5	4.7	±0.7	427	±210	342	±35	43.1	±4.8	16.0	±1.1
	LR	43.8	±4.9	5.1	±0.7	283	±90	345	±42	40.6	±4.6	15.1	±1.4
15-Oct-12	control	48.6	±6.2	4.8	±1.0	431	±116	365	±52	32.3	±3.7	13.2	±1.2
	LR	56.7	±5.2	6.7	±0.9	467	±107	372	±31	30.8	±5.7	11.2	±1.0
05-Dec-12	control	38.6	±5.0	6.4	±0.8	17.1	±5.0	127	±10	49.7	±6.1	27.1	±3.7
	LR	34.9	±2.9	5.7	±0.5	12.7	±8.1	106	±7	37.6	±3.3	21.8	±2.5
05-Feb-13	control	38.3	±3.2	6.2	±0.6	182	±65	273	±39	35.3	±3.7	22.8	±1.9
	LR	35.9	±3.6	5.9	±0.6	94.5	±27.2	256	±27	28.7	±4.1	19.0	±1.8

C_{mic} and N_{mic} microbial carbon and nitrogen, *WSS* water-soluble sugars (mg Glucose-equivalents m^{-2})

Data are means ± SE with $n = 12$ for each treatment. Bold values indicate significant difference between treatments (*t*-test; $P < 0.05$)

Table 2 Results from two-way ANOVA showing effects of time and litter removal on soil gas fluxes, soil parameters, and microbial groups detected by phospholipid fatty acid (PLFA) analysis. *T_{soil}* soil temperature at 5 cm, *VWC* soil volumetric water content, *C_{mic}* microbial carbon, *N_{mic}* microbial nitrogen, *WSS* water-soluble sugars

	Time		Litter removal		Time x Litter removal	
	F	p	F	p	F	p
CO_2	51.3	***	146	***	0.95	ns
CH_4	4.71	***	12.4	***	2.27	**
N_2O	1.92	*	22.8	***	0.62	ns
T_{soil}	107649	***	0.02	ns	46.2	***
VWC	52.8	***	2.91	ns	0.51	ns
C_{mic}	4.72	***	0.26	ns	1.73	ns
N_{mic}	1.92	ns	0.67	ns	2.00	ns
NO_3^-	108	***	0.01	ns	4.97	ns
NH_4^+	24.7	***	2.45	ns	2.31	*
PO_4^{3-}	21.9	*	0.16	ns	5.20	ns
WSS	2.45	*	0.38	ns	1.41	ns
pH	5.33	***	0.09	ns	2.10	*
Total PLFAs	2.20	ns	0.50	ns	3.49	*
Gram + bacteria	1.36	ns	0.68	ns	3.18	*
Gram- bacteria	2.36	ns	0.15	ns	4.06	*
Fungi	3.28	*	0.32	ns	0.59	ns

Soil gas fluxes and soil parameters, $n = 12$; microbial groups, $n = 3$. *Asterisks* indicate levels of significance (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

removed. At the other sampling dates between July and October 2012, soil CH_4 uptake was of similar magnitude in control and LR plots.

Average N_2O fluxes were 117.5 % lower in LR than control plots (Fig. 1d), with control plots acting as N_2O sources (5.72 ± 1.38 μg N_2O-N h^{-1} m^{-2}), while LR led to an uptake of atmospheric N_2O of 1.00 ± 1.16 μg N_2O-N h^{-1} m^{-2}. However, soils under both treatments switched between being N_2O sources and N_2O sinks during the study period. In control plots, we observed three N_2O emission peaks on 01-Aug-2012, 05-Sep-2012 and 17-Oct-2012, where VWC had rapidly increased after periods of dry conditions. Although VWC was similar in LR plots on these dates, N_2O fluxes did not increase. Furthermore, on 13-Jan-2013 high N_2O emissions were detected in both treatments under a thin snow and ice cover (~1 cm). On the other sampling dates, N_2O fluxes were below the LoD (Fig. 1d).

Carbon dioxide fluxes were positively correlated with soil temperature in both treatments (control: $r = 0.86$, $P < 0.01$; LR: $r = 0.84$, $P < 0.01$) and negatively correlated with VWC in LR plots ($r = -0.51$, $P < 0.05$). Methane fluxes were positively related to VWC only in control plots ($r = 0.53$, $P < 0.05$), whereas N_2O fluxes did not reveal any significant correlations with soil temperature or VWC. Soil temperature and VWC were negatively correlated over the study period in both control and LR plots (both $r = -0.64$, $P < 0.01$).

Temperature sensitivities of CO_2 fluxes (Q_{10}, Fig. 2) decreased with increasing soil temperature in both treatments. At 11 °C, which was the mean soil temperature during the study period, the Q_{10} calculated from CO_2 fluxes at 11 and 21 °C was 2.45 ± 0.07 in control plots and 2.86 ± 0.09 in LR plots. Calculated over the observed T_{soil} range (4–18 °C), removing the litter significantly increased Q_{10} values (t-test, $t = -13.7$, $p < 0.001$).

Soil microbial community composition

One week after removing the litter layer, C_{mic} increased by 72.6 % due to litter removal (Table 1). At all following sampling dates, C_{mic} in LR plots was not significantly different from controls. Furthermore, seasonal changes of C_{mic} were observed, with highest values in October, whereas N_{mic} was relatively stable throughout the study period.

Similarly to C_{mic}, the total sum of microbial PLFAs was affected by litter removal at the first sampling date and increased by 37.3 % 1 week after litter removal (Fig. 3). On this date, PLFA markers for gram+ (+36.9 %) and gram- (+30.9 %) bacteria were also significantly increased in LR plots. At the other samplings dates, no significant differences between treatments were found. However, seasonal changes in PLFA groups were detected in control plots, with highest concentrations of bacterial PLFA markers in August and February and lowest concentrations in July. The fungal PLFA marker 18:2ω6,9 constantly increased from July to February.

The influence of environmental parameters on total microbial community variation as expressed

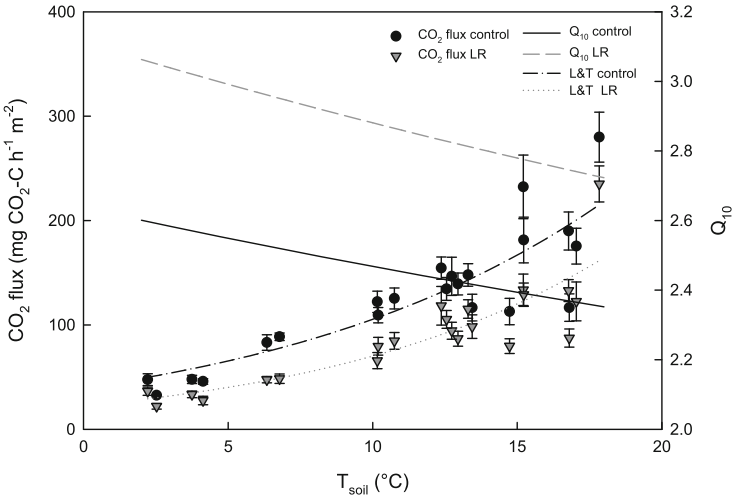

Fig. 2 CO_2 flux (mean ± SE, $n = 12$) in control (●) and litter-removal (▽) plots, and temperature sensitivity (Q_{10}) of CO_2 flux in control (*black solid line*) and litter-removal (*grey dashed line*) plots. Relationship between CO_2 flux and T_{soil} was best described by a Lloyd & Taylor (F&T) function ($r^2 = 0.74$, $P < 0.001$ for control plots, *black dashed-dotted line*; $r^2 = 0.73$, $P < 0.001$ for litter-removal plots, *grey dotted line*)

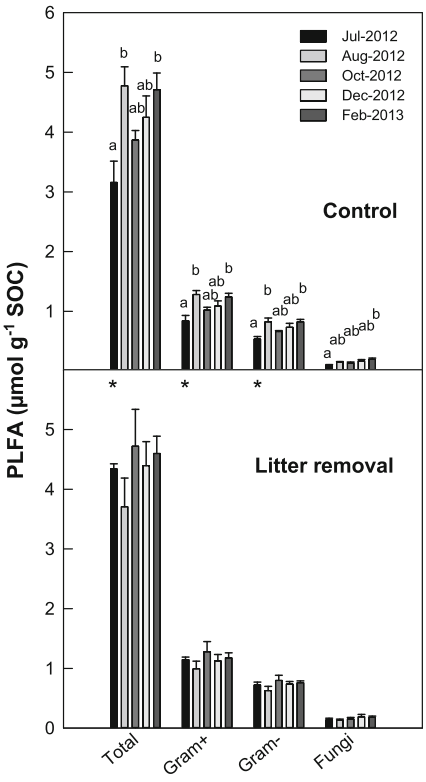

Fig. 3 Concentrations of total, gram + bacterial, gram- bacterial and fungal phospholipid fatty acid (PLFA) markers in soil from control (*upper panel*) and litter-removal (*lower panel*) plots from July 2012 to February 2013. *Asterisks* indicate significant differences between treatments at the respective time points (*t*-test; *, $P < 0.05$), *letters* indicate significant differences between time points for the respective treatment (one-way ANOVA, no time effect for litter removal was found). Given are means ± SE ($n = 3$)

by the constrained variability of the CCA was 67.9 %, split in 41.1 % and 17.6 % for the CCA1 and CCA2, respectively. Both CCA1 and CCA2 were significant ($P < 0.001$, permutation test). The abundance (mol%) of the fungal PLFA 18:2ω6,9 was positively related to VWC and PO_4^{3-} and negatively to C_{mic} and N_{mic}, SOC, TN and NO_3^- (Fig. 4a). The abundance of bacterial PLFAs (gram+, gram- and general bacteria) was positively related to soil temperature, NO_3^-, NH_4^+, SOC and TN, and negatively to pH, VWC and DaLR. PLFA scores (Fig. 4b), an indicator of species composition, showed that differences between treatments were only significant at the first two sampling dates. There was a clear separation between sampling time points showing a shift from July 2012 to February 2013.

Discussion

Soil properties

We hypothesized that litter removal affects concentrations of mobile C and nutrients in the mineral soil (Hypothesis i) because litter is a major source for soil nutrients, and depolymerization of litter compounds yields mobile molecules like sugars, phenols, amino acids and NO_3^- which are water-soluble and prone to leaching into the mineral soil. However, our results did not confirm this assumption. We only found a temporary increase of NH_4^+ at the first sampling date, which presumably was a disturbance effect of the litter removal in the week before. Surprisingly, we found no changes in NO_3^-, NH_4^+, PO_4^{3-} or WSS at any other sampling date. Similar results were reported by Xu et al. (2013), who conducted a meta-analysis on 70 *in situ* litter manipulation experiments across various ecosystems and climatic regions. They discovered that litter removal had no influence on concentrations of DOC, extractable inorganic N (EIN) and extractable P in mineral soils of temperate forests. Litter-derived DOC can be quickly mineralized by soil microbial communities (Kalbitz et al. 2003) and adsorbed to the soil mineral matrix (Guelland et al. 2013). Mobile N forms like NO_3^-, NH_4^+ and amino acids are quickly immobilized by microorganisms and plant roots in the mineral soil (Inselsbacher et al. 2010). Litter-derived P can be adsorbed to the mineral matrix (Tiessen 2008) or taken up by plant roots before it enters the mineral soil (Attiwill and Adams 1993). It is therefore possible that because DOC, inorganic N and P were either adsorbed to the mineral matrix or turned over quickly, in the present study changes in these pools caused by litter manipulation were not detectable with standard soil extraction methods that target plant-accessible compounds.

Soil greenhouse gas fluxes

In the present study, litter removal significantly changed soil fluxes of all three measured GHGs. In agreement with hypothesis ii, CO_2 fluxes were reduced by 29.9 % in LR plots, and litter-induced contribution to total CO_2 flux ranged from 15.6 to 46.1 %. This is in line with previous studies that have reported a litter-induced contribution of 5–45 % total soil CO_2 flux in temperate forests (Borken and Beese 2005; Bowden et al. 1993;

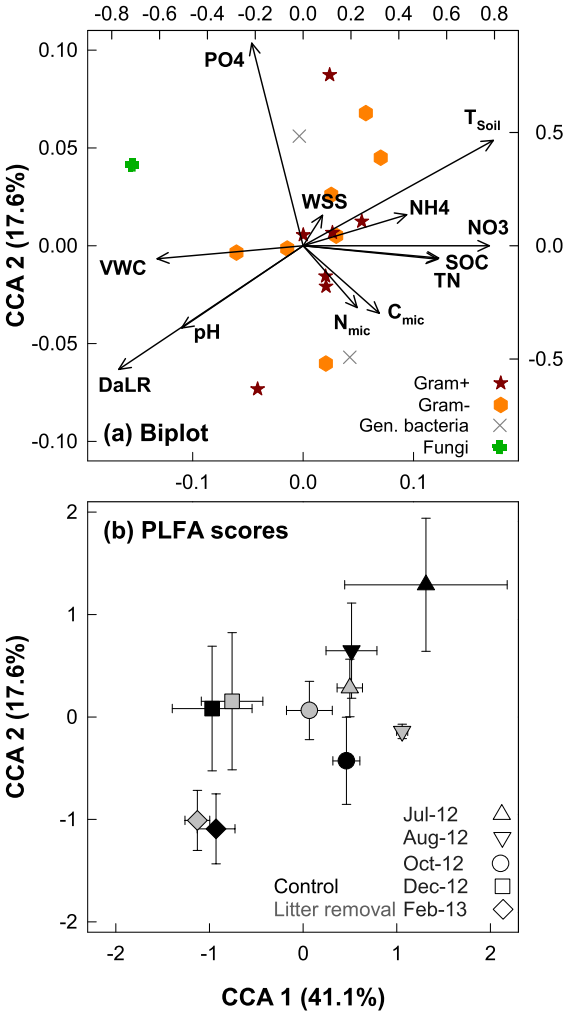

Fig. 4 Influence of soil parameters and time on microbial community composition as determined by canonical correspondence analysis (CCA). **a**, Biplot with microbial groups (gram+, gram-positive bacterial PLFAs; gram-, gram-negative bacterial PLFAs; gen. bacteria, unspecific bacterial PLFAs; fungi, fungal PLFA 18:2ω6,9) and explaining environmental variables as factor loadings (*arrows*). We used relative abundances (%mol) of single PLFA markers as soil microbial community matrix, and soil parameters (pH; VWC, soil volumetric water content; T_{Soil}, soil temperature; SOC, soil organic carbon; TN, total nitrogen; C_{mic}, microbial carbon; N_{mic}, microbial nitrogen; NH4, ammonium-N; NO3, nitrate-N; PO4, phosphate-P; WSS, water-soluble sugars) and time (DaLR, day after litter removal) as constraining variables. **b**, Distribution of samples collected at 5 time points in 2 treatments according to the PLFA species matrix (mean ± 95 % CI, $n = 3$)

Vose and Bolstad 2007). High contributions of the litter layer to total soil CO_2 fluxes can be explained by the active decomposition of litter material, which is rich in easily available C and nutrients. In the present study, the amount of C stored in the litter layer was estimated to be 0.55 kg C m^{-2}, which represents 21 % of the total soil C stock (litter-C + mineral soil-C in 0–5 cm soil depth). Carbon dioxide fluxes of both LR and control plots were closely related to T_{soil}. Temperature sensitivity as expressed by Q_{10} was higher in LR plots, indicating that CO_2 flux from mineral soil was more temperature-sensitive than litter-induced CO_2 flux. Similar results were reported by Creamer et al. (2015) for an Australian native woodland, who reported that the temperature sensitivity of litter-C was lower than that of soil-C. This supports the theory that with decreasing substrate quality, temperature sensitivity of soil CO_2 flux increases because more enzymatic steps are required to break down low-quality organic matter, and each of these steps in turn is temperature sensitive due to microbial enzyme kinetics (Bosatta and Ågren 1999; Fierer et al. 2005; Yuste et al. 2007). However, because we have not tested the temperature sensitivity of litter-induced CO_2 flux alone, we cannot prove this assumption.

Methane fluxes were negative during the entire study period, which indicates constant uptake of atmospheric CH_4 by soils of both treatments. Well-aerated soils of upland forests have been shown to act mostly as CH_4 sinks due to high activity of methanotrophic bacteria that oxidize CH_4 under aerobic conditions to produce energy (Blais et al. 2005; Le Mer and Roger 2001). In our study, litter removal increased average CH_4 uptake by 16.0 %, which corroborates hypothesis iii. We found highest litter-induced contributions to total CH_4 fluxes between November and January, where CH_4 uptake was between 19.9 and 100.9 % higher in LR plots than in control plots. This period was characterized by steadily increasing VWC due to frequent rainfalls. Soil VWC was similar in both treatments at all sampling dates and can therefore not explain different CH_4 fluxes in the two treatments. However, we assume that the wet litter layer itself acted as a barrier against diffusion of atmospheric CH_4 into the soil and, therefore, reduced CH_4 uptake in control plots. This has also been suggested for subtropical forests (Wang et al. 2013) and temperate forests, especially broad-leaved forests like beech (Brumme and Borken 1999). Nevertheless, we cannot test this assumption because we measured only net CH_4 fluxes but not CH_4 diffusion. Furthermore, leachates such as monoterpenes from litter have been described to suppress CH_4 consumption in mineral soils (Amaral and Knowles 1997, 1998), from which we conclude that

litter removal increases CH_4 consumption and that the inhibitory effect of the litter layer might be stronger in the wet season. We found a positive correlation between CH_4 fluxes and VWC in control plots, which indicates lower CH_4 uptake rates (i.e. less negative CH_4 fluxes) at high VWC in the presence of an intact litter layer. If soil VWC is high, soil O_2 levels are low, which can reduce CH_4 oxidation by methanotrophic bacteria and decrease CH_4 uptake rates.

In accordance with hypothesis iv, litter removal decreased average N_2O fluxes by 117.5 % and turned soils from N_2O sources (5.72 μg N_2O-N h^{-1} m^{-2} in controls) to moderate N_2O sinks (−1.00 μg N_2O-N h^{-1} m^{-2} in LR). Nitrous oxide uptake by soils of various ecosystems has frequently been reported and was reviewed by Schlesinger (2013) but has also been challenged as measurement error (Cowan et al. 2014). In the present study, three N_2O emission peaks were measured in control plots between August and October, which all coincided with rapid increases in soil VWC due to heavy rainfalls after dry periods. Interestingly, these peaks only occurred in control plots, although VWC was not different between treatments. It is, however, possible that after rainfall the wet litter layer acted as diffusion barrier for O_2 and created anoxic microsites in control plots where N_2O was produced. Another possible explanation is that increased runoff due to litter removal led to higher local aeration and therefore reduction of N_2O to N_2 in aerobic microsites, although VWC was not lower in LR plots. It is also conceivable that N_2O was produced in the wet litter layer itself, which is rich in C and N to support nitrification and denitrification, and which after rainfalls might contain enough moisture to form anoxic microsites. Dong et al. (1998) reported that 50 % of emitted N_2O in a German beech forest originated from the leaf litter/humus layer. In the present study, we observed high N_2O emissions from both control and LR plots in January 2013. This could be explained by the presence of a thin snow and ice layer that might have acted as diffusion barrier against O_2 and thus created anoxic conditions in both treatments. This was corroborated by low CH_4 consumption rates in control and LR plots at this particular date. Furthermore, although negative soil temperatures in 5 cm depth were not recorded on any of the gas sampling dates, a preceding freeze-thaw event on the soil surface could have led to elevated N_2O fluxes on this date, as has been observed earlier (e.g., van Bochove et al. 2000; Teepe et al. 2001; Wolf et al. 2012; Butterbach-Bahl et al. 2013). We found no correlations between N_2O fluxes and T_{soil} or VWC, which indicates that N_2O formation and consumption was limited by low N content and acidic pH at our study site, as has also been reported for other temperate forests (Butterbach-Bahl et al. 1998; Castro et al. 1992; Hahn et al. 2000).

Soil microbial community composition

Our data suggest an initial transient effect of litter removal on soil microbial abundance and community composition. At the first sampling 1 week after litter removal, C_{mic} as well as PLFAs of gram+and gram- bacteria increased in LR plots, whereas we found no difference between treatments at the consecutive samplings. This immediate increase in bacterial PLFAs could be a consequence of the litter removal at the beginning of the experiment. Although we took great care to completely remove the litter layer, we cannot rule out that some remains of fine debris were left on the LR plots. This remaining fine debris would probably be slightly damaged and also well-aerated because the litter layer on top was removed. Because fragmentation increases litter decomposability (David and Handa 2010; Hassall et al. 1987), this might have led to a flush of available C and nutrients, which could have supported fast-growing bacteria and led to increased concentrations of bacterial PLFAs at the first sampling. In the long term, however, we did not find any influence of litter removal on the contribution of fungi to the soil microbial community, which refutes hypothesis v. This is in line with a study of Brant et al. (2006), which studied the influence of above- and below-ground litter manipulation on soil microorganisms at 3 different sites in the USA and Hungary. They reported no influence of aboveground litter removal after 4, 7 and 13 years, respectively. Similar to our results, Creamer et al. (2015) reported that bacterial community composition analysed by terminal restriction fragment length polymorphism (T-RFLP) was not different in mineral soils compared to mineral soils mixed with pre-incubated eucalyptus litter. In a study that used ^{14}C-labelled leaf litter, Kramer et al. (2010) discovered

that recent (<4 year old) leaf litter made up <10 % microbial-C in mineral soil of a temperate oak forest, whereas greatest inputs to microbial-C originated from roots. Our results corroborate that removal of aboveground litter does not influence microbial community composition of mineral soils within 8 months.

Similar to previous studies, we found a significant influence of seasonality on soil microbial community composition (Kaiser et al. 2010; Koranda et al. 2013; Rasche et al. 2011). From summer to winter, bacterial and fungal PLFA markers increased slightly. CCA analysis of single PLFA composition showed that differences between sampling time points were larger than between treatments. Our results indicate that seasonal differences in microbial community composition seem to be linked to soil pH, T_{soil} and VWC. In a study in an Austrian beech forest similar to our site, Kaiser et al. (2010) also found a significant influence of soil moisture and temperature on soil microbial community composition. This seems plausible, as water availability and temperature are well-known determinants of microbial metabolism. Overall, our data confirm the importance of seasonal changes in temperature, moisture availability and soil nutrient cycling for the composition of microbial communities in temperate forest soils.

Conclusions

The litter layer contributes largely to soil GHG fluxes and influences temperature sensitivity of soil CO_2 fluxes. This should be accounted for in climate change models as litter represents a major component of total C input to soils. Our results suggest that in the short term, the litter layer controls soil GHG fluxes mainly via physical processes and C chemistry and not via nutrient leaching into the mineral soil. Furthermore, our data indicate that nutrient leaching from litter does not determine microbial community composition in the mineral soil in the short term. Our results are relevant for the basic understanding of forest biogeochemical cycles and should be taken into account when assessing GHG budgets in forests.

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3.2. PAPER #2: SHORT-TERM SOIL MINERAL AND ORGANIC NITROGEN FLUXES DURING MODERATE AND SEVERE DRYING-REWETTING EVENTS

Sonja Leitner[1], Pia Minixhofer[1], Erich Inselsbacher[2], Katharina M. Keiblinger[1], Michael Zimmermann[1,3], Sophie Zechmeister-Boltenstern[1]

[1] Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

[2] Department of Geography and Regional Research, University of Vienna, Vienna, Austria

[3] Federal Office for Agriculture (BLW), Bern, Switzerland

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Contributions:

SL selected the sampling site and designed the study, performed microdialysis measurements, calculated statistics, wrote the manuscript

PM performed microdialysis measurements and laboratory analysis, wrote the manuscript

EI supervised and performed microdialysis measurements, wrote the manuscript

KK supervised amino acid analysis

MZ selected the sampling site and designed the study

SZB supervised the study, wrote the manuscript

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Short communication

Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying–rewetting events

Sonja Leitner[a,*], Pia Minixhofer[a], Erich Inselsbacher[b], Katharina M. Keiblinger[a], Michael Zimmermann[a,c], Sophie Zechmeister-Boltenstern[a]

[a] University of Natural Resources and Life Sciences Vienna (BOKU), Institute of Soil Research, Peter Jordan-Straße 82, 1190 Vienna, Austria
[b] University of Vienna, Department of Geography and Regional Research, Althanstraße 14, 1090 Vienna, Austria
[c] Federal Office for Agriculture (BLW), Mattenhofstraße 5, 3003 Bern, Switzerland

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ABSTRACT

Nitrogen (N) availability to plants in dry soil is limited by diffusive flux of N compounds through the soil solution towards the root surface. Conventional soil extraction procedures only provide information about bulk soil N concentrations, which can be distorted during soil sampling, transport, storage and extraction, and hence are of limited use to detect short-term N dynamics. Soil microdialysis is a new tool to monitor diffusive flux of mineral and organic N compounds *in situ* in high temporal and spatial resolution with minimal disturbance, and is therefore well-suited to determine dynamic fractions of plant-available N in soil microsites.

We investigated N availability and mobilization during a drying–rewetting event in a temperate beech forest using soil microdialysis and soil extractions with water. While water extracts mainly revealed mineral N in the form of NH_4^+ and NO_3^-, diffusive N fluxes *in situ* were dominated by amino acids. Microdialysis showed that rewetting of dry soil led to a fast but short-lived mobilization of NO_3^- and some neutral hydrophilic amino acids (lysine, glutamine, cysteine, glycine), which was not detected in water extracts, and the rewetting N flush was larger with increasing drought duration. Our results suggest that at our temperate forest site plant-available N was dominated by amino acids, a fraction of N that might be missed using conventional soil extraction methods. Considering expected increases in the frequency of extreme climatic events, the observed release of mobile N forms bears the potential of N loss from soil if severe drought is followed by a heavy rain event.

1. Introduction

Almost all ecosystems experience periods of drought followed by rewetting events. An increase in the frequency and severity of extreme weather events due to climate change is expected to aggravate the negative effects associated with drought (IPCC, 2014). One of the most obvious adverse effects is that plants as well as microorganisms may be impacted by severe drought stress. At the same time, the lack of water leads to nutrient limitation since the capacity of soils to supply sufficient nutrients is determined by the availability of water. When soils dry out, diffusion of nutrients through the soil towards root surfaces and soil microorganisms is inhibited by reduced water-filled pore space and increased tortuosity of water films around solid particles (Moldrup et al., 2001). Furthermore, when plants experience drought stress, stomatal conductance and consequently transpiration is reduced, which decreases mass flow of water and dissolved nutrients to the root surfaces, further decreasing the supply of nutrients for plant uptake. With ongoing soil drying, water films are disrupted and roots and microorganisms get physically separated from nutrients. Taken together, these drought effects lead to an accumulation of nutrients in soils during extended dry periods because they are not taken up by plants or immobilized by microorganisms. During rewetting of dry soil these accumulated nutrients can be mobilized rapidly and are prone to leaching. This nutrient flush during rewetting results in temporary pulses of increased microbial activity (Manzoni et al., 2014) and high rates of nutrient turnover (Birch, 1958; Evans et al., 2016).

* Corresponding author. Tel.: +43 1 47654 91142.
E-mail address: sonja.leitner@boku.ac.at (S. Leitner).

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Plant nitrogen (N) acquisition is a complex process involving both transport of N in the soil and across root membranes and mycorrhizal hyphae (hereafter referred to as roots) (Leadley et al., 1997; Tinker and Nye, 2000), but several studies indicate that soil N supply rates, not root uptake rates, critically determine plant N acquisition (Clarkson and Hanson, 1980; Lambers et al., 2008; Leadley et al., 1997). Generally, the supply of N by diffusion becomes increasingly important at times when mass flow is low or absent and cannot meet the N demand of plants, as is the case at reduced transpiration rates and low soil water contents (Clarke and Barley, 1968; Comerford, 2005; Gerber and Brookshire, 2014).

Besides soil water content, N diffusion is controlled by a variety of other factors including bulk density, buffering capacity and ion exchange capacity (Jungk and Claassen, 1997; Lipson and Näsholm, 2001; Van Rees et al., 1990). Mobility of different forms of N in the soil solution depends on solute charge because the predominantly negative surface charge of clay minerals and soil organic matter leads to a retention of cations like ammonium (NH_4^+) and basic amino acids, whereas anions like nitrate (NO_3^-) and acidic amino acids or neutral hydrophilic amino acids can move more easily through the soil solution (Rothstein, 2010). On the other hand, hydrophobic compounds have been shown to be more easily adsorbed to soil particles compared to hydrophilic substances (Kaiser and Zech, 2000). Therefore, it is important to not only estimate soil factors such as pH and ion exchange capacity, but also to estimate the relative abundance of individual N compounds in undisturbed soils directly in the field. However, until now, this task remained challenging owing to inadequate soil sampling techniques.

Over the last three decades, several attempts have been made to estimate soil N pools and turn-over rates *in situ*. The most prominent are the use of ion-exchange resin bags or resin columns, soil solution collection by different kinds of lysimeters, soil centrifugation, and *in-situ* water perfusion and extraction (Andersson, 2003; Binkley et al., 1992; Chen and Williams, 2013; Giesler and Lundström, 1993; Raison et al., 1987; Weihermüller et al., 2007). The primary objective of all these studies was to best approximate N under field conditions.

One promising approach, based on soil microdialysis, has recently been established as a novel tool to monitor soil N fluxes *in situ* at high spatial and temporal resolution (Inselsbacher and Näsholm, 2012a; Inselsbacher et al., 2011). In contrast to conventional soil extracts, which have been criticized for altering soil N concentrations during soil sampling, transport, storage, sieving and shaking (Černohlávková et al., 2009; Inselsbacher, 2014; Jones and Willett, 2006; Rousk and Jones, 2010; Warren and Taranto, 2010), monitoring soil N fluxes by microdialysis directly reflects N availability to plant roots (Inselsbacher and Näsholm, 2012a). Because of the small size of the microdialysis membrane (1 cm long with an outer diameter of 0.5 mm), its installation causes minimal disturbance of the soil matrix (Inselsbacher et al., 2011) and allows the continuous measurement of N diffusion from the bulk soil across the membrane surface for hours or days.

In the present study we combined a conventional soil water extraction method with soil microdialysis to detect mobilization of mineral (NH_4^+ and NO_3^-) and organic (amino acids) N in the first 72 h after a rewetting pulse *in situ*. We hypothesized that (i) rewetting of dry soil mobilizes both mineral and organic N and that soil microdialysis reveals short-term N patterns which are not reflected in conventional soil extracts, and (ii) that the size of the mobilization flush is larger when the preceding drought is longer. To this end, a precipitation manipulation experiment in a temperate forest was used, and N mobilization was monitored at high temporal resolution following irrigation of dry soil in the autumn of 2014.

2. Material and methods

2.1. Study site and experimental design

The study was conducted in a temperate beech forest (*Fagus sylvatica* L., stand age 120 years) at the ILTER-site "Rosalia Lehrforst" in Lower Austria (47°42′26.33″N, 16°17′58.15″E, 600 m asl). The mean annual temperature is 6.5 °C and the mean annual precipitation 796 mm. The soil was classified as pseudo-gleyic cambisol over granitic bedrock with 4.1% SOC, 0.18% N, pH 4.0, and 0.595 g cm^{-3} bulk density (Leitner et al., 2016).

At the study site a rainfall manipulation experiment had been set up in May 2013 (Schwen et al., 2014). In short, 8 experimental plots of 2 m × 2 m each were covered with 4 m × 4 m transparent acrylic roofs 1.2 m above the ground surface to exclude rainfall. Two parallel manipulation treatments ($n = 4$) were conducted during the vegetation period (May until October): i) a moderately stressed four-week drought (4WD) treatment, which experienced six drying–rewetting cycles, each consisting of four weeks of precipitation exclusion followed by irrigation with 75 mm decalcified tap water, and ii) a severely stressed eight-week drought (8WD) treatment that received three drying–rewetting cycles, each consisting of eight weeks of precipitation exclusion followed by irrigation with 150 mm decalcified tap water. Both manipulation treatments were repeated in 2013 and 2014. In each plot, soil sensors were buried in 10 cm depth to measure soil volumetric water content (VWC, TDR theta.ML2x probes, UMS, Germany) and temperature (T_{soil}, thermistor Th2-f probes, UMS, Germany).

2.2. Soil analysis

In October 2014 at the end of the experimental rainfall manipulation, soil samples were taken in each plot 1 h before, and 24 h and 72 h after irrigation in triplicates with a steel soil corer (4 cm diameter, 10 cm length) and homogenized into one composite soil sample per plot. Soil was transported to the lab on ice and immediately sieved (<2 mm) and stored at 4 °C over night. On the next day, aliquots of 2.5 g field-moist soil were extracted with 25 ml high-purity deionized water (MilliQ) for 1 h on a rotary shaker, filtered with acid-free filter paper (Whatman Type 40, pore size 8 μm), and stored at −20 °C for further analysis.

To determine *in-situ* N diffusion before and during the first 20 h after irrigation we deployed two microdialysis systems, each consisting of a syringe infusion precision pump (CMA 400) equipped with four gas-tight microsyringes (5 ml, Hamilton, Bonaduz, Switzerland) which provided the perfusate solution. Each syringe was connected via 50 cm FEP tubing to a microdialysis probe with a polyarylethersulphone membrane (CMA 20, 10 mm length, 500 μm outer and 400 μm inner diameter, 20 kDa molecular weight cut-off). Membranes were installed 2 h prior to the irrigation at least 50 cm within the plots to a soil depth of 1.5 cm. In detail, the litter layer was lifted and a guiding channel was prepared by a steel cannula (800 μm outer diameter). The membranes were inserted carefully into the prepared channels and were then left in the soil throughout the experiment. The membranes were perfused with MilliQ water at a flow rate of 5 μl min^{-1} for 9 h, after which the flow rate was switched to 1 μl min^{-1} over night. Samples were collected continuously in 300 μl vials in a refrigerated microfraction collector (6 °C; CMA 470), transported to the lab on ice and stored at −20 °C until analysis. All equipment is commercially available at CMA Microdialysis AB (Solna, Sweden). Membrane calibration and calculation of N diffusion rates based on microdialysis membrane surface and time was done according to Inselsbacher and

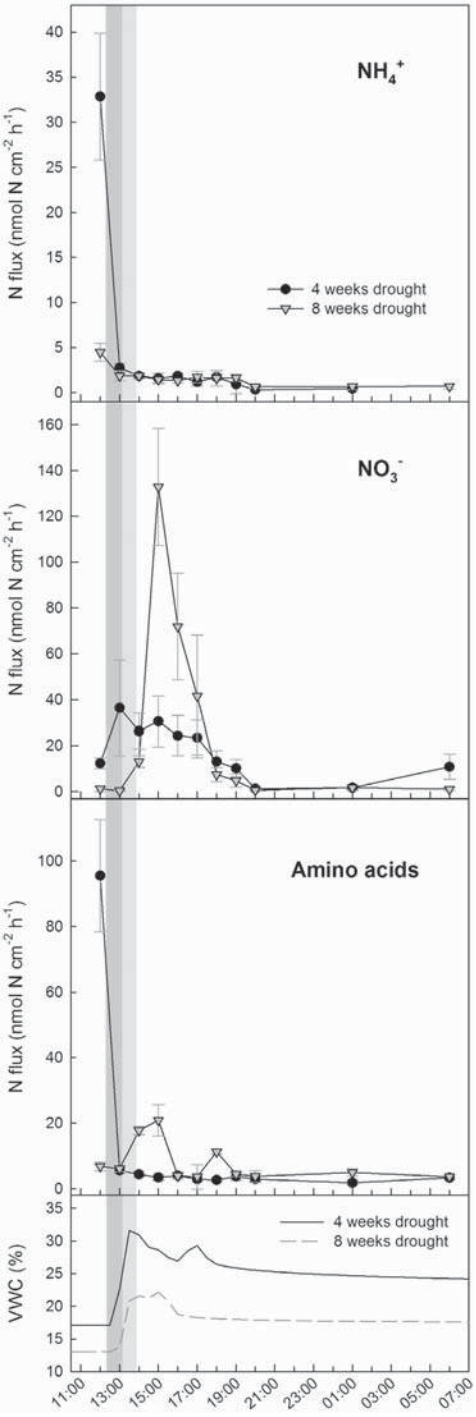

Fig. 1. Microdialysis flux of NH_4^+, NO_3^-, and sum of 19 amino acids after four and eight weeks of experimental drought, and soil volumetric water content (VWC, bottom panel) in 10 cm depth. Vertical bars show the duration of the manual irrigation (light gray: 150 mm irrigation following eight weeks of drought; dark gray: 75 mm irrigation following four weeks of drought). Shown are averages ± SE of four microdialysis membranes (upper three panels) and averages of four VWC sensors (bottom panel).

Näsholm (2012b). Briefly, membrane functionality was tested before and after field use by determining the relative recovery (RR) of sampled N compounds. To this end, membranes were placed in a beaker containing a standard solution of 100 μmol l^{-1} NO_3^-, NH_4^+ and 19 amino acids (asp, aspartic acid; glu, glutamic acid; his, histidine; arg, arginine; lys, lysine; asn, asparagine; ser, serine; gln, glutamine; thr, threonine; tyr, tyrosine; cys, cysteine; gly, glycine; ala, alanine; val, valine; met, methionine; trp, tryptophan; phe, phenylalanine; ile, isoleucine; leu, leucine) in MilliQ water on a magnetic stirrer and samples were collected over the course of 3 h at a flow rate of 5 μl min^{-1}. Relative recovery of individual N compounds was then calculated as given in Eq. (1),

$$RR\,(\%) = c_{dial}/c_{std} \times 100 \quad (1)$$

where c_{dial} is the concentration of N compound in the dialysate, and c_{std} is the concentration of N compound in the standard solution.

Nitrogen diffusion rates over the membrane surface were calculated according to Inselsbacher et al. (2014) following Eq. (2)

$$F_{MD} = c \times V/(A \times t) \quad (2)$$

where F_{MD} is the diffusive flux rate of N compound over the membrane surface given in nmol N cm^{-2} h^{-1}, c is the concentration of N compound in nmol N μl^{-1}, V is the sample volume (300 μl), A is the membrane surface area (0.159 cm^2), and t is the sampling time in h that is required to obtain 300 μl of sample (e.g., 1 h at a flux rate of 5 μl min^{-1}, 5 h at a flux rate of 1 μl min^{-1}).

Microdialysis samples and soil water extracts were analyzed colorimetrically for NO_3^- and NH_4^+ concentrations (Hood-Nowotny et al., 2010). Furthermore, concentrations of 19 individual amino acids were measured on an Agilent 1200 HPLC system equipped with a precolumn and an Eclipse Plus RP C18 3.5 μm column (4.6 × 150 mm), coupled to a fluorescence detector (FLR, exc. 230 nm, em. 450 nm, PMT 11) and a diode array detector (DAD, signal A: 338 nm, 10 nm; Ref 390, 20 nm; Signal B: 262, 16 nm; Ref 224, 8 nm, Signal C: 230 nm, 16 nm; Ref 360, 100 nm; all purchased from Agilent Technologies, Vienna, Austria). As mobile phase, borate-phosphate buffer (solvent A, 10 mM sodium hydrogenphosphate and 10 mM sodium borate wit 8 ppm NaN_3, pH 8.2) and methanol:acetonitrile:water (9:9:2, solvent B) were used at a flow rate of 1.5 ml min^{-1} in a gradient (start at 2% solvent B, increase to 57% solvent B from 0.5 min to 21 min, then 100% solvent B to 22 min, then 3 min post-run equilibration back to 2% solvent B) at 40 °C column temperature. For online pre-column derivatization, 1 μl sample or standard were derivatized with 0.5 μl o-phthaldehyde (OPA) and 0.4 μl 9-fluorenylmethyl chloroformate (FMOC) in 2.5 μl borate buffer (pH 10.2) using 32 μl solvent A with 1.5% (v/v) phosphoric acid to stop the reaction. As standards, a mixture of 23 amino acids (purchased from Agilent) was employed at concentrations of 225, 90, 45, 22.5, 9 and 4.5 μM. For more details on the HPLC method please refer to Woodward et al. (2007) and Frank and Powers (2007). Samples under the limit of detection (LOD) were excluded from data analysis (Armbruster and Pry, 2008).

2.3. Statistics

To determine the effects of drought treatment and time on N concentrations and fluxes, we used two-way ANOVA followed by Tukey's HSD *post hoc* test. Homogeneity of variance was tested with Levene's test, and if necessary data were log-transformed.

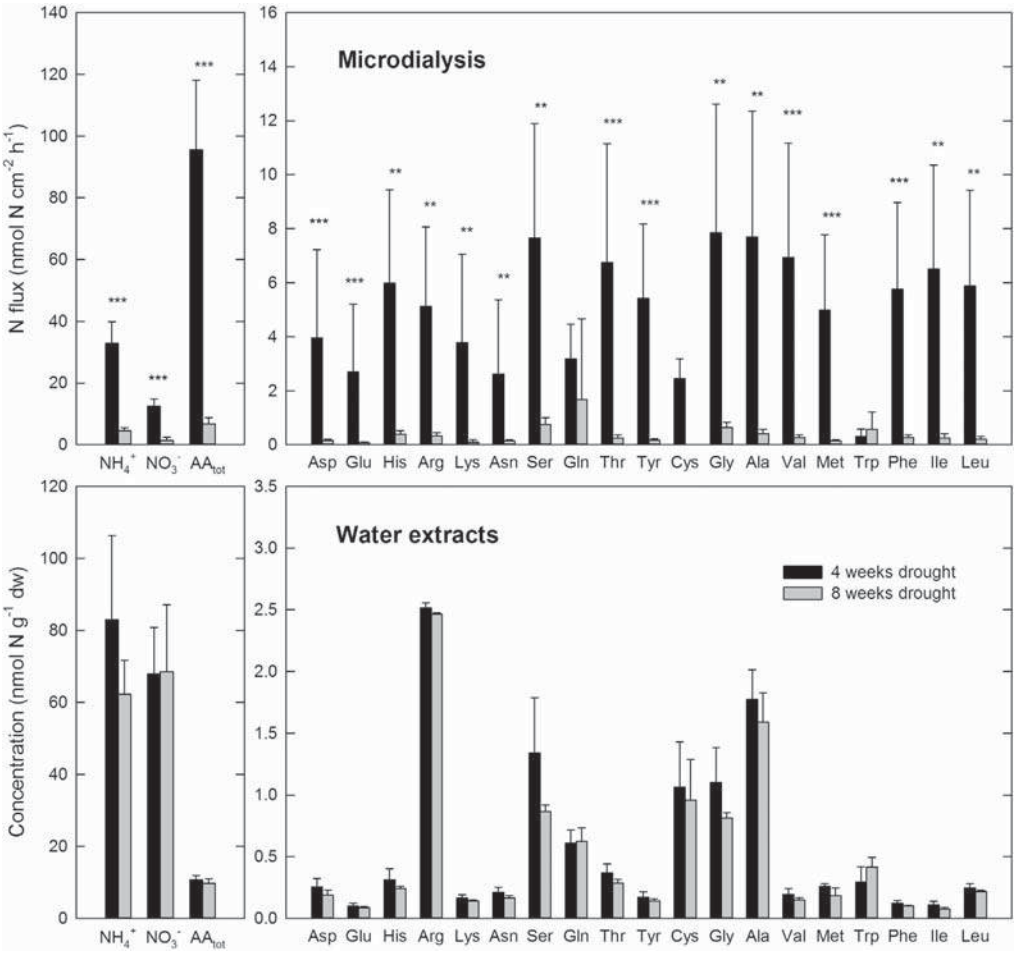

Fig. 2. Comparison of NH_4^+, NO_3^-, total amino acids (AA_{tot}) and 19 individual amino acids determined by microdialysis (upper panel) and water extracts of soil samples (lower panel) after four and eight weeks of experimental drought. Shown are averages ± SE ($n = 4$) of the first sampling time point (before irrigation). Please note the different scales of *y*-axes. Asterisks denote significant differences between drought treatments (two-way ANOVA, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Statistical analysis was performed with R 3.3.2 (www.r-project.org, packages "afex" and "lmerTest").

3. Results and discussion

Plant-available N compounds are primarily limited by their rate of diffusion through the soil to the root surface and not by bulk soil N contents (Nye, 1980) or root uptake capacity (Oyewole et al., 2016). Diffusion of N towards root surfaces is a process that integrates multiple factors like soil solution concentration, root uptake rates, viscosity of the diffusion medium, distance from source to sink and tortuosity of the pore space (Moldrup et al., 2001), and interactions of N compounds with minerals and organic matter (Gardner, 1965). Microdialysis has recently been established as a feasible tool to measure diffusive fluxes of N compounds through the soil solution and over the microdialysis membrane, and has been successfully employed in boreal forests (Inselsbacher and Näsholm, 2012b; Inselsbacher et al., 2014; Oyewole et al., 2014) and agricultural systems (Brackin et al., 2015). Here we present the first study to employ this technique in a temperate forest soil to assess short-term changes in N availability after experimental rainfall manipulation.

Nitrogen pools in soils are highly dynamic and depend on various processes including enzymatic depolymerization of proteins, input via root exudation, N transformation (e.g. ammonification, nitrification), uptake by plants (Näsholm et al., 2009) and immobilization by microorganisms (Schimel and Bennett, 2004; Warren and Taranto, 2010). These processes are variable in both time and space and can create microsites with disproportionately high concentrations or reaction rates relative to the surrounding soil matrix (Hagedorn and Bellamy, 2011; Leon et al., 2014). Soil extraction integrates N concentrations over a soil volume in the range of a couple of cm^3 that are relatively static over hours or days. In contrast, the small size of microdialysis membranes and high sampling intervals enable a high spatial (≤1 mm) and temporal (≤1 h) resolution of N availability, which is especially relevant in organic-rich heterogeneous forest soils (Inselsbacher and Näsholm, 2012b). Locally isolated patches of high N concentrations can be generated when N accumulates due to a number of reasons: (i) when microbial activity has ceased due

to lack of water but enzymatic decomposition of organic matter is still sustained (Lawrence et al., 2009), (ii) when microorganisms are physically separated from their substrates due to the disruption of water films in dry soil (Manzoni et al., 2014), or (iii) when microsites are not explored by roots.

Our microdialysis results showed large differences in NH_4^+ and amino acid flux between the 4WD and 8WD treatment with flux rates that varied by a factor of 10 (Fig. 1). When implanting microdialysis membranes into the soil it is possible to sample microsites that had not been accessed by living roots or microorganisms and where N had accumulated. When evaluating temporal dynamics, the high flux of NH_4^+ and total amino acids in the 4WD treatment was not sustained but dropped after 1 h to fluxes similar to the 8WD treatment (Fig. 1). As expected (hypothesis i), irrigation of the soil led to a mobilization of NO_3^- and some neutral hydrophilic amino acids (lys, gln, cys, gly, Supplementary Fig. S1). Overall, more N was mobilized in the 8WD treatment than in the 4WD treatment, suggesting that during the longer drought period more labile N had accumulated (hypothesis ii). This is in line with Bimüller et al. (2014) who reported that prolonged summer drought led to an accumulation of more labile N fractions in a beech forest in southern Germany. Similarly, Williams and Xia (2009) found that the drier the soil before rewetting, the more microbial and soluble organic matter pools increase in soil. In addition to the mobilization of accumulated N, rewetting leads to a burst in microbial activity (Placella et al., 2012) and increased N transformation rates (Borken and Matzner, 2009) within minutes and hours, which might have added additional free amino acids and NO_3^- to the soil solution as water content increased.

In microdialysis samples from both treatments at the first time point (before rewetting) available N was dominated by organic N, which contributed 54-68% to total N flux, while NH_4^+ accounted for 23–35% and NO_3^- for only 9–10% (Fig. 2, upper panel). This corresponds to microdialysis results from boreal forests, where amino acids contributed 74-89% of the total N flux (Inselsbacher and Näsholm, 2012a). There is growing evidence that plants take up organic N forms even in competition with soil microbes (Ganeteg et al., 2017; Lipson and Näsholm, 2001; Näsholm et al., 2009; Schmidt et al., 2014). Since beech trees are able to use amino acids as source for their N demand (Leuschner et al., 2006; Scott and Rothstein, 2011), the high abundance of amino acids at our temperate beech forest site could represent an important source for N plant nutrition. In contrast to microdialysis samples, water extracts were dominated by mineral N, with NH_4^+ contributing 44–51% and NO_3^- contributing 42–49%, whereas total amino acids amounted to only 6–7% of total N in the present study (Fig. 2, lower panel). Effects of drought length as well as short-term ($\leq$24 h) changes in NO_3^-, NH_4^+ and AA_{tot} concentrations were not detected in water extracts (Supplementary Fig. S2 and Supplementary Table 1). Soil water extracts are thought to represent bulk N concentration, which includes both available unbound N from the soil solution plus protected N that was made available by destruction of soil aggregates during sampling, sieving and shaking. Sieving and extracting also lead to a loss of amino acids due to ongoing mineralization and subsequent nitrification during sample handling (Inselsbacher, 2014; Rousk and Jones, 2010). Results from water extraction of soil samples may therefore overestimate the contribution of inorganic N forms compared to amino acids to total plant-available N. The significant discrepancy between results gained by water extraction and microdialysis sampling in the present study highlights the importance of estimating soil N fluxes with as little disturbance of the natural soil structure as possible. Our results further show that, similar to boreal forests (Inselsbacher and Näsholm, 2012a), amino acids may constitute a more important source for plant N nutrition than previously assumed in temperate beech forests.

In conclusion, our results suggest that the increased release of N during and following rewetting was likely caused by an immediate but short-lived mobilization of N that had accumulated during drying, and that it is the chemical nature (i.e., charge and polarity) of a compound that determines whether it is mobilized upon rewetting or not. Furthermore, we showed that not only in boreal but also in temperate forests N availability can be dominated by amino acid and not mineral N, a conclusion that might be missed when using conventional soil water extracts. We suggest that microdialysis is well suited to monitor both short- and long-term changes in soil N dynamics during drying–rewetting cycles *in situ*. This technique is therefore of particular interest for future studies examining the effects of drought stress and extreme events on plant and microbial N nutrition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.02.014.

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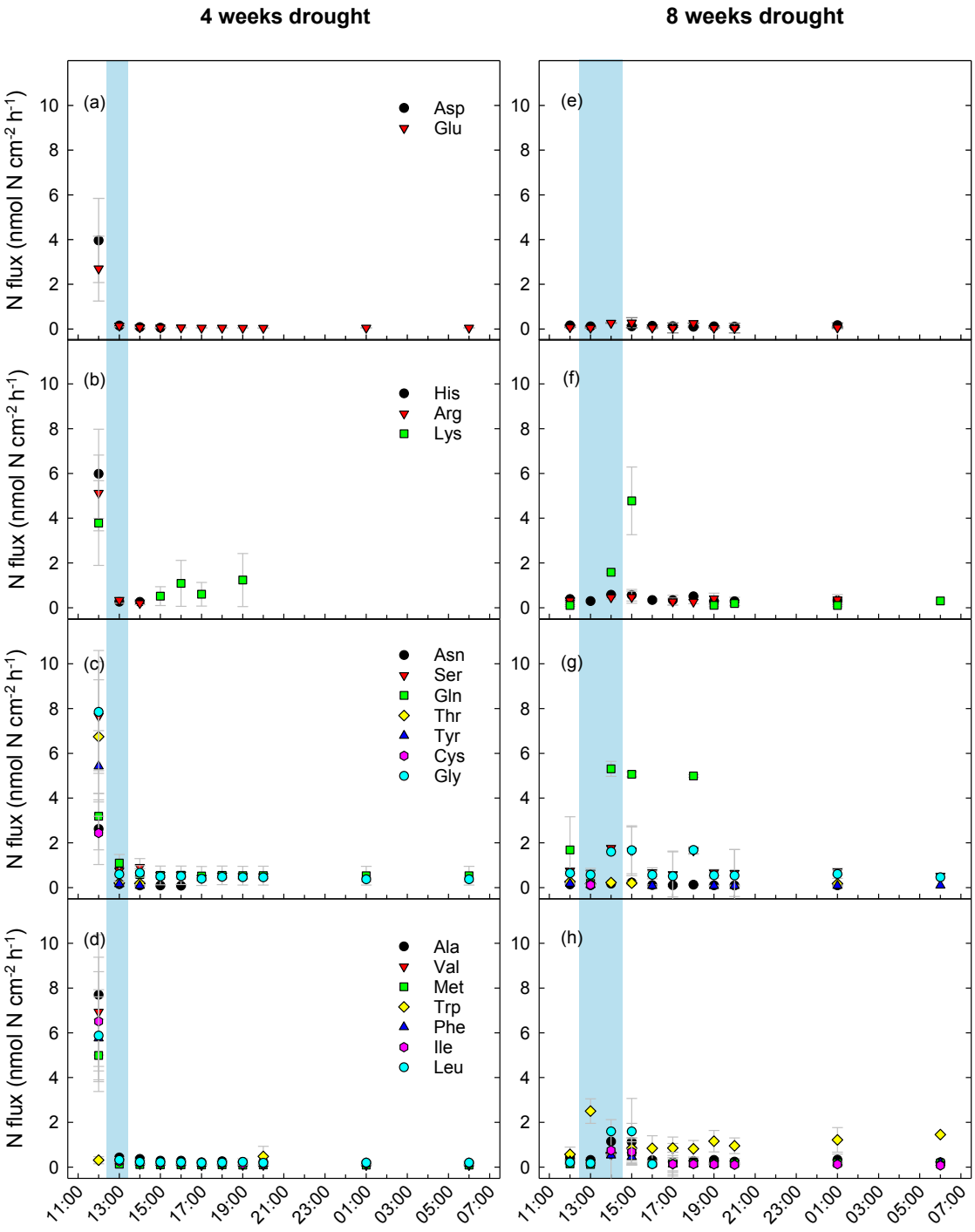
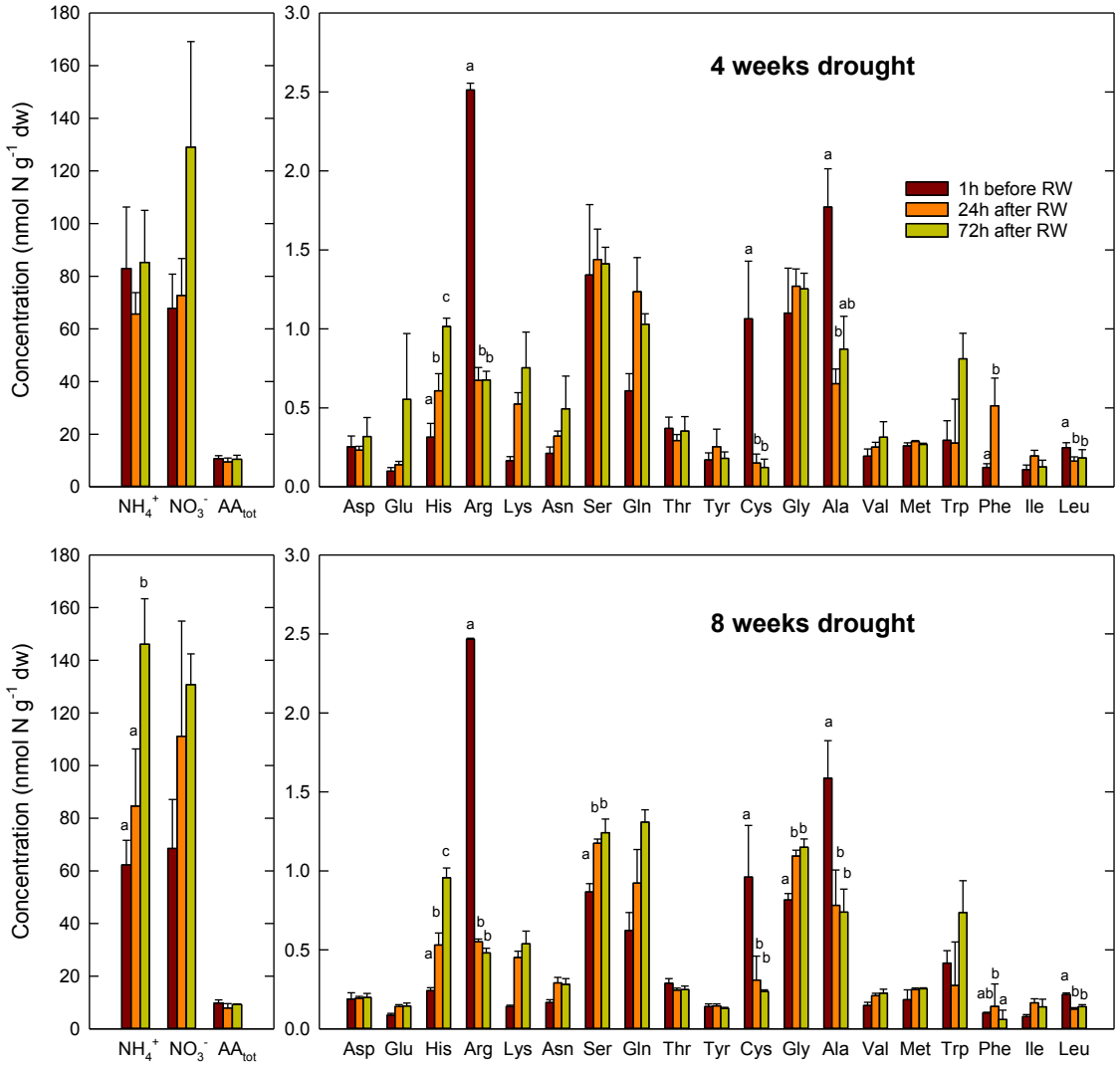
Supplementary material:



Supplementary Figure S1: Microdialysis flux of 19 individual amino acids (*a* + *e* acidic, *b* + *f* alkaline, *c* + *g* neutral, *d* + *h* hydrophobic) in 2 experimental drought treatments. Vertical blue bars indicate duration of manual irrigation (75 mm following 4 weeks drought, 150 mm following 8 weeks drought). Shown are mean ± SE of 4 microdialysis membranes. Samples below LOD are not shown.



Supplementary Figure S2: Concentrations of NH_4^+, NO_3^-, total amino acids (AA_{tot}) and 19 individual amino acids in water extracts of soil samples taken 1 h before, and 24 h and 72 h after manual rewetting (RW) after 4 and 8 weeks of experimental drought. Shown are mean ± SE (n = 4). Please note the different scales of y-axes. Letters indicate significant differences between time points (2-way ANOVA with Tukey's HSD, $P < 0.05$).

Supplementary Table 1: Results from 2-way ANOVA showing effects of time (1 h before, 24 h and 72 h after rewetting) and treatment (4 and 8 weeks drought) on N concentrations in soil water extracts.

	Time		Treatment		Time x Treatment	
	F	**p**	**F**	**p**	**F**	**p**
NH_4^+	3.71	*	1.85		2.64	
NO_3^-	3.26		0.28		0.39	
AA_{total}	1.44		1.13		0.05	
Asp	0.44		1.97		0.21	
Glu	1.19		0.92		0.99	
Asn	1.92		0.93		0.82	
Ser	5.60	*	2.36		0.11	
Gln	0.00		2.83		1.68	
His	71.19	***	0.51		0.32	
Gly	7.79	**	2.54		0.08	
Thr	0.15		1.82		0.20	
Arg	186.85	***	0.32		1.38	
Ala	4.38	*	0.05		0.25	
Tyr	0.66		1.46		0.56	
Cys	11.47	**	0.30		0.07	
Val	1.32		1.83		0.09	
Met	0.75		0.10		2.27	
Trp	3.37		0.06		0.02	
Phe	5.82	*	1.29		3.50	
Ile	2.77		0.21		0.20	
Leu	6.31	*	1.25		0.06	
Lys	0.58		1.18		1.25	

AA_{total}, sum of 19 individual amino acids. Asterisks indicate levels of significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n = 4)

3.3. PAPER #3: REPEATED EXTREME DROUGHT AND RAINFALL EVENTS REDUCE SOIL RESPIRATION AND AFFECT ITS TEMPERATURE AND MOISTURE SENSITIVITY

Sonja Leitner[1], Johannes Kobler[2], Eugenio Díaz-Pinés[1], Nermina Saronjic[1], Michael Zimmermann[1,3], Sophie Zechmeister-Boltenstern[1]

[1] Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

[2] Environmental Agency Austria, Vienna, Austria

[3] Swiss Federal Office for Agriculture (BLW), Bern, Switzerland



Contributions:

SL Designed the study, performed soil respiration measurements and gas flux calculations, calculated statistics, wrote the manuscript

JK Calculated statistics

EDP Wrote the manuscript

NS Helped with rewetting experiments, performed soil analysis

MZ Designed the study, performed soil respiration measurements and gas flux calculations

SZB Designed the study, wrote the manuscript

Repeated extreme drought and rainfall events reduce soil respiration and affect its temperature and moisture sensitivity

Sonja Leitner[a], Johannes Kobler[b], Eugenio Díaz-Pinés[a], Nermina Saronjic[a], Michael Zimmermann[a,c], Sophie Zechmeister-Boltenstern[a]

[a] Institute of Soil Research, University of Natural Resources and Life Sciences, Vienna, Austria

[b] Environmental Agency Austria, Vienna, Austria

[c] Swiss Federal Office for Agriculture, Bern, Switzerland

Keywords

CO_2, climate change, extreme events, Q10, temperature sensitivity, moisture dependence, temperate forest

Abstract

Most temperate forest ecosystems experience repeated drying and rewetting events and most climate models predict an increase in the intensity and frequency of both severe droughts and heavy rainfalls in northern mid-latitudes. It still remains unclear how repeated extreme droughts and heavy precipitation events affect ecosystem C cycling in general and soil respiration (R_s) in particular. It has been stressed recently that current soil moisture responses of R_s cannot be extrapolated to soil moisture conditions under changed precipitation regimes. While drought generally leads to a decrease in R_s, rewetting of dry soil can cause a disproportionate pulse of CO_2 within minutes to days. However, whether this rewetting CO_2 pulse compensates or even outweighs the drought-induced decrease in R_s is unknown. Furthermore, it is not clear whether increased frequency and/or intensity of drying-rewetting cycles lead to overall increased or decreased R_s rates compared to constantly moist conditions or moderate dry-wet cycles. Most studies investigating impacts of drying-rewetting on ecosystem processes where either conducted in the laboratory, or only investigated single or few rewetting events during the year. In the present study, we simulated repeated moderate (6 cycles of 1-month drought followed by 75 mm irrigation) and severe (3 cycles of 2-month drought followed by 150 mm irrigation) drying-rewetting events during the vegetation period (from May until October) in a temperate forest while measuring R_s, soil temperature and soil moisture at high (<daily) temporal

resolution for two years. We then fitted soil temperature and moisture response curves to the R_s data to examine the impact of the different drying-rewetting stress scenarios on the temperature and moisture sensitivity of R_s. Compared to controls that received natural precipitation, moderate drying-rewetting stress did not affect R_s. However, repeated severe droughts and heavy rainfall events led to a ~30 % decrease in R_s during the vegetation period and increased its temperature (Q_{10}) and moisture sensitivity. Furthermore, at soil moisture levels <30 % WFPS, there was a shift from temperature to moisture dependence of R_s in controls and both stress treatments. Our findings corroborate the importance of accounting for both temperature and moisture dependence in order to accurately predict R_s and assess ecosystem C balance under changing precipitation regimes. Furthermore, we reemphasize that extreme experimental manipulations in the field are needed to quantify ecosystem responses to extreme climatic events.

Introduction

Soil respiration (R_s) is the second largest C flux after photosynthesis between terrestrial ecosystems and the atmosphere (Xu and Shang 2016), comprising ~100 Pg C yr^{-1} and making it a critical component of the global C cycle. Furthermore, it has been widely agreed that feedback effects between global warming and R_s could intensify climate change, and might annihilate all human effort to reduce CO_2 emissions (Reichstein et al. 2013). According to projections accounting for a positive climate feedback, the turnover rate of soil C is expected to increase on average by 2 to 10 % per 1 °C global warming (Bonan 2008). This increase would represent an additional C release of up to 10 Pg C yr^{-1}, a number which equals the global C emissions from fossil fuels and land use change combined (Ciais et al. 2013). However, despite the relevance of R_s as C source, our knowledge about its sensitivity to changing climatic conditions remains incomplete (Wu et al. 2011).

One of the predicted consequences of climate change is an increase in severe droughts and heavy rainfalls (Kirtman et al. 2013). In this context, a shift of summer rainfall patterns in the temperate climate zone is expected, which will lead to extended summer droughts followed by stronger rainfall events in Central Europe (Kromp-Kolb et al. 2014). Even if the total amount of precipitation does not change in this region, changes in precipitation patterns might constrain soil water availability during the growing season. Soil moisture is the second-most important driver of CO_2

emissions from soils after soil temperature (T_{soil}) (Raich and Schlesinger 1992, Davidson et al. 1998, Moyano et al. 2013), especially when above or below the optimum moisture range (Skopp et al. 1990).

The dependence of R_s on soil moisture and temperature can be described with various response functions, as reviewed for moisture (Rodrigo et al. 1997, Moyano et al. 2013) and for temperature (Fang and Moncrieff 2001, Tuomi et al. 2008). Depending on which functions are used, the prediction of R_s under different climatic conditions can vary substantially. Furthermore, while many studies have investigated how soil moisture controls ecosystem processes within present-day climatic windows (Beier et al. 2012), it has been stressed that current moisture responses cannot be extrapolated to predict R_s under changing precipitation patterns (Vicca et al. 2014). We specifically lack information about the impact of repeated extreme drying-rewetting events like consecutive extended drought periods followed by heavy rainfall (Harmon et al. 2011). While laboratory studies have provided some insight into the underlying mechanisms (Franzluebbers et al. 2000, Fierer and Schimel 2003, Iovieno and Baath 2008, Evans and Wallenstein 2012, Göransson et al. 2013), field observations are urgently needed to determine ecosystem responses *in situ* (De Boeck et al. 2015). Manipulation experiments that apply alterations that extends beyond the historical ranges of environmental conditions are a feasible way to examine ecosystem responses to extreme events and to identify thresholds that lead to shifts in process control (Kayler et al. 2015).

At suboptimal soil moisture, osmotic stress and low substrate diffusion decrease microbial activity and, thus, heterotrophic R_s (Moyano et al. 2013, Manzoni et al. 2014), and low stomatal conductance decreases the amount of recent photosynthetic assimilates that are transported to the soil, resulting in a decrease in autotrophic R_s (Casals et al. 2011, Zhou et al. 2014). While drought generally reduces R_s, the effects of rewetting are still a matter of debate (Borken and Matzner 2009). Rewetting of dry soil can cause a pronounced pulse of CO_2 within minutes and elevated CO_2 emissions exceeding pre-wetting rates for days (e.g., Franzluebbers et al. 2000, Fierer and Schimel 2003, Haney et al. 2004, Parton et al. 2012, Xu and Luo 2012), a phenomenon that was termed the “Birch” effect after its discoverer (Birch 1958). These rewetting CO_2 pulses originate from either soil biomass turnover (Kieft et al. 1987) or release of previously protected soil organic matter (Adu and Oades 1978), and they can contribute substantial amounts to total annual R_s. For example, Lee et al. (2004) estimated that the soil CO_2 flush caused by a single intensive storm can be equivalent to 5-10 % of the annual net ecosystem production of mid-latitude forests. It was suggested that the magnitude of the rewetting

response of R_s depends on both duration and intensity of the preceding drought (Unger et al. 2010). However, it remains unclear whether rewetting CO_2 pulses outweigh or even exceed the drought-induced reduction of R_s, and whether repeated extreme drying-rewetting cycles lead to a decrease or an increase in total R_s compared to constantly moist conditions or moderate dry-wet cycles. Furthermore, given that rewetting CO_2 pulses are only short-lived, R_s measurements with high temporal resolution (<1 day) are required to accurately quantify the contribution of rewetting CO_2 pulses to annual R_s.

In the present study, we conducted a two-year precipitation manipulation field experiment simulating two scenarios of repeated extreme drying-rewetting events in a temperate forest in Austria, while measuring soil moisture, T_{soil} and R_s in high temporal resolution. A Gauss temperature model was then fitted to the R_s data either alone, or in combination with a quadratic soil moisture function in order to evaluate the influence of different precipitation patterns on the temperature and moisture sensitivity of R_s. We hypothesized that (i) repeated extreme drying-rewetting events would lead to a decrease in R_s compared to soil receiving natural precipitation, and (ii) that consecutive severe drought and heavy precipitation events would result in a shift in the temperature and moisture sensitivity of R_s.

Material and methods

Study site

The study was conducted at the International Long Term Ecological Research Network (ILTER) site "Rosalia" in the forest of the University of Natural Resources and Life Sciences Vienna, Austria (47°42'26.33" N/16°17'58.15" E). Mean annual temperature at the site is 6.5 °C and mean annual precipitation is 796 mm. For our experiment, we selected a pure mature beech stand (*Fagus sylvatica* L., 80-100 yrs. stand age) that was located along a west-exposed hillslope (slope ~16°) at 600 m asl. The soil is a dystric cambisol over granitic bedrock with 4.1 % SOC, 0.18 % N, pH 4.0 and 0.595 g cm^{-3} bulk density (Leitner et al. 2016) with sandy loam soil texture (Schwen et al. 2014). Air temperature (T_{air}) and humidity were recorded at the study site every 30 min (RFT-2 sensor, METER ENVIRONMENT, Germany). Rainfall was recorded using a tipping bucket rain gauge (ARG 100, Campbell Scientific, Germany) at a height of 2.0 m on an open meadow adjacent to the study site.

116 *Experimental design*

117 A precipitation manipulation experiment was conducted over two years from April 2013 until
118 March 2015. Before start of the experimental manipulation, a total of 12 sampling plots with a size of 2
119 m x 2 m were established in late summer 2012. In each plot, soil sensors were buried in 10 cm depth to
120 measure T_{soil} (thermistor Th2-f probes, METER ENVIRONMENT, Germany) every 30 min. Soil moisture
121 was measured in 10 cm depth using time-domain reflectometry (theta.ML2x probes, METER
122 ENVIRONMENT, Germany) every 30 min and converted to water-filled pore space (WFPS) using a total
123 soil porosity of 0.61 m^3 m^{-3} (Schwen et al. 2015).

124 In 2013 and 2014, 8 of the 12 sampling plots were covered with transparent plastic roofs (4 m x
125 4 m, installed at a height of 1.2 m) to exclude rainfall during the vegetation period (May to October). To
126 prevent lateral water flow along the hillslope to enter the drought stressed plots, 40 cm deep trenches
127 were dug on the uphill side of the plots, lined with plastic foil and filled with gravel. Underneath the
128 roofs an irrigation system with sprinklers (axial-flow full cone nozzles, Series 460, Lechler GmbH,
129 Germany) was installed, which was connected to a water tank via a water pump. Roofs were
130 implemented from 30-Apr to 22-Oct in 2013 and from 29-Apr to 17-Oct in 2014.

131 On the roof-covered plots, we simulated 2 different drought stress scenarios (n = 4): (i) a
132 "moderate stress" treatment, where plots were irrigated once a month with 75 mm descaled tap water,
133 resulting in 6 drying-rewetting cycles per year, and (ii) a "severe stress" treatment, where plots were
134 irrigated every 2 months with 150 mm descaled tap water, resulting in 3 drying-rewetting cycles per
135 year. In total, both drought-stress scenarios received 450 mm irrigation applied between May and
136 October, which corresponds to the 10-year average amount of rainfall during the vegetation period in
137 this region. The remaining 4 plots were kept uncovered during the entire experiment and received
138 natural rainfall ("natural control").

139 *Soil respiration measurements*

140 Soil respiration was measured using an automated soil-atmosphere gas flux detection system
141 (Butterbach-Bahl et al. 1998) purchased from Karlsruhe Institute of Technology, Institute of
142 Atmospheric Environmental Research (KIT-IFU, Garmisch-Partenkirchen, Germany). In the center of
143 each sampling plot an automated static flux chamber (non-steady-state, non-flow-through) with a
144 basal area of 0.5 m x 0.5 m and a height of 0.15 m was installed on top of an equally-sized stainless

steel frame that had been inserted 5 cm into the ground. The chambers consisted of a stainless steel frame and transparent acryl glass and were equipped with fans to ensure homogeneous air mixing. Chambers were opened and closed pneumatically. Three chambers (one of each treatment) were closed at a time for 45 min during which 4 air samples were taken from each chamber, followed by 2 air samples from a known calibration standard (400 ppm CO_2, purchased from Linde Gas, Austria). A full measurement cycle during which all 12 chambers were measured lasted 3 hours; thus, eight R_s measurements per chamber and day were obtained. Chambers were connected with stainless steel tubes to a central valve switching unit and gas was transferred to a non-dispersive infrared CO_2 analyzer (LI-840A CO_2/H_2O analyzer, LI-cor, NE, USA) via a gas pump (flow rate 250 ml min^{-1}, NMP 830 KNDC, KNF Neuberger GmbH, Germany). Soil respiration was calculated from the slope of the linear increase of the 4 headspace CO_2 concentrations over the closure time corrected for air temperature and pressure and is given in mg C m^{-2} h^{-1} (Metcalfe et al. 2007). Detection limit of the system was 0.144 mg C m^{-2} h^{-2} (Parkin et al. 2012), and R_s measurements with a regression coefficient (R^2) <0.9 were discarded. This resulted in a dataset of approx. 42,000 data points of 3-hourly R_s flux rates.

Climate sensitivity of soil respiration

To express the temperature sensitivity of R_s, a Gauss function according to Tuomi et al. (2008) was used (Equ. 1):

$$R_s = a * e^{bT_{soil}+cT_{soil}^2} \quad \text{(Equ. 1)}$$

where R_s is soil respiration (mg C m^{-2} h^{-1}), T_{soil} is soil temperature (°C), and a, b and c are fitted parameters. This temperature function is a generalization of the Arrhenius function (Arrhenius 1898) and was proposed to give a better and unbiased relationship between R_s and T_{soil} by defining a maximum temperature T_m beyond which R_s decreases (Fang and Moncrieff 2001).

To express the sensitivity of R_s to soil moisture, a quadratic function (Martin and Bolstad 2005) was used (Equ. 2):

$$R_s = d * WFPS + e * WFPS^2 \quad \text{(Equ. 2)}$$

where R_s is soil respiration (mg C m^{-2} h^{-1}), WFPS is soil water-filled pore space (%), and d and e are fitted parameters. This soil moisture function is based on the assumption that the optimal soil moisture where R_s is highest is at intermediate WFPS levels, where macropores are mostly air-filled,

thus facilitating O_2 diffusion, while micropores are mostly water-filled, thus facilitating diffusion of soluble substrates (Moyano et al. 2013).

To account for both temperature and moisture sensitivity of R_s simultaneously, we combined the Gauss temperature function from Equ. 1 with the quadratic moisture function from Equ. 2 as a product in a multiple non-linear regression (Equ. 3):

$$R_s = (a * e^{bT_{soil}+cT_{soil}^2}) * (d * WFPS + e * WFPS^2) \quad \text{(Equ. 3)}$$

with R_s as soil respiration (mg C m^{-2} h^{-1}), T_{soil} as soil temperature (°C), *WFPS* as soil water-filled pore space (%), and *a*, *b*, *c*, *d* and *e* as fitted parameters,.

To express differences in the temperature sensitivity of R_s between treatments, Q_{10} as the rate of change in R_s caused by a change in soil temperature by 10 °C was calculated for each treatment. Q_{10} is known to vary over different temperature ranges with, in general, greater temperature sensitivities at lower temperatures (Kätterer et al. 1998, Janssens and Pilegaard 2003). Therefore, Equ. 1 and Equ. 3 were used to calculate R_s at temperatures ranging from 1-30 °C, and then Q_{10} values were calculated using Equ. 4:

$$Q_{10} = \frac{R_{T_0+10}}{R_{T_0}} \quad \text{(Equ. 4)}$$

where R_{T_0+10} and R_{T_0} are the soil respiration rates at soil temperatures T_0 and T_0+10 °C, respectively, with T_0 ranging from 1-20 °C. To determine the moisture sensitivity of R_s and to test whether it was affected by the applied drought stress treatments, we calculated $Q_{moisture}$ as the quotient of change in R_s caused by a change in soil WFPS by 10 %. To this end, Equ. 3 was used to calculate R_s at WFPS levels ranging from 10-70 %, and then $Q_{moisture}$ was calculated using Equ. 5:

$$Q_{moisture} = \frac{R_{WFPS_0+10}}{R_{WFPS_0}} \quad \text{(Equ. 5)}$$

where R_{WFPS_0+10} and R_{WFPS_0} are the soil respiration rates at $WFPS_0$ and $WFPS_0$+10 %, respectively, with $WFPS_0$ ranging from 10-60 %.

Statistics

All temperature and moisture models (Equ. 1, 2 and 3) where parameterized for each treatment (control, moderate stress, and severe stress) using the mean of the 4 replicate plots per treatment. The functions were fitted to the measured R_s values by means of the damped least squares method, minimizing the sum of squares of residuals through the Levenberg-Marquardt algorithm (Moré 1978,

Bates and Watts 1988). As goodness-of-fit (GOF) parameters, coefficient of determination (R^2), root mean square error (RMSE), Akaike's Information Criterion (AIC), and the Bayesian Information Criterion (BIC) where compared. To detect differences between treatments, data were averaged over sampling periods (vegetation period and winter) and then one-way ANOVA followed by LSD *post hoc* test was employed and differences were considered statistically significant at $p < 0.05$. To detect changes in soil moisture over time, April WFPS values of each year were compared using one-way ANOVA followed by LSD *post hoc* test. Data were tested for homogeneity of variance using Levene's test and log-transformed if necessary. Statistical analyses were calculated using R 3.4.0, packages "stats" (R Core Team 2017), "hydroGOF" (Zambrano-Bigiarini 2017), and "minpack.lm" (Elzhov et al. 2016).

Results

Climate

Total precipitation at the study site was similar in 2013 (873 mm) and 2014 (903 mm), but its distribution between vegetation period and winter differed between the two years (Figure 1): in 2013, control plots received 437 mm of rainfall during the vegetation period (May until Oct), which was in the same range as the amount of irrigation water that was applied to both moderately and severely stressed plots during this time (450 mm). In contrast, the vegetation period of 2014 was much wetter, with control plots receiving 628 mm of rain, whereas both moderately and severely stressed plots again received 450 mm irrigation water (Table 1).

Mean T_{air} during the vegetation period was 14.8 ± 1.9 °C and did not differ significantly between 2013 and 2014 (Figure 1). The same was true for T_{soil} that averaged 12.3 ± 0.1 °C during the vegetation period and did not differ between treatments (Figure 1).

Before start of the precipitation manipulation, soil WFPS was slightly but not significantly higher in moderately stressed (45.0 ± 2.2 %) and severely stressed plots (44.1 ± 2.3 %) compared to control plots (37.1 ± 4.5 %) (Figure 1). During the vegetation period in 2013 and 2014, severely stressed plots (20.1 ± 1.8 % in 2013, 23.4 ± 1.7 % in 2014) were, on average, drier than control plots (24.8 ± 2.9 % in 2013, 29.4 ± 2.5 % in 2014) and moderately stressed plots (25.8 ± 2.4 % in 2013, 28.8 ± 2.5 % in 2014). Furthermore, severely stressed plots became significantly drier over time, and this effect was not outweighed by natural rainfall in winter: when comparing soil moisture before roof installation in each year, WFPS in severely stressed plots significantly decreased from 44.1 ± 2.3 % in

April 2013, over 41.0 ± 3.4 % in April 2014, down to 30.7 ± 4.8 % in April 2015, whereas April-WFPS did not change significantly over time in control (36.1 ± 3.0 %) and moderately stressed plots (42.6 ± 3.4 %).

Soil respiration

During the vegetation period, control plots emitted on average 90.7 ± 6.5 mg C m^{-2} h^{-1} in 2013 and 109.1 ± 3.2 mg C m^{-2} h^{-1} in 2014 (Table 2). Emissions from moderately stressed plots were in the same range and not significantly different from control plots, with 74.4 ± 8.8 mg C m^{-2} h^{-1} in 2013 and 96.5 ± 8.5 mg C m^{-2} h^{-1} in 2014. In severely stressed plots, the precipitation manipulation led to a significant decrease in average R_s during the vegetation period in both 2013 (65.2 ± 6.3 mg C m^{-2} h^{-1}) and 2014 (77.2 ± 6.7 mg C m^{-2} h^{-1}). This decrease did not persist during the winter when roofs where removed.

Climate sensitivity of soil respiration

To describe the temperature sensitivity of R_s, a Gauss model was fit to the R_s data of each treatment (Figure 2, Table 3). According to visual inspection of the data, inside a temperature window between 1-15 °C, this Gauss model fit the R_s data well, but at T_{soil} >15 °C, the data showed two distinct groups: R_s measurements on days where soil WFPS was >20 % (black circles in Figure 2) were underestimated by the temperature model, while R_s measurements on days with soil WFPS ≤20 % (gray circles in Figure 2) were overestimated by the temperature model. This was also visible when comparing measured R_s values to R_s estimated by the temperature model (Supplementary Figure S1): the model fit was good for R_s <80 mg C m^{-2} h^{-1}, but for higher fluxes during the summer, there was greater uncertainty of the temperature model. More specifically, in the dry summer of 2013 the model overestimated R_s, while it underestimated R_s in the wet summer of 2014 (Figure 3).

Residuals of the temperature model were dependent on soil WFPS for all three treatments (Supplementary Figure S2): When WFPS was >30 % residuals were close to zero. At WFPS <30 %, however, residuals became negative, indicating that the temperature model overestimated R_s at low soil moisture levels. There was a relationship between residuals of the temperature model and WFPS which was best described by a quadratic model (indicated by a purple line in Supplementary Figure S2). We defined a "soil moisture threshold" (THR) as the first y intercept of the quadratic curve, i.e. by setting y (the residuals) equal to 0 in the equation of the curve and solving for x. This THR describes

the WFPS level below which R_s switches from being temperature-controlled to being moisture-controlled. In control plots, THR was 29.98 % WFPS, in moderately stressed plots THR was lower with 27.84 % WFPS, and in severely stressed plots THR was higher, equaling 32.91 % WFPS.

The relationship between R_s and WFPS was best described by a quadratic function (Figure 4). To account for both temperature and moisture sensitivity of R_s, we combined both the Gauss temperature function and the quadratic soil moisture function in a multiple non-linear regression, which describes the temperature and moisture dependence of R_s in a 3-dimensional space (Figure 5). This 3D temperature-moisture model was fit to the dataset of each treatment (Table 3). Model fit of the temperature-moisture model was better than that of the temperature-only model (Table 3), which was also supported by a better relationship between measured and estimated R_s of the temperature-moisture model (Supplementary Figure S3), and a better estimation of R_s during summer (Figure 6). Furthermore, there was no relationship between residuals of the temperature-moisture model and WFPS (Supplementary Figure S4).

The combined influence of soil moisture and temperature on R_s was also apparent for Q_{10} values (Figure 7): according to the temperature model, Q_{10} at 10 °C was 1.18 for control plots, 1.15 for moderately stressed plots, and 0.84 for severely stressed plots (Table 4). When the combined temperature-moisture model was used to estimate R_s, Q_{10} values at 10 °C increased by a factor of 2 for control plots (2.57) and moderately stressed plots (2.72), and even by a factor of 3 for severely stressed plots (2.84).

To determine the influence of soil moisture on R_s, we calculated $Q_{moisture}$ as the factor by which R_s changes for an increase in 10 % WFPS (Figure 8). At WFPS levels >30 %, $Q_{moisture}$ was close to 1 (range 0.95-1.24), indicating no influence of a change in soil moisture on R_s (Table 4). However, when WFPS increased from 10 to 20 %, $Q_{moisture}$ was 1.79 for control plots, 1.77 for moderately stressed plots, and 1.88 for severely stressed plots.

Discussion

Climate

Extreme changes in precipitation patterns (increase in severe droughts and heavy rainfalls) decreased soil moisture at our study site during the vegetation period, when roofs were installed, and this decrease in soil moisture persisted during the winter after removal of roofs, leading to soil

desiccation in the long run. Despite the fact that all plots received approximately the same amount of water along the observation period, the soil at the study site had a high sand content and was, thus, likely not able to fully absorb the amount of water applied during intense irrigation events. Furthermore, repeated extreme drought and rainfall events had been shown to increase soil hydrophobicity at our study site, which resulted in low wettability of the soil (Schwen et al. 2015). Hydrophobic substances (e.g. waxes, alkanes, fatty acids) can develop at critically low soil moisture conditions (Goebel et al. 2011) and inhibit the infiltration of water and solutes into the soil matrix (Bachmann et al. 2008). Apart from a direct effect of decreased soil moisture on R_s, which can inhibit R_s if soil water content drops below optimum moisture, an increase in hydrophobicity and, thus, water repellency of dry soil was shown to reduce the availability of soil organic matter (SOM) to microbial decomposition (Lamparter et al. 2009) and might, therefore, also affect R_s indirectly.

Soil respiration

While drought usually decreases R_s in temperate forest soils (Burton et al. 1998, Davidson et al. 1998, Rey et al. 2002, Curiel Yuste et al. 2003, Borken et al. 2006), the "Birch effect" causes pronounced pulses of CO_2 emissions after rewetting. Given that these CO_2 pulses are only short-lived (hours to days), only studies employing high-resolution gas flux measurements (<daily) can accurately quantify the effect of extreme drying-rewetting on total R_s (Vicca et al. 2014). Most previous studies examining the impact of drying-rewetting on forest soil R_s where either conducted in the lab excluding autotrophic respiration (Sørensen 1974, Pulleman and Tietema 1999, Borken et al. 2003, Fierer and Schimel 2003, Rey et al. 2005, Muhr et al. 2010, Göransson et al. 2013), employed low temporal resolution (>weekly) R_s measurements (Kim et al. 2010, Schindlbacher et al. 2012), or only covered single (Borken et al. 1999, Shi et al. 2011) or few (Unger et al. 2010, Wu and Lee 2011) rewetting events during the year. In the present study, we measured R_s at 3-hourly resolution over two full years and followed the impact of extreme changes in rainfall patterns on R_s. We hypothesized that repeated extreme drying-rewetting events would lead to a decrease in R_s compared to soil receiving natural precipitation. In support of this, we found that the severe stress treatment, which was subjected to repeated droughts of two months duration, led to a reduction in R_s compared to control plots, indicating that rewetting CO_2 pulses were not large enough to compensate for the drought-induced reduction in R_s. A drought-induced reduction in R_s can be caused by microbial C limitation due to

decreased substrate diffusion in soil (Manzoni et al. 2014), and by the down-regulation of belowground C allocation via plant roots (Blessing et al. 2016). In support of the latter, we found that in drought-stressed plots, fine root activity (O_2 uptake) was significantly reduced compared to control plots (Boris Rewald, personal communication). However, because we did not distinguish between heterotrophic and autotrophic R_s, we cannot quantify the respective impacts of drought-stress on these two components of R_s.

While severe stress decreased R_s in our study, the moderate stress treatment, which, after all, experienced six consecutive one-month drought periods, did not lead to a significant reduction in R_s. This was a surprising result for a temperate forest that rarely experiences an entire month without any rainfall and underlines the importance of extreme experimental manipulations that exceed ecosystem stress resistance and resilience in order to identify thresholds of disturbance that lead to a change in ecosystem functions (Whitford et al. 1999, Côté and Darling 2010).

Climate sensitivity of soil respiration

Our results showed that R_s was best described by a soil temperature-moisture function, indicating a combined temperature and moisture limitation of R_s. When using only T_{soil} but not soil moisture to predict R_s, the model exhibited great uncertainty for R_s fluxes during the summer, indicating that at cold temperatures, R_s was primarily controlled by T_{soil}, whereas at warm temperatures, there was a co-limitation of R_s by T_{soil} and soil moisture. Similarly, when soil moisture dropped below a threshold of ~30 %, there was a shift towards increasing moisture dependence of R_s. Furthermore, repeated severe dry-rewetting events increased the soil moisture threshold from 30 % WFPS in control plots to 33 % WFPS in severely stressed plots. This indicates a higher sensitivity of drought stressed soil towards a reduction in soil moisture, perhaps due to cumulative effects of repeated severe drying-rewetting cycles that decreased the resistance of microorganisms and roots to further drying. While ecosystems might be able to withstand even severe disturbances once, repeated disturbances represent additive stress, which can decrease ecosystem functioning and, ultimately, lead to system failure (Suding et al. 2004). This was also supported by the change in $Q_{moisture}$ (the change of R_s for a change in 10 % WFPS) after repeated drying-rewetting events. While moderate drought stress did not affect moisture sensitivity of R_s, severe stress lead to an increase in $Q_{moisture}$ that was most pronounced at low WFPS, but still apparent even at 50 % WFPS. This is in line with our second

hypothesis that consecutive severe drought and heavy precipitation events would result in a shift in the temperature and moisture sensitivity of R_s and further corroborates our theory that repeated severe drying-rewetting cycles enhance the moisture dependence of R_s. One possible explanation for this might be that severe drought can lead to C limitation of soil microorganisms because it decreases substrate diffusion through soil water towards sites of microbial uptake (Schjønning et al. 2003), leading to reduced microbial activity and starvation (Manzoni et al. 2014). Furthermore, as the matric potential in the remaining soil water increases, microorganisms have to allocate more C towards the production of osmo-regulatory substances (e.g. osmolytes, extracellular polysaccharides) to maintain their osmotic equilibrium (Schimel et al. 2007) and to buffer variations in soil moisture (Or et al. 2007). In addition, repeated drying-rewetting cycles reduce the amount of available substrate released by rewetting (Fierer et al. 2003, Carbone et al. 2011), which could further aggravate the C limitation of heterotrophic soil microorganisms after repeated drying-rewetting events.

Repeated severe drying-rewetting events also affected temperature sensitivity of R_s, which translated into a change in Q_{10} values. However, the response of Q_{10} to drying-rewetting strongly depended on the applied R_s function. When the Gauss function, which accounted only for temperature but not moisture, was used to estimate R_s, Q_{10} values were not affected by moderate drought stress, and decreased due to severe stress. When the combined temperature-moisture R_s function, was used to calculate R_s, Q_{10} showed opposite responses, with both moderate and severe drought stress increasing Q_{10} values. This is in line with a study from semi-arid grasslands that showed that repeated drying-rewetting cycles increased the temperature sensitivity of R_s, which was attributed to a proportional increase in rewetting-pulse substrate coming from a more complex soil C pool rather than from microbial cell lysis, likely due to depletion of labile substrates and microbial adaptation to repeated drying-rewetting (Chatterjee and Jenerette 2011). The temperature sensitivity of R_s can be affected by soil moisture through effects on enzyme activity kinetics (Jenerette et al. 2008) and substrate availability (Davidson et al. 2006). Therefore, it is important to account for the moisture sensitivity of R_s when assessing its temperature dependence; otherwise severe over- or underestimation of R_s can occur.

Conclusions

Our results provide deeper insight into the impact of consecutive extreme drought and rainfall events on R_s. Extreme events can severely affect R_s and shift its climate sensitivity from temperature-dependence to moisture-dependence. Furthermore, the impact of drought on R_s increases with the length of the drought period, with longer drought periods leading to a stronger reduction in R_s. Thus, models that predict ecosystem C balance under a changing climate need to account for the duration and frequency of drought periods. Our high-resolution R_s data can help modelers to refine existing soil and climate models.

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Tables

Table 1: Sum of water (precipitation and irrigation, mm) that control plots, moderately stressed plots, and severely stressed plots received before (prestart, mean of Apr 2013), during (vegetation period, May-Oct), and after (winter, Nov-Apr) precipitation manipulation treatments.

	Control	Moderate stress	Severe stress
Prestart	28	28	28
Vegetation period 2013	437	450	450
Winter 2013/14	370	370	370
Vegetation period 2014	628	450	450
Winter 2014/15	217	217	217

Table 2: Mean soil respiration (mg C m^{-2} h^{-1}) for controls, moderately stressed and severely stressed plots before (prestart, mean of Apr 2013), during (vegetation period, May-Oct), and after (winter, Nov-Apr) precipitation manipulation treatments. Data are mean ± SE (n = 4). Superscripts indicate significant differences between treatments (one-way ANOVA, $p < 0.05$).

	Control	Moderate stress	Severe stress
Prestart	52.2 ± 4.9	57.9 ± 6.1	55.9 ± 5.3
Vegetation period 2013	90.7 ± 6.5 ^{b}	74.4 ± 8.8 ^{ab}	65.2 ± 6.3 ^{a}
Winter 2013/14	42.9 ± 1.2	48.9 ± 6.2	43.3 ± 3.0
Vegetation period 2014	109.1 ± 3.2 ^{b}	96.5 ± 8.5 ^{ab}	77.2 ± 6.7 ^{a}
Winter 2014/15	41.6 ± 2.6	53.1 ± 3.6	44.3 ± 4.5

Table 3: Calculated equation parameters for the best-fit temperature functions after Gauss alone, and in combination with a quadratic soil moisture function with model statistics (*RMSE*, root mean square error; *AIC*, Akaike Information Criterion; *BIC*, Bayesian Information Criterion; *n*, number of cases). R_s, soil respiration (mg C m^{-2} h^{-1}); T_{soil}, soil temperature (°C); *WFPS*, soil water-filled pore space (%).

Treatment	Calculated best-fit parameters	R^2	RMSE	AIC	BIC	n
	Temperature model (Gauss)					
Control	$R_S = 6.35*\exp(0.38* T_{soil}-0.01* T_{soil}^2)$	0.76	19.9	31025	31049	3519
Moderate stress	$R_S = 7.71*\exp(0.34* T_{soil}-0.01* T_{soil}^2)$	0.73	18.2	30552	30577	3537
Severe stress	$R_S = 7.86*\exp(0.36* T_{soil}-0.01* T_{soil}^2)$	0.68	20.3	31765	31790	3584
	Temperature-moisture model (Gauss*quadratic fit)					
Control	$R_S = 6.76*\exp(0.30*T_{soil}-0.007* T_{soil}^2)*(0.08*WFPS-0.001*WFPS^2)$	0.89	13.7	28412	28443	3519
Moderate stress	$R_S = 6.89*\exp(0.26*T_{soil}-0.005* T_{soil}^2)*(0.08*WFPS-0.007*WFPS^2)$	0.86	13.0	28169	28200	3537
Severe stress	$R_S = 7.83*\exp(0.26*T_{soil}-0.005* T_{soil}^2)*(0.07*WFPS-0.007*WFPS^2)$	0.82	15.2	29665	29696	3584

Table 4: Q_{10} of soil respiration for temperature range from 10-20 °C calculated using a best-fit temperature function after Gauss alone, and in combination with a quadratic soil moisture function. $Q_{moisture}$ values were calculated using the combined Gauss*quadratic fit model and indicate the change in respiration for an increase in soil WFPS from 10-20%, 30-40%, and 50-60% WFPS, respectively.

Treatment	Q_{10} (10-20°C)	$Q_{moisture}$ (10% WFPS)	$Q_{moisture}$ (30% WFPS)	$Q_{moisture}$ (50% WFPS)
	Temperature model (Gauss)			
Control	1.18	-	-	-
Moderate stress	1.15	-	-	-
Severe stress	0.84	-	-	-
	Temperature-moisture model (Gauss*quadratic fit)			
Control	2.57	1.79	1.15	0.98
Moderate stress	2.72	1.77	1.14	0.95
Severe stress	2.84	1.88	1.24	1.11

Figures

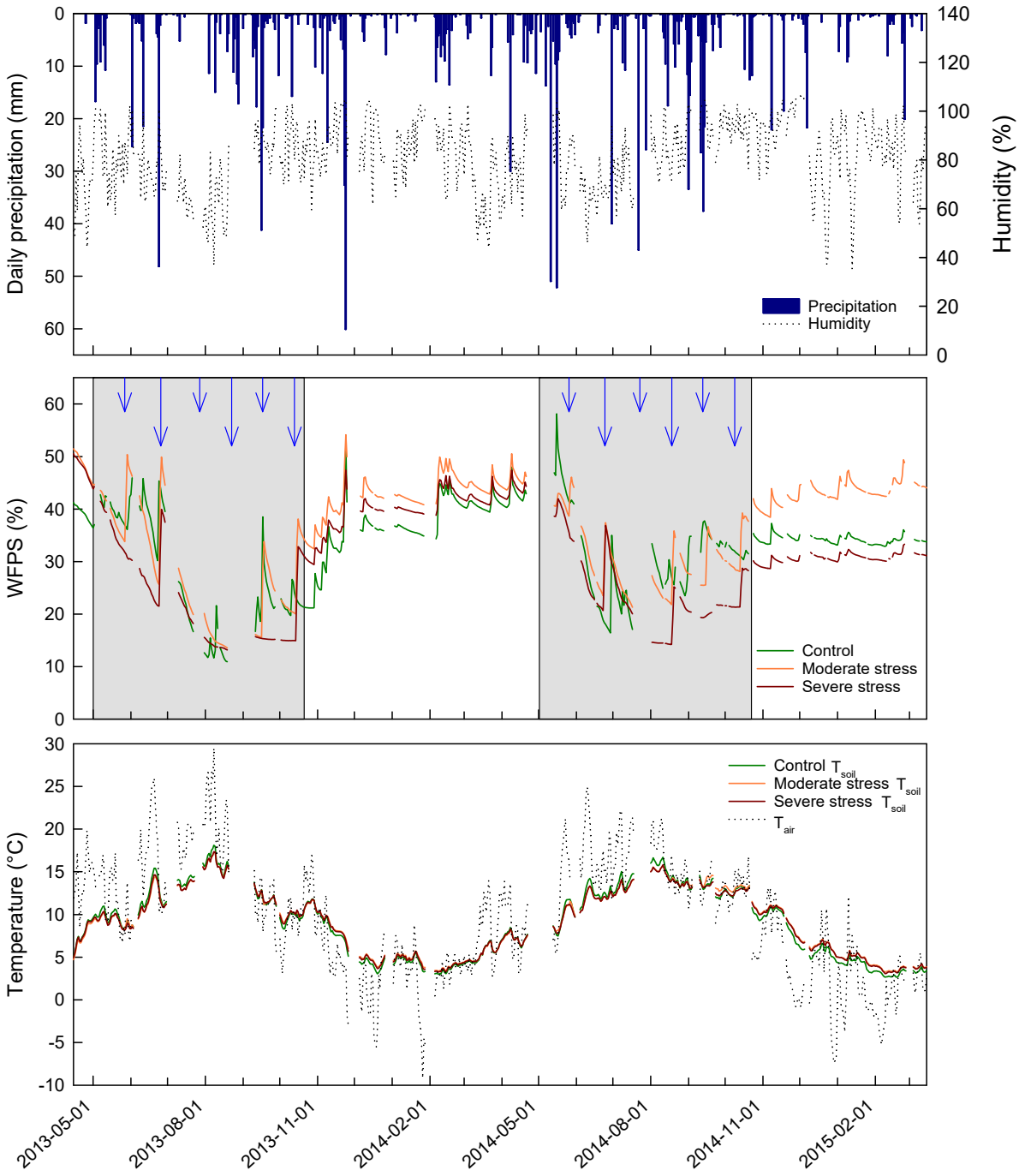
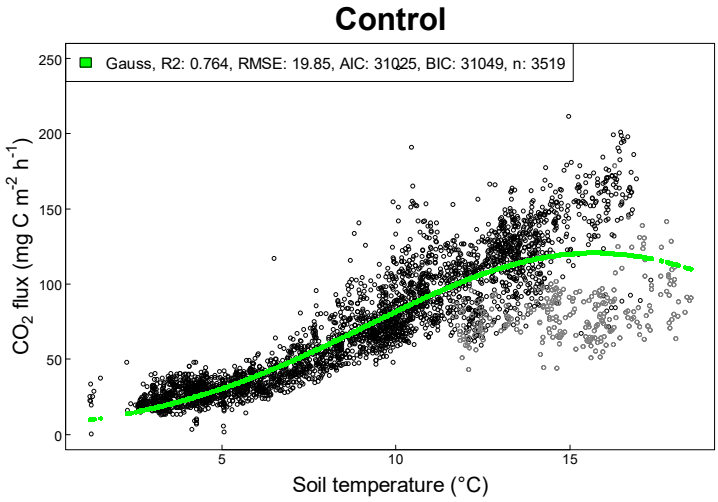


Figure 1: Air and soil climate at the Rosalia field station from April 2013 until March 2015. *WFPS*, soil water-filled pore space (10 cm depth); T_{soil}, soil temperature (10 cm depth); T_{air}, air temperature. Data are daily means (n = 4). Grey areas in the central panel indicate the period where moderately stressed and severely stressed plots were covered by roofs and did not receive natural rainfall, but where manually irrigated instead. Blue arrows indicate the timing of irrigation events, with small arrows indicating dates where only moderately stressed plots were irrigated (75 mm per irrigation), and large arrows indicating dates where both moderately (75 mm per irrigation) and severely stressed plots (150 mm per irrigation) were irrigated.



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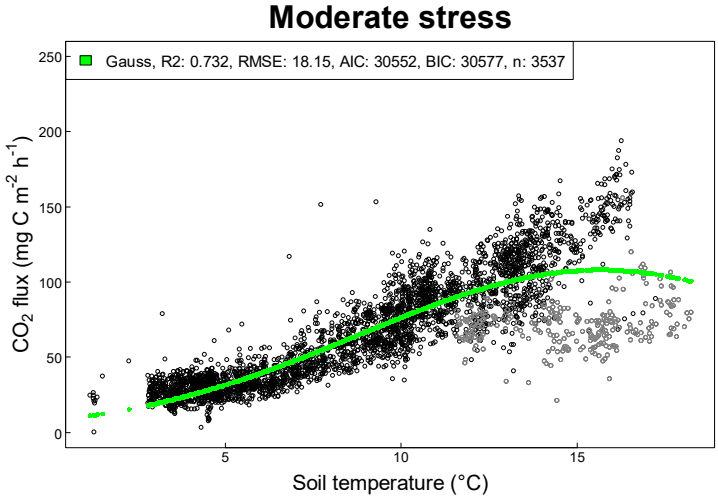

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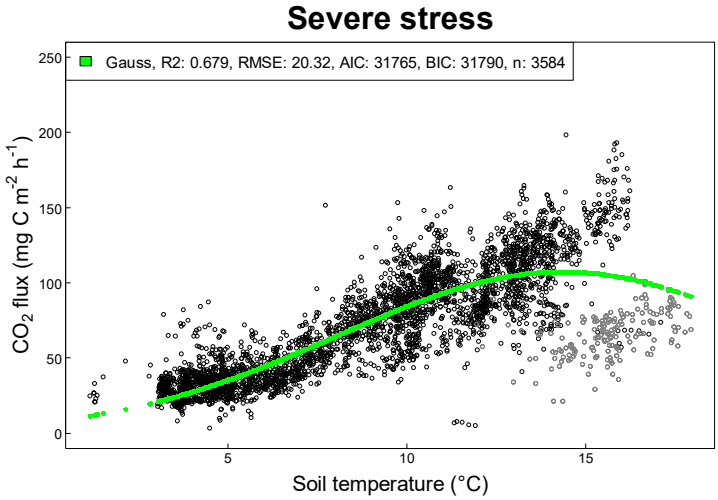
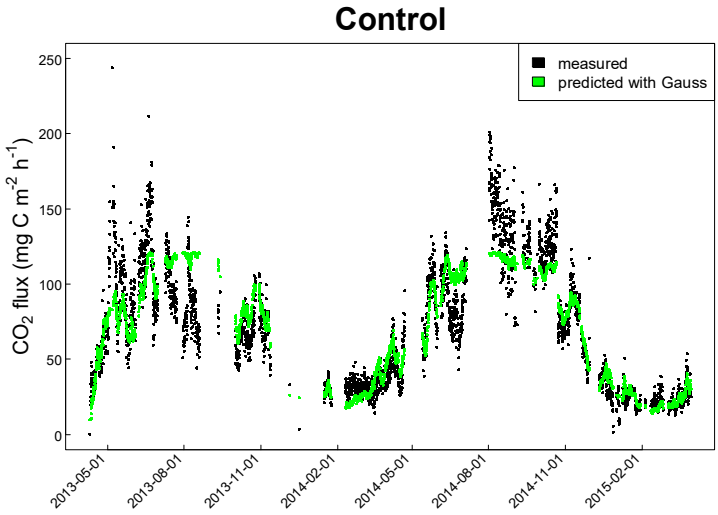


629

630 **Figure 2:** Soil CO_2 flux (black circles, mg C m^{-2} h^{-1}) plotted against soil temperature (10 cm depth) of
631 control plots, moderately stressed plots, and severely stressed plots (data are means of 3-hourly flux
632 measurements, n = 4). Gray dots indicate soil CO_2 flux values measured at WFPS ≤ 20%. Green dots
633 show the relationship between soil CO_2 flux and soil temperature as estimated by a Gauss model
634 (*RMSE*, root mean square error; *AIC*, Akaike Information Criterion; *BIC*, Bayesian Information Criterion;
635 *n*, number of cases).



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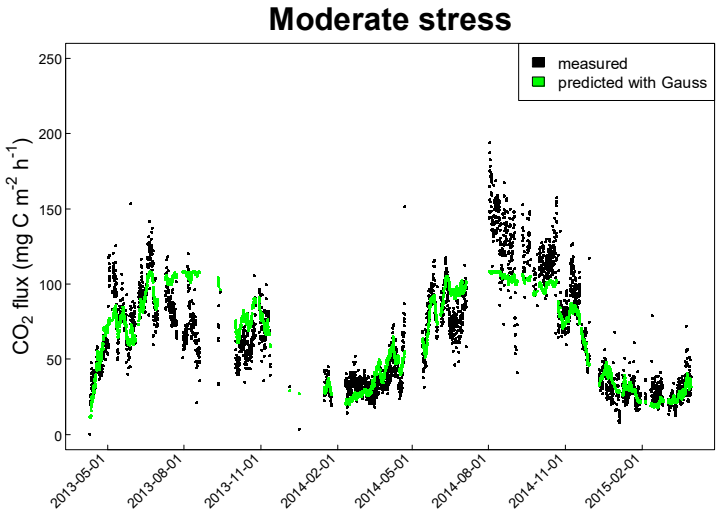

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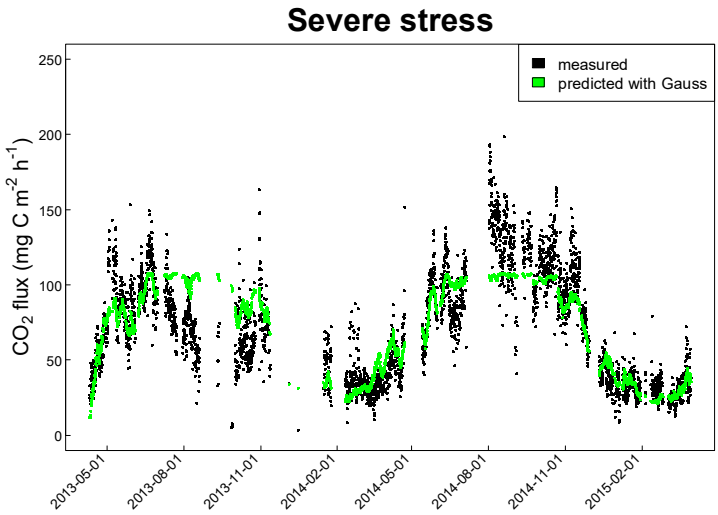
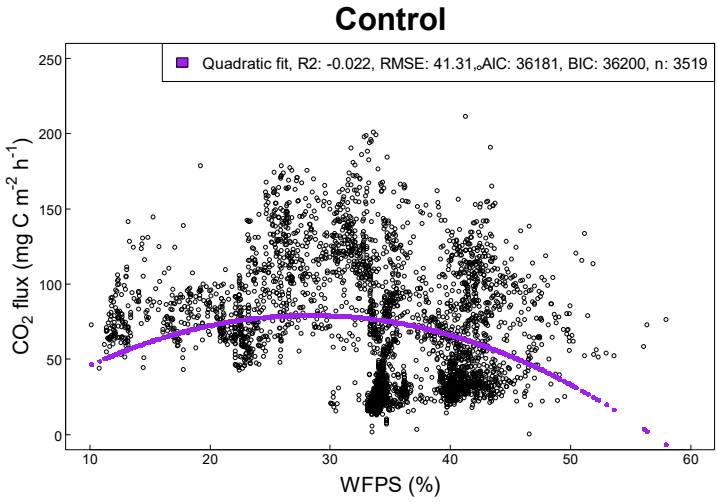


638

639 **Figure 3:** Comparison of measured (black dots) soil CO_2 flux, and soil CO_2 flux predicted using a
640 temperature-Gauss model (green dots). Soil CO_2 flux was measured between April 2013 and March
641 2015 in control plots, moderately stressed plots, and severely stressed plots. Shown are daily means of
642 3-hourly flux measurements (n = 4).



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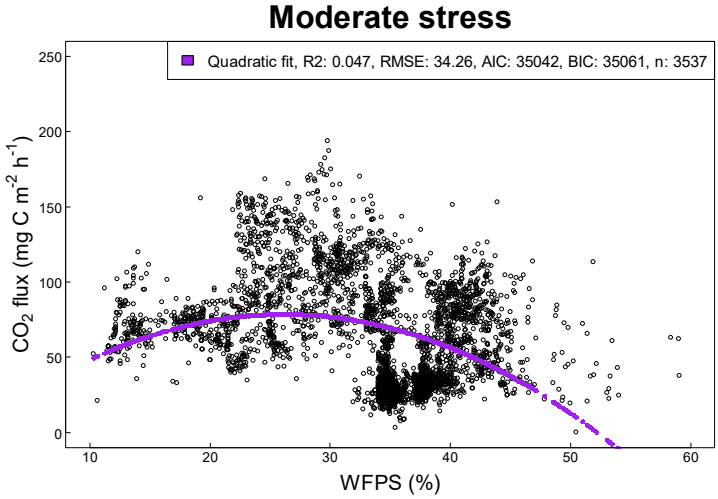

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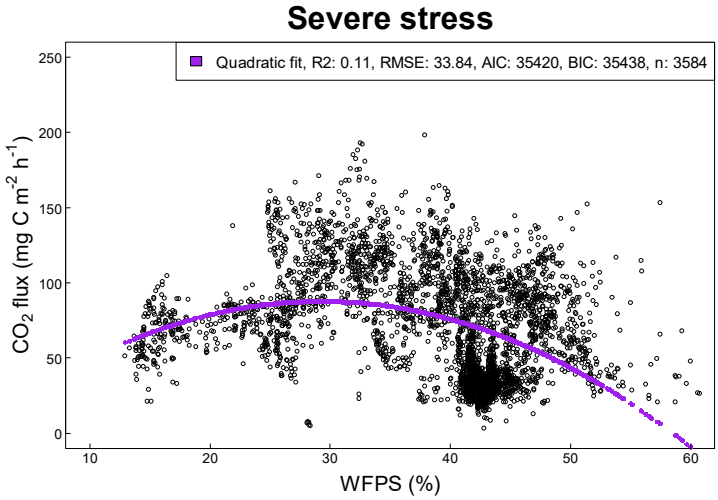


645

646 **Figure 4:** Soil CO_2 flux (black circles, mg C m^{-2} h^{-1}) plotted against soil water-filled pore space (WFPS,
647 10 cm depth) of control plots, moderately stressed plots, and severely stressed plots (data are means
648 of 3-hourly flux measurements, n = 4). Purple dots show the relationship between soil CO_2 flux and soil
649 WFPS as estimated by a quadratic model (*RMSE*, root mean square error; *AIC*, Akaike Information
650 Criterion; *BIC*, Bayesian Information Criterion; *n*, number of cases).

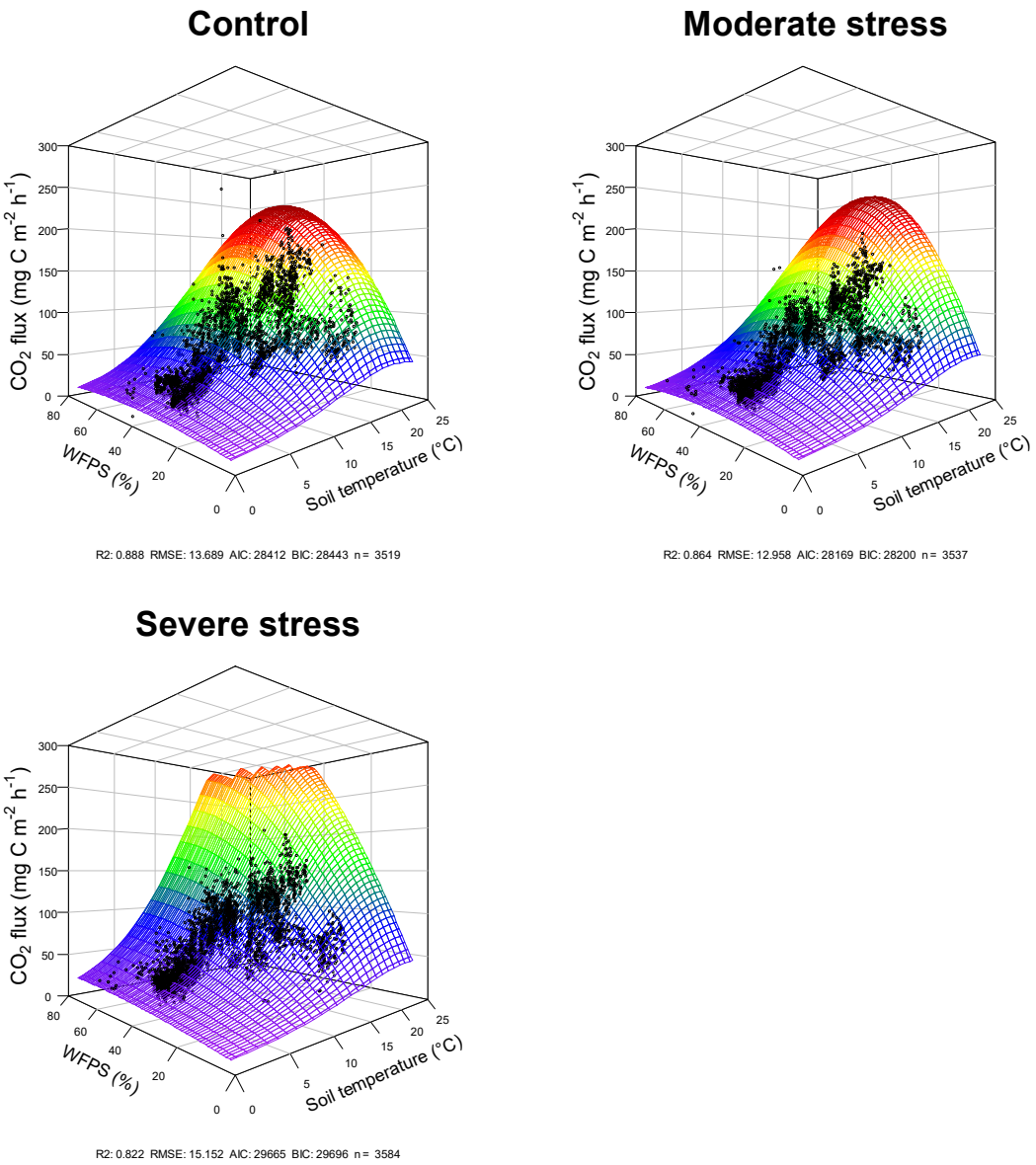


Figure 5: Measured soil CO_2 flux values (black circles, mg C m^{-2} h^{-1}) of control, moderately stressed, and severely stressed plots, showing the dependence of soil CO_2 flux on soil temperature (T_{soil}, °C) and soil water-filled pore space (WFPS, %), with the 3D best-fit model (surface) for the formula CO_2 flux = $a*\exp(b*T_{soil}+c*T_{soil}^2)*(d*WFPS+e*WFPS^2)$, where *a*, *b*, *c*, *d*, and *e* are fitted parameters. Model fit indicators are shown at the bottom of each graph (*RMSE*, root mean square error; *AIC*, Akaike Information Criterion; *BIC*, Bayesian Information Criterion; *n*, number of cases). Data are means of 3-hourly measurements (n = 4).

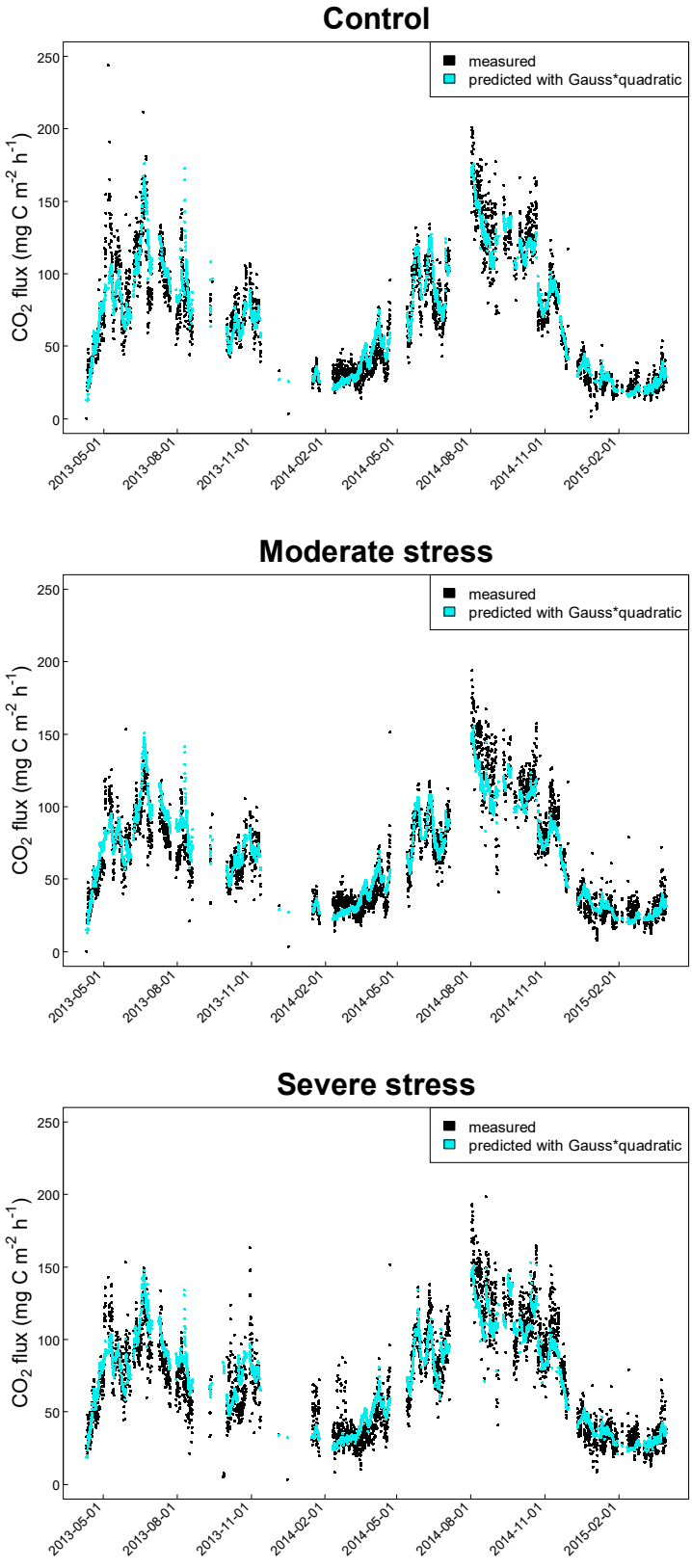
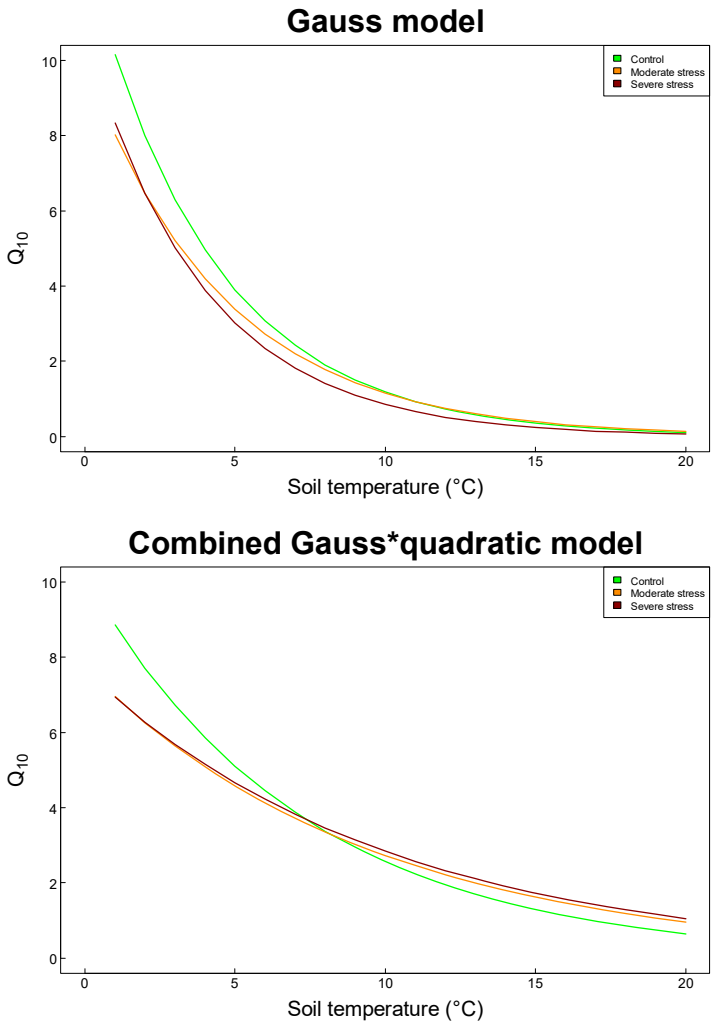


Figure 6: Comparison of measured soil CO_2 flux (black dots), and predicted soil CO_2 flux (cyan dots) using a combined Gauss temperature model in combination with a quadratic soil moisture function of the formula CO_2 flux = $a*\exp(b*T_{soil}+c*T_{soil}^2)*(d*WFPS+e*WFPS^2)$, where *a*, *b*, *c*, *d*, and *e* are fitted parameters. Soil CO_2 flux was measured between April 2013 and March 2015 in control plots, moderately stressed plots, and severely stressed plots. Shown are daily means of 3-hourly flux measurements (n = 4).



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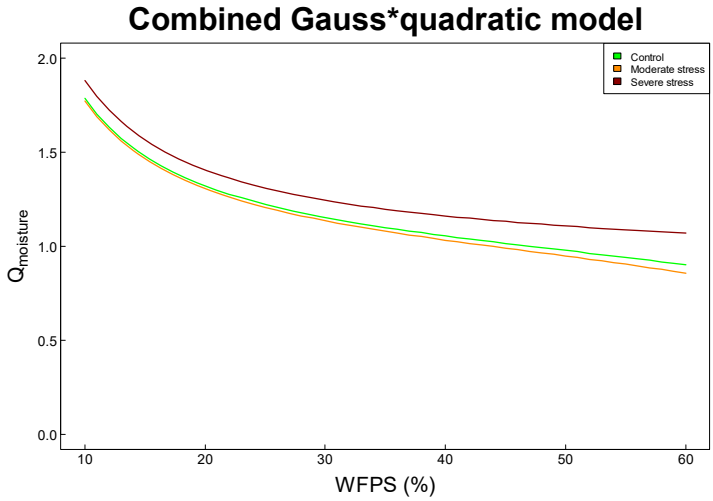
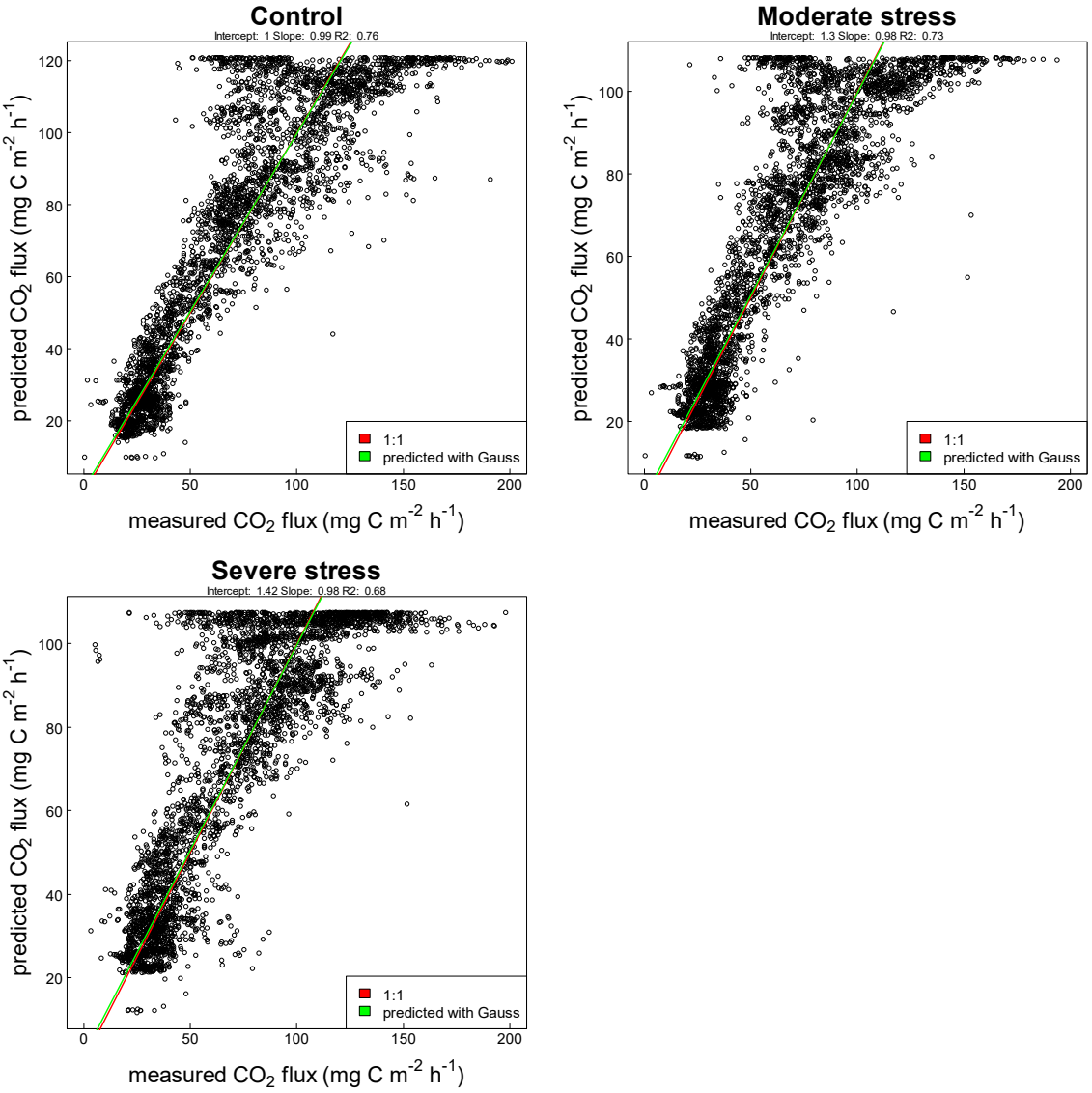
671 **Figure 7:** Comparison of Q_{10} values estimated using a Gauss model (upper panel) and a combined
672 Gauss*quadratic model (bottom panel) for control plots, moderately stressed plots, and severely
673 stressed plots.

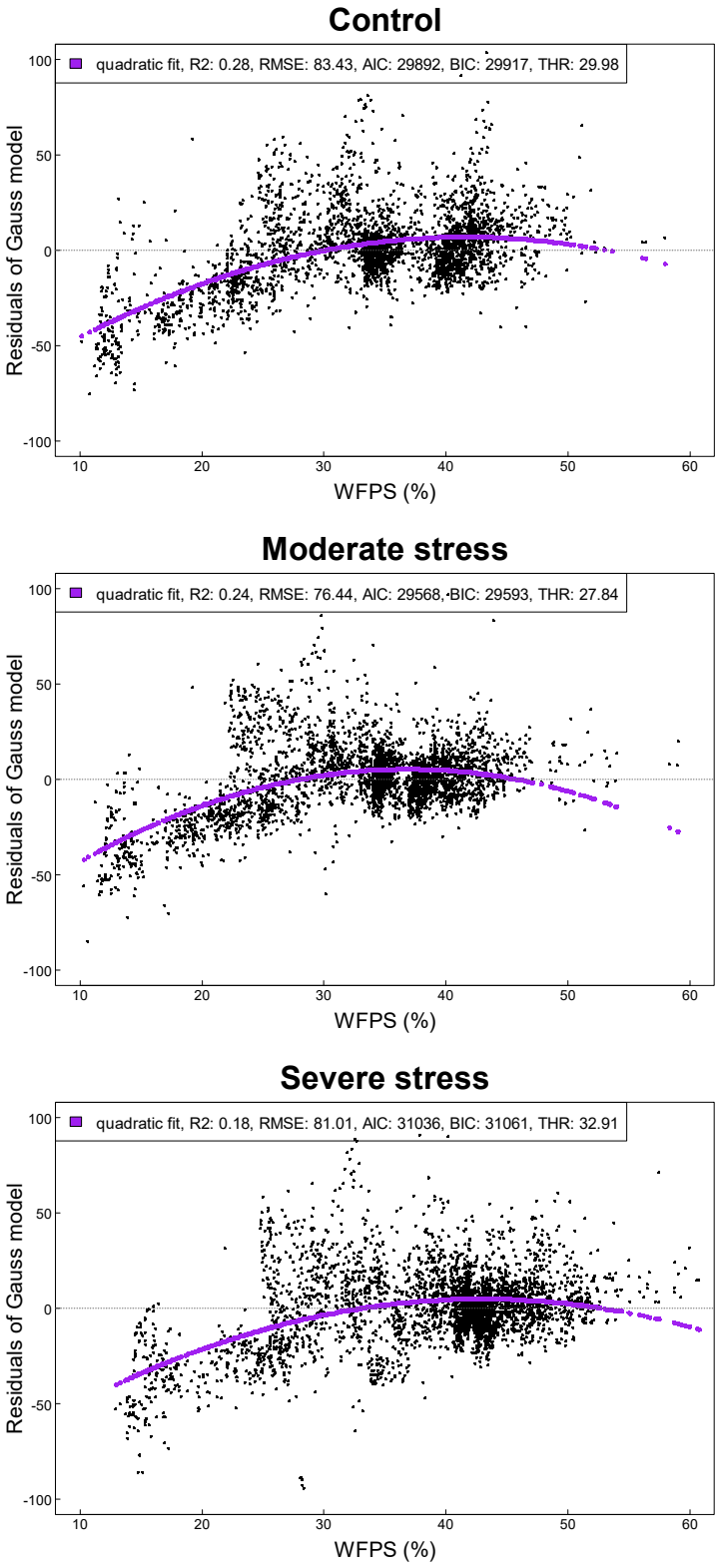


674
675 **Figure 8:** Comparison of $Q_{moisture}$ values of soil CO_2 flux estimated using a combined Gauss*quadratic
676 model for control plots, moderately stressed plots, and severely stressed plots.

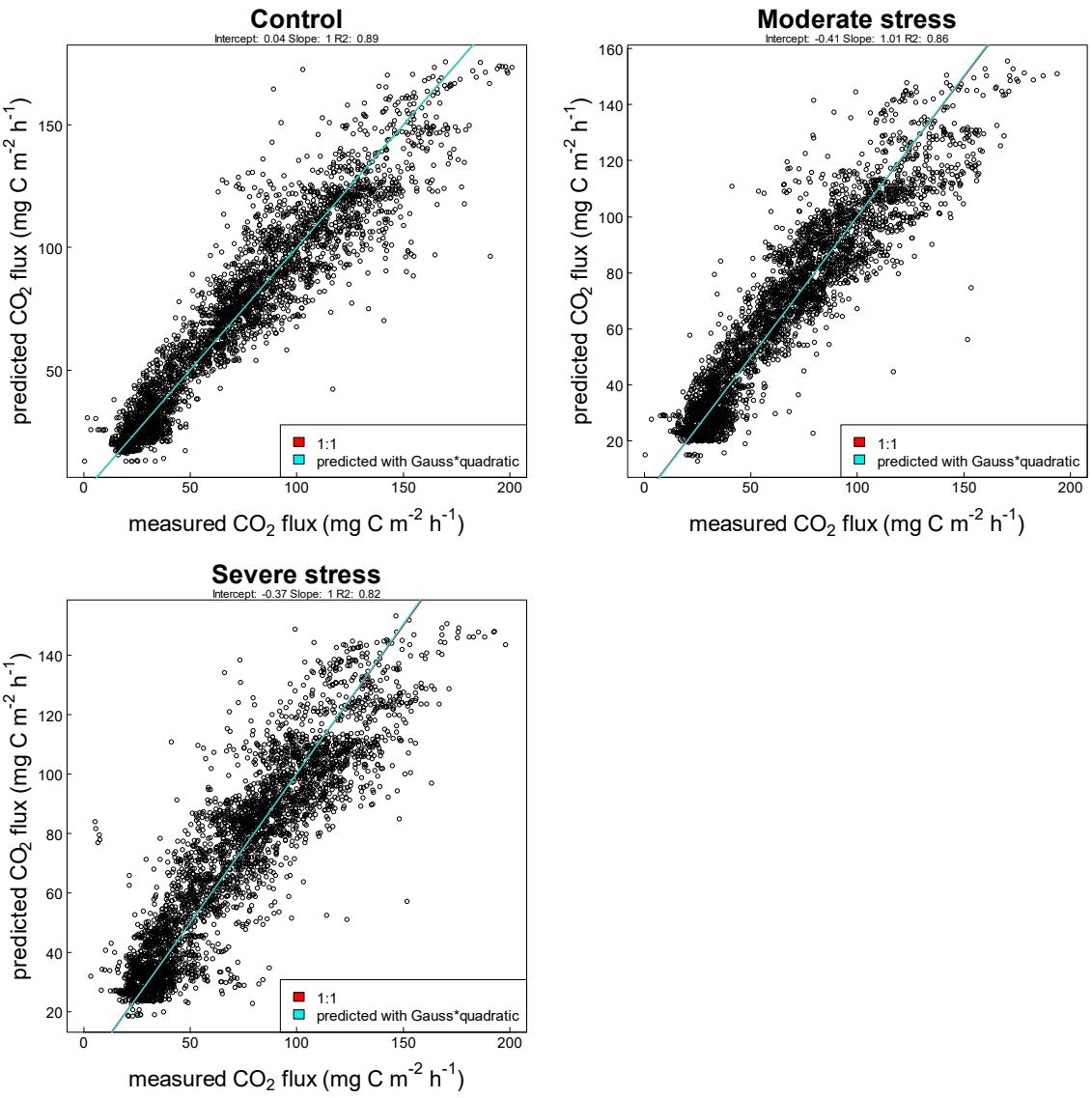
Supplementary figures:



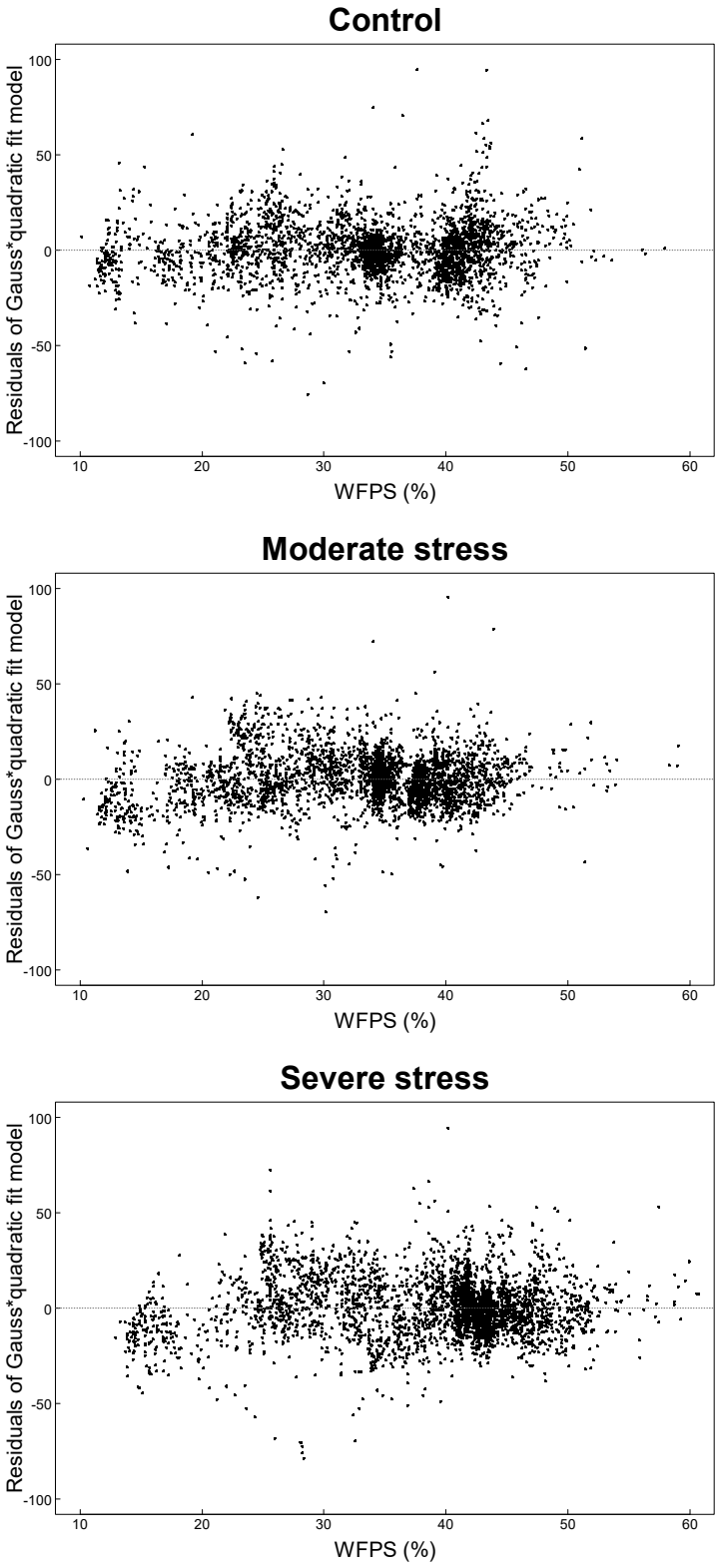
Supplementary Figure S1: Comparison of measured soil CO_2 flux data, and soil CO_2 flux data predicted with a temperature model (Gauss) for control plots, moderately stressed plots, and severely stressed plots (data are means of 3-hourly flux measurements, n = 4). Intercept and slope as well as R^2 of the linear correlation between measured and predicted values are shown on the top of each plot. The red line indicates a perfect (1:1) correlation.



Supplementary Figure S2: Shown are residuals of a Gauss model predicting the relationship between soil CO_2 flux and soil temperature, plotted against soil water-filled pore space (WFPS). The residuals show a curved pattern that approaches a quadratic relationship (purple dots; *RMSE*, root mean square error; *AIC*, Akaike Information Criterion; *BIC*, Bayesian Information Criterion; THR, soil moisture threshold). The soil moisture threshold was defined as the value of WFPS where the quadratic curve crosses the zero-line (dotted line), i.e. left of which residuals become negative.



Supplementary Figure S3: Comparison of measured soil CO_2 flux data, and soil CO_2 flux data predicted with a combined temperature-moisture model (Gauss*quadratic) of the formula CO_2 Flux = a*exp(b*T_{soil}+c*T_{soil}^2)*(d*WFPS+e*$WFPS^2$), where *a*, *b*, *c*, *d*, and *e* are fitted parameters. Data are means of 3-hourly flux measurements (n = 4) for control plots, moderately stressed plots, and severely stressed plots. Intercept and slope as well as R^2 of the linear correlation between measured and predicted values are shown on the top of each plot. The red line indicates a perfect (1:1) correlation.



Supplementary Figure S4. Shown are residuals of a combined temperature-moisture model (Gauss*quadratic) to estimate CO_2, plotted against soil water-filled pore space (WFPS). The curved pattern of residuals (see Supplementary Figure S2) has now disappeared.

3.4. PAPER #4: LINKING NO AND N2O EMISSION PULSES WITH THE MOBILIZATION OF MINERAL AND ORGANIC N UPON REWETTING DRY SOILS

Sonja Leitner[1,2], Peter M. Homyak[2,3], Joseph C. Blankinship[2,4], Jennifer Eberwein[5], G. Darrel Jenerette[5], Sophie Zechmeister-Boltenstern[1], Joshua P. Schimel[2]

[1] Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

[2] Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA, USA

[3] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

[4] Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ, USA

[5] Department of Botany and Plant Sciences, University of California, Riverside, CA, USA

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Contributions:

SL Designed the study, performed microdialysis measurements and laboratory analysis, calculated statistics, wrote the manuscript

PH Designed the study, performed gas flux measurements, wrote the manuscript

JB Helped with the rewetting experiment, performed soil sampling

JE Performed gas flux measurements

GDJ Provided N_2O laser equipment, wrote the manuscript

SZB Designed the study, wrote the manuscript

JS Selected the sampling site and designed the study, wrote the manuscript

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Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils

Sonja Leitner [a, b, *], Peter M. Homyak [b, c], Joseph C. Blankinship [b, d], Jennifer Eberwein [e], G. Darrel Jenerette [e], Sophie Zechmeister-Boltenstern [a], Joshua P. Schimel [b]

[a] Institute of Soil Research, University of Natural Resources and Life Sciences, Vienna, Austria
[b] Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA, USA
[c] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA
[d] Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ, USA
[e] Department of Botany and Plant Sciences, University of California, Riverside, CA, USA

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ABSTRACT

Drying and rewetting of soils triggers a cascade of physical, chemical, and biological processes; understanding these responses to varying moisture levels becomes increasingly important in the context of changing precipitation patterns. When soils dry and water content decreases, diffusion is limited and substrates can accumulate. Upon rewetting, these substrates are mobilized and can energize hot moments of intense biogeochemical cycling, leading to pulses of trace gas emissions. Until recently, it was difficult to follow the rewetting dynamics of nutrient cycling in the field without physically disturbing the soil. Here we present a study that combines real-time trace gas measurements with high-resolution measurements of diffusive nutrient fluxes in intact soils. Our goal was to distinguish the contribution of different inorganic and organic nitrogen (N) forms to the rewetting substrate flush and the production of nitric oxide (NO) and nitrous oxide (N_2O). Diffusive flux of N-bearing substrates (NO_2^-, NO_3^-, NH_4^+ and amino acids) was determined *in situ* in hourly resolution using a microdialysis approach. We conducted an irrigation experiment in a semi-arid California grassland at the end of the dry season, and followed soil N flux and N trace gas emissions over the course of 30 h post-wetting. Upon rewetting, both inorganic and organic N diffused through the soil, with inorganic N contributing most to the rewetting N flush. Emissions of NO and N_2O rapidly increased and remained elevated for the duration of our measurements, whereas diffusive soil N flux was characterized by large temporal variation. Immediately after rewetting, NO_3^- contributed 80% to the total diffusive N flux but was consumed rapidly, possibly due to fast microbial uptake or denitrification. Ammonium flux contributed only ~10% to the initial diffusive N flux, but it dominated total N diffusion 27 h post-wetting, coinciding with peak N-gas emissions. This suggests nitrification may control most of the N trace gases produced during the late stages of a rewetting pulse. Nitrite contributed only 1% to total N diffusion and did not show a clear temporal pattern. Amino acids contributed roughly as much as NH_4^+ to the initial diffusive N flux, but the organic N pulse was short-lived, indicating that organic N did not contribute substantially to N-gas formation shortly after rewetting at our study site. Our results support the hypothesis that in semi-arid environments N-bearing substrates concentrate during dry periods and, upon rewetting, can lead to pulses of NO and N_2O when they react chemically or are transformed by microorganisms.

1. Introduction

Periods of drought are common in most terrestrial ecosystems; hence the influence of drying and rewetting on soil processes has been central in ecosystem research. This becomes even more important with projected increases in extreme weather events (IPCC, 2014). Rewetting triggers a cascade of responses in soil

* Corresponding author. University of Natural Resources and Life Sciences, Institute of Soil Research, Peter Jordan-Str. 82, 1190 Vienna, Austria.
E-mail address: sonja.leitner@boku.ac.at (S. Leitner).

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0038-0717/

physical and chemical processes (Homyak et al., 2016) and shifts in microbial physiology (Placella and Firestone, 2013). For nitrogen (N), drying concentrates N-containing substrates in hydrologically disconnected microsites which, upon rewetting, can produce both nitric oxide (NO), an air pollutant, and nitrous oxide (N_2O), a powerful greenhouse gas (Galbally et al., 2008). When soils rewet, it is thought that a flush of inorganic N [ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-)] governs both the abiotic and biotic transformations that produce N emission pulses. In drylands it has been suggested that the most important processes of NO production following a rewetting event are i) flushing and rapid abiotic chemo-denitrification of NO_2^- and ii) biotic nitrification of NH_4^+ (Davidson, 1992; Homyak et al., 2016). Nitrous oxide is assumed to be mainly produced via nitrification in dry soils (Davidson, 1992; Beare et al., 2009), but after rewetting when high microbial activity leads to O_2 depletion, denitrification of nitrate (NO_3^-) or NO_2^- can also contribute to N_2O emissions (Venterea and Rolston, 2000; Ruser et al., 2006; Galbally et al., 2008). While the processes that lead to N-gas formation have largely been identified (Butterbach-Bahl et al., 2013; Pilegaard, 2013), we know little about the temporal dynamics of inorganic N accumulation and flushing upon rewetting, and how these substrates may synchronize to sustain trace gas emission pulses. Even less attention has been given to the dynamics of organic N and whether it contributes to these emission pulses.

Determining the rewetting dynamics of N compounds has been challenging because it is difficult to monitor minute-to hour-scale changes in N supply in intact soil. Studies have mostly relied on destructive sampling, but disturbances during soil collection and analysis can alter microbial processes (Dumont et al., 2006; Lee et al., 2007) and N concentrations (Rousk and Jones, 2010; Warren and Taranto, 2010; Inselsbacher, 2014). For instance, destructive sampling may overestimate N availability because bulk soil extractions may release protected N in organo-mineral complexes that had not been available for microbial uptake (Van Gestel et al., 1991; Fierer and Schimel, 2003). The N that actually diffuses to microbes and reactive microsites upon rewetting is not well quantified, leading us to ask: What are the dominant forms of inorganic and organic N that are bioavailable during a rewetting pulse? How do the concentrations of these substrates vary across time? And does peak diffusive N flux coincide with peak NO and N_2O emission pulses?

We answered these questions in intact soils by using microdialysis to capture N diffusion dynamics (Inselsbacher et al., 2011) coupled with measurements of NO and N_2O emissions. Similarly to microorganisms or plant roots, microdialysis probes collect substrates diffusing through the soil solution (Ginige et al., 2004), allowing us to determine diffusive N fluxes upon rewetting. We hypothesized that: i) rewetting would cause a NO_2^- flush coinciding with rapid emissions of N gases, ii) substrates consumed by biological processes would decrease after the rewetting pulse, and iii) available N-bearing organic substrates would decrease after wetting.

2. Materials and methods

The study site was located in a seasonally-dry oak savanna in the University of California Sedgwick Reserve (N 34.7120, W 120.0388; 370 m asl). Vegetation is dominated by Mediterranean annual grasses (*Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*). The soil is a thermic Pachic Haploxeroll (pH 6.9, 2.2% C, 0.21% N, 1.2 g cm^{-3} BD, upper 10 cm) on flat slopes (Blankinship et al., 2016). The mean annual temperature is 16.8 °C. Annual precipitation averages 380 mm, with most falling between November and April.

In early November 2015, before onset of the winter growing season, we irrigated a soil plot (2 m × 1 m) with 30 L (corresponding to 15 mm rainfall) of local well water (0.003 mg NH_4^+-N L^{-1}, 1.6 mg NO_3^--N L^{-1}, 0.4 mg DON L^{-1}). Fluxes of NO and N_2O were determined by chamber methodology (Davidson et al., 1991) 1 h before and every 1–4 h post-wetting over the course of 30 h, with a pause between 11 h and 24 h post-wetting. One portable dynamic chamber (30.5 cm diameter, 10 cm height) was connected to a chemiluminescent NO analyzer (Scintrex LMA-3, Canada) and an Off-axis ICOS N_2O laser analyzer (Los Gatos Research, CA, USA). During gas flux measurements, the chamber was consecutively placed in each corner of the experimental plot, with at least 15 cm distance to the plot edges. Closure time was 5 min for each location, and between measurements the chamber was vented until concentrations returned to ambient levels (~60 s). To ensure airtight sealing and to dampen pressure fluctuations inside the chamber, the chamber was equipped with a 20 cm long polyethylene skirt at its base (Parkin and Venterea, 2010). Fluxes were calculated based on the rate of change in gas concentration inside the chamber after correcting for air temperature and air pressure (Homyak et al., 2016).

Microdialysis probe calibration and soil sampling was performed according to Inselsbacher et al. (2011). Four flow-through polyarylethersulphone probes (CMA 20, 10 mm long, 500 μm diameter, 20 kDa molecular weight cut-off; CMA Microdialysis AB, Sweden) were installed vertically down to 2.5 cm soil depth after creating a pilot hole with a cannula. The probes were positioned in a square at the center of the plot, with 50 cm distance between each probe, and left at the same location for the duration of our measurements. High-purity deionized water (MilliQ) was pumped through the system using a syringe infusion pump (CMA 400, flow rate 5 μl min^{-1}), and samples were collected hourly in a refrigerated microfraction collector (6 °C; CMA 470), with a pause between 11 and 24 h after rewetting. Every 3 h, samples were taken out of the microfraction collector and stored in a cool box on ice until frozen (−20 °C) within 24 h of collection. Diffusive N fluxes from the soil solution were calculated based on membrane surface area and time and expressed as μg N cm^{-2} h^{-1} (Inselsbacher and Näsholm, 2012). We also sampled the upper 5 cm of soil using a 4-cm diameter corer within 20 cm of the microdialysis probes prior to wetting and 1, 8, 24 and 30 h post-wetting. In the lab, soil was extracted in either 0.5 M K_2SO_4 or MilliQ water. Microdialysis and soil extract samples were analyzed colorimetrically for NO_2^- (Homyak et al., 2015), NO_3^- and NH_4^+ on a plate reader (Hood-Nowotny et al., 2010). Seventeen amino acids were analyzed by reverse-phase liquid chromatography (see Supplementary methods for details) on a UPLC system equipped with a fluorescence detector (Waters Corp., MA, USA). Statistical analysis was done with R 3.3.2 (www.r-project.org).

3. Results

Nitric oxide emissions increased 25-fold upon rewetting, from 0.29 ± 0.05 ng N m^{-2} s^{-1} pre-wetting to 7.47 ± 1.26 ng N m^{-2} s^{-1} 1 h post-wetting (Fig. 1a), and they continued to increase until reaching a peak at 41.6 ± 2.0 ng N m^{-2} s^{-1} 8 h post-wetting. Following this peak, fluxes declined to approximately 25 ng N m^{-2} s^{-1} drifting to values below 20 ng N m^{-2} s^{-1} by the end of our measurements. Nitrous oxide emissions also increased immediately after rewetting, from −0.04 ± 0.10 ng N m^{-2} s^{-1} pre-wetting to 5.57 ± 1.27 ng N m^{-2} s^{-1} within 1 h post-wetting (Fig. 1b). Compared to NO, the increase in N_2O emissions was slower and highest after 27 h (12.95 ± 6.03 ng N m^{-2} s^{-1}).

Microdialysis requires moist conditions for substrates to diffuse into the probes; therefore, we were unable to determine pre-wetting diffusive fluxes. Nitrate accounted for ~80% of the total

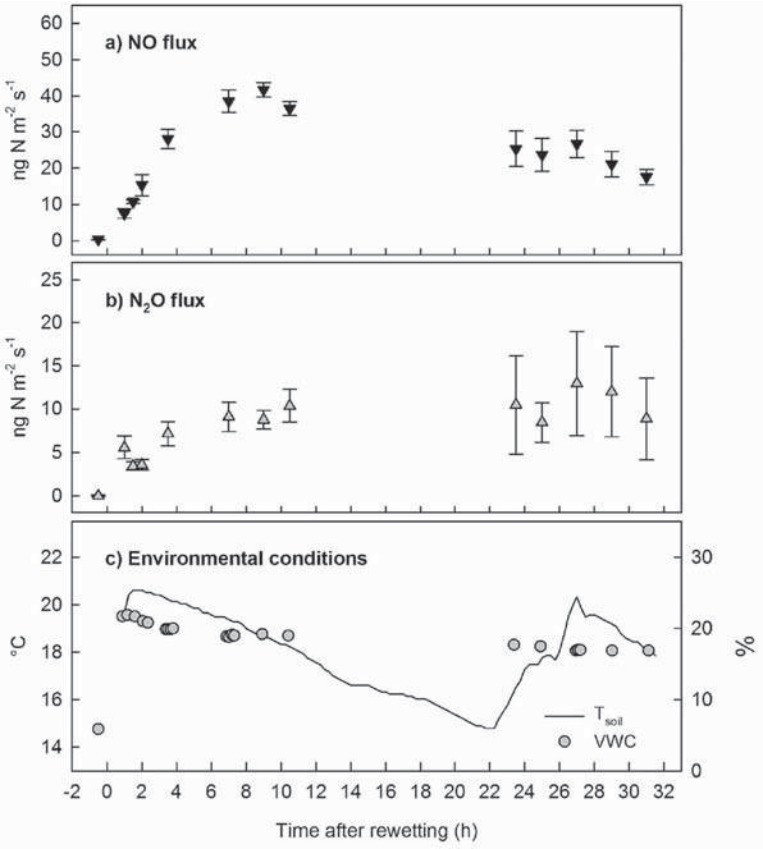

Fig. 1. Fluxes of a) nitric oxide (NO) and b) nitrous oxide (N_2O), and c) soil temperature (T_{soil}) and volumetric water content (VWC). The first time point was measured in dry soil; all consecutive times were measured after irrigating soils with 15 mm of water. Data are average ± SE (n = 4).

diffusive N flux immediately after the rewetting pulse (Fig. 2a); fluxes were highest during the first 2 h post-wetting (0.70 ± 0.47 μg N cm^{-2} h^{-1}), but decreased rapidly and remained low for the duration of our measurements (Fig. 3a). Nitrite amounted to only 1% of the total diffusive N flux (Fig. 2a). During the first 10 h after rewetting, diffusive NO_2^- flux averaged 0.03 ± 0.004 μg N cm^{-2} h^{-1} (Fig. 3b), but decreased by ~50% by the second day. Ammonium represented 9% of the total diffusive N flux immediately after rewetting (Fig. 2a). Similarly to NO_3^-, we observed an initial flush of NH_4^+ during the first 2 h post-wetting, when fluxes averaged 0.07 ± 0.04 μg N cm^{-2} h^{-1}, but the pulse was short-lived (Fig. 3c). After ~27 h, NH_4^+ diffusive fluxes increased to a high of 3.56 ± 2.84 μg N cm^{-2} h^{-1}, the highest diffusive N flux we measured. Initially, the amino acid flux was in the same range as initial NH_4^+ and contributed 10% to total diffusive N flux (Fig. 2a); flux was highest in the first 2 h post-wetting (0.07 ± 0.03 μg N cm^{-2} h^{-1}) but then rapidly dropped by ~75% to around 0.02 ± 0.01 μg N cm^{-2} h^{-1}, where flux remained into the second day of measurement (Fig. 3d).

The distribution of inorganic N species in water-extracted soils was similar to that of microdialysis, with NO_3^- accounting for 63% of the total N pool, 18% NO_2^-, 7% NH_4^+, and 12% amino acids (Fig. 2b). In K_2SO_4 extracts, NH_4^+ concentrations where higher compared to water extracts (*t*-test, $P < 0.01$); NH_4^+ made up the largest fraction of the exchangeable N pool (53%), compared to 7% NO_2^-, 28% NO_3^-, and 12% amino acids (Fig. 2c). In contrast to microdialysis, bulk soil N concentrations did not change significantly between pre- and post-wetting conditions (one-way ANOVA, $P > 0.05$).

4. Discussion

During dry periods, mineral and organic N substrates are hypothesized to accumulate in soil because of (i) decreased plant N uptake, and (ii) because soil microsites where decomposition and N mineralization take place become hydrologically disconnected from microsites of microbial N immobilization (Parker and Schimel, 2011; Homyak et al., 2016). Our results show that N-bearing substrates were rapidly mobilized upon rewetting, and that this mobilization coincided with rapid increases in N trace gas emission pulses.

Both NO and N_2O emissions increased rapidly within the first hour after rewetting, and remained elevated over the next 30 h. Theory suggests that in arid and semi-arid ecosystems when soils are at low to intermediate water contents NO and N_2O are primarily produced via nitrification (Davidson, 1992). However, rapid chemical reactions involving NO_2^- (chemodenitrification) contribute to these emissions (Medinets et al., 2015; Heil et al., 2016). At our study site, nitrification potentials increase during the dry season

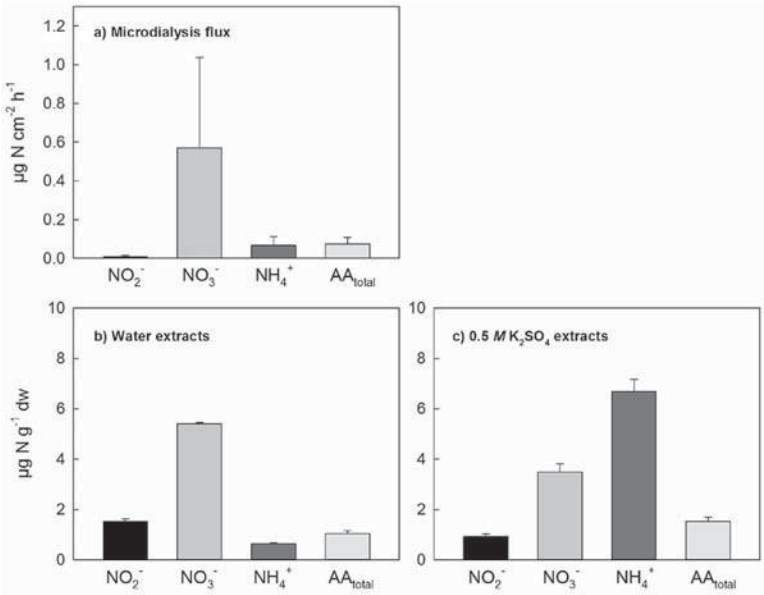

Fig. 2. Contribution of inorganic and organic N to a) diffusive N flux measured by microdialysis at the first sampling time point 1 h after rewetting (upper panel, average ± SE, n = 4), and concentrations determined by extracting soils with b) MilliQ water or c) 0.5 *M* K_2SO_4 (lower panels, average ± SE, n = 3). AA_{total}, sum of 17 amino acids.

(Parker and Schimel, 2011), and chemodenitrification upon rewetting is responsible for generating rapid NO emission pulses (Homyak et al., 2016). Consistent with this understanding, there was ongoing diffusive NO_2^- flux throughout our measurements, which could have stimulated chemodenitrification, especially upon rewetting. Furthermore, diffusive NH_4^+ flux increased 27 h after the rewetting pulse, which coincided with the period of highest N gas emission. This suggests that as microbes recover from drought-induced stress, increasing mineralization and NH_4^+ supply may contribute to N gas emission pulses via nitrification.

Immediately after rewetting, NO_3^- made up the majority of the initial diffusive N flux but then rapidly disappeared, suggesting it was immobilized or denitrified. In drylands, denitrification is usually low because soils are well-aerated but denitrification is anaerobic (Venterea and Rolston, 2000; Galbally et al., 2008). Although we did not measure nitrification and denitrification rates, Parker and Schimel (2011) found that potential denitrifying enzyme activity increased during the dry season at our study site, suggesting denitrification may be important immediately post-wetting. Indeed, N_2O emissions increased within minutes of wetting soils to a maximum of 13 ng N m^{-2} s^{-1} 27 h post wetting. Moreover, we found a negative correlation between NO_3^- diffusion and N_2O emissions ($r = -0.89$, $p < 0.001$, Supplementary Table 1), perhaps suggesting that NO_3^- was in fact reduced to N_2O. It has been suggested that bursts of microbial respiration following a rewetting pulse can rapidly deplete soil oxygen levels, allowing denitrification to occur in anoxic soil microsites and leading to substantial N_2O emissions in dry lands (Hu et al., 2017). Therefore, it seems likely that the increase in soil moisture together with the initial NO_3^- flush during the rewetting pulse favored denitrification, which could have contributed to the observed N gas emissions and the drawdown of NO_3^- in soil.

We acknowledge that the drawdown in NO_3^- could have been caused by diffusional depletion zones forming around the microdialysis membranes over time; depletion zones may develop if N diffuses through the membranes at a faster rate than it could be replenished by the soil (Inselsbacher et al., 2011). However, our observations suggest diffusional depletion of NO_3^- was unlikely to develop for at least two reasons: i) membranes are more likely to develop diffusion depletion zones for cations (e.g., NH_4^+) than for anions because cations can bind to negatively charged clay minerals and soil organic matter. Yet, NH_4^+ diffusivity increased over time in membranes kept in fixed locations for >26 h; hence diffusional depletion of more mobile substrates (e.g. NO_2^-, NO_3^-, or even amino acids) is unlikely; and ii) depletion zones are less likely to form if substrate production rates are high enough to maintain concentration gradients. Since NO and N_2O emissions were high after rewetting, NO_2^- and NO_3^- supply rates should have also been high.

We also note the irrigation water used in our study contained 1.6 mg NO_3^--N L^{-1}, corresponding to 0.24 kg N ha^{-1}. This is similar to NO_3^- concentrations in rainwater for the southwestern US (0.5–1.5 kg NO_3^--N ha^{-1}; Holland et al., 2005) and within the range of expected atmospheric N deposition for our site (5–7 kg N ha^{-1} yr^{-1}; Fenn et al., 2010). Based on a soil porosity of 54%, we expected the irrigation water to infiltrate to a depth of at least 3 cm, wetting 66 kg of soil. Under these conditions, and assuming steady state, irrigating soils would have raised the NO_3^- content of the soil (4–6 µg NO_3^--N g^{-1} dw) by only 0.7 µg NO_3^--N g^{-1} dw, or by at most 18%. Therefore, well water addition, alone, is unlikely to explain the NO_3^- patterns detected using microdialysis.

Considering the role of organic N, amino acids contributed about as much to initial N flux as NH_4^+, but the amino acid flush was short-lived. The dominant amino acids were methionine, valine and tyrosine (Supplementary Figs. S1 and S2), but we found no indication of known microbial osmolytes like proline, which may be synthesized by microorganisms experiencing drought stress (Killham and Firestone, 1984; Csonka, 1989). Our findings are consistent with previous studies that have reported no *in-situ* osmolyte accumulation in drying soils (Boot et al., 2013; Göransson et al., 2013; Kakumanu et al., 2013). It has been suggested, however,

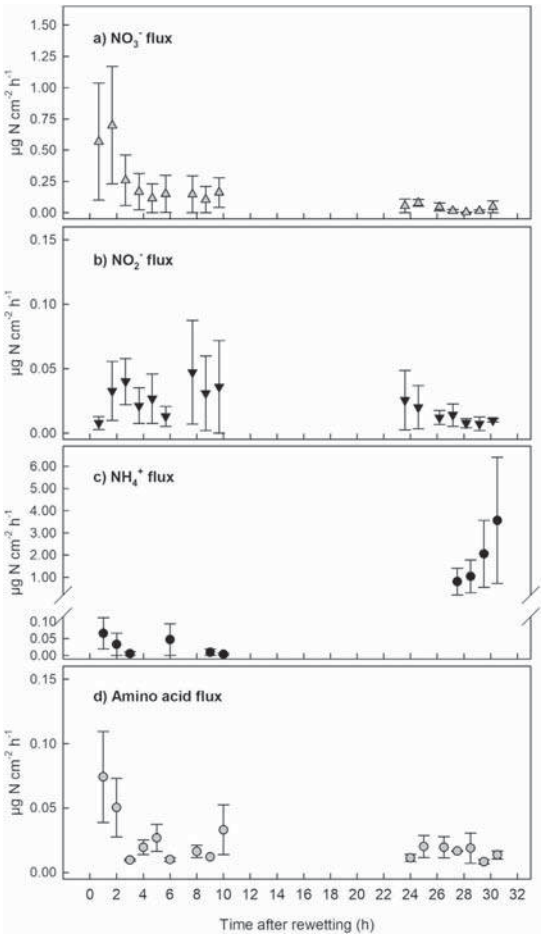

Fig. 3. Diffusive fluxes of a) nitrate (NO_3^-), b) nitrite (NO_2^-), c) ammonium (NH_4^+) and d) sum of 17 amino acids determined *in situ* with microdialysis over the course of 30 h after irrigating soils with 15 mm of water (average ± SE, n = 4).

that even if osmolytes are produced by drought-stressed microorganisms, they may be less likely to be disposed into the soil solution after rewetting; instead, osmolytes may be mineralized intracellularly since they represent a valuable source of C and N (Warren, 2014). In the context of N trace gas formation, organic N has been proposed to directly contribute to N_2O formation via heterotrophic nitrification (Müller et al., 2014). However, because amino acids were unavailable beyond the first 2 h post-wetting, they may not account for a significant fraction of N_2O production at our site.

In contrast to microdialysis results, soil extracts did not capture differences in N availability before and after rewetting, likely because bulk extractions integrate N pools that turn over at different rates (i.e., slow and fast cycling pools). These differences highlight the potential of the microdialysis approach, since it allows measuring rapidly turning-over bioavailable N pools at high temporal resolution—a prerequisite for studying short-term changes in N availability during pulsed events (e.g., Homyak et al., 2017). Our study emphasizes soil N diffusive fluxes can change rapidly and that these changes can be detected *in situ* while bypassing artifacts introduced when measuring N dynamics in the laboratory (e.g, disturbances associated with sample collection and/or sieving).

In conclusion, we show that soil N transformations following a rewetting pulse can be rapid, including an immediate draw-down of NO_3^- and amino acids followed by NH_4^+ production 24 h following rewetting. These shifts in the availability of different N-forms corresponded to shifts in the emission of NO and N_2O. Observations of both soil N diffusion and trace gas emissions were enabled by the combination of new microdialysis and trace-gas measurements that allowed evaluation of short-term dynamics of N transformations. In this semi-arid grassland, microbial processes controlled emissions of N gases both by generating substrates that concentrate in dry soils and that may react chemically upon rewetting (i.e., NO_2^-), and by generating substrates that can stimulate biological N gas evasion as microbes recover from drought stress.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2017.09.005.

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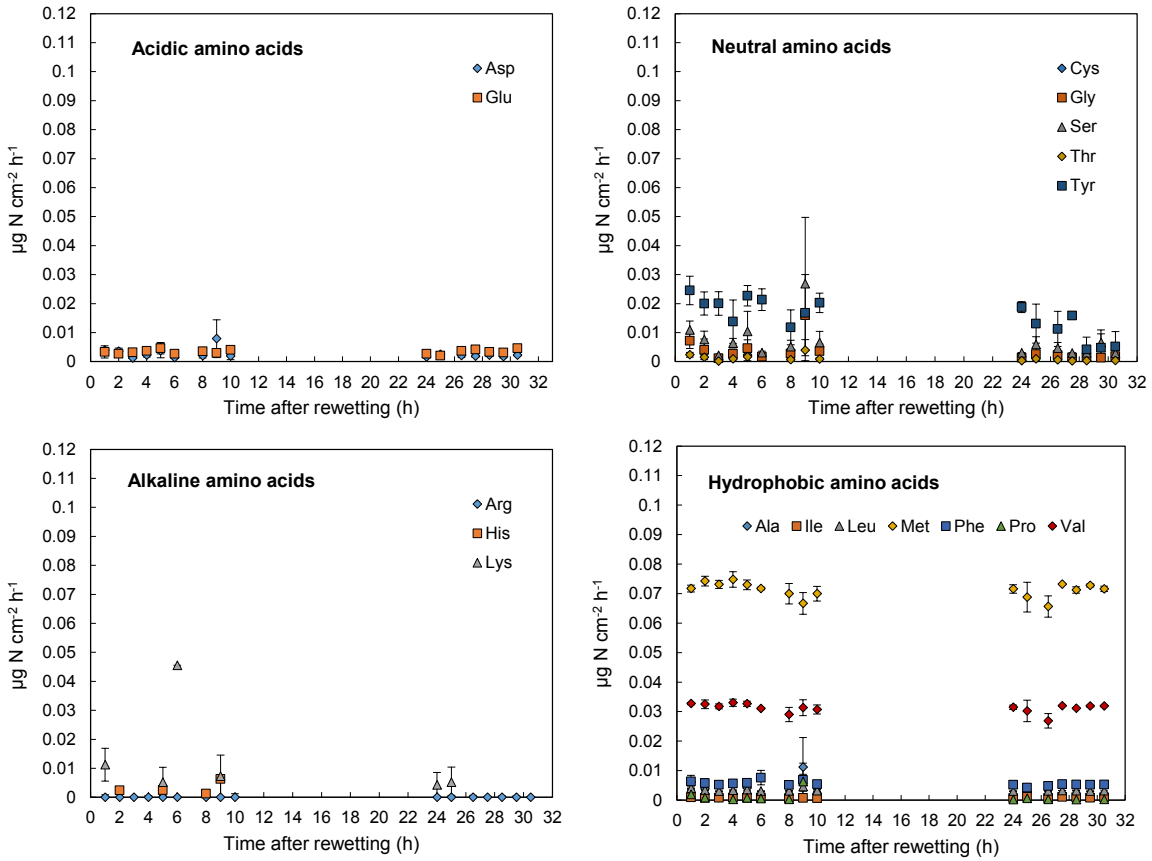
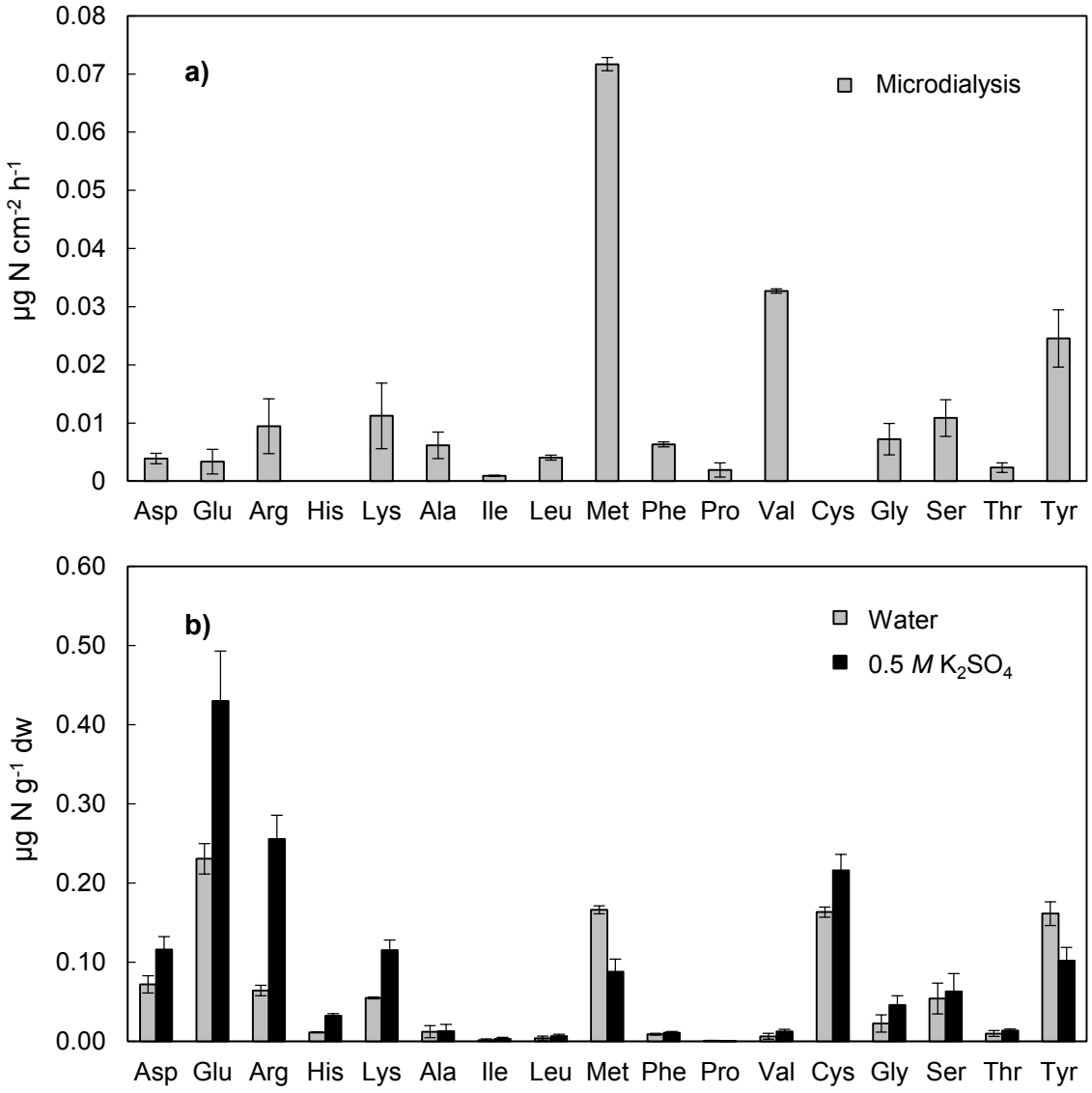
Supplementary material: Amino acid detection

17 individual amino acids (ala, alanine; arg, arginine; asp, aspartic acid; cys, cysteine; glu, glutamic acid; gly, glycine; his, histidine; ile, isoleucine; leu, leucine; lys, lysine; met, methionine; phe, phenylalanine; pro, proline; ser, serine; thr, threonine; tyr, tyrosine; val, valine) were analyzed by reversed-phase liquid chromatography on a Waters Ultra High Performance Liquid Chromatography (UPLC) system equipped with a Waters Fluorescence (FLR) detector (Waters Corp., MA, USA). 40 µl aliquots of samples, standards and blanks were derivatized with 10 µl of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AQC Fluor reagent) in 50 µl borate buffer at 55 °C for 10 min, and an aliquot of 2 µl was injected. Individual amino acids were separated on a Waters AccQ-Tag Ultra C-18 1.7 µm column (2.1 x 100 mm) by elution with a mixture of AccQ Tag Ultra Eluent A (aqueous buffer) and AccQ Tag Ultra Eluent B (organic phase) at the following gradient: 0-5.74 min isocratic 99.9 % Eluent A, declining to 90.0 % Eluent A from 5.74 to 7.74 min, to 78.8 % Eluent A at 7.74 min, to 40.4 % Eluent A at 8.04 min, to 10 % Eluent a from 8.05 to 8.73, then re-equilibration at 99.9 % Eluent A until the end of the run at 9.50 min. Flow rate was 0.7 ml min^{-1} and column temperature was set to 57 °C. For calibration, an Amino Acid Hydrolysate Standard (Thermo Scientific, IL, USA) containing a 2.5 mM mixture of 17 amino acids in 0.1 M HCl, with the exception of cysteine (1.25 mM) was prepared freshly every day in the range of 100-0.1 µM N by serial 1:2 dilution with MilliQ water.

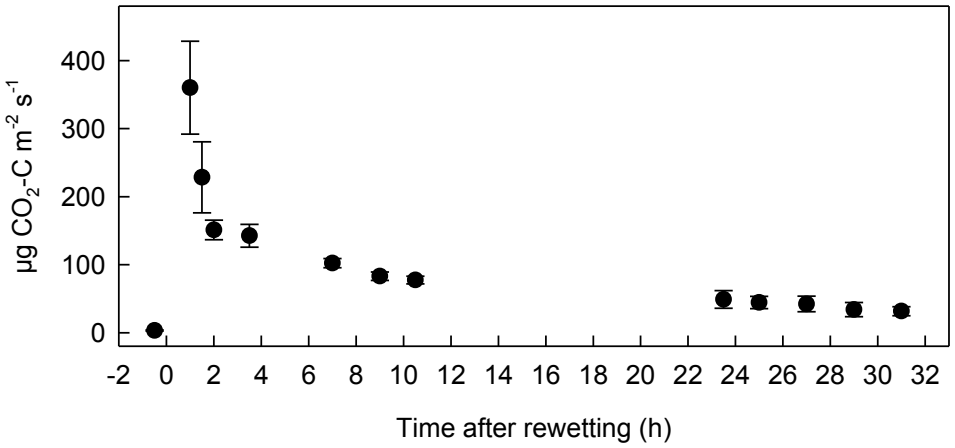
Supplementary material: Figures



Supplementary Figure S1: Microdialysis flux rates of 17 individual amino acids after rewetting of dry soil. Flux rates below limit of detection are not shown. Data are mean ± SE (n = 4).



Supplementary Figure S2: Comparison of a) microdialysis flux rates of amino acids 1 h after rewetting, and b) amino acid concentrations determined by water and 0.5 *M* K_2SO_4 extracts. Data are mean ± SE (n = 4).



Supplementary Figure S3: Soil respiration following the wetting of dry soil. The first time point was measured in dry soil; all consecutive times were measured after irrigating soils with 15 mm of water. Data are mean ± SE (n = 4).

	NO	NH_4^+	NO_3^-	NO_2^-	AA_{tot}	VWC	T_{soil}
N_2O	0.42	0.35	**-0.89** ***	-0.29	**-0.64** *	**-0.82** **	-0.31
NO		-0.34	-0.51	0.43	**-0.61** *	-0.24	0.1
NH_4^+			-0.48	-0.56	-0.35	**-0.68** *	-0.38
NO_3^-				0.23	**0.81** ***	**0.84** ***	0.24
NO_2^-					-0.07	0.41	0.16
AA_{tot}						**0.68** **	-0.03
VWC							0.24

Supplementary Table 1: Pearson's correlation coefficients and corresponding significance levels (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) for NO and N_2O gas fluxes per soil area (ng N m^{-2} s^{-1}), diffusive soil fluxes of NH_4^+, NO_3^-, NO_2^- and total amino acids (AA_{tot}) per microdialysis membrane area (µg N cm^{-2} h^{-1}), and environmental parameters (VWC, volumetric water content in %, T_{soil}, soil temperature in °C, both measured at 5 cm depth).

4. CONCLUSIONS AND REMARK

My first research objective was to determine how much of forest soil GHG flux is litter-induced, *i.e.* the role of the litter layer in the production and consumption of GHGs. We were able to show that the litter layer contributes substantially to forest soil GHG emissions and influences the temperature sensitivity of soil respiration. At our forest site, litter produced 30 % of soil CO_2 emissions, and it modulated CH_4 uptake and N_2O fluxes of the soil. After litter removal, CH_4 uptake increased by 16 % and N_2O emission peaks after rainfall events disappeared. These findings have strong implications for forest biogeochemistry, especially in the context of the intensification of forest biomass utilization for energy and heat production (Merilä *et al.*, 2014). Furthermore, these findings highlight that litter GHG emissions should be specifically accounted for in future climate change studies. Both rising temperatures and changes in precipitation will likely affect litter microclimate, which in turn could influence decomposer activity and litter-induced GHG fluxes (Baldrian *et al.*, 2013; Schlesinger *et al.*, 2016). In addition, extreme changes in climate like severe droughts in combination with heat waves can alter timing and amount of litter inputs to the soil (Liu *et al.*, 2015). In the long run, climate change in combination with ecosystem disturbances like bark beetle attacks on drought-stressed trees could trigger large-scale tree mortality and lead to shifts in plant species composition in Central Europe (Seidl *et al.*, 2016), which will translate into changes in litter quality. This will affect ecosystem processes beyond local scales and could have implications for national GHG budgets. Therefore, it is important to explicitly include litter as a component in forest C models.

My second research objective was to determine how frequency and intensity of drought periods and rain events affect soil N availability. I was able to show that rewetting of dry soil led to a pronounced pulse in NO_3^- diffusion even after multiple drying-rewetting cycles, and this pulse was larger if the preceding drought period had been longer. Further, we showed that short-term dynamics of N mobilization can be detected and quantified with state-of-the-art equipment like soil microdialysis that enables measurements in undisturbed soil and at high temporal resolution. Extreme weather events, even a single rewetting event, can trigger rapid and disproportionate flushes of NO_3^- that are short in duration but can contribute substantially to ecosystem N cycling. If this NO_3^- flush is not immobilized by soil microorganisms or taken up by plant roots, this bears substantial risk of N leaching and groundwater pollution, especially if repeated severe droughts are followed by heavy rainfalls (Dirnböck *et al.*, 2016). The soil microdialysis approach can help to elucidate these highly dynamic process responses. Furthermore, in contrast to conventional soil extractions that integrate over fast-cycling and intermediate nutrient pools and extract nutrients from a relatively large soil volume (Van Gestel *et al.*, 1991; Černohlávková *et al.*, 2009), microdialysis enables us to measure nutrients diffusing through soil, which is the fraction that can actually be accessed by plant roots and microorganisms (Inselsbacher & Näsholm, 2012). This provides us with a more realistic picture of plant and microbial nutrition, and it enables us to take a closer look at the functioning of the active part of the soil

microbial community, which constitutes only a small percentage of total soil microbial biomass but catalyzes the majority of soil processes (Blagodatskaya & Kuzyakov, 2013). It should, however, be noted that while the small size of microdialysis membranes allows us to be spatially explicit in the detection of soil microsites of high biochemical activity, it can also be challenging to account for spatial heterogeneity. Nevertheless, this technique has great potential to deepen our understanding of soil processes under natural conditions and in the context of climate change.

In the third paper of my thesis, I wanted to know to which extent GHG emissions are reduced by severe drought periods, and whether potential rewetting pulses could compensate or even outweigh the drought-induced reduction, leading to increased or decreased overall GHG emissions. We found that repeated severe droughts led to a strong reduction in soil respiration that was not compensated by rewetting CO_2 pulses, resulting in an overall decrease of soil respiration during the vegetation period. Besides the alterations of total CO_2 efflux, severe drying-rewetting stress affected the temperature and moisture sensitivity of soil respiration. Furthermore, we found that extreme manipulation treatments are needed if we aim at detecting thresholds of process control. Contrary to our expectations, we observed that the moderate drying-rewetting stress treatment—which after all experienced six consecutive drought periods of an entire month without rainfall—did not change average soil respiration rates compared to controls receiving natural precipitation. We suggest that climate change experiments in the field should be conducted over multiple years to account for inter-annual variation, and they should apply repeated stress events to account for cumulative effects. Furthermore, we highlight that mean annual precipitation might not be a good predictor of future soil GHG emissions: despite the fact that the different stress treatments in the forest precipitation experiment received approximately the same amount of water during the vegetation period, uneven temporal distribution of rainfall significantly reduced soil respiration. And while the reduction in respiration was not permanent and recovered with natural precipitation inputs during winter, repeated severe droughts increased hydrophobic compounds in the soil and led to poor wettability, which resulted in soil desiccation over the two years of precipitation redistribution (Schwen *et al.*, 2015). Therefore, future climate manipulation studies should try to exhaust ecosystem stress resistance in order to identify "tipping points" beyond which changes become non-reversible.

My fourth research objective was to link soil nutrient cycling dynamics with pulses of GHG emissions after a rewetting event. This study was conducted in a semi-arid grassland in California, where we irrigated soil after six months of drought as it usually happens during the transition from dry to rainy season. We found that rewetting led to a pulse in both NO and N_2O emissions within minutes that coincided with increased N diffusion in soil. Right after the irrigation, NO_3^- mobilization dominated N diffusion in soil, but this NO_3^- flush disappeared quickly. After 27 h, NH_4^+ diffusion increased and contributed most to total N diffusion, which coincided with elevated NO and N_2O emissions, indicating that at this time point, nitrification was the most important process leading to NO and N_2O production. This study highlights that the combination of microdialysis with high-resolution GHG flux measurements is well suited to link aboveground and belowground N cycling processes. Furthermore, our results corroborate the idea that N compounds accumulate in dry soil, and, upon rewetting, can be

mobilized and fuel GHG emissions from soil when they react chemically or are transformed biologically.

We are living in a changing world. What we consider "normal" today is already beyond natural variations of the pre-industrial era less than 200 years ago. This is true for climate (2015 was the first time in recorded history that global mean temperatures were more than 1 °C above pre-industrial levels; Hawkins *et al.*, 2017) as well as C and nutrient cycles (today's atmospheric concentrations of CO_2, CH_4 and N_2O are at the highest level in 800.000 years; WMO, 2017), and it already affects all aquatic and terrestrial ecosystems. This is a monumental change that challenges us all, and never before has the world been changed at such a breathtaking rate. Combating global change requires a concerted effort of all countries of the world, on all political, economic, societal and personal levels. And it is the duty of the scientific community to develop solutions and provide the basis for the decision-making process.

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7. CURRICULUM VITAE

Personal information

Name	Sonja Leitner
Date of birth	15 Dec 1984
Nationality	Austria
Website	http://sonjaleitner.wordpress.com

Research interests

- Greenhouse gas exchange, formation and consumption processes
- Impact of climate change and extreme events on C and N cycles
- Mobilization of N in soil and water
- C and N turnover in terrestrial ecosystems

Academic career

Since 2013	**PhD Natural Resources and Life Sciences (Forestry),** University of Natural Resources and Life Sciences (BOKU), Vienna, Austria Institute of Soil Research
2012	**Research assistant** University of Natural Resources and Life Sciences, Vienna, Austria Institute of Soil Research
2011	**Research assistant** University of Natural Resources and Life Sciences, Vienna, Austria Institute of Silviculture
2003 - 2010	**MSc Biology (Terrestrial Ecology)** University of Vienna, Austria Department of Microbiology and Ecosystem Science Diploma thesis: *"Influence of litter chemistry and stoichiometry on glucan depolymerization during decomposition of beech litter"*
2009	**Study assistant** University of Vienna, Austria Department of Microbiology and Ecosystem Science

Fellowships & acquired funding

2015	4-month exchange scholarship at the University of California, Santa Barbara, USA, (Prof. Josh Schimel), awarded by the Austrian Marshall Plan Foundation (AT), €6'500
2013	First Austrian recipient of a 3-year PhD fellowship from the AXA research fund (FR), €120'000
2014	Proposal co-writer of the funded DRAIN project (PI Dr. Michael Zimmermann), Kommunalkredit Public Consulting (AT), KR13AC6K11008 (ACRP6), €279'000
2012	Proposal co-writer of the funded MINT WABO project (PI Prof. Sophie Zechmeister-Boltenstern), BMWF (AT), €611'800

Language skills

German (native speaker), English (C1), Portuguese (B1), Spanish (A2), French (A1)

Scientific community service & professional memberships

Reviewer for Soil Biology & Biochemistry (2015, 2016, 2017), Journal of Food Science and Agriculture (2017), Geoderma (2016), Forests (2016)
Member of the European Geosciences Union and the Austrian Soil Science Society
Participant in the ClimMani network (Climate Change Manipulation Experiments in Terrestrial Ecosystems) and the iLTER network (International Long-Term Ecosystem Research)

Teaching experience

Co-supervision of 6 master theses at BOKU University Vienna:

- Pia Minixhofer: Impact of extreme weather events on plant-available nitrogen and amino acids using microdialysis (2015)
- Markus Schartner: Impact of repeated dry-wet cycles on macro-aggregate stability and organic carbon stabilization of a beech forest soil (2015)
- Jan Bockholt: Rainfall partitioning and solute fluxes in a pure beech stand and the effect of droughts and heavy rain events at plot scale (2014)
- Paul Brugner: Influence of weather extremes on forest-soil nutrient cycling (2014)
- Orracha Sae-Tun: Impact of litter-removal and seasonality on soil microbial community composition in a beech forest (2014)
- Lukas Kranzinger: Impact of litter removal and seasonality on soil greenhouse gas fluxes and nutrient cycling in an Austrian beech forest (2014)

Tutoring:

- 2010: “Interaction of Terrestrial and Aquatic Ecosystems”, University of Vienna, Department of Microbiology and Ecosystem Science
- 2010: “C-cycling in alpine ecosystems”, University of Vienna, Department of Microbiology and Ecosystem Science
- 2008: “Plant anatomy”, University of Vienna, former Department of Ecophysiology and Functional Anatomy of Plants

Other work experience

2003 - 2004	**Waitress** at Do & Co Catering Service (Vienna and Lower Austria, Austria)
2004 - 2009	**Sales assistant** at Intersport Eybl Vösendorf (Lower Austria, Austria)
2006	**Freelancer** at Europcar Car Rental (Vienna, Austria)

Publications

Peer-reviewed publications (ORCID ID 0000-0002-1276-8071):

Leitner S, Homyak PM, Blankinship JC, Eberwein J, Jenerette D, Zechmeister-Boltenstern S, Schimel JP (2017): Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. Soil Biology & Biochemistry 115: 461-466, doi: 10.1016/j.soilbio.2017.09.005.

Leitner S, Minixhofer P, Inselsbacher E, Keiblinger KM, Zimmermann M, Zechmeister-Boltenstern S (2017): Short-term soil mineral and organic nitrogen fluxes during moderate and severe drying–rewetting events. Applied Soil Ecology 114: 28-33, doi:10.1016/j.apsoil.2017.02.014.

Mooshammer M, Hofhansl F, Frank AH, Wanek W, Hämmerle I, **Leitner S**, Schnecker J, Wild B, Watzka M, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2017): Decoupling of microbial carbon, nitrogen and phosphorus cycling in response to extreme temperature events. Science Advances 3 (5): e1602781, doi:10.1126/sciadv.1602781.

Leitner S, Sae-Tun O, Kranzinger L, Zechmeister-Boltenstern S, Zimmermann M (2016): Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest. Plant and Soil 403: 455-469, doi:10.1007/s11104-015-2771-3.

Liu D, Keiblinger KM, **Leitner S**, Mentler A, Zechmeister-Boltenstern S (2016): Is there a convergence of deciduous leaf litter stoichiometry, biochemistry and microbial population during decay? Geoderma 272: 93-100, doi:10.1016/j.geoderma.2016.03.005

Schwen A, Zimmermann M, **Leitner S**, Woche SK (2015): Soil Water Repellency and its Impact on Hydraulic Characteristics in a Beech Forest under Simulated Climate Change. Vadose Zone Journal 14(12), doi:10.2136/vzj2015.06.0089.

Leitner S, Wanek W, Wild B, Hämmerle I, Keiblinger KM, Zechmeister-Boltenstern S and A Richter (2012): Influence of litter chemistry and stoichiometry on glucan depolymerization during decomposition of beech (Fagus sylvatica L.) litter. Soil Biology & Biochemistry 50: 174-187, doi:10.1016/j.soilbio.2012.03.012.

Keiblinger KM, Schneider T, Roschitzki B, Schmid E, Eberl L, Hämmerle I, **Leitner S**, Richter A, Wanek W, Riedel K and S Zechmeister-Boltenstern (2012): Effects of stoichiometry and temperature perturbations on beech litter decomposition, enzyme activities and protein expression. Biogeosciences 9: 4537-4551, doi:10.5194/bg-9-4537-2012.

Mooshammer M, Wanek W, Schnecker J, Wild B, **Leitner S**, Hofhansl F, Blöchl A, Hämmerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S and A Richter (2012): Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 93 (4): 770–782, doi:10.1890/11-0721.1.

Manuscripts in preparation:

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

Díaz-Pinés E, **Leitner S**, Holtermann C, Zimmermann M, Zechmeister-Boltenstern S: Impact of repeated extreme drought and heavy rainfall events on soil-atmosphere fluxes of nitrous oxide and methane.

Leitner S, Kobler J, Zechmeister-Boltenstern S, Dirnböck T: Impact of drought and windthrow on nitrogen leaching of two forests in a Karst watershed.

Selected conference contributions:

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

Leitner S, Minishofer P, Inselsbacher E, Saronjic N, Zechmeister-Boltenstern S, Zimmermann M, Díaz-Pinés E: DRAIN – Impact of Droughts and heavy RAIN on greenhouse gas emissions and soil nitrogen cycling. Climate day of the Climate Change Centre Austria. Vienna, Austria, 22-24 May, 2017. [Talk]

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

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, keine anderen als die angegebenen Quellen verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Die vorliegende Arbeit wurde bisher in gleicher oder ähnlicher Art noch nicht vorgelegt.

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