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“Effects of summer drought on
carbon and nutrient dynamics
in mountain grasslands“

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“The world is full of obvious things
which nobody by any chance ever observes.”

Sherlock Holmes in
“The Hound of the Baskervilles”
(Sir Arthur Conan Doyle)

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Summary

Although climate models for many European Regions predict only minor changes of mean temperature and precipitation, there is evidence that the temporal dynamics of precipitation will become more variable leading to a higher frequency of drought periods and an increase in the intensity of precipitation events. Drought periods have been shown to affect plant productivity, but also microbe-mediated processes such as carbon (C) and nitrogen (N) cycling in soils. However, much less is known about indirect effects of drought that are driven by altered plant belowground C allocation and its consequences for microbial communities in soils. It is also unclear if recurring drought periods could induce lasting effects on plant-microbe C dynamics. Moreover, little is known about the sensitivity of microbial specialist groups, such as bacterial (AOB) and archaeal ammonia oxidizers (AOA) during, and their recovery after drought. In my PhD project I addressed these uncertainties combining a drought field-experiment with state of the art stable-isotope, chemical and molecular techniques.

Chapter one of this thesis focuses on effects of drought on plant belowground C allocation and the distribution of newly assimilated (^{13}C labelled), plant derived C within the microbial community determined by phospholipid fatty acid (PLFA) analysis. During drought the uptake and amount of C transferred to plant belowground compartments decreased significantly. While the amount of PLFAs, specifically of Gram-positive bacterial markers, was increased in drought plots, the incorporation of labelled C into microbial PLFAs appeared to be disrupted, and ^{13}C accumulated in the extractable organic C fraction in the soil. Thus, drought has the potential to weaken the link between plant and microbial C turnover.

In chapter two of this thesis I report on responses of plant C allocation and soil microbial community dynamics to a short dry spell in experimental sites with extended drought-history compared to control plots receiving ambient

amounts of precipitation. Plants with drought-history showed decreased shoot N content and lower C uptake and belowground allocation. PLFA content and composition, in turn were found to be stronger affected by short-term soil moisture variations than by drought-history. Thus, recurring drought periods could alter the response to drought of both, plants and microorganisms, but on different time scales.

Chapter three concentrates on effects of drought on the N-turnover and on ammonia-oxidizing bacteria and archaea in soils of meadows with different land management, an abandoned and an extensively managed site. Nitrogen-mineralization, a physiologically 'broad' process was not affected by drought, while nitrification, a comparably 'narrow' process showed less variation during drought simulation, but only at the abandoned site. Bacterial *amoA* gene-abundances were stable during drought at both sites, while archaeal *amoA* abundances were strongly reduced at managed site, accompanied by sharp increases of ammonium concentrations in the soil solution. Thus, the differential responses of ammonia-oxidizing organisms in soils could have been rather related to a niche differentiation at high levels of NH_4^+ , than only to decreases of soil water content.

Taken together, drought affected plant C uptake, belowground allocation and uptake into microbial PLFAs and induced a community shift towards microbial groups that could tolerate lower osmotic pressure. Nonetheless the community composition was resilient and approached to the controls after the end of drought. Recurring drought periods caused a lasting imprint in plant N content, and decreased C turnover compared to ambient controls. The microbial community, in turn responded rather to short term moisture variations, than that they were affected by drought-history. However, low land-management intensity could buffer effects of drought on soil microbes and could alter the responses of microbe-mediated processes in soils to drought.

Introduction, thesis outline & experimental setup



Introduction

Terrestrial carbon and nitrogen cycling

Globally, the largest gross exchange of carbon occurs between the atmosphere and the terrestrial biosphere (IPCC, 2007). The key processes in this carbon cycling are (i) gross primary production, i.e. the assimilation of carbon into biomass (about 120 Pg of carbon per year), which is almost balanced by (ii) the release of CO₂ back to the atmosphere through either autotrophic or heterotrophic respiration. Differences between uptake and release of CO₂ define, whether an ecosystem is a source, or a sink for atmospheric CO₂ (LeQuéré *et al.*, 2009; IPCC, 2013), and this net exchange could provide a positive or negative feedback to increasing atmospheric CO₂ concentrations (Friedlingstein *et al.*, 2006; Heimann & Reichstein, 2008) and thus on climate. Climate, in turn is an important control over carbon and nutrient (e.g. nitrogen) cycling. Both, temperature and precipitation play an important role in carbon turnover (Carvalhais *et al.*, 2014), as they exert a strong control on carbon assimilation by plants, as well as on the decomposition of organic matter in soils by microbes (Davidson & Janssens, 2006; Litton & Giardina, 2008; Frey *et al.*, 2013).

Nitrogen (N) is one of the most important, often growth limiting nutrient for primary

production, particularly in non-fertilized terrestrial ecosystems. Although molecular nitrogen (N₂) constitutes the major component of the earth's atmosphere, nitrogen is only available for plant growth either after microbial fixation by symbiotic or free living bacteria, or after microbial decomposition of organic material (LeBauer & Treseder, 2008). In soils the rate-limiting step in nitrogen cycling is the break-down and depolymerisation of N-containing polymers to smaller molecules by extracellular enzymes (Schimel & Bennett, 2004). These smaller molecules can then be taken up by either plants or microorganisms and can be further mineralized to inorganic nitrogen forms, such as ammonium or nitrate (Booth *et al.*, 2005). Plants actively compete with microbes for assimilable nitrogen forms such as peptides, amino acids, ammonium and nitrate (Kuzakov & Xu, 2013). Within a plant nitrogen is used for biomass production, specifically for structural and non-structural proteins, e.g. enzymes such as the photosynthetic enzyme Ribulose 1-5-Bisphosphate oxygenase/carboxylase (RuBisCO), which concentrations strongly correlate with photosynthesis and primary production (see review by Walker *et al.*, 2014).

Global climate change – local extreme weather events

Over the last one and a half centuries an unprecedented increase in atmospheric CO₂ (and other greenhouse gases) was observed, surpassing 400 ppm for the first time in record in May 2013 (www.noaa.gov.us), which with extreme likelihood can be attributed to human activity (IPCC, 2007; IPCC, 2013). Simultaneously, global mean surface temperatures have been increasing by 0.3°C to 0.6°C over the last century indicating a gradual ‘global warming’ with the five warmest years on global-average occurring in the last twenty years. Based on earlier observation climate projections suggest a further increase in the global mean surface temperature of up to +2°C by 2100, which is even greater than that seen over the past 10,000 years (IPCC, 2013).

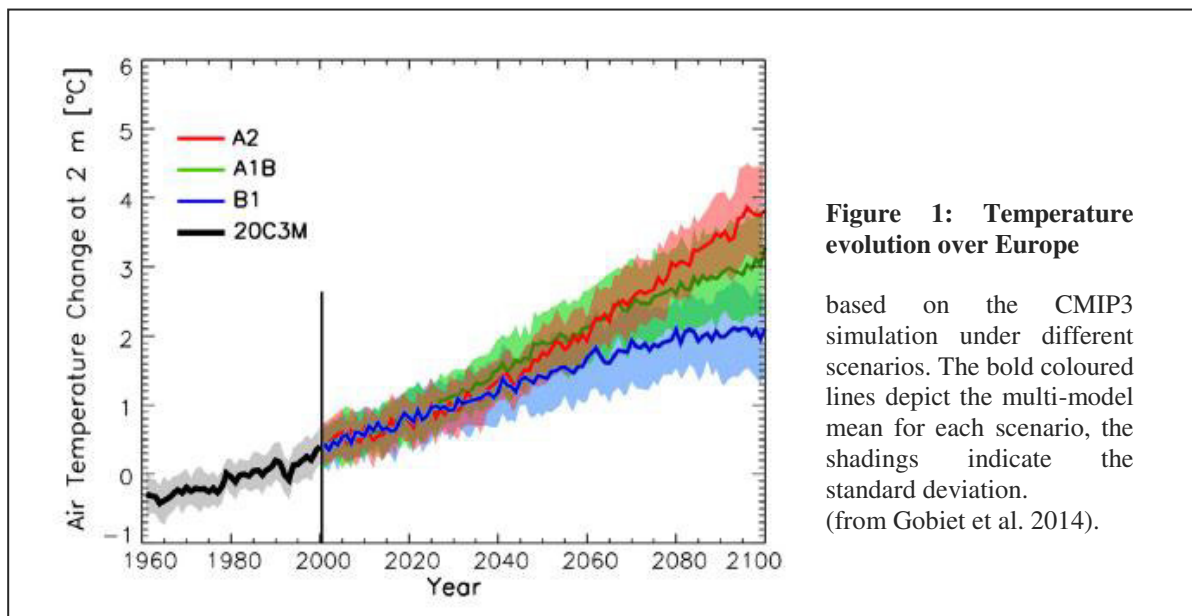
The continuing changes of climate in the future increase the likelihood of the occurrence of extreme weather phenomena, such as heat waves, dry spells, heavy rainfalls and storms. Extreme weather events are defined as being spatially and temporally rare in statistical terms (rarer than the 10th or 90th percentile of a probability density function, see Seneviratne, 2012). They may not directly be linked to anthropogenic activities, but rather appear as secondary effects of a globally changed climate. Extreme weather events can have severe impacts on nature and human health, as well as at the socio-economic level, by influencing e.g. agriculture, energy production, or demography

(IPCC SREX., 2012, and citations therein). The characteristics and the probability of the occurrence of such extreme weather events can however strongly vary between regions and ecosystems (Seneviratne *et al.*, 2006; Seneviratne, 2012; IPCC SREX, 2012).

In the European Alpine Region mean surface temperatures are predicted to increase by up to +4°C by the end of 2100 (see Figure 1; Gobiet *et al.*, 2013), thus warming may take place at higher rates than in many other parts of Europe. At present the Alps are perceived as “*Water towers for Europe*” (EEA Report, 2009), and are characterized as well water supplied ecosystems, receiving a major proportion of precipitation as rain during the summer (growing season), and as snow during winter (Wieser *et al.*, 2008). Climate projections however also predict an increased irregularity of precipitation timing, enhancing the likelihood and frequency of drought periods and torrential rains in European mountainous regions (Schär *et al.*, 2004; Beniston, 2005; Seneviratne *et al.*, 2006; IPCC, 2007; IPCC SREX, 2012 *et al.*, 2012). The predicted increasing frequency of drought and heavy rainfall events shows a large potential to affect ecosystem structure and functioning and thus biogeochemical cycles (van Oijen *et al.*, 2013; Reichstein *et al.*, 2013).

How to define ‘drought’?

Although there are several definitions for drought and although drought periods may have a range of different triggers (e.g. soil moisture



drought, hydrological drought), they can be generally characterized as “*recurring extreme climate event over land, characterized by below-normal precipitation over a period of months to years*” (Dai, 2011) or as “*periods of abnormally dry weather long enough to cause a serious hydrological imbalance*” (Definition: IPCC SREX., 2012). Hence, even in wet and humid regions drought periods can occur relative to local normal conditions (Dai, 2011). The main reasons for droughts are therefore reduced precipitation and/or increased rates of evapotranspiration. However, not only the immediate water availability can be affected by drought; as depending on the ecosystem, water can be stored as soil moisture, groundwater, and surface waters, they have a characteristic temporal memory and respond faster or slower to reduced precipitation (e.g. Seneviratne *et al.*, 2010). This means that effects of drought may have a different persistence depending on the affected system, which could also lead to carry-over effects from one year to the next (e.g. van der Molen *et al.*, 2011).

Soil responses to drought – physical alterations

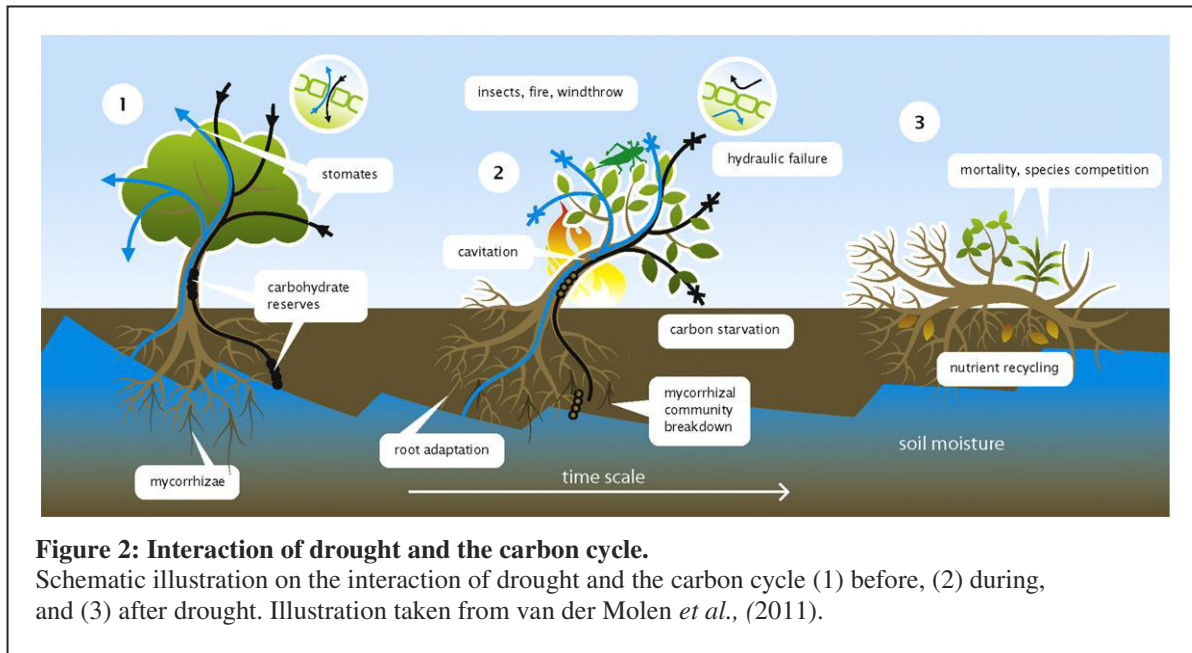
Soils are a diverse, highly structured and complex environment representing the matrix for plants and microorganisms. Soils have a highly complex arrangement of spatially distributed, aggregated solids forming tortuous pore spaces. This consequently leads, unlike in open water, to an inhomogeneous distribution of nutrients, and preferential flow paths, which channel the water flow and transfer of soluble or particulate matter, forming diverse niches and ‘hot-spots’ (Bundt *et al.*, 2001; Crawford *et al.*, 2005). Thus soils are spatially heterogeneous and display numerous interfaces between solid, liquid and gaseous media (Or *et al.*, 2007).

During drought evaporation and evapotranspiration exceed water inputs, which leads to a continuously decreasing water content. In soils, drying occurs sequentially from macropores ($>80\ \mu\text{m}$) to micropores ($<30\ \mu\text{m}$) until the water films around individual soil particles become disconnected from each other. Concomitantly the proportion of gaseous pore space increases. As a consequence, the amount of soil water and solute diffusivity decreases and nutrient concentrations increase (Fierer & Schimel, 2002; Schimel *et al.*, 2007; Moyano *et al.*, 2013;

Manzoni *et al.*, 2014b). Moreover, drying can trigger structural changes in the soil by affecting the size and stability of soil aggregates (Denef *et al.*, 2001). In particular, macroaggregates are destabilized by shrinking; in clayic soils, in turn, the stability of micropores increases due to the cohesion of slightly soluble components with soil particles (Borken & Matzner, 2009). In soils with high soil organic matter the organic matter could either act as a buffer against drying (Franzluebbers, 2002), or drying could even be enhanced by the higher presence of hydrophobic compounds, e.g. waxes, which could increase water repellency. This could in particular decrease the re-wettability of soils after drying. Generally, the longer a soil is dry the longer it takes for full rewetting (Goebel *et al.*, 2011).

How do plants cope with drought? – From individuals to the landscape level

Extreme climatic events, such as drought or flooding have a stronger impact on physiological processes than changes in mean climate (Reyer *et al.*, 2013). During rain-free periods, when soil moisture content gradually decreases, plants have to avoid losing water by sustaining a water potential below the water potential of the surrounding environment. The onset of drought can be delayed by hydraulic redistribution (hydraulic lift), which refers to the transport of water through the roots from wetter into dryer soil areas and can facilitate water movement through the soil-plant-atmosphere system (Siqueira *et al.*, 2008). Nonetheless at some point plants reach the so-called wilting point, where water is held too strong by the soil matrix and cannot be taken up by plants. This wilting point is variable and depends on vegetation type and soil texture (see e.g. Seneviratne *et al.*, 2010). In temperate ecosystems this wilting point for plants occurs approximately at $-1.5\ \text{MPa}$ ($pF\ 4.2$), and equals the point, where all water-containing pores are smaller than $0.2\ \mu\text{m}$ (Rodriguez-Iturbe, 2000), but in Mediterranean ecosystems this point can be as low as $-5\ \text{MPa}$ (Seneviratne *et al.*, 2010). To sustain dry conditions and avoid wilting plants therefore have evolved complex physiological, biochemical and morphological adaptations (Osakabe *et al.*, 2014). They can either minimise water loss, typically by closing their stomata, or sustain and maximise water uptake at low soil water potential by



adjusting their osmotic potential (Jackson *et al.*, 2000; Chaves *et al.*, 2003; see Figure 2).

Stomatal conductance (i.e. opening and closing) is controlled by a complex set of factors (e.g. tissue pH, root signals, abscisic acid, xylem sap flow) that finally result in changes of turgor in guard cells relative to epidermal cells (see review by Osakabe *et al.*, 2014). Nonetheless, stomata rather respond to the rate of water loss in leaves, driven by changes in evaporative demand than by air humidity changes (Chaves *et al.*, 2003). Exposed to mild water deficit plants tend to increase their water-use-efficiency, which follows a non-linear relationship between carbon assimilation and stomatal conductance, as stomatal conductance declines before photosynthesis does (Cowan & Farquhar, 1977). However, decreased stomatal conductance to save water implies a trade-off for carbon assimilation. There is still an ongoing debate if plants rather save water at the expense of carbon starvation (isohydric plants), or if they do not regulate stomatal conductance, but risk hydraulic failure (anisohydric plants; e.g. see Lawlor & Cornic, 2002; McDowell *et al.*, 2008). Carbon starvation happens when respiration, growth or defence exceed photosynthesis and mobilization of stored non-structural carbohydrates. During drought plant growth ceases before photosynthesis, therefore initially non-structural carbohydrates are available in a surplus. This surplus however declines over time, when maintenance respiration exceeds carbon uptake. The hydraulic failure hypothesis, on the other hand

predicts that low soil water availability coupled with a high evapotranspiration demand leads to cavitation within the xylem, which means to become air-filled. Thus, the water transport from roots over the xylem to leaves cannot be maintained, which leads to desiccation (McDowell *et al.*, 2008). However, both carbon starvation and hydraulic failure may not be occurring exclusively; more likely these two mechanisms are coupled and interrelated (McDowell, 2011).

Generally, root, and autotrophic soil respiration are strongly fuelled by recently assimilated belowground allocated carbon (e.g. Kuzyakov, 2006; Jones *et al.*, 2009, see Figure 2). Carbon allocated belowground connects source (leaves) with sink (roots) organs (Brüggemann *et al.*, 2011; Epron *et al.*, 2012); thus changes in belowground carbon allocation can reflect both altered source or sink activity. Despite of reductions in CO₂ uptake (indicating decreased source activity), drought has commonly been found to decrease root respiration (Burton *et al.*, 1998; Huang & Fu, 2000), indicating decreased sink activity, which could slow down belowground carbon allocation during drought (Ruehr *et al.*, 2009). The proportion of recent carbon allocated belowground has however been shown to remain constant or even to increase during drought (Ruehr *et al.*, 2009; Burri *et al.*, 2014; Hasibeder *et al.*, 2014). This could indicate a higher sink demand of roots for growth and maintenance, or for the synthesis of osmotically active compounds to allow water uptake (Chaves *et al.*, 2002; Chen & Jiang,

2010). In addition, mutualistic relationships of plant roots with soil microorganisms, in particular with mycorrhizal fungi, could enhance the water uptake of plants. In trade for plant-derived carbohydrates fungal hyphae may exploit water and nutrients from smaller soil pores and could delay the onset of drought stress in plants (Allen, 2007, see next section). Nonetheless, changing allocation by significantly enhancing the root system might result in suboptimal growth after restoration, thus strong shifts of allocation pattern may only be triggered by extreme drought (Poorter *et al.*, 2012).

Drought can not only alter the carbon, but also the nitrogen budget in plants. The increasing water deficit during drought could lead to reduced uptake of inorganic nitrogen by plant roots (Lambers *et al.*, 2008; Miller, 2010), which in turn could lead to nitrogen starvation and limit the production of photosynthetic enzymes. Moreover, by closing stomata to reduce water loss plants decrease the transfer of nutrients from belowground to aboveground organs, and also decrease the mass flow towards fine roots, i.e. the transfer of water and nutrients (Cramer *et al.*, 2009). However, plants may have a decreased nitrogen demand, as they may have decreased rates of photosynthesis and therefore a lower demand for the production of photosynthetic proteins, such as RuBisCO. These factors could eventually lead to altered concentrations of both carbon and nitrogen in plant biomass, and subsequently in plant litter, which in turn could impact on litter or soil organic matter decomposition by microbes (e.g., Sanaullah *et al.*, 2012).

Irrigation manipulation experiments have been shown that plants respond to drought with physiological adaptations to maintain productivity (Jentsch *et al.*, 2011). Despite plant species-specific adaptations, drought can on a larger scale significantly decrease gross primary production, i.e. assimilation of CO₂, of whole ecosystems. This was dramatically visible during the extreme Europe-wide summer drought period in 2003, which has been documented as the year with the lowest productivity for more than 100 years and turned Western Europe to an anomalous source of carbon to the atmosphere (Ciais *et al.*, 2005). Primary production was not only reduced during drought but also did not entirely recover during the rest of the growing season and overall abolished

the amount of carbon that was sequestered during the four preceding years (Ciais *et al.*, 2005).

Thus, drought periods not only lead to immediate responses, but may lead to lagged or carry-over effects (see also Box1: Resistance, resilience). Lagged or delayed stress effects are often detrimental, and become apparent only after some time, e.g. when a community collapses, or become visible as increased plant mortality in the next growing season, which may happen even several years after the disturbance (Bigler *et al.*, 2007). In plants, this could involve e.g. the depletion of carbon reserves, which could make them more vulnerable to further disturbances or to pathogens, and which could delay their recovery after drought and prolong resilience (van der Molen *et al.*, 2011). On the other hand, recurring stresses, such as drought, could lead to an improved response to future stresses, in the sense that plants can respond quicker and more adequate to a recurrent stress event. This concept is still under discussion and has been termed ecological stress memory (Walter *et al.*, 2011). Moreover, the timing of extreme drought periods appears to significantly alter the response of productivity. Hence during drought periods that occurred in the early growing period the decline of productivity was stronger than during droughts in the late vegetation period (Craine *et al.*, 2012).

Drought impacts on soil microorganisms

Soil microorganisms play a central role in the decomposition of soil organic matter mediating many biogeochemical processes including carbon and nitrogen cycling. In soils, microorganisms are often organised attached to surfaces, e.g. as colonies embedded in extracellular polymeric substances or bound to soil particles forming microaggregates together with other components of the soil matrix, rather than they follow a plankton-like free swimming lifestyle (Or *et al.*, 2007). Likely because of the complex structures and numerous different niches in soils, soil microbes are more abundant and also more diverse than e.g. in the ocean (Whitman *et al.*, 1998). They are however not uniformly distributed in the soil and are found to form 'hot spots' of activity, diversity and abundance e.g. along preferential flow paths (Bundt *et al.*, 2001) or in the rhizosphere of plants (Jones *et al.*, 2009). Together with substrate availability and temperature, soil moisture is an

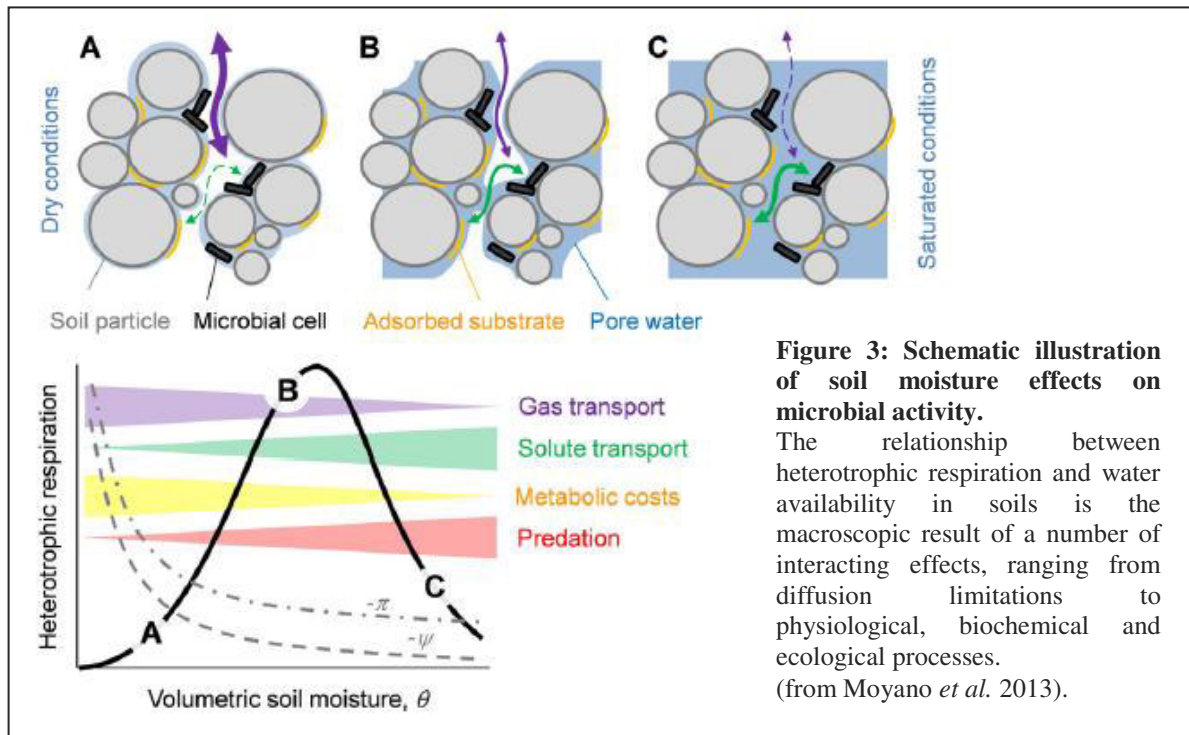
important determinant of microbial activity and of the rate at which soil carbon is mineralized to CO₂ (Davidson & Janssens, 2006; Frey *et al.*, 2013), as well as of the rate at which other greenhouse-active gasses, e.g. CH₄ and N₂O are released into the atmosphere (Conrad, 1996). However microbial communities must have adapted to variable moisture (and oxygen) levels as water distribution and subsequently nutrient diffusion vary on small and larger temporal and spatial scales (Or *et al.*, 2007). Nonetheless, changes in one of these controlling parameters could either directly or indirectly affect microbe-mediated processes, which then in turn could feed back on climate (Davidson & Janssens, 2006; Bardgett *et al.*, 2008).

On the ecosystem level drought can be considered as a press disturbance that develops over longer time periods, rather than as a pulse disturbance. At the microbial level this distinction is not as straightforward and drought could represent both a press and a pulse disturbance (Schimel *et al.*, 2007; Shade *et al.*, 2012). However, the physical alterations during drought within the soil matrix, such as decreasing water content, disconnection of water films and concomitantly restricted diffusion, limited mobility and strongly negative soil water potentials also directly impact on microorganisms (Skopp *et al.*, 1990; Moyano *et al.*, 2013; see Figure 3). Drought could also indirectly affect soil microbes and microbial functioning. Above and belowground processes are tightly linked over recent carbon inputs by plants, in particular in the close vicinity of plant roots, the rhizosphere (Jones *et al.*, 2004). Plants allocate a large proportion of recently assimilated carbon downwards and release up to 40% as low molecular organic compounds, such as sugars or organic acids either actively over fine roots or mycorrhizal symbionts, or passively (by leakage, or during root growth as root cap or border cell losses) into the rhizosphere (Kuzyakov & Cheng, 2001; Jones *et al.*, 2009; Brüggemann *et al.*, 2011; Epron *et al.*, 2012; Kuzyakov & Xu, 2013). These labile plant inputs have been shown to stimulate microbial activity, and to enhance, but sometimes also to suppress decomposition of more stable and older soil organic matter into smaller polymers, processes termed ‘rhizosphere priming effects’ (Kuzyakov & Cheng, 2001; Fontaine *et al.*, 2003). During drought, plants alter their belowground allocation pattern, which could

subsequently lead to altered inputs to soils as root exudation. The altered input or altered availability of labile carbon could therefore have significant indirect impacts on soil microbes specifically in the rhizosphere, and might also feed-back on nutrient availability for plants (Bardgett *et al.*, 2008; van der Putten *et al.*, 2013). Also plant litter type and quantity (e.g. higher input of fine root necromass) or litter stoichiometry can be changed by enduring drought periods, thereby shifting the substrate quantity and quality for soil microbes (van der Molen *et al.*, 2011; Bardgett *et al.*, 2013). A further indirect effect of drought driven by the increased spatial isolation with decreasing soil water content is less competition between (but may increase the competition within) microbial populations, but also between microbes and plants. In addition, a lower predation pressure e.g. by nematodes or other soil micro- and mesofauna (Or *et al.*, 2007).

Nevertheless, to survive drought periods microbes must respond to a lower water availability to avoid dehydration (Schimel *et al.*, 2007; Or *et al.*, 2007). The degree to which decreasing water availability causes physiological stress in microbes is highly dependent on the response, i.e. the activation of protective or adaptive mechanisms for surviving, of individual cells (Griffiths & Philippot, 2012; see Box Resistance, resilience). Thus drought can affect microbial activity, which is often estimated as a decrease in heterotrophic respiration rates (Manzoni *et al.*, 2012; Moyano *et al.*, 2012), but the mechanisms driving this decline are still not clear (Manzoni *et al.*, 2014a).

Microbial drought adaption strategies include changing resource-allocation from growth to survival pathways. One possibility for microbes to sustain dry periods is to maintain envelope integrity by increasing the production of extracellular polymeric substances. These polymers embedding cells in colonies change in viscosity from high to low soil moisture and could have a protective function to avoid desiccation, crystallization and finally lysis. A further microbial strategy to avoid dehydration is to adapt their internal water potential to the decreasing matrix water potential (Csonka, 1989; Schimel *et al.*, 2007; Or *et al.*, 2007). This can be achieved by taking up or producing osmolytic compounds; bacteria, use preferentially nitrogen containing compounds such as amines (proline, glutamine and glycine betain, (Csonka, 1989), while fungi, preferentially use carbon containing compounds



e.g. polyols, such as glycerol, erythol and mannitol (Witteveen & Visser, 1995). However, in arid ecosystems nitrogen-containing osmolytes in the microbial biomass were found to be unaffected by drought (Boot *et al.*, 2013), which could be due to limited substrate availability (Manzoni *et al.*, 2014b).

Another strategy of microbes to sustain periods of adverse environmental conditions or to deal with environmental variability is shifting to dormancy (Lennon & Jones, 2011). Dormancy is a phylogenetically widespread microbial trait in soils. Even under non-stressed conditions approximately only up to 5% of the microbial biomass is active, but up to 40% are potentially active and ready to become active when conditions become favourable for growth (Blagodatskaya & Kuzyakov, 2013). Dormant cells can have altered differentiation to spores or cysts, a reduced cell size, reduced DNA or decreased or increased RNA content, or an altered quantity and composition of fatty acids (Lennon & Jones, 2011; Blagodatskaya & Kuzyakov, 2013). The duration of how long a microbial cell can remain dormant depends on the maintenance energy costs. Records on decade- or even century-old spores that are still able to resuscitate from dormant or persisting states are reported (review by Lennon & Jones, 2011). Nevertheless, all adaptive mechanisms incur energy costs, and could alter microbial functioning

and microbe mediated processes (Schimel *et al.*, 2007; see Figure 3).

The level of adaption of a microbial community to changing environmental conditions, such as drought, are a function of the potential rate of change in a community composition (Wallenstein & Hall, 2012). Thus the inherent capabilities and responses from individual microorganisms finally determine the response of a microbial community (Griffiths & Philippot, 2013). For instance, fungi-dominated food webs and energy channels appear more tolerant to drought than bacteria-dominated food webs (Yuste *et al.*, 2011; de Vries *et al.*, 2013). Filamentous fungi have a spatially large vegetative mycelium and an extended hyphal network, which allows them to grow through air and bridge soil pores to exploit and allocate water and nutrients over comparably long distances (Ritz & Young, 2004; Allen, 2007). Thus, during drought fungi can actively grow to sites where water is still available and so avoid dehydration. Mycorrhizal fungi maintain a mutualistic relation with plant roots; by enlarging the rooting surface and nutrient and water uptake for plants. Thus mycorrhiza could delay the onset of water stress for plants, as mycorrhizal hyphae have access to smaller soil pores than plant fine roots (Allen, 2007). Within bacterial groups, gram-positive bacteria have a stronger binding between their peptidoglycan

layers and cytoplasmic membrane, and are more effective in maintaining a constant cell turgor and are thus more drought-resistant than gram-negative bacteria (Csonka, 1989; Or *et al.*, 2007). Hence, fungi, gram-positive and gram-negative bacteria may occupy different niches in water availability. Some microbial groups, e.g. some gram-negative taxa such as e.g. Bacteroidetes and Proteobacteria, prefer a narrow water potential, while others, such as some fungal taxa and gram-positive Firmicutes are broader in their occurrence and have also a more negative water potential optimum (Lennon *et al.*, 2012). However, not only the phylogenetic, but also the functional composition e.g. the distribution of r- and K-strategist microbes, could determine and modulate responses of a microbial community to drought periods (de Vries & Shade, 2013; Krause *et al.*, 2014). Fast growing r-strategists are likely to be less resistant but more resilient than slower growing K-strategists (Allison & Martiny, 2008; de Vries & Shade, 2013).

Whether community changes lead to a change of ecosystem processes strongly depends on the degree of functional redundancy of microbes. ‘Broad’ or aggregate processes are characterized by a high degree of functional redundancy within a microbial community, as they are brought about by many if not all microorganisms, as for example CO₂ production. ‘Narrow’ processes, in contrast, are conducted by microbial specialists or are very specific biochemical pathways as for example the individual steps in the process of nitrification. Thus, according to the manifold of different organisms and individual processes that contribute to broad processes they could be more stable during disturbances. Narrow processes, conducted by highly specialized microbes could therefore respond more sensitive, when optimum parameters change (Allison & Martiny, 2008; Schimel & Schaeffer, 2012).

An example for a comparably physiological narrow process within nitrogen cycling is nitrification. The term nitrification combines a cascade of oxidative steps from ammonia (NH₃) over hydroxylamine (NH₂OH) to nitrite (NO₂⁻) and further to nitrate (NO₃⁻). Within this cascade the first and rate-limiting step is the oxidation of NH₃ to NH₂OH, which is catalysed by the enzyme ammonia-monooxygenase (AMO). AMO is a membrane-bound enzyme composed of three subunits, of which the subunit A (amoA) is

highly conserved in ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Treusch *et al.*, 2005). AOB are autotrophic, obligate aerobes belonging to monophyletic groups within the β- and γ- subclasses of Proteobacteria (Kowalchuk & Stephen, 2001). AOA, in turn are found within the phylum Thaumarchaeota (Könneke *et al.*, 2005). Thus, in theory AOB and AOA are functional redundant in ammonia-oxidation (Leininger *et al.*, 2006; Schauss *et al.*, 2009). AOB and AOA differ in their ecophysiology (Erguder *et al.*, 2009), but, with exception of soil pH, there is no single factor discriminating between AOB and AOA, as both groups appear physiologically diverse (Prosser & Nicol, 2012). Nonetheless, in many soils archaeal *amoA* gene copies outnumber bacterial ones (Leininger *et al.*, 2006; Alves *et al.*, 2013), but this does not necessarily imply that AOA dominate ammonia-oxidation (Prosser & Nicol, 2012). Cultivated AOA have been found to have a significantly smaller size and therefore higher surface to volume ratio (Jung *et al.*, 2011), they have a higher substrate affinity, but also a much lower saturation constant than AOB (Martens-Habbena *et al.*, 2009). Thus AOA were found to be more efficient in substrates or environments with low NH₃/or NH₄⁺ concentrations, while AOB were rather present at high NH₃/or NH₄⁺ concentrations and were found to dominate nitrification in N-rich grasslands (Di *et al.*, 2009, 2010). However, due to the small number of cultivated AOA there is still little known about their physiological optimum conditions and their metabolic versatility (Schleper, 2010).

There is however, still a large uncertainty, whether AOB and AOA respond differentially to drought. In grassland soils neither AOB, nor AOA *amoA* abundances were affected by drought (Hartmann *et al.*, 2013), whereas in soil-mesocosm incubations AOA strongly decreased, maybe enforced by higher concentrations of NH₄⁺ in the remaining soil solution (Thion & Prosser, 2014). However, in seasonal dry grasslands drought has been found to lead to only minor effects on gene copy numbers of AOB and AOA, but rRNA-based methods indicated strong decreases of activities during drought and pulses of activities after rewetting (Placella & Firestone, 2013; Barnard *et al.*, 2013). Thus, theoretically drought should lead to decreased nitrification rates, but effects of drought appeared to be strongly context dependent

and do not show a consistent picture: nitrification was found to be highest at intermediated levels of water filled soil pore space (Gleeson *et al.*, 2010), to decrease at low water potentials likely limited by substrate diffusion (Stark & Firestone, 1996; Emmett *et al.*, 2004) or not to be affected by drought treatments (Chen *et al.*, 2011; Hartmann *et al.*, 2013; Auyeung *et al.*, 2013).

Rewetting – repeated drying rewetting – lagged effects

After long dry periods soils can develop high water repellency, as drought has been shown to decrease the proportion of macroaggregates, which brings forth a higher proportion of hydrophobic surfaces. This may alter preferential flow paths and impede rewetting of soils. In

addition, the disruption of macroaggregates can also increase nutrient availability (Denef *et al.*, 2001; Schimel *et al.*, 2011; Goebel *et al.*, 2011).

For soil microbes, rewetting events after dry periods are particularly challenging. Microbes have to quickly adjust their osmotic potential by releasing or metabolizing osmotically active compounds that they have previously accumulated to avoid cell burst (Csonka, 1989). Upon rewetting isolated soil pores become re-connected, and accumulated organic compounds, either derived from broken-up aggregates, dead soil microbes or inactive extracellular enzymes become available for microbial metabolism and dormant cells can quickly resuscitate (Or *et al.*, 2007; Borken & Matzner, 2009; Placella *et al.*, 2012; Barnard *et al.*, 2013). Thus, despite of a strong physical release of

BOX 1: The concept of ecological stability – resistance and resilience

Ecological stability can be measured in many ways. This includes the persistence of a system, of populations or of ecological attributes through time. Both, the degree of resistance and resilience determine an (eco-) system's stability. Stability therefore determines a system's ability to continue functioning under changing conditions or during disturbances (e.g. Pimm, 1984; Shade *et al.*, 2012).

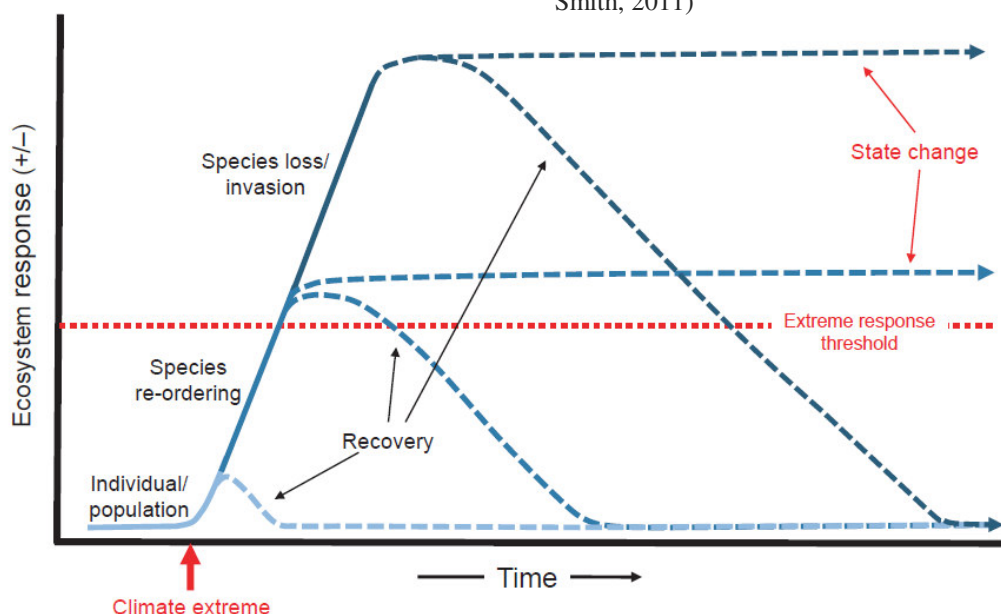
Resistance can be defined as the degree to which a community or any other ecological attribute withstands changes facing disturbances (Pimm, 1984; Allison & Martiny, 2008). The inverse of resistance is sensitivity.

Sensitivity therefore describes the degree to which a community, or any ecological attributes responds to a disturbance.

Resilience is the speed with which a system returns to its pre-disturbance level after a disturbance, thus resilience is particularly a function of time (Pimm, 1984; Orwin & Wardle, 2004).

Based on the degree of resistance and resilience ecosystems or communities could shift from one to another, an alternative stable state (Beisner *et al.*, 2003).

Figure 4: A mechanistic framework for assessing responses to climate extremes (from Smith, 2011)



CO₂ when water replaces gaseous pore spaces, water pulses have been shown to induce, a carbon mineralization peak (Birch-effect, Birch, 1958; Fierer & Schimel, 2003), and to stimulate nitrogen mineralization rates (Fierer & Schimel, 2002; Austin *et al.*, 2004; Borken & Matzner, 2009). These activity and mineralization peaks have been found to occur within hours after the water pulse and could last for a couple of days (Birch, 1958; Fierer & Schimel, 2002; Austin *et al.*, 2004; Saetre & Stark, 2005; Landesman & Dighton, 2011). However, after frequent drying-rewetting cycles, these pulses in CO₂ release have been shown to decrease (Fierer & Schimel, 2002).

Eventually, recurring drought periods could lead to shifts in plant species composition (Allen & Breshears, 1998; Chaves *et al.*, 2003; Harte *et al.*, 2006; Metcalfe *et al.*, 2010; McDowell, 2011; van der Molen *et al.*, 2011). Similarly, on the microbial level repeated drying and rewetting cycles have been found induce shifts in microbial community composition (Landesman & Dighton, 2011) and likely form a new steady state and baseline community (Fierer *et al.*, 2003; Evans & Wallenstein, 2012; see Box1: Resistance - resilience).

Land-management Change in European Alpine Regions

Mountainous areas are facing severe local and regional changes in land management practices and intensity. In mountain or alpine regions the

predominant pattern of changes in land-use is the cessation of agriculture, hence increased abandonment of previously extensively (i.e. animal grazing and hay production) managed meadows and pastures (Tenhunen *et al.*, 2009; Zimmermann *et al.*, 2010). As a result of changes in land-use the composition of plants species can shift and the amount and quality of organic matter input to the soils and nutrient availability in soils is changed (Tasser *et al.*, 2005; Meyer *et al.*, 2012), likely inducing changes in microbial community composition (Zeller *et al.*, 2001; de Vries *et al.*, 2012; Grigulis *et al.*, 2013; van der Putten *et al.*, 2013) and substantially altering grassland carbon fluxes (Bahn *et al.*, 2006; Schmitt *et al.*, 2010). Extensively managed grasslands have often been found to inhabit a larger fungal (or mycorrhizal) biomass, compared to bacteria-dominated food webs in intensively managed agricultural soils (de Vries *et al.*, 2012; de Vries & Shade, 2013). AS fungal-dominated food-webs are thought to be more resistant to drought ecosystems subjected to low or intermediate moderate land-management could subsequently also be more resistant to drought (de Vries *et al.*, 2012) and resilient in terms of carbon storage than intensively managed grassland (Klump *et al.*, 2011). Changes in land management could thus strongly alter the responses of carbon and nutrient dynamics in mountain grassland.

Thesis outline & experimental setup

Thesis outline

The main results of my thesis are presented in chapters one to three.

Drought periods are predicted to become more frequent in many European Regions including to date well-watered systems such as the European Alps. Drought affects ecosystem carbon cycling on different levels as outlined above. For instance, drought might negatively affect plant productivity and carbon uptake due to decreased stomatal conductivity to save water. This could lead to decreased rhizodeposition and decrease the availability of labile carbon for soil microbes. Moreover, the decreasing soil water content during drought periods may directly affect diffusive processes and therefore nutrient availability and affect the physiology of soil microbes. The aim of this thesis therefore was to investigate *in situ* the extent to which drought affects the carbon and nitrogen dynamics in soils of mountain grasslands with different land-management history backgrounds.

In **chapter 1**, I report a study that aims at understanding the effects of a simulated drought period on the carbon turnover in a mountain meadow, as well as on microbial biomass and community structure during the drought period. During drought simulation canopies of control and drought-treated (for eight weeks) meadow plots

were pulse-labelled with ^{13}C , which was traced from aboveground to belowground sections of plants, as well as into microbial groups (by phospholipid fatty acid PLFA analysis). This study has been published in *New Phytologist* 201 (3), 916-927, 2014.

Chapter 2 investigates how recurring summer drought periods (here termed as plots with drought-history) could alter effects of drought on plant and microbial carbon dynamics, as drought may not only immediately affect ecosystem's carbon cycling, but may also induce effects that persist after the end of a disturbance. To study this we conducted a ^{13}C pulse labelling experiment at a mountain meadow that had been exposed to summer drought simulations in three subsequent years. However, in this field study a dry spell occurred at the time of pulse-labelling, allowing us to test whether drought-history modulated the responses of plants and soil microbes to further drying and rewetting.

In **chapter 3**, I report on effects of drought on soil nitrogen cycling and on bacterial and archaeal ammonia-oxidizers. The aim of this study was to investigate effects of drought and rewetting under field conditions on the microbial potential to produce and take up inorganic nitrogen, as well as on the abundances of bacterial and archaeal ammonia-oxidizers (AOB and AOA)

in mountain grassland with different preconditions such as e.g. soil organic matter content or organic and inorganic nitrogen concentrations. To study this, we conducted a ten week drought experiment by setting up rain-out shelters during the growing season at both an extensively managed meadow, and at an abandoned mountain meadow, and analysed potential microbial nitrogen turnover rates and *amoA* gene copy numbers before, during and after drought simulation. This study has been published in *Biogeosciences* 11, 6003-6015, 2014.

Experimental setup

The study sites were located close to the village of Neustift in the Stubai Valley about 60 km south-west from Innsbruck (Austria) in the Central European Alps (47°7'45''N, 11°18'20''E). These sites were part of a multi-year drought experiment within the EU CARBO-Extreme network program (European Community's Seventh Framework Programme, FP/2997.2013, grant agreement no. 226701). The work that has been conducted in this thesis was funded by the Austrian Science Fund (FWF; P22214-B17).

For the studies reported in this thesis, drought experiments were conducted in 2010 and 2011 on two sites with different land-management intensity. The first site was an extensively managed mountain meadow, where aboveground biomass was mown and harvested once per year for hay production and partly grazed by cattle in late summer. The vegetation is a *Trisetetum flavescens* community, dominated by annual grasses and herbs, (e.g. *Anthoxanthum odoratum*, *Festuca rubra*, *Alchemilla vulgaris*, *Leontodon hispidus*, and *Trifolium repens*) and was shown to be comparably productive (Bahn *et al.*, 2009). The

second site was a former meadow site that was abandoned from agricultural management, i.e. cessation of mowing, harvesting, grazing and fertilization more than thirty years ago. The vegetation at this site is a *Seslerio-Caricetum* community also dominated by grasses and herbs (*Festuca rubra*) with dwarf shrubs (*Calluna vulgaris*, *Vaccinium myrtillus*, *Vaccinium vitis-idea*). Both sites were at a similar elevation (1850 to 1970 m above sea level, meadow and the abandoned site, respectively) with a mean annual temperature of about 3°C and a mean annual precipitation of 1100 mm, mainly during summer as rain.

Drought events were simulated by installing rain-out shelters (see Figure 5) for eight to ten weeks during the vegetation period from the end of May to mid of August. After drought simulation all plots were rewetted mimicking a severe heavy precipitation event by adding 20 mm of water over five minutes. To study the effects of drought and drought-history on the carbon transfer from plants to soil microorganisms we pulse-labelled canopy sections of plants at the meadow site with $^{13}\text{CO}_2$ over a period of 90 minutes (see Chapter 1 and 2). Then we traced the assimilated ^{13}C from plant shoots over fine roots and extractable organic carbon pool to soil microbes that were quantified using phospholipid fatty acid analysis (PLFAs) over a period of 200 hours and analysed the samples in the SILVER-lab (Stable Isotope Laboratory Vienna for Environmental Research) at the University of Vienna (see Chapter 1). In an additional approach, effects of drought on nitrogen cycling were investigated. Towards this end, we analysed organic and inorganic nitrogen pools, as well as gross rates N mineralization (NH_4^+ production) and NH_4^+ uptake and gross rates on nitrification (NO_3^- production) and NO_3^- uptake using ^{15}N pool dilution assays. In addition, in cooperation with the Helmholtz-Center in Munich abundances of functional genes for ammonia-oxidation (*amoA*) in bacteria and archaea were examined (funded by 'Nachwuchsförderung 2011 der Universität Innsbruck', no. 134753, and by the 'Tiroler Wissenschaftsfonds', TWF UNI-0404/1121) (see Chapter 3).



Figure 5: Rain-out shelter used for drought-simulation

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All photographs in this section were taken by Lucia Fuchslueger

Manuscripts

Chapter 1

“Experimental drought reduces the transfer of recently fixed plant C to soil microbes and alters the bacterial community composition in a mountain meadow”

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Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow

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Summary

- Drought affects plants and soil microorganisms, but it is still not clear how it alters the carbon (C) transfer at the plant–microbial interface. Here, we tested direct and indirect effects of drought on soil microbes and microbial turnover of recent plant-derived C in a mountain meadow.
- Microbial community composition was assessed using phospholipid fatty acids (PLFAs); the allocation of recent plant-derived C to microbial groups was analysed by pulse-labelling of canopy sections with ¹³CO₂ and the subsequent tracing of the label into microbial PLFAs.
- Microbial biomass was significantly higher in plots exposed to a severe experimental drought. In addition, drought induced a shift of the microbial community composition, mainly driven by an increase of Gram-positive bacteria. Drought reduced belowground C allocation, but not the transfer of recently plant-assimilated C to fungi, and in particular reduced tracer uptake by bacteria. This was accompanied by an increase of ¹³C in the extractable organic C pool during drought, which was even more pronounced after plots were mown.
- We conclude that drought weakened the link between plant and bacterial, but not fungal, C turnover, and facilitated the growth of potentially slow-growing, drought-adapted soil microbes, such as Gram-positive bacteria.

Introduction

Drought has the potential to lead to severe imbalances in the terrestrial carbon (C) cycle, by affecting the production of organic matter by plants and decomposition by microorganisms (Ciais *et al.*, 2005; Schwalm *et al.*, 2010; Reichstein *et al.*, 2013). Drought directly alters the physical environment for soil microorganisms and plants: it decreases soil water content and concomitantly increases the proportion of oxygen-filled soil pores (Schimel *et al.*, 2007; Manzoni *et al.*, 2012a; Moyano *et al.*, 2013); it reduces the mobility of nutrients in the soil, thereby disconnecting organisms from substrates, while nutrient concentrations in the remaining soil water increase (Schjønning *et al.*, 2003; Schimel *et al.*, 2007). Thus, drought has a strong but equivocal effect on nutrient availability.

The response of a microbial community to drought depends on the physiological tolerance and metabolic flexibility of the constituent microbes (Allison & Martiny, 2008). Generally, fungi are thought to be more tolerant to dry periods than bacteria (Schimel *et al.*, 2007; Strickland & Rousk, 2010; Manzoni *et al.*, 2012a). They are able to create large hyphal networks that facilitate nutrient and water transfer over long distances, and to explore water-filled soil pores not accessible for plant roots (Allen, 2007; Joergensen & Wichern, 2008), and they have

lower nutrient requirements than bacteria (Strickland & Rousk, 2010). Mycorrhizal fungi are directly connected to plant roots from which they obtain recently assimilated C (10–30% of the net primary production; Allen, 2007; Jones *et al.*, 2009; de Deyn *et al.*, 2011). Mycorrhizas can even enhance water supply for plants during drought by taking up water from smaller soil pores (Wardle *et al.*, 2004; Allen, 2007). Soil ecosystems dominated by fungi may therefore be considered to be less sensitive to drought than bacteria-dominated soils (Yuste *et al.*, 2011; de Vries *et al.*, 2012).

Bacteria, however, are expected to inhabit smaller soil pores, and may be protected for longer from desiccation (Moyano *et al.*, 2013); nonetheless, they have to balance the increasing osmotic potential of the soil solution (Schimel *et al.*, 2007). Alternatively, they can endure unfavourable conditions by shifting to dormancy or producing cysts (Schimel *et al.*, 2007; Lennon & Jones, 2011). Gram-positive bacteria appear to be more resistant to drought than Gram-negative bacteria, because of their thicker peptidoglycan cell wall layer (Schimel *et al.*, 2007; Manzoni *et al.*, 2012a). Genera of Gram-positive bacteria (e.g. *Firmicutes*) have been termed ‘drought-adapted generalists’ (Lennon *et al.*, 2012). Overall, drought may select for more resistant microbial groups, which can result in the shift of an existing microbial community (Castro *et al.*, 2010), and affect microbial-driven ecosystem functions by

changing their activities (Schimel *et al.*, 2007; Allison & Martiny, 2008; Lennon & Jones, 2011; Wallenstein & Hall, 2012).

In addition to direct physical effects, drought can affect soil microbes by altering the input of plant C into the rhizosphere (Bardgett *et al.*, 2008). On the one hand, drought may alter fine-root turnover (Chaves *et al.*, 2003), and on the other hand drought may affect the input of root exudates (including secretions, lysates from border cells and mucilage; see Jones *et al.*, 2009). Root exudates represent an important source of organic C for soil microorganisms in the rhizosphere and enhance soil organic matter decomposition (i.e. the 'priming effect'; e.g. Fontaine *et al.*, 2003; Kuzyakov, 2010), which in turn makes nutrients accessible for microbial as well as for plant uptake. During drought periods, plants may alter belowground C allocation (Chaves *et al.*, 2003; Ruehr *et al.*, 2009; Albert *et al.*, 2011; McDowell, 2011; Manzoni *et al.*, 2012b). This may severely affect the quantity and quality of C available for soil microbes in the rhizosphere, such as fungi and Gram-negative bacteria, which seem to be tightly connected to recently assimilated plant C (Denef *et al.*, 2009; de Deyn *et al.*, 2011; Bahn *et al.*, 2013). Nevertheless, it is not clear how drought affects processes at the root–soil interface (Bardgett *et al.*, 2008; Compant *et al.*, 2010; Sanaullah *et al.*, 2011). Moreover, it is poorly understood how drought affects belowground C allocation in usually well-watered ecosystems, such as grasslands in the European Alps (Wieser *et al.*, 2008), which are predicted to experience more frequent drought periods in the near future (Schär *et al.*, 2004; IPCC, 2007; Seneviratne *et al.*, 2010).

In many European grasslands, mowing, that is, clipping and subsequent harvesting of aboveground plant biomass, is a common management practice and shapes plant and microbial communities, as well as nutrient composition in the soil (Bardgett *et al.*, 2001; Klumpp *et al.*, 2011; de Vries *et al.*, 2012; Meyer *et al.*, 2012; Shahzad *et al.*, 2012). In contrast to drought, mowing is an immediate disturbance, abruptly changing the soil microclimate (Bahn *et al.*, 2006) and C input, as plant roots release a pulse of low-molecular-weight compounds (Paterson & Sim, 1999; Hamilton *et al.*, 2008; Henry *et al.*, 2008). This has been shown to induce a transient increase in microbial nitrogen (N) mineralization, allowing the higher N demand of plants to be met to rebuild biomass (Paterson & Sim, 1999; Hamilton *et al.*, 2008; Henry *et al.*, 2008; Cheng *et al.*, 2011; Shahzad *et al.*, 2012). It is, however, still unexplored whether drought alters such effects of mowing, but it may be speculated that plants under drought conditions may have lower nonstructural C reserves, potentially decreasing the intensity of the C pulse.

In this study, we therefore aimed to assess the direct and indirect plant-mediated effects of a severe summer drought on microbial processes and community composition in a mountain meadow. We asked the questions of how drought under field conditions affects the biomass of plants and the abundance of microbial groups, how it affects the transfer of C from plants to microbes, and how it alters short-term C turnover in grasslands after mowing. Specifically, we hypothesized that drought decreases plant C pools, thereby reducing the availability of recently fixed plant C for microbes in the rhizosphere; and that,

overall, drought decreases microbial biomass, reducing the abundance of bacteria more strongly than that of fungi. Finally, we hypothesized that drought reduces the pulse of recently assimilated C to the soil after mowing compared with controls. We experimentally simulated an extended drought period in a mountain meadow in the Austrian Central Alps. Soil microbial biomass and community composition were determined using phospholipid fatty acids (PLFAs). To investigate the allocation of recently assimilated C to microbial biomass, we pulse-labelled plants with $^{13}\text{CO}_2$ and traced labelled C from plants via the extractable organic C (EOC) pool to microbial PLFAs. Both controls and drought plots were mown towards the end of experimental drought and after labelling.

Materials and Methods

Study site

The study area was located in the Austrian Central Alps near Neustift, Stubai Valley (47°07'45"N, 11°18'20"E); the sampling site, a mountain meadow, was situated at 1850 m above sea level. Mean annual temperature was *c.* 3.0°C; mean annual precipitation was *c.* 1097 mm. Soils were classified as dystric cambisols (Food and Agriculture Organization of the United Nations, soil classification system) with a pH (in CaCl_2) of 4.9 (Meyer *et al.*, 2012). The study site is characterized by high plant productivity, with peak above- and belowground biomasses ranging between 240 and 440 g m^{-2} and between 420 and 980 g m^{-2} , respectively (Bahn *et al.*, 2006; Schmitt *et al.*, 2010), as well as comparatively high soil respiration rates (Bahn *et al.*, 2010). The experimental site was part of a meadow that is briefly grazed by cattle in spring and autumn, and cut for haying once a year at the end of July. The vegetation is dominated by perennial grasses and herbs including *Anthoxanthum odoratum* L., *Festuca rubra* L., *Alchemilla vulgaris* L., *Leontodon hispidus* L. and *Trifolium repens* L. (Bahn *et al.*, 2009; Schmitt *et al.*, 2010).

Experimental set-up

We simulated an extended summer drought event by installing rain-out shelters (3.0 × 3.5 m; *n* = 3) with light- and UV-B-permeable plastic foil (UV B Window; Folitec GmbH, Westerbürg, Germany; light permeability *c.* 95%; UV-B permeability > 70%) to exclude any precipitation. In the centre of each shelter as well as in nearby established controls, plastic frames (1 × 1 m) were carefully placed into the upper 3 cm of soil. Rain-out shelters were left on the plots for 8 wk (16 June 2010 to 11 August 2010; Fig. 1); to detect the effect of mowing and to maintain the usual land-management practice in this meadow (Schmitt *et al.*, 2010), the aboveground biomass of the complete site was cut and removed before the end of the drought treatment (8 and 9 August 2010). After plots had been mown (11 August 2010), they received 20 l m $^{-2}$ of previously collected rainwater (= 20 mm precipitation) over a time period of 3 min, mimicking a heavy precipitation event, and subsequently rain-out shelters were removed. Precipitation, air temperature (T_{air}) and soil

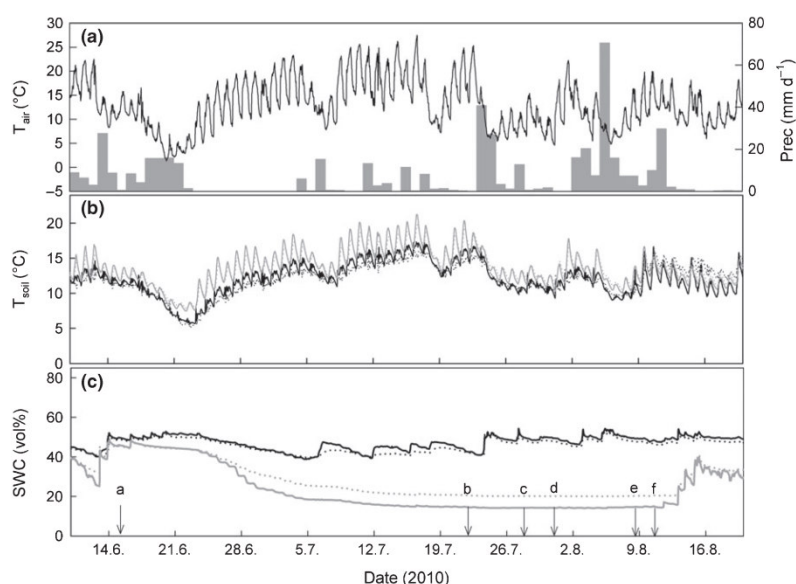


Fig. 1 Microclimate during the drought experiment: (a) air temperature (T_{air} ; in °C 0.5 h^{-1}) and precipitation (prec; in mm d^{-1}); (b) soil temperature (T_{soil} ; in °C 0.5 h^{-1}) and (c) volumetric soil water content (SWC; in %) at 5 (solid lines) and 10 cm soil depth (dotted lines) in control (black) and drought (grey) plots. Arrows with lowercase letters in (c) indicate dates of action: 'a' marks the beginning of the drought treatment; 'b', 'c' and 'd' indicate dates of pulse-labelling; 'e' indicates mowing and 'f' indicates rewetting and thus the end of the drought treatment.

temperature (T_{soil}) (S-TMB-M006 with HOBO Micro Station Data Logger H21-002; Onset Computer Corp., Bourne, MA, USA) and soil water content (SWC; ECH2O EC-5 with data Logger EM50; Decagon Devices Inc., Pullman, WA, USA) at 5 and 10 cm soil depth were continuously monitored throughout the experiment. A similar drought simulation was conducted at the same plots in the previous year.

Labelling procedure

Pulse labelling with $^{13}\text{CO}_2$ was conducted after at least 6 wk of drought simulation on three individual days with high radiation to ensure high uptake of labelled CO_2 into plants (Supporting Information Table S1). Per labelling day, a set of drought and control plots were equipped with plexiglass chambers ($1 \times 1 \times 0.7\text{ m}$; 95% light permeability), which were placed on previously installed plastic frames (see Bahn *et al.*, 2009, 2013). After 10 min of equilibration, $^{13}\text{CO}_2$ (>99.9% CO_2 with 99 atom-% ^{13}C ; Cambridge Isotope Laboratories, Andover, MA, USA) was added over a period of 90 min, which has been shown to ensure sufficient ^{13}C uptake for detecting ^{13}C in microbial PLFAs (Bahn *et al.*, 2013).

Sample collection

Samples of plant biomass (aboveground biomass and fine roots) and soil were taken 1, 6, 7 and 8 wk (which was after mowing) after installation of rain-out shelters, as well as 1 wk after rewetting. For the pulse-labelling experiment, we took plant and soil samples 1 h before labelling to determine the natural background $\delta^{13}\text{C}$ of plant biomass and soil (Table S2). We started sampling directly after the labelling procedure (at 0, 2 and 6 h), and continued on the following days (at 24, 48, 96 and 192 h). Then the study site was mown and aboveground biomass harvested. We

took soil samples after mowing, and subsequently all plots were rewetted and sampling was continued (24, 72 and 192 h after rewetting). For each plant and soil sample, we pooled material from two soil collars with an area of $5 \times 7\text{ cm}$ and a depth of 10 cm. The aboveground biomass was cut, and immediately treated by microwave for 3 min to interrupt any metabolic activities in plants (Popp *et al.*, 1996). Soil was carefully sieved to 2 mm, manually freed from roots, which were collected (fine-root biomass), washed and also treated by microwave. Aliquots of soil samples for extraction of PLFAs were immediately frozen at -80°C , and remaining soil was stored at 4°C until further processing. Plant material and soil aliquots were dried (for 72 h at 60°C) and finely ground for subsequent analyses of bulk ^{13}C content by EA-IRMS (elemental analysis-isotope ratio mass spectrometry; EA 1110 (CE Instruments, Milan, Italy), coupled to a Finnigan MAT Delta Plus IRMS (Thermo Fisher Scientific, Waltham, MA, USA)).

Determination of C and N pools in the soil

Extractable organic carbon (EOC) and total extractable nitrogen (TEN) were measured in K_2SO_4 extracts (2 g of fresh soil was extracted with 20 ml of 0.5M K_2SO_4) using a Total Organic Carbon/Total Nitrogen (TOC/TN) analyser (TOC-V CPH E200V/TNM-122V; Shimadzu, Vienna, Austria). Aliquots of K_2SO_4 extracts were used to determine the $\delta^{13}\text{C}$ of EOC, measured by direct injection (without column, direct mode) on high-performance liquid chromatography-isotope ratio mass spectrometry (HPLC-IRMS) (Dionex Corporation, Sunnyvale, CA, USA) linked to a Finnigan Delta V Advantage Mass Spectrometer connected by a Finnigan LC-IsoLink Interface (both Thermo Fisher Scientific, Waltham, MA, USA) at a flow of $0.5\text{ ml water min}^{-1}$. NH_4^+ was determined photometrically by a modified indophenol reaction method (Kandeler & Gerber,

affected by the drought treatment, where mean aboveground biomass was $168 (\pm 35) \text{ g m}^{-2}$ and mean root biomass was $381 (\pm 64) \text{ g m}^{-2}$. Similar to biomass, neither the C content of aboveground biomass nor that of fine roots was altered by drought (Fig. S1a,d, Table 1a). Among all studied soil parameters, only NO_3^- was significantly decreased during drought, by 55.4%, but this effect was not detectable after mowing (Fig. S1, Table 1a,b).

Effects of drought on soil microbes

In drought-treated plots, total PLFAs were already significantly higher (+116.2%) compared with controls 1 wk after the beginning of the experiment and stayed at a constant higher level until mowing (Fig. 2a, Table 1a). This was similar for all microbial groups, but most pronounced for Gram-positive bacteria, which increased almost threefold compared with controls (+181.7%; Table 1a). The amounts of Gram-negative bacteria and total fungi were two-fold higher in drought plots than in controls (Fig. 2g, Table 1a); within fungal PLFAs, the marker 16:1 ω 5 showed the highest increases (Fig. 2d, Table 1a). Nevertheless, the ratio of fungal:bacterial PLFAs was not affected by the drought treatment (Fig. 2e, Table 1a). Interestingly, we found a strong decrease of total PLFAs by 37% (t -test; $P=0.045$) after mowing in the drought plots, while PLFAs in control plots were not affected (Fig. 2). Rewetting affected PLFAs neither in drought nor in control plots (Fig. 2, Table 1b).

We conducted a CCA to assess the effects of drought on the microbial community composition (Fig. 3). We used the relative abundances of all sampled PLFAs as the community matrix and DOY, DOD, SWC, T_{soil} , and EOC, TEN, NH_4^+ and NO_3^-

pools as the environmental matrix. The contribution of the environmental variables (constrained variability) to the total variability of the microbial community matrix was 35%, of which CCA axis 1 (CCA1) accounted for 60% ($P=0.005$) and CCA axis 2 (CCA2) for 15% ($P=0.13$); thus, CCA1 accounted for 21% and CCA2 for 5% of total variability. We found that CCA1 showed a high correlation with DOY, T_{soil} and NH_4^+ (Fig. 3a); therefore, CCA1 could describe a seasonal gradient during the experiment, reflected by a distribution of the samplings along CCA1 (weeks 1–8 of drought, RW, 1 wk after rewetting; Fig. 3b). CCA2 displayed a gradient of SWC and showed a high correlation with DOD (Fig. 3a,b), showing the effects of the drought treatment on the microbial community composition. The drought-induced shift (CCA2) of the soil microbial composition was mainly driven by the distribution of Gram-positive and Gram-negative bacterial PLFA markers. Gram-positive bacteria correlated with higher EOC and TEN, and lower SWC, while Gram-negative bacteria correlated with higher SWC and NO_3^- . Fungal markers, by contrast, were not separated by SWC, but by NH_4^+ and T_{soil} (Fig. 3a).

Effects of drought on C dynamics of plant, soil and microbial C pools

In both controls and drought-treated plots, ^{13}C in aboveground biomass peaked within 24 h after labelling (432.1 ± 151.0 and $263.7 \pm 135.2 \text{ mg } ^{13}\text{C m}^{-2}$, respectively; mean $\pm$ SE, $n=3$) and then decreased exponentially over time (Fig. 4a). There was a trend towards higher mean residence time of ^{13}C in aboveground biomass in drought plots, but differences compared with controls

Table 1 Effects of drought, sampling time and the interaction term (drought $\times$ time) on carbon (C) and nitrogen (N) pools (a) before mowing, also given as percentage of controls, calculated from mean values for data obtained during drought treatment until mowing, and (b) after mowing, evaluated by two-way repeated-measures ANOVA

	(a) Before mowing							(b) After mowing						
	Drought			Time		Drought $\times$ time		Drought		Time		Drought $\times$ time		
	(%)	F(1)	P	F(2)	P	F(1, 2)	P	F(1)	P	F(1)	P	F(1, 1)	P	
Aboveground biomass	−17.4	1.96	ns	0.01	ns	0.01	ns							
Fine roots	+43.8	2.99	ns	0.05	ns	0.32	ns	1.34	ns	0.00	ns	0.02	ns	
Soil	−0.8	0.03	ns	0.83	ns	0.20	ns	0.44	ns	1.91	ns	1.28	ns	
EOC	+26.9	2.35	ns	1.76	ns	0.79	ns	4.02	ns	0.87	ns	2.41	ns	
TEN	+22.4	1.31	ns	2.26	ns	1.25	ns	1.30	ns	1.52	ns	0.00	ns	
NH_4^+	+20.9	1.41	ns	12.77	**	2.60	ns	5.20	ns	1.45	ns	0.06	ns	
NO_3^-	−55.4	4.70	*	1.24	ns	0.14	ns	2.64	ns	0.04	ns	0.11	ns	
Total PLFAs	+116.2	33.57	***	1.33	ns	0.97	ns	1.27	ns	0.02	ns	0.46	ns	
Fungi _{tot}	+107.3	37.28	***	0.72	ns	1.10	ns	0.36	ns	0.26	ns	0.12	ns	
Fungi _{gen}	+102.1	37.79	***	0.78	ns	0.79	ns	0.01	ns	0.42	ns	0.01	ns	
16:1 ω 5	+117.2	28.82	***	2.66	ns	1.37	ns	1.54	ns	0.08	ns	0.34	ns	
Bacteria _{tot}	+121.5	31.20	***	1.39	ns	0.80	ns	1.40	ns	0.01	ns	0.56	ns	
Gram-negative	+100.7	29.39	***	1.17	ns	0.72	ns	2.54	ns	0.00	ns	0.57	ns	
Gram-positive	+181.7	30.21	***	1.86	ns	1.24	ns	0.48	ns	0.00	ns	0.42	ns	
Fungi:bacteria	−9.1	2.20	ns	3.68	ns	0.78	ns	1.06	ns	1.60	ns	1.67	ns	

Before mowing, $n=9$ (for fine roots, $n=6$); after mowing, $n=6$. After mowing, no data for aboveground biomass were available. Values in bold show significant effects of drought calculated by one-way ANOVA. Asterisks indicate levels of significance (ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

Bacteria_{tot}, total bacterial PLFAs; EOC, extractable organic carbon; Fungi_{gen}, general fungal PLFAs; Fungi_{tot}, total fungal PLFAs; PLFA, phospholipid fatty acid; TEN, total extractable nitrogen.

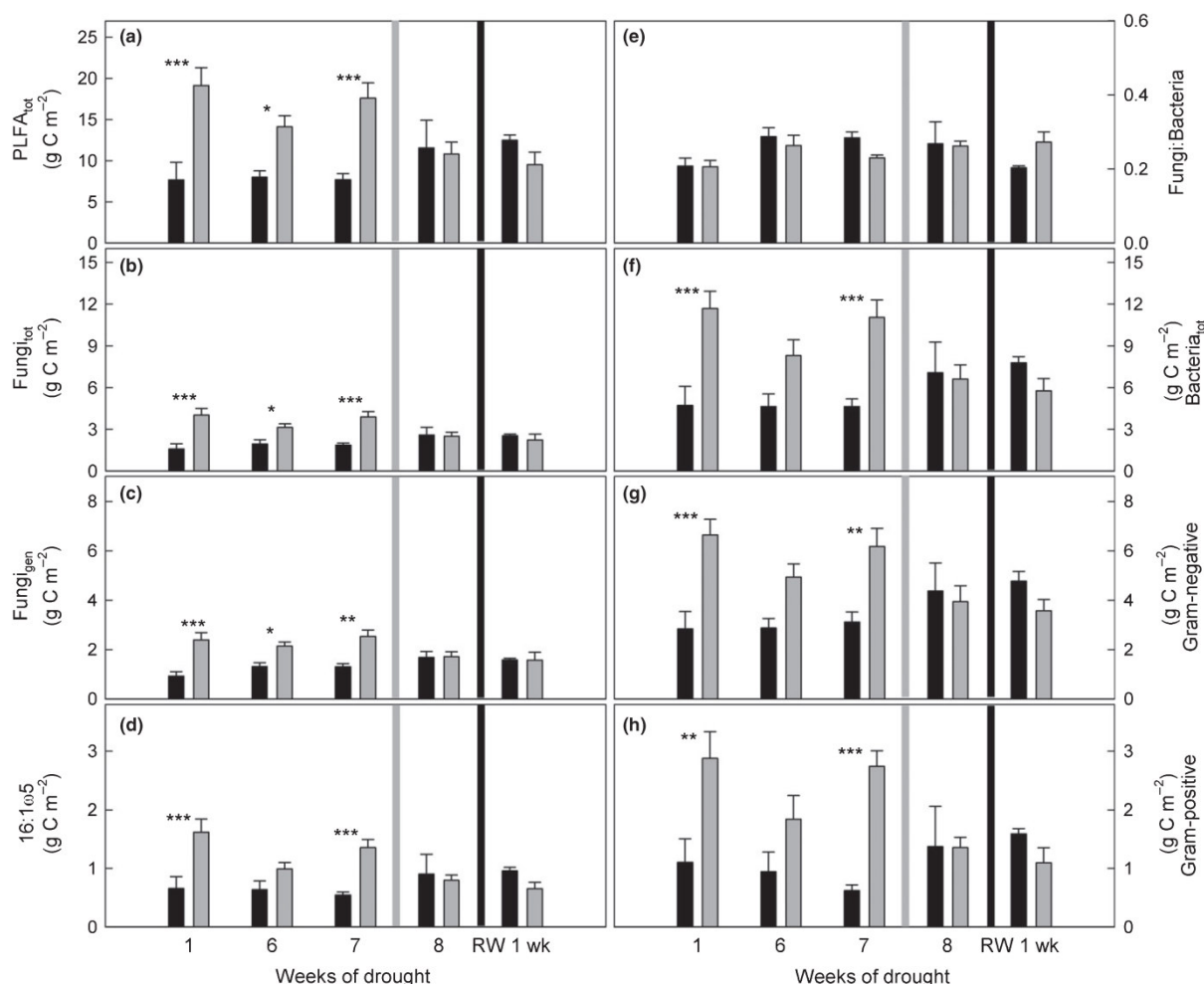


Fig. 2 Microbial biomass over the course of the experiment (in g PLFA-C m⁻² calculated for the uppermost 10 cm of soil; $n = 3$; error bars show $\pm$ SE) in control (black bars) and drought (grey bars) plots. (a) Total phospholipid fatty acids (PLFA_{tot}) were divided into (b) total fungal (Fungi_{tot}) and (f) total bacterial (Bacteria_{tot}) PLFAs. Fungal PLFAs were further separated into (c) general fungal PLFAs (Fungi_{gen}) and (d) 16:1 ω 5. Bacterial PLFAs were subdivided into (g) Gram-negative and (h) Gram-positive markers. (e) The ratio of Fungi_{tot} to Bacteria_{tot}. Light grey lines indicate mowing of aboveground biomass, and black lines rewetting. Asterisks indicate significant differences between treatments at single samplings (one-way ANOVA; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

were not significant (Fig. 4a, Table 2a). Because mowing has to be considered as a major disturbance of C dynamics, ¹³C turnover was analysed separately before and after mowing by two-way repeated-measures ANOVA (Fig. 4b–d). During drought, before mowing, fine roots received significantly less ¹³C, while after mowing there was no difference compared with controls (Fig. 4c, Table 2a). Before mowing, ¹³C increased to the same extent in bulk soil of control and drought plots. After mowing and rewetting, however, the amount of ¹³C in the bulk soil increased further in the controls and became significantly higher than in drought plots (Fig. 4b). In contrast to the controls, where the amount of ¹³C in EOC increased only slightly, there was a significant accumulation of ¹³C in EOC in drought plots. Moreover,

we detected a pulse of ¹³C in EOC directly after mowing in drought plots, which decreased after rewetting, while the amount of ¹³C in the controls constantly stayed at a low level (Fig. 4d).

Although microbial biomass was higher in drought plots (Fig. 2), the absolute amount of ¹³C incorporated into total PLFAs was significantly lower in drought plots compared with controls (Fig. 5a). This reduction during drought was most pronounced for Gram-negative bacteria, followed by the fungal marker 16:1 ω 5 and Gram-positive bacteria (Fig. 5d,g,h). For the general fungal marker, in contrast, similar amounts of ¹³C were incorporated in drought plots as in the controls (Fig. 5c). In both control and drought plots, we found a remarkably high ratio of fungal:bacterial ¹³C uptake (Fig. 5e), in contrast to the lower

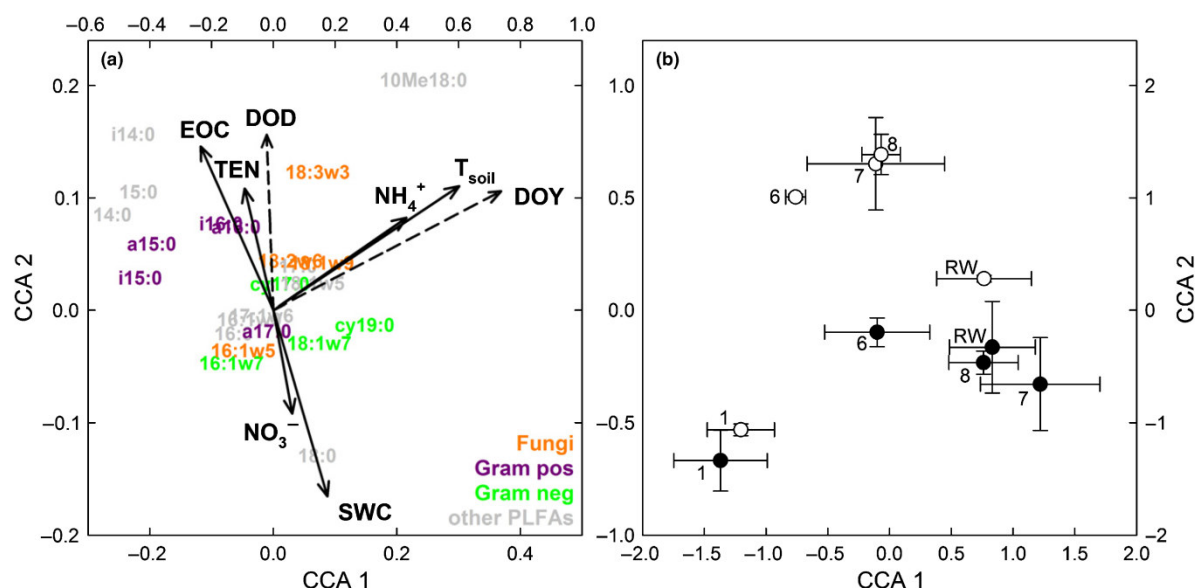


Fig. 3 The effects of soil parameters and time on soil microbial community composition, as determined by canonical correspondence analysis (CCA). Relative abundances of phospholipid fatty acids (PLFAs) were used as the soil microbial community matrix, and soil parameters (gravimetric soil water content (SWC); soil temperature at 5 cm (T_{soil}); extractable organic carbon (EOC); total extractable nitrogen (TEN); ammonium-N (NH_4^+); nitrate-N (NO_3^-)) and time (day of the year (DOY); days of drought (DOD) (dashed arrows)) were used as the environmental data matrix. Single PLFAs are coloured according to their attribution to microbial groups. The CCA is based on data from samples collected during drought (1, 6, 7 and 8 (after mowing) weeks of drought), as well as 1 wk after rewetting (RW). The contribution of constrained variability to total variability was 35%, of which CCA1 accounted for 60% and CCA2 for 15%. The significances of CCA1 and CCA2 were $P = 0.005$ and $P = 0.15$, respectively (permutation test). (a) Biplot of the distribution of single PLFAs and environmental parameters. (b) The distribution of samples collected during drought and after rewetting in control (closed circles) and drought (open circles) plots; characters next to symbols identify the time of sampling (error bars indicate SE).

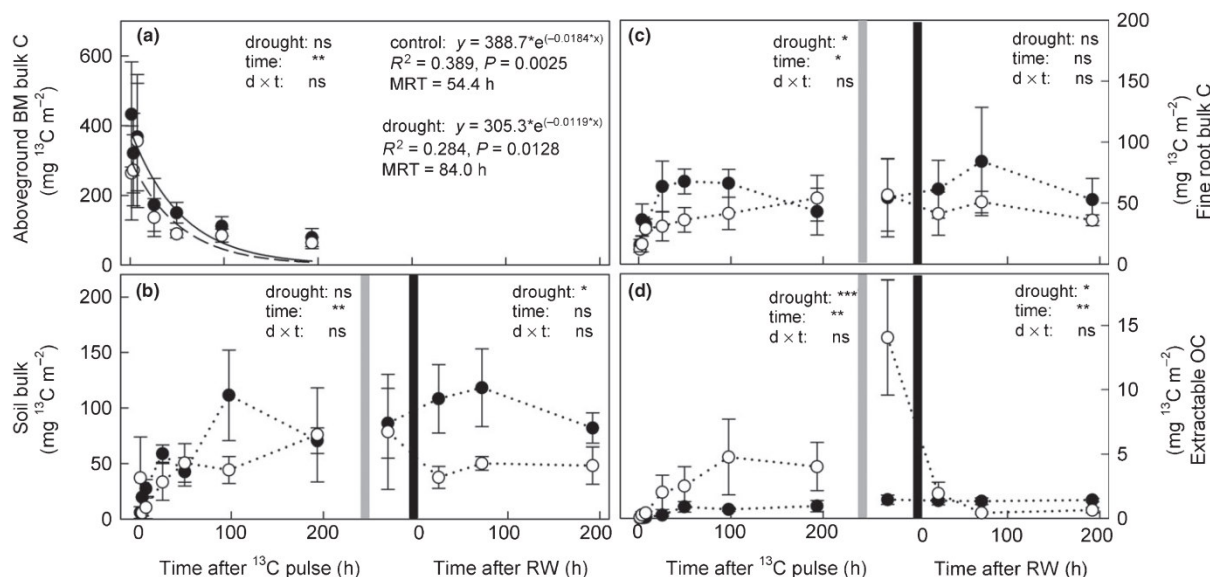


Fig. 4 ^{13}C excess (in $\text{mg } ^{13}\text{C m}^{-2}$) after pulse-labelling in (a) aboveground plant biomass, (c) fine-root bulk biomass, (b) bulk soil and (d) the extractable organic carbon (C) pool in control (closed circles) and drought (open circles) plots; ^{13}C excess in (a) followed an exponential decay function ($y = a \times e^{(-\lambda \times x)}$, with a = initial pool size, λ = decay constant and x = time; control, solid line; drought, dashed line; mean residence time of ^{13}C (MRT) = $1/\lambda$). ^{13}C excesses for (b) and (d) were calculated for the uppermost 10 cm of soil. Light grey lines indicate mowing, and black lines indicate rewetting. Effects of drought on ^{13}C excess were analysed separately before and after cutting by two-way repeated-measures ANOVA with time and drought treatment as factors (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; for further details see Table 2).

Table 2 Effects of drought on ^{13}C excess in all carbon (C) pools (a) before and (b) after mowing were analysed separately by two-way repeated-measures ANOVA

	(a) Before mowing						(b) After mowing					
	Drought		Time		Drought $\times$ time		Drought		Time		Drought $\times$ time	
	F(1)	P	F(6)	P	F(1, 5)	P	F(1)	P	F(3)	P	F(1,1)	P
Aboveground biomass	1.53	ns	4.89	**	0.23	ns						
Fine roots	4.97	*	2.95	*	0.86	ns	0.75	ns	0.37	ns	0.24	ns
Soil	1.72	ns	3.81	**	1.38	ns	8.71	*	0.23	ns	0.52	ns
EOC	7.93	**	2.35	ns	1.05	ns	6.85	*	9.08	**	8.89	**
Total PLFAs	11.39	**	5.73	***	0.64	ns	17.26	**	0.13	ns	0.52	ns
Fungi _{tot}	3.631	ns	6.18	***	0.27	ns	16.44	**	0.54	ns	0.44	ns
Fungi _{gen}	1.52	ns	5.53	***	0.10	ns	14.43	**	0.85	ns	0.45	ns
16:1 ω 5	17.32	***	5.90	***	2.17	ns	21.52	***	0.10	ns	0.50	ns
Bacteria _{tot}	14.67	***	4.46	**	0.91	ns	17.39	**	0.08	ns	0.71	ns
Gram-negative	30.81	***	8.40	***	2.04	ns	17.05	**	0.14	ns	0.70	ns
Gram-positive	5.37	*	2.46	ns	1.51	ns	17.82	***	0.20	ns	0.88	ns
Fungi:bacteria	0.48	ns	3.32	*	1.51	ns	0.31	ns	5.11	*	1.12	ns

Before mowing, $n = 42$; after mowing, $n = 12$. After mowing, no data for aboveground biomass were available. Asterisks indicate levels of significance (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Bacteria_{tot}, total bacterial PLFAs; EOC, extractable organic carbon; Fungi_{gen}, general fungal PLFAs; Fungi_{tot}, total fungal PLFAs; PLFA, phospholipid fatty acid.

fungi:bacteria PLFA ratio (Fig. 2e), indicating that fungi received a higher portion of plant-derived C compared with bacteria.

After mowing, ^{13}C in PLFAs in drought-treated plots stayed at a significantly lower level than in controls (Fig. 5a). This pattern was similar for all microbial groups, and also for fungal markers, where ^{13}C incorporation had not been affected by drought before mowing (Fig. 5b,c). This indicated that mowing caused a pulse of plant C in the soil, which in control plots was incorporated into PLFAs, but in drought plots was not taken up by microbes and accumulated in the EOC pool. However, mowing caused a significant decrease in microbial biomass in drought-treated plots and therefore dead microbes may also have substantially contributed to this label peak in the EOC (Figs 2a, 4d).

Discussion

The probability of extreme events and drought periods is predicted to increase in European mountain regions (IPCC, 2012), with potentially severe consequences for ecosystem C dynamics (Reichstein *et al.*, 2013). However, the effects of drought on ecosystems that are usually well supplied with water are not well understood. In this study, we demonstrate that under field conditions a severe summer drought period has the potential to weaken the coupling of plant and microbial C turnover in a mountain meadow.

Effects of drought on plant belowground C allocation

Drought is well known to strongly affect plant biomass and C pools, by increasing the proportion of fine-root biomass to sustain water uptake and photosynthesis, at the expense of aboveground C stocks (Chaves *et al.*, 2003; McDowell, 2011). However, in our experiment, drought neither affected plant biomass, nor their C pools, probably because most of the plant

biomass was already largely established at the onset of drought. Nonetheless, drought significantly reduced the absolute amount of recently assimilated C in fine roots, without decreasing C uptake at the time of pulse labelling (Fig. 4c). Thus, drought may have slowed down plant C turnover, which was also found by others (Ruehr *et al.*, 2009; Barthel *et al.*, 2011; Brüggemann *et al.*, 2011). However, ^{13}C significantly increased in the EOC pool during drought, which suggests that plant roots may have maintained, or even increased root exudation or the release of mucilaginous material to decrease the friction resistance in the soil and keep the contact between roots and soil (Walker *et al.*, 2003).

Effects of drought on soil microbes

In contrast to our expectations, both fungal and bacterial PLFAs were higher in drought-treated plots than in controls. In addition, we detected a shift of the microbial community composition that occurred mainly within soil bacteria. We also observed that drought showed a distinct effect on the incorporation of plant-derived C into microbial PLFAs, specifically a reduction of label in bacteria. Fungal PLFAs were increased during drought and incorporated similar amounts of recent plant-derived C in drought plots as in controls (Fig. 5b). This corresponds well with the finding of Bahn *et al.* (2013) at the same study site, where even extended reductions in photosynthesis did not decrease the amount of recent plant C in fungal PLFAs. Thus, the transfer of ^{13}C label from plants to fungi seemed not to be interrupted by experimental drought. Of the four PLFAs used as fungal markers, only 16:1 ω 5, often used as a marker for arbuscular mycorrhizas, showed a significant reduction in ^{13}C incorporation during drought. This was in contrast to our expectations, as mycorrhizal fungi are a strong sink for plant-derived C in meadows (Denef *et al.*, 2009; Balasooriya *et al.*, 2012) and may even promote

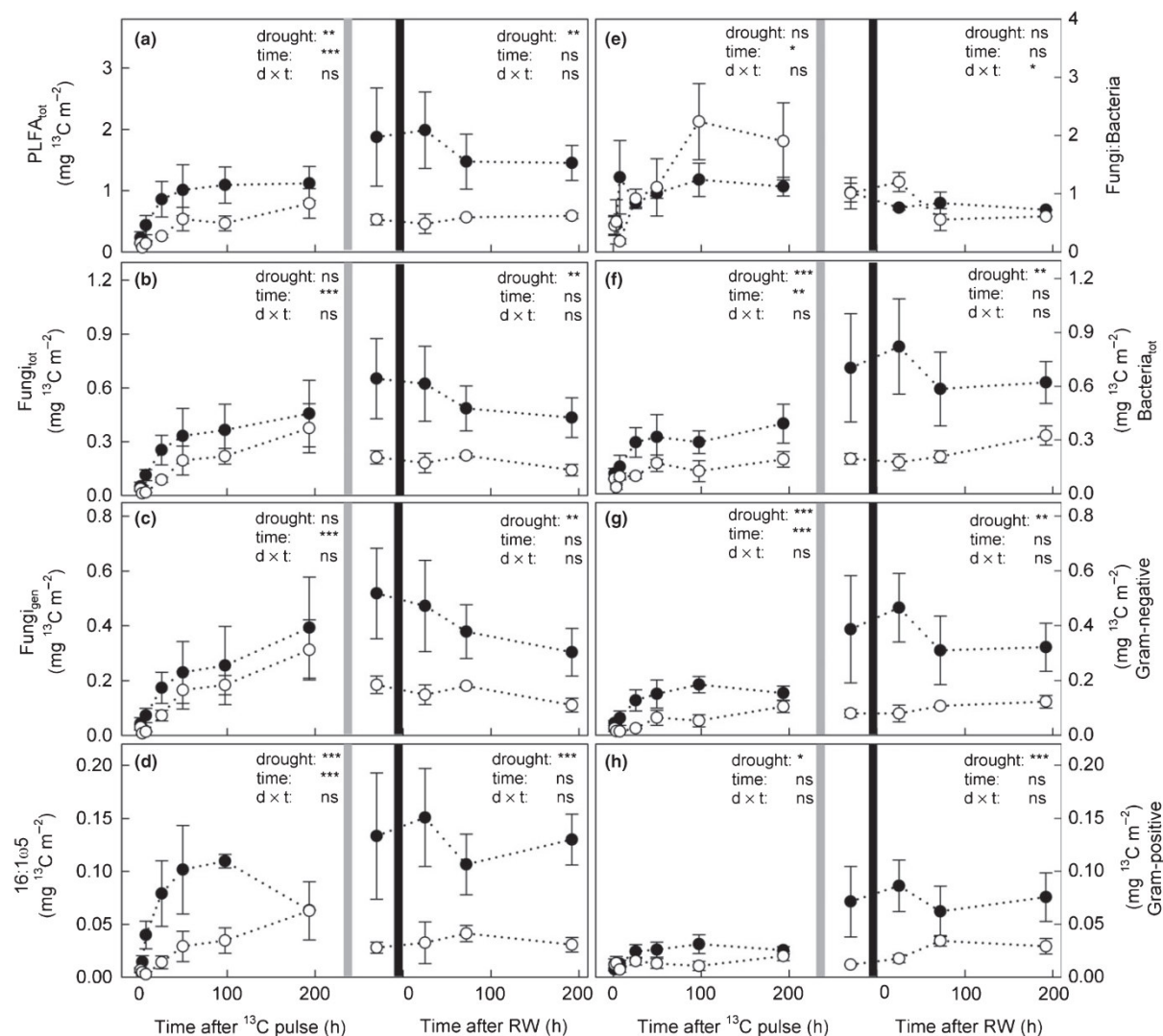


Fig. 5 ^{13}C excess (in $\text{mg } ^{13}\text{C m}^{-2}$) after pulse-labelling in (a) total phospholipid fatty acids (PLFA_{tot}), and (b) total fungal ($\text{Fungi}_{\text{tot}}$) and (f) total bacterial ($\text{Bacteria}_{\text{tot}}$) PLFAs, and (e) the ratio of fungal to bacterial ^{13}C uptake in control (closed circles) and drought (open circles) plots over time. Fungal PLFAs were grouped into (c) general fungal marker and (d) $16:1\omega5$ PLFAs, and bacterial PLFAs into (g) Gram-negative and (h) Gram-positive PLFAs. Values were calculated for the uppermost 10 cm of soil. Mowing is indicated by light grey lines and rewetting is indicated by black lines. Effects of drought before and after cutting were analysed separately by two-way repeated-measures-ANOVA (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; further details are given in Table 2).

water uptake for plants during drought conditions (Wardle *et al.*, 2004; Allen, 2007). However, this biomarker might not solely be representative of arbuscular mycorrhiza, as it was shown also to occur in bacterial taxa (Olsson, 2006).

All bacterial PLFAs were increased in experimental drought plots, but Gram-positive bacteria became relatively more abundant compared with Gram-negative bacteria. Gram-positive bacteria are considered to be inherently more resistant to drought regarding their cell wall composition (Schimel *et al.*, 2007; Manzonei *et al.*, 2012a), and their abundance correlated with higher EOC and TEN (Fig. 3a). This is further evidence that, in

contrast to Gram-negative bacteria, Gram-positive bacteria may prefer more complex substrates and therefore could be classified as 'dry-adapted generalists' (Lennon *et al.*, 2012). Many Gram-negative bacteria in turn seem to benefit from root or fungal exudates (Treonis *et al.*, 2004; Olsson, 2006; Denef *et al.*, 2009; Balasooriya *et al.*, 2012; Bahn *et al.*, 2013) and incorporated almost tenfold more recent plant C than Gram-positive bacteria, but both received significantly less ^{13}C during drought.

Thus, drought weakened the link between both Gram-positive and Gram-negative bacteria and plants, as indicated by a significantly lower absolute bacterial uptake of label and a concurrent

increase of ^{13}C in the EOC. This could have been caused by decreased bacterial activity and a higher proportion of dormant microbes (Schimel *et al.*, 2007) and consequently lower uptake of plant-derived C during drought, which is, however, in contrast to the observed strong increase in bacterial PLFAs. It is more likely that drought may not have caused a decrease in microbial activity, which was also reported by Steinweg *et al.* (2013), but restricted it to water-containing, but disconnected, soil pores (Schimel *et al.*, 2007; Moyano *et al.*, 2013). These small soil pores may represent 'hot spots' for bacterial decomposers, and may not be accessible to soil mesofauna grazing on microbes, or competing plant roots or fungal hyphae (Strickland & Rousk, 2010). Microbes in these disconnected microsites may be separated from root exudates, which would explain the reduced microbial uptake of plant-derived ^{13}C from EOC.

We rejected our hypothesis that drought decreases microbial biomass and showed that, in the studied grassland, beneficial effects on microbes may have exceeded detrimental ones, possibly also reflecting a carry-over effect from the drought experiment performed in the same plots in the previous year (but see results on recovery responses after rewetting). This is in contrast to other studies in Mediterranean or semi-arid grasslands, where drought induced a decrease in microbial biomass (Alster *et al.*, 2013) or diversity, especially for bacteria (Yuste *et al.*, 2011; Sheik *et al.*, 2011). Microbial biomass also decreased when a drought treatment was applied to barren soil, but increased in soils with mixed plant vegetation (Sanaullah *et al.*, 2011). This illustrates that effects of drought are strongly context-dependent, but also suggests that the presence of plants is crucial for microbial responses to drought.

Mowing and rewetting

Mowing has been shown to lead to a short-term pulse of plant-released C into the rhizosphere (Paterson & Sim, 1999; Hamilton *et al.*, 2008; Henry *et al.*, 2008). While we expected that this mowing-induced C pulse would be reduced by drought, we observed an increase of ^{13}C in both drought plots and controls. In controls, this pulse of C after mowing was visible as a significant increase of ^{13}C in microbial PLFAs, whereas in drought plots ^{13}C peaked in the EOC pool and not in the microbes. This further indicates that microbes may have been unable to take up the C released by plants during drought. Alternatively, the steep increase of ^{13}C in EOC in drought plots may have been fuelled by lysis of dead microbes or by an increase in fine-root turnover after mowing. However, such C pulses after mowing have been shown to induce a short-term increase in nutrient mineralization, which in turn increases the availability of nutrients for plants (Hamilton *et al.*, 2008). Thus, smaller C pulses might lead to lower nutrient mineralization rates and may increase the competition for nutrients between plants and microbes, and so hamper plant growth.

In contrast to mowing, rewetting did not affect microbial biomass either in controls or after drought treatment. The ^{13}C peak in the EOC in drought plots after mowing quickly disappeared, but was not used for new biomass production, as evidenced by a

lack of ^{13}C incorporation into PLFAs. In turn, after rewetting, soil respiration rates steeply increased (Ladreitner *et al.*, data not shown; 'Birch-effect' (Birch, 1958)), which suggests that the accumulated ^{13}C might have become available for microbes after plots were rewetted, or it may have leached out of the system. Nonetheless, we observed a shift of the microbial community composition in the drought-treated plots towards that of the controls already 1 wk after rewetting, indicating that the microbial community in this mountain grassland is highly resilient, even to strong drought.

Conclusions

Severe experimental drought, surprisingly, did not reduce microbial biomass, but altered the microbial community composition in a mountain meadow in favour of Gram-positive bacteria. Drought did not interrupt the C transfer from plants to fungi, but decreased the uptake of label into bacterial groups, especially when the meadow was mown. From our study, we therefore conclude that (1) drought has the potential to weaken the link between plant and bacterial C turnover and (2) the microbial community in the studied grassland is highly adaptable to altered soil moisture conditions, responding to severe drought by shifting to slow-growing, drought-adapted soil microbes, such as Gram-positive bacteria, and quickly recovers after rewetting.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Plant biomass carbon pools, and soil carbon and nitrogen pools over the course of the drought experiment.

Table S1 Date and time, and abiotic conditions during pulse-labelling, for individual sites

Table S2 Natural abundance of ¹³C of plant biomass, soil and extractable organic C pool in the soil

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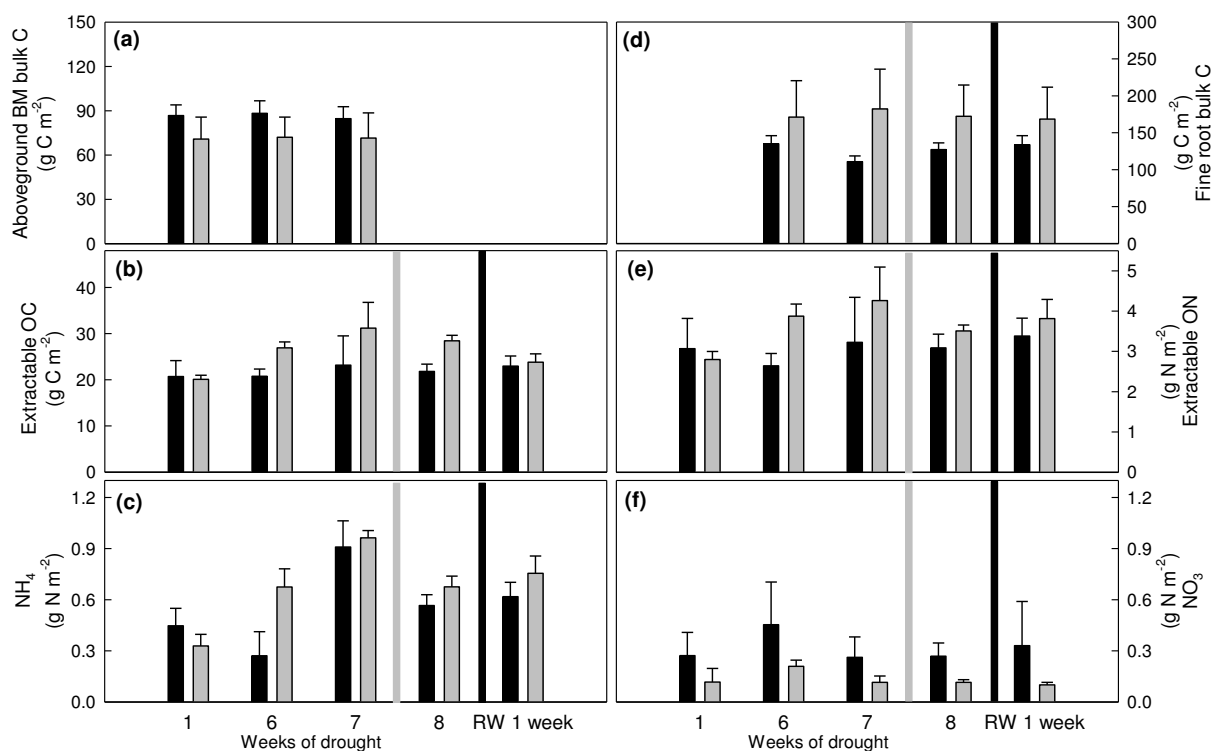


Figure S1: Aboveground biomass and fine root bulk C content (**a, d**), as well as extractable organic C (**b**) and organic (**c**) and inorganic N pools (**e, f**) in control (black bars) and drought plots (grey bars) over the time of the drought experiment. Bars display mean values ($n=3$, error bars = SE). Grey lines indicate mowing, black lines indicate rewetting. Asterisks display significant differences between treatments at single sampling points (derived by one-way ANOVA, Fisher's LSD: * $p<0.05$; ** $p<0.01$ * $p<0.05$).

Table S1: *Date* and time of the day (*time*) of labelling events for drought and control plots respectively. $[CO_2]_{start}$ and $[CO_2]_{labelling}$ show the CO_2 -concentrations in the labelling chamber before and during labelling. $^{13}CO_2$ describes the isotopic composition in atom% during labelling (values are means of 5 min intervals $\pm$ SE) analysed by Quantum cascade laser (QCL, Aerodyne Research Inc., Billerica, MA, USA), the average amount of $^{13}CO_2$ added is given in $ml\ min^{-1}$. Photosynthetic active radiation (*PAR*) during labelling as well as air temperature inside (T_{in}) and outside (T_{out}) the chambers are displayed (minimum – maximum temperature). CO_2 concentration in the labelling chamber was monitored with infrared-gas-analysers (Vaisala Ajj, Vantaa, Finland and EGM-4, PP Systems, Hitchin, UK);

<i>Date</i>	<i>Treatment</i>	<i>Time</i> (MET)	$[CO_2]_{start}$ (ppm)	$[CO_2]_{labelling}$ (ppm)	$^{13}CO_2$ (atom%)	$^{13}CO_{2\ added}$ ($ml\ min^{-1}$)	<i>PAR</i> ($\mu mol\ m^{-2}\ s^{-1}$)	T_{in} ($^{\circ}C$)	T_{out} ($^{\circ}C$)
22.07.2010	drought	11:54–13:24	237	552 $\pm$ 19	18.3 $\pm$ 1.1	12.9	1848–1908	26.7–37.7	26.0–27.3
	control	12:49–14:19	248	556 $\pm$ 5	23.2 $\pm$ 0.1	38.3	1423–1908	29.7–39.9	26.5–28.8
28.07.2010	drought	10:40–12:10	253	519 $\pm$ 18	22.6 $\pm$ 0.2	23.3	652–1185	19.1–24.1	15.7–19.4
	control	11:11–12:41	256	496 $\pm$ 28	18.9 $\pm$ 0.6	21.7	652–1185	15.0–25.8	14.9–21.2
31.07.2010	drought	12:05–13:35	280	520 $\pm$ 12	23.5 $\pm$ 0.3	34.2	1840–2026	23.4–33.2	21.1–24.2
	control	12:49–14:19	216	555 $\pm$ 28	22.9 $\pm$ 0.5	43.2	1759–1993	24.9–30.1	20.5–24.4

Table S2: Natural abundance ^{13}C of several components sampled from drought and control plots. Values represent means ($n=3$) $\pm$ SE. Differences between treatments were tested using t-tests, if data was not homoscedastic Welch-test was used instead. Isotopic composition of biomass samples are reported in $\delta^{13}\text{C}$ (‰): $\delta^{13}C_{\text{sample}} = 1000 * \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}$ with R describing the ratio of $^{13}\text{C}/^{12}\text{C}$. The $\delta^{13}\text{C}$ values were calculated relative to the VPDB standard (R_{standard}).

	<i>Control</i>			<i>Drought</i>			<i>p</i>
	<i>mean</i>	$\pm$	<i>SE</i>	<i>mean</i>	$\pm$	<i>SE</i>	
Aboveground BM bulk	-28.12	$\pm$	0.09	-27.89	$\pm$	0.25	ns
Fine root bulk	-27.89	$\pm$	0.16	-26.89	$\pm$	0.37	ns
Soil bulk	-26.53	$\pm$	0.08	-26.40	$\pm$	0.25	ns
EOC	-26.84	$\pm$	0.48	-26.51	$\pm$	0.15	ns

Chapter 2

“Drought-history modulates carbon allocation during drought in mountain grassland”

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Drought-history modulates plant carbon allocation during drought in mountain grassland

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Key words: ¹³C pulse-labelling, belowground carbon allocation, drought, microbial community composition, nutrient cycling, phospholipid fatty acid, PLFA, plant-soil interactions, resilience

Abstract

1. Drought periods are projected to become more frequent in many European regions and show a strong potential to affect plant and microbial carbon (C) dynamics even beyond the end of drought. How a history of recurring droughts could affect plant carbon and soil microbial community dynamics is however still little understood.
 2. To address this question we measured *in situ* plant, soil and microbial parameters before, during and after a naturally occurring dry spell on sites with drought-history and ambient precipitation regime within a multi-year drought-simulation experiment on mountain grassland. We labelled canopy sections of plants with ^{13}C and belowground allocation and its incorporation into soil microbial biomass using phospholipid fatty acid analysis (PLFAs).
 3. Plants were more strongly affected by drought-history than by short-term moisture variation. Plant shoots in drought-history plots had a lower N content, and assimilated and allocated less ^{13}C belowground compared to plants in control plots. Fine root respiration rates were lower and, in contrast to ambient controls, not fuelled by recently assimilated carbon.
 4. Soil parameters and PLFA content and composition strongly responded to short-term moisture changes, but were less affected by drought-history. During the dry spell the uptake of plant derived ^{13}C into microbial groups occurred later, but steeply increased after a strong rain pulse, in drought-history plots, overall reaching the same amount as in ambient controls.
 5. *Synthesis:* We conclude that in the studied grassland drought could have induced lasting effects on the plant C uptake and belowground allocation dynamics and on litter quality. The microbial community in this ecosystem appears however to be highly dynamically responding to short-term moisture changes, but still resilient even to extreme recurring drought.
-

Introduction

Climate projections for Central Europe predict severe alterations in rainfall timing at constant annual precipitation. Based on these projections the frequency of rain-free periods that could develop into drought, but also that of heavy precipitation events is going to increase (IPCC 2007; IPCC. 2012; Schär et al., 2004; Seneviratne et al., 2006), causing temporally inhomogeneous soil water availability. Water is crucial for physiological processes and controls substrate diffusion and nutrient availability for plants and microbes (Goebel et al., 2011) and thus their activity (Chaves, et al., 2003; Or et al., 2007). Water shortage during drought can therefore severely affect carbon (C) and nutrient turnover in terrestrial ecosystems (Bardgett et al., 2013; Or et al., 2007; Reichstein et al., 2013). However, the impact of drought not only depends on the drought tolerance or resistance (i.e. the performance during drought) of a plant or a microbial community. Alterations induced by drought could enhance or decrease the sensitivity to recurring disturbances, and lead to long term, carry over, or lagged effects and eventually may alter ecosystem functioning (Smith 2011). Hence, the impact of drought also depends on the resilience capabilities (i.e. the recovery after disturbances) of a system (Allison & Martiny, 2008; de Vries & Shade, 2013; Griffiths & Philippot, 2012; Shade et al., 2012), with possible distinct responses of aboveground and belowground organisms (Bardgett et al., 2013).

Plants are considered to be more vulnerable to increased climatic variability than to changes of the mean climate (Reyer *et al.* 2013). They respond to drought with physiological or morphological adaptations to reduce water loss, such as closing stomata, the production of thicker leaves, or by maximizing water uptake by investing in fine roots to avoid hydraulic failure (Chaves et al., 2003; Manzonei et al., 2012b; Reyer et al., 2013). Such adaptations could lead to reduced carbon uptake and have been shown to alter the dynamics of belowground allocation by lowering the transfer of recent carbon to soil microorganisms (Ruehr *et al.* 2009; Sanaullah, Chabbi & Rumpel 2012; Hasibeder *et al.* 2014). Moreover, drought periods can disrupt the plants' phenology or increase plant mortality (Chaves et al., 2003; Reyer et al., 2013; Walter et al., 2011; Walter et al, 2013). Hence, acclimations or adaptations of plants to drought could be irreversible and could persist even after the end of drought and may raise or alleviate the susceptibility to further drought periods (Walter *et al.* 2013).

Compared to plants soil microorganisms have shorter generation times and higher turnover rates. They may therefore be more resistant, respond quicker and also recover faster after disturbances (Wolters *et al.* 2000; Prosser *et al.* 2007), e.g. resuscitate from dormancy and reassemble similar as undisturbed communities (Lennon & Jones, 2011; Schimel et al., 2007). The mechanisms driving microbial responses to drought under *in situ* conditions however are still not

fully understood (Bardgett *et al.* 2013). Drought periods have been shown to promote drought-tolerant generalists e.g., some gram-positive bacterial, or fungal groups (de Vries *et al.*, 2012a; Lennon *et al.*, 2012; Yuste *et al.*, 2011). Rewetting of dried soils in turn induces pulses of water and nutrient availability by dissolving nutrients released from broken up aggregates and lysed dead microbes. Such pulses stimulate fast-growing microbes feeding on newly available substrate (Borken & Matzner, 2009; Denef *et al.*, 2001; Fierer & Schimel, 2003.; Schimel *et al.*, 2007) and fuel – mostly short-lived – activity peaks of microbial C and nitrogen mineralization (Birch, 1958; Borken & Matzner, 2009; Placella *et al.*, 2012). Nonetheless, repeated drying and rewetting of soils has been shown to foster a more drought-tolerant microbial community (de Vries *et al.*, 2012b; Fierer *et al.*, 2003), which could even succeed into a new baseline community structure (Evans & Wallenstein, 2014). Community changes might as well be accompanied by functional shifts (reviewed by Krause *et al.*, 2014), specifically when functionally narrow specialist microbes profit or are hampered by the altered conditions (Schimel & Schaeffer, 2012).

Indirectly, altered input of plant-derived C during drought could enforce direct effects of water limitation on soil microorganisms (Bardgett *et al.*, 2008). Specifically fungal mycorrhizae, which are also involved in water uptake of plants (Allen 2007), have been shown to receive plant-derived C even during extreme drought, while the uptake into bacterial groups was stronger reduced (Fuchslueger *et al.*, 2014). Such differences occurring during drought could possibly influence the resilience of the soil microbial community after drought and modulate its response to further drought periods. It is however still not clear whether recurring drought periods could substantially alter plant belowground C allocation and hence microbial community dynamics. We therefore aimed at investigating whether preceding and recurring drought periods have the potential to induce lasting imprints on the C uptake by plants and alter the C transfer pattern from plants to soil microbes, or if these dynamics are resilient and perform similar as in control plots receiving ambient amounts of precipitation. Towards this aim we conducted a ^{13}C pulse-labelling experiment under *in situ* conditions on repeatedly drought-stressed (drought-history) and ambient control plots in a mountain meadow in the Central Austrian Alps and chased the assimilated ^{13}C from plants over the extractable soil organic carbon pool, to soil microbial biomarker (phospholipid fatty acids). However, a naturally occurring dry spell shortly before and during the ^{13}C labelling-experiment significantly decreased the soil water content in both drought-treated and ambient plots. The occurrence of this short dry spell allowed us comparing responses of the plant-C allocation and the soil microbe consortium to short term moisture fluctuation in plots with drought-history and ambient meadow soils. We hypothesized to detect a lasting imprint of the recurring droughts on plants with drought history that will increase their susceptibility the dry spell lead to stronger

responses, such as decreased carbon uptake and belowground allocation, compared to plants in ambient plots. Moreover, we hypothesized that the soil microbial community in the studied grassland is highly resilient even after recurring droughts and responds to short term moisture fluctuations regardless of drought-history.

Material and Methods

Site description

The study area was located in the Austrian Central Alps near Neustift, Stubai Valley (47°07'45"N, 11°18'20"E). The sampling site was situated at 1850 m and part of a mountain meadow that is briefly grazed in spring and autumn, and aboveground plant biomass is cut annually for hay production. The vegetation was dominated by high productive perennial grasses and herbs (Bahn et al., 2006; Schmitt et al., 2010) (and high rates of soil respiration (Bahn et al., 2010). The mean annual temperature was 3°C and mean annual precipitation summed up to 1100 mm. The soil was classified as dystric cambisols (FAO-soil classification system) with a pH (in CaCl₂) of 5.4.

Experimental setup

We conducted a ¹³C pulse-labelling experiment on plots that were repeatedly exposed to extreme drought periods, which will in the following be referred to as 'drought-history' plots, as well as on ambient control plots, which received natural occurring amounts of precipitation. On drought-history plots rain-out-shelters ($n=3$) had been installed for eight to ten weeks between May and August in 2009, 2010 and 2011, respectively, excluding approximately one third of the annual precipitation. During these periods the total meadow area, including drought and controls plots, of the meadow was mown to sustain the traditional land-management. After eight to ten weeks of rain-exclusion all plots received 20 mm of previously collected rain-water over a period of ca. 3 min to simulate a heavy precipitation event, the rain-out shelters were removed and all plots received natural occurring precipitation. In 2011, rain-exclusion shelters had been re-installed on May 31. After ten weeks of rain exclusion plots were rewetted and rain-out shelters were removed on August 9; two weeks after rewetting aboveground sections of sets of drought and control plots were labelled with 99.9% ¹³CO₂ over a period of 90 minutes on three consecutive days with high photosynthetic active radiation (August 23-25, see Table 1; Figure 1 shows a chronological order of events). A detailed description of the labelling set-up and procedure is given in Bahn et al. (2013) and Fuchslueger et al. (2014).

Sampling of plant biomass and soil

To capture recovery dynamics of soil parameters after the simulated drought period soil sampling started one week after rewetting (August 16 2011). Sampling of plant material and soil continued during the dry spell, at the time of pulse labelling. Samples of plant biomass (shoots and fine roots) and soil were taken one hour before labelling, to determine the natural abundance of ^{13}C to calculate ^{13}C excess, and then after labelling (1.5, 3.5, 6.5, 25.5, 48, 96 and 144 h after ^{13}C pulse). During this sampling the dry spell was interrupted by a heavy precipitation event (on August 27 2011; between 48 and 96 h after the labelling pulse). Per sampling the material of two soil collars with an area of 5x7 cm and 10 cm soil depth were pooled. From each collar aboveground plant material (shoots) was cut and collected, the uppermost organic material removed and the soil was carefully sieved to 2 mm and manually freed from fine roots. Aliquots of soil samples were immediately frozen at -80°C for the determination of PLFAs, and the remaining soil was stored at 4°C until further processing. Fine root material was also collected, carefully washed and coarsely dried with paper towels. Aliquots of fine roots were further used to determine fine root respiration (for a detailed description see Hasibeder *et al.* 2014). Then shoots and remaining fine roots were treated by microwave for 3 min to interrupt metabolic activity (Popp *et al.* 1996). Plant material and soil aliquots were dried at 60°C for 72 h, weighed and finely ground for subsequent analyses of bulk ^{13}C , ^{15}N and total C and N contents by EA-IRMS (EA 1110 (CE Instruments, Milan, Italy), coupled to a Finnigan MAT Delta Plus IRMS (Thermo Fisher Scientific, Waltham, MA, USA)).

Determination of extractable organic carbon and soil microbial biomass

Extractable organic carbon (EOC) and total extractable nitrogen (N) fractions were determined in K_2SO_4 extracts (2 g of fresh soil was extracted with 20 ml of 0.5 M K_2SO_4) by a TOC/TN analyser (TOC-V CPH E200V/TNM-122V; Shimadzu, Vienna, Austria). The $\delta^{13}\text{C}$ of the EOC pool was measured by direct injection (without column, direct mode) and at a flow of $0.5\text{ ml water min}^{-1}$ on a HPLC (High Performance Liquid Chromatograph, Dionex Corporation, Sunnyvale, CA, USA) linked to a Finnigan Delta V Advantage Mass Spectrometer, connected by a Finnigan LC-IsoLink Interface (both Thermo Fisher Scientific, Waltham, MA, USA). NH_4^+ concentrations were measured photometrically from K_2SO_4 extracts using a modified indophenol reaction method (Kandeler & Gerber 1988). NO_3^- was measured from water extracts (2g of fresh soil were extracted with 20 ml of MilliQ water) by chemically suppressed ion-chromatography (DX500, Dionex, Austria) on a Dionex AS11 column. Extractable organic nitrogen (EON) was calculated by subtracting inorganic N pools (NH_4^+ and NO_3^-) from total extractable N.

Soil microbial biomass and microbial community composition, as well as microbial incorporation of plant-derived carbon were estimated by extracting phospholipid fatty acids (PLFAs) from frozen soil samples using the same procedure as described in Fuchslueger et al. (2014). Total lipids were extracted from soil using a chloroform/methanol/citric acid buffer and cleaned from neutral lipids. After an internal standard (19:0) was added PLFAs were converted to fatty acid methyl esters (FAMES) by alkaline methanolysis. Samples were analysed using a Trace GC Ultra connected by a GC-IsoLink to a Delta V Advantage Mass Spectrometer (all Thermo Fisher Scientific, Waltham, MA, USA). Samples were injected in splitless mode (injector temperature 220°C) and separated on a DB23 column (60 m x 0.25 mm x 0.25 µm; Agilent, Vienna, Austria) with 1.5 ml min⁻¹ He as the carrier gas (GC programme: 1.5 min at 70°C, 30°C min⁻¹ to 150°C, 1 min at 150°C, 4°C min⁻¹ to 230 °C, 15 min at 230 °C). FAMES were identified using mixtures of bacterial and fungal FAMES (bacterial acid methyl ester mix and 37 Comp. FAME Mix; Supelco). FAMES were quantified against the internal standard (19:0) and corrected for the methyl group that was added during methylation. We used the markers 18:1ω9, 18:2ω6,9, 18:3ω3,6,9 and 16:1ω5 for total fungi. However, 16:1ω5 is either a marker for arbuscular mycorrhizal fungi or for gram-negative bacteria (Zelles 1997; Olsson 2006). The sum of i15:0, a15:0, i16:0 a16:0 and a17:0 were used as gram-positive bacterial markers, and 16:1ω7, 18:1ω7, cy17:0(9/10) and cy19:0(9/10) as gram-negative bacteria. Gram-positive, gram-negative, as well as 15:0, 17:1ω6, 17:9, 18.1ω5, and 10Me18:0 markers were summed to give total bacterial PLFAs.

Calculation of ¹³C excess and turnover

The excess ¹³C, i.e. the pulse labelling-derived amount of ¹³C, (given in µmol or nmol ¹³C g⁻¹ dm soil) was calculated for the available carbon pools as

$$excess^{13}C = (atom\%_{sample} - atom\%_{nat.ab}) * \frac{C_{pool}}{100} \quad (1)$$

with $atom\%_{sample}$ describing the $atom\%$ ¹³C of the labelled sample, $atom\%_{nat.ab}$ representing the natural ¹³C abundance of these samples (taken before labelling), and C_{pool} being the C concentration of the respective sampled pool (i.e., shoots, fine roots, fine root respiration, EOC and PLFAs). The C turnover in plant, soil and microbial pools underlies different temporal dynamics. We therefore used different models to calculate the mean residence time of ¹³C in respective compartments. A detailed description of the different models is given in the supplement.

Statistical analyses

Effects of drought-history and moisture level on plant biomass, as well as on plant, soil and microbial carbon and/or nitrogen pools were assessed by two-way ANOVA, with a Tukey-HSD post-hoc test. If data did not meet ANOVA-assumptions they were either log-transformed or rank-normalized. To aggregate dynamics of microbial communities in relation to soil parameters during the dry spell and to assess possible differences between ambient controls and drought-history plots a canonical correspondence analysis (CCA) was conducted using the relative abundances of single PLFA markers as species matrix and available soil parameters (including SWC, T_{soil} at 10 cm, EOC, EON, C_{mic} , N_{mic} , NH_4^+ and NO_3^- concentrations) as environmental matrix. Effects of drought-history and short-term moisture variability on species (PLFA composition), as well as constrained scores (environmental matrix) scores were identified by two-way ANOVA. Differences in ^{13}C -turnover dynamics after pulse-labelling were analysed by two-way repeated measures ANOVA with treatment and time after labelling pulse as factors. In addition, paired t-tests were calculated for single time points after pulse-labelling (after Bonferroni-correction). All statistical analyses were performed in R.3.0.3 (R Development Core Team, 2013) using the vegan package to compute the CCA (Oksanen *et al.* 2013) and in Sigmaplot 11.

Results

Microclimatic parameters

During the first week after the end of rain-exclusion the meadow received in total 58 mm of precipitation, leading to an initial increase of the SWC from 15% to 39% in the drought-history plots, which was however still significantly lower than in ambient controls (Figure 1).

Subsequently, a short rain-free and warm period before pulse-labelling decreased the SWC from 65% to 25% in controls, and from 39% to 16% in drought-history plots, while T_{soil} at 10 cm soil depth increased significantly by 3 °C in both drought-history and ambient controls. After ten days of dry conditions a heavy precipitation event of 22mm, accompanied by a sudden fall in temperature induced a strong increase of the SWC at both sites. Because of these strong microclimatic dynamics we grouped our samples to three different categories: pre (after the rain exclusion, but before the dry spell), dry (during the dry spell) and wet (after the end of the dry spell; Figure 1 Table 2.)

Plant responses to moisture fluctuations related to drought-history effects

Shoot plant biomass was affected neither by drought-history, nor by the dry spell ranging between 212 and 249 g dry mass m⁻². Also fine root biomass was similar in drought-history (148 ±7 g dry mass m⁻²) and ambient control plots (144±7 g dry mass m⁻²), but significantly increased at both sites after the rain pulse (to 169±12 g dry mass m⁻² and 165±12 g dry mass m⁻², respectively; Table 2). Regardless of drought-history, plant shoot C contents decreased significantly at higher soil water levels, but fine root C contents remained unchanged. The N contents in contrast were significantly lower in shoots, but enriched in ¹⁵N, while in they were higher in fine roots with similar ¹⁵N signatures in drought-history plots as compared to ambient controls independent of fluctuating soil moisture levels. This consequently resulted in a higher shoot, but lower fine root C:N ratio in drought-history plots than in ambient controls (Figure 2, Table 2). In drought-history plots fine root respiration was significantly decreased during the dry spell compared to the ambient controls, indicating lower metabolic activity, but after the end of the dry spell this effect was negligibly smaller (Figure 2, Table 2).

The amount of ¹³C label taken up into shoots was significantly lower in post-drought than in ambient control plots. However, in both treatments the ¹³C peaked within the first 6.5 h after the labelling pulse, followed by an exponential decrease over time resulting in similar mean residence times (MRT) of ¹³C of about 69 h (Figure 4a, Table 3). In fine root biomass of drought-history plots the ¹³C peaked earlier (after 3.5 h), but the peak was significantly lower, and the MRT of ¹³C was 3.5 times longer than in ambient plots (Figure 4b, Table 3). 48 h after pulse-labelling the proportion of ¹³C detected in fine roots compared to total ¹³C in plant material (i.e. the sum of excess ¹³C in shoots and fine roots) was significantly lower in post-drought than in ambient plots (Figure 4c, Table 3). However, no ¹³C was detectable in fine root respiration in drought-history plots, while in ambient controls the ¹³C peaked already after 3.5 h after the labelling pulse and then followed a linear decrease (Figure 4d, Table 3).

Soil microbial and nutrient responses to moisture fluctuation related to drought-history effects

Extractable organic carbon (EOC) and nitrogen (EON) concentrations increased during the dry spell in drought-history but not in ambient plots. The imprint of drought-history on the concentrations of inorganic N pools (NH₄⁺ and NO₃⁻) was negligible: regardless of drought-history they strongly decreased during the dry spell and slightly increased after the rain pulse (Figure 3, Table 2). The soil PLFA content was not affected by drought-history, but responded dynamically to moisture fluctuations and was higher after the rain-pulse in both drought-history and ambient controls (Figure

3, Table 2). The fungi:bacteria ratio, in turn, was highest before, and decreased during the dry spell in both drought-history and ambient controls. While in ambient controls the ratio remained at a lower level after the rain pulse, in drought-history plots the fungi:bacteria ratio increased driven by a stronger increase of fungal biomarkers (Figure 3, Table 2).

A canonical correspondence analysis confirmed that the variations in moisture levels led to significant changes in microbial community composition estimated by PLFAs, while effects of drought-history were less pronounced. The environmental matrix (constrained variability) accounted for 57.5%, and the PLFA matrix (species scores) explained 42.5% of the total variability, of which within the species matrix the CCA axis 1 explained 49.2% and CCA axis 2 explained 3.9%. Drought-history had no significant effects on either environmental, nor species scores along CCA axes 1, but on environmental scores along CCA axis 2. The fluctuations of the moisture-level in turn significantly affected both environmental, and species distribution leading to three clusters according to moisture levels (pre, dry, wet, Figure 5, Table 4). The species scores indicated fungi were more abundant under ambient conditions before the dry spell, while bacterial, specifically gram-positive bacterial marker became more abundant under drier conditions regardless of drought-history. Even after the end of the dry spell under more moist conditions the community composition was different than before the short dry spell.

A biplot showed that T_{soil} ($R^2=0.48$, p^{***}), SWC ($R^2=0.44$, p^{***}), NH_4^+ ($R^2=0.41$, p^{***}) and EON ($R^2=0.13$, p^*) contributed most to the distribution of site scores. Fungal markers were related to higher EOC and EON concentrations, but also to higher SWC, while bacterial, specifically gram-positive marker tended to be dominant at lower SWC (Figure 5).

Effects of drought-history and moisture fluctuation on microbial turnover of recent plant carbon

In the EOC pool of both drought-history and ambient control plots the plant derived ^{13}C peaked 3.5 h after labelling and remained at this level for the next 45 h. After the rain pulse, however, the amount of ^{13}C decreased in drought-history plots, but not in ambient controls leading to an overall significant effect of drought-history on EOC ^{13}C concentrations (Figure 4, Table 3). In drought-history plots the plant-derived ^{13}C in the EOC pool remained slightly longer (138.9 h) than in ambient controls (123.5 h; Table 3).

The amounts of ^{13}C recovered in total PLFAs, as well as in all microbial groups (fungi_{tot}, gram-positive, gram-negative, as well as bacteria_{tot}), were similar in drought-history and ambient controls, but followed significant different temporal dynamics (Figure 6, Table 3). In drought-history plots the ^{13}C in soil microbial groups increased initially only slowly, but after the rain pulse

(between 96 and 144 h after the labelling pulse) steeply increased. In ambient controls, in contrast, the ^{13}C peak time was more variable, ranging between 3.5 and 48 h after the labelling-pulse, and the MRT of ^{13}C in the microbial groups was ranging from 66.7 h in fungi up to 332.6 h in gram-positive bacteria (Figure 6, Table 2).

Discussion

Effects of drought-history on the response of plant carbon turnover during a dry spell

Even three recurring severe drought periods did not induce lasting imprints on either biomass and or carbon content of plant shoots compared to ambient controls. Moreover plant biomass did not respond to the short-term water shortages during the dry spell or to the subsequent heavy rainfall event. This indicates a high resistance of the present plant community against alterations in water availability in the studied grassland. Nonetheless, we cannot rule out that plants in post-drought plots may have acclimated to low soil moisture by producing thicker leaves and increasing their leaf mass density thereby reducing the specific leaf area (SLA; i.e. Leaf area per unit leaf mass) and leaf water content, without changing their total biomass (Jentsch *et al.*, 2011; Lambers *et al.*, 2008; Reich *et al.*, 1997).

However, the N content was lower in shoots and higher in fine roots in plants with drought-history compared to ambient controls, which could hint to physiological adaptations of plants to recurring droughts. Despite the fact that at lower water content diffusion and substrate supply towards roots may have been restricted (Chaves *et al.* 2002), in soils with drought history the concentrations of extractable organic and inorganic N were either unaffected or tended to be increased compared to ambient soils. The higher N content in fine roots of plants with drought-history therefore suggests that plants may not have been limited in N available in the soil, or restricted in N uptake (but see Durand *et al.*, (2010)). On the other hand, plants may have allocated N from shoots to belowground organs to store and produce N-containing osmolytic compounds to enhance water or nutrient uptake (Miller 2010). Alternatively, less N may have been allocated from fine roots over the xylem to leaves and shoots, due to stomata closure to reduce evapotranspiration and water loss (Chaves *et al.* 2003; Reyer *et al.* 2013). Plant leaf N concentrations have been shown to correlate with photosynthesis, as large amounts of N are invested and stored in the Ribulose 1-5-Bisphosphate oxygenase/carboxylase (RuBisCO) protein. Thus, the translocation of N from soil to photosynthetic tissues is crucial to sustain photosynthesis and plant growth (Pons & Pearcy 1994; Chaves *et al.* 2002; Lambers *et al.* 2008; Miller 2010). RuBisCO concentrations were found to be significantly correlated with carbon assimilation globally (meta-analysis by Walker *et al.* (2014), as well as more specifically in mountain grasslands (Wohlfahrt *et al.*, 1999). Thus, the lower shoot N

content observed in post-drought plots could therefore indicate drought-induced reductions of carbon assimilation (Chaves *et al.* 2003; He & Dijkstra 2014), which can be underpinned by the reduced uptake of ^{13}C in plant shoots during pulse-labelling. However, the altered N distribution within drought-history plants could also indicate shifts of the plant species composition in the studied meadow.

Despite lower uptake of ^{13}C into shoot biomass, the proportion of ^{13}C translocated to fine roots was lower in drought-history compared to ambient controls. This is in contrast to other studies in grassland systems, where during drought plants have been shown to either increase (Burri *et al.*, 2014; Sanaullah *et al.*, 2012) or at least maintain the proportion of carbon allocated belowground (Hasibeder *et al.* 2014), likely related to a higher sink demand of fine roots for recent carbon (Cramer *et al.*, 2009). However, in drought-history plots fine root respiration rates were significantly lower during the dry spell and contained almost no recently assimilated C indicating decreased metabolic activity. This indicates that root respiration of plants with drought-history plots was mainly fuelled by carbon compounds stored before labelling (at least older than 144h) and suggests a reduced belowground sink for newly assimilated carbon. Nonetheless, recently assimilated C translocated belowground, could have been allocated to osmotic compounds (Chaves *et al.* 2002) or being exuded as rhizodeposits.

Effects of drought-history on the response of soil and soil microbial carbon turnover during a dry spell

Drought-history affected the response of extractable organic carbon and nitrogen pools visible as increased concentrations during and after the dry spell. Increased dissolved organic matter concentrations during dry periods have often been related to decreased microbial activity because of limited substrate diffusion or higher microbial mortality (Fierer *et al.*, 2003; Manzoni *et al.*, 2012a; Schimel *et al.*, 2007). It is therefore likely that extractable organic material, including extracellular enzymes (Manzoni *et al.*, 2014), or recent plant derived carbon (Fuchslueger *et al.* 2014) may have accumulated during dry periods. In our study however less plant derived ^{13}C was detectable in the EOC pool. Nonetheless in drought-history plots, the proportion of ^{13}C in EOC was comparably high related to the amount of ^{13}C detected in fine roots. This suggests that plants could have increased root exudation or the production of protective or osmotic mucilage (Manzoni & Katul, 2014; Walker *et al.*, 2003) or may have had a higher fine root biomass turnover (Pregitzer, Hendrick & Fogel 1993).

Alternatively, the activity of microbes could have been decreased or substrate diffusion towards microbes could have been discontinued during the dry spell (Manzoni *et al.*, 2014; Moyano

et al., 2013; Schimel & Schaeffer, 2012). This was corroborated by a lower incorporation of plant derived ^{13}C into microbial, in particular into bacterial groups during the first 48 hours after the labelling pulse in drought-history plots, whereas in ambient controls ^{13}C was incorporated and turned over faster in PLFAs even during dry conditions. In drought-history plots the rain pulse, which occurred between 48 and 96 hours after labelling, led to a strong increase of ^{13}C in all microbial groups and the accumulated plant derived carbon became available and/or microbes regained activity (Or *et al.* 2007; Borken & Matzner 2009; Placella *et al.* 2012; Barnard, Osborne & Firestone 2013). However, the amount and response of total PLFAs was similar regardless of drought-history, therefore the preceding drought apparently had induced lasting imprints on the microbial turnover of plant-derived carbon. The inorganic N pools, in contrast, also responded to short term-variations of soil moisture, but showed no imprint of drought-history. During the dry spell particularly NH_4^+ concentrations decreased significantly regardless of drought-history, which could indicate either decreased microbial production rates or increased plant or microbial uptake, e.g. to produce osmotic compounds (Schimel et al., 2007). Thus, drought-history appears to distinctly affect carbon and nitrogen cycling in soils.

At the onset of the dry spell the microbial community composition might not have fully recovered from the preceding extreme summer drought, indicated by lower fungal marker abundances in soils with drought-history compared to ambient control plots. This could indicate that, although fungi are considered to be more resistant to drought (Allen 2007; Yuste *et al.* 2011), when they eventually are affected by drought they may recover more slowly than e.g. bacterial groups, which may be more susceptible to drought, but also recover faster (Allison & Martiny, 2008; de Vries et al., 2012). However, the dry spell induced highly similar responses irrespectively of drought-history, suggesting that the drought-history may not have improved or hampered the fitness of certain microbial taxa. Thus, the microbial community in the studied grassland appears to be to a certain extent resilient to drought (Fuchslueger *et al.* 2014), but similar to other multi-year irrigation manipulation studies (e.g Cruz-Martínez *et al.* 2009; Landesman & Dighton 2010) the microbial community composition was more strongly affected by short-term moisture variations than by drought-history. However, in the longer term (after more than ten years), repeated drying-rewetting cycles could induce shifts of microbial taxa: in prairie soils with drought-history for examples drought-tolerant bacterial taxa were found to become more abundant, while opportunistic and drought-sensitive taxa decreased (Evans & Wallenstein, 2014).

Implications and Conclusion

- We conclude from our study that recurring drought periods induced lasting effects on plant carbon uptake and turnover that could be driven by limiting N allocation from below- to aboveground plant compartments, restricting photosynthetic activity. Moreover, we conclude that plants with drought-history shifted their belowground carbon allocation pattern and used preferentially ‘older’ carbon compounds to fuel root metabolic activities.
- Belowground carbon and microbial community composition in turn seemed to be more responsive to short-term moisture variations and less sensitive to the long-term drought-history.

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Figures and Tables:

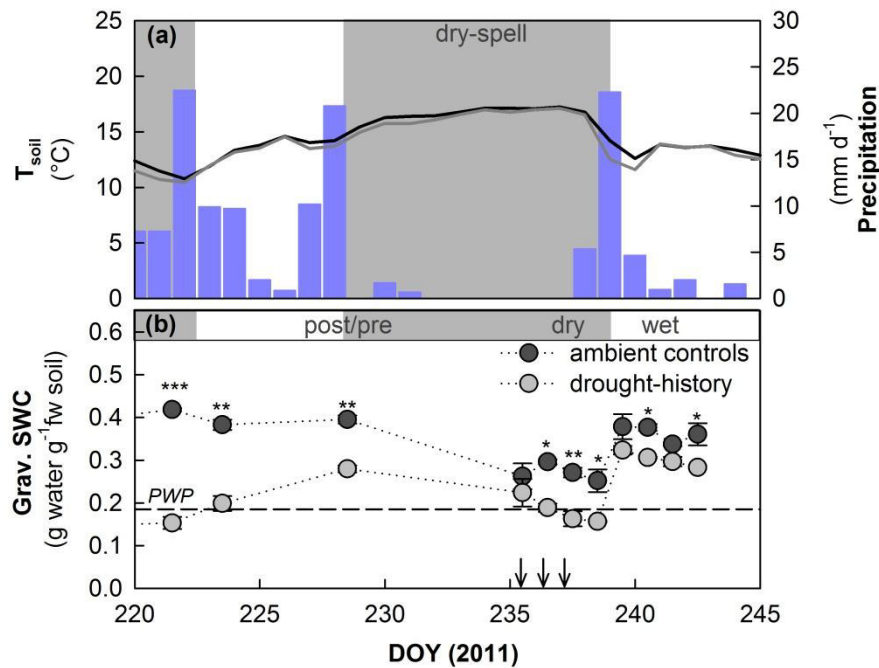


Figure 1: Microclimatic conditions during the experimental period. **(a)** Daily means of soil temperature in 10 cm soil depth in ambient control (black line) and drought-history plots (grey line) given in $^{\circ}\text{C}$ (T_{soil}) and daily sums of precipitation in mm (blue bars). **(b)** Mean gravimetrically determined soil water content (SWC, $\text{g water g}^{-1} \text{fw soil}$) in ambient control plots (black circles) and plots with drought-history (grey circles); ($n=3$, $\pm\text{SE}$); differences at single time points were assessed by paired t-test after a Bonferroni correction (asterisks mark levels of significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$). The dashed line marks the SWC equalling to the permanent wilting point (PWP) for plants at a pF of 4.2. Arrows indicate three subsequent days of ^{13}C pulse-labelling. The grey background marks periods of low soil water content either because of experimental rain-exclusion, or induced by the naturally occurring dry spell.

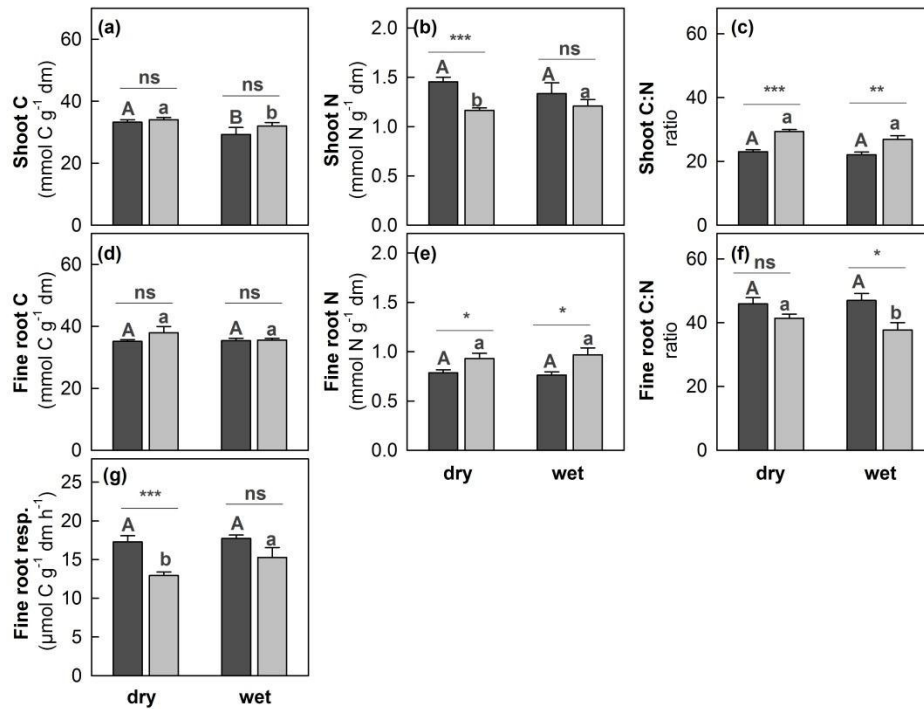


Figure 2: Plant parameters at ambient control (black bars) and drought-history plots (grey bars) during (dry) and after (wet) a short, naturally occurring dry spell. (a) Mean plant shoot carbon and (b) nitrogen contents, and (c) shoot C:N ratios, as well as (d) fine root carbon (e) and nitrogen contents and fine root C:N ratios, and (i) fine root respiration rates (dry: $n=15$; wet: $n=9$) for wet conditions; $\pm$ SE). Asterisks mark differences between ambient controls and drought-history at single sampling times (ns, not significant; * $p<0.05$; ** $p<0.01$); differences at a 0.05 significance level between dry and wet conditions within ambient controls are marked with upper case letters, while differences within drought-history are marked with lower case letters; see Table 2 for more statistical details

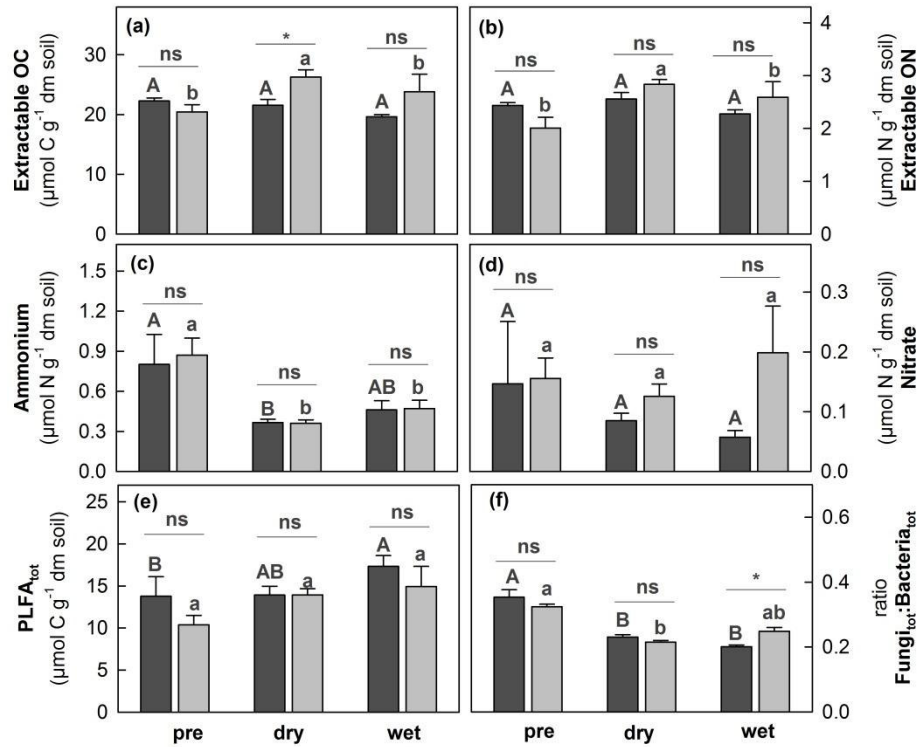


Figure 3: Soil parameters in ambient controls (black) and drought-history (grey) plots before (pre), during (dry) and after (wet) a short, naturally occurring dry spell. Means of (a) soil extractable organic carbon and (b) extractable organic nitrogen, (c) ammonium and (d) nitrate concentrations, as well as (e) total microbial phospholipid fatty acids (PLFA_{tot}) and the (f) ratio of fungal (Fungi_{tot}) to bacterial PLFA (Bacteria_{tot}) markers were determined (pre: n=3; dry: n= 15; wet: n=9). Asterisks mark differences between ambient controls and drought-history at single sampling times (ns not significant; *p<0.05; **p<0.01); differences at a 0.05 significance level between pre, dry and wet conditions within ambient controls are marked with upper case letters, while differences within drought-history are marked with lower case letters; see Table 2 for more statistical details.

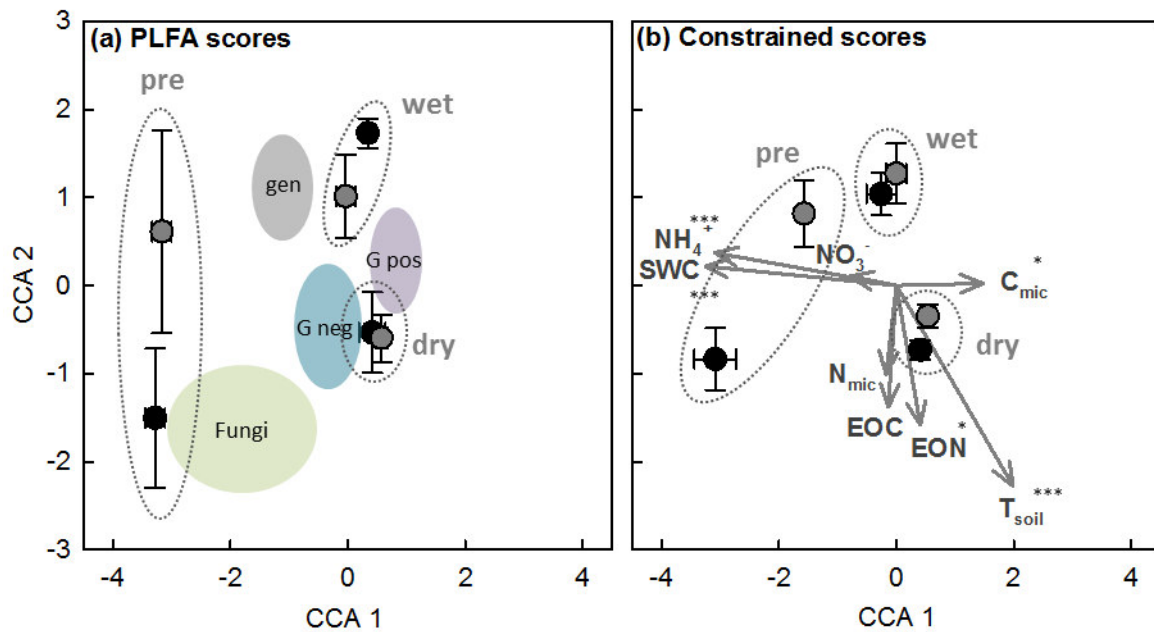


Figure 4: (a) Site scores of soil microbial community composition and (b) biplot (vectors and site scores) of the constraining environmental factors determined by canonical correspondence analysis (CCA). Relative abundances of PLFAs were used as community matrix and combined with an environmental matrix including T_{soil} (Soil temperature at 10 cm soil depth; $R^2 = 0.48$), SWC (gravimetric soil water content; $R^2 = 0.44$), EOC (extractable organic C; $R^2 = 0.08$), EON (extractable organic N; $R^2 = 0.13$), C_{mic} (microbial C; $R^2 = 0.08$), N_{mic} (microbial N; $R^2 = 0.04$), NH_4^+ ($R^2 = 0.41$) and NO_3^- concentrations ($R^2 = 0.03$), asterisks mark significant vectors (* $p < 0.05$; *** $p < 0.001$). The averaged distribution of microbial groups ($\pm$ SE) in (a) is indicated by different colours (Fungi: green; Gram-positives: purple; Gram-negatives: blue; general markers: grey). Site scores for (a) PLFA distribution and (b) environmental constraints of ambient controls (black circles) and drought-history plots (grey circles) cluster to three groups (pre, dry and wet). Further information is given in Table 3

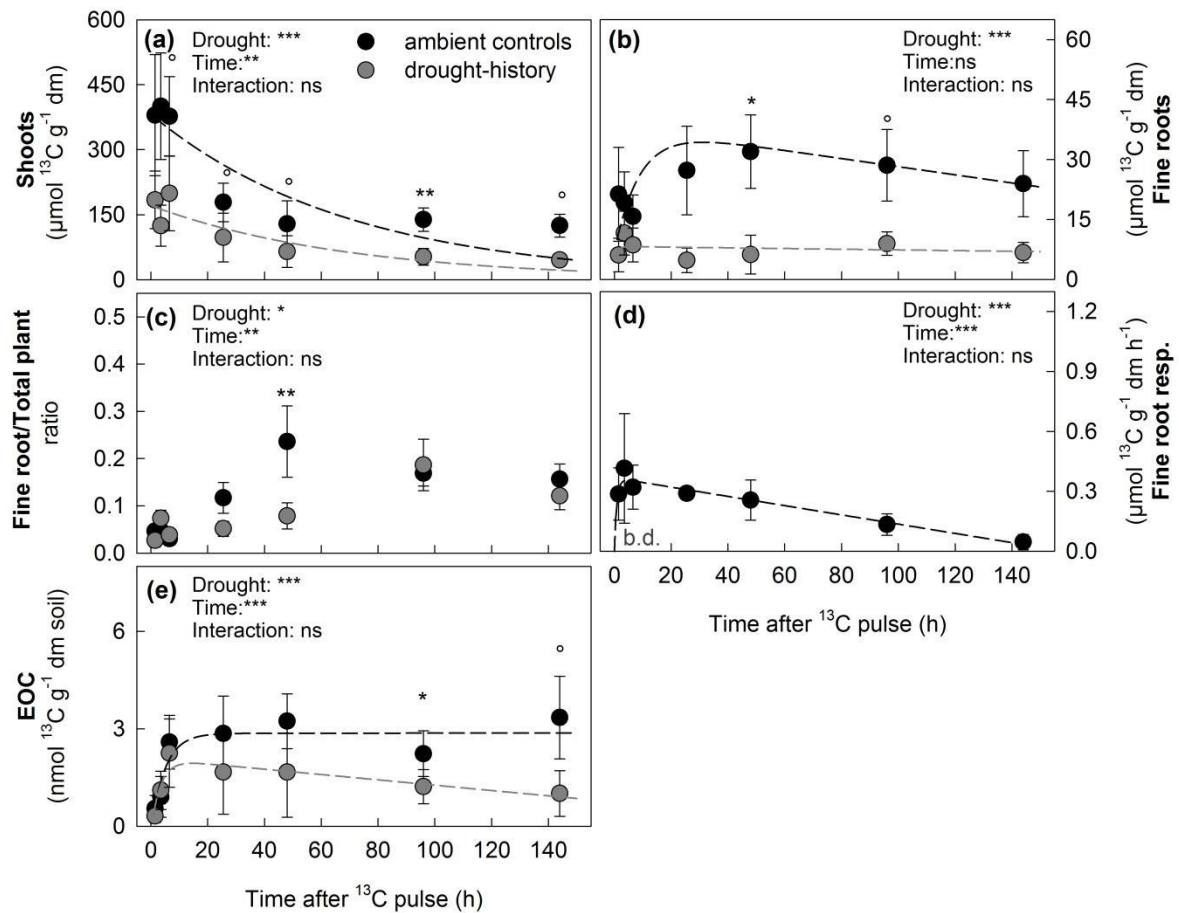


Figure 5: Pulse labelling derived ^{13}C (i.e. excess ^{13}C) in (a) plant shoots and (b) fine root biomass, as well as (c) the proportion of ^{13}C excess in fine roots per total plant ^{13}C excess (sum of shoot and fine roots); moreover ^{13}C excess in (d) fine root respiration and in the (e) soil extractable organic C pool in ambient controls (black circles) and drought-history plots (grey circles) (means; $n=3$, $\pm\text{SE}$). Nonlinear regressions (dashed lines) describe the ^{13}C dynamics in ambient controls (black) and drought-history plots (grey). Note that in (d) fine root respiration of drought-history plots the excess ^{13}C was below the detection limit (b.d.). Effects of drought-history and time after labelling was assessed by two-way repeated measures ANOVA; Asterisks mark significant differences at single labelling points computed by paired t-tests (Bonferroni corrected; $^{\circ}p<0.1$, $*p<0.05$, $**p<0.01$; $***p<0.001$). See Table 4 and Table S1 in the supplementary information for detailed statistical information and model fittings, respectively.

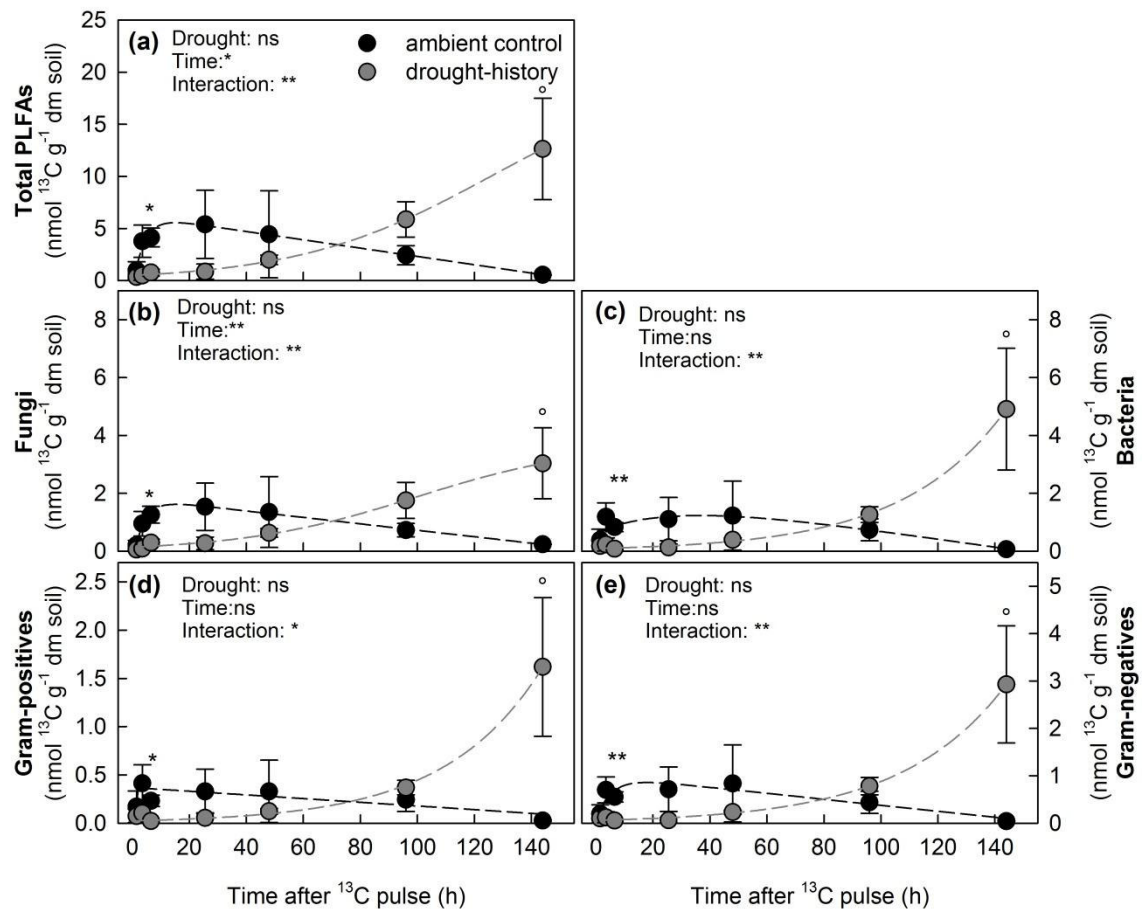


Figure 6: Pulse labelling derived ^{13}C (i.e. excess ^{13}C) in (a) total PLFAs and separately for (b) total fungal and (c) total bacterial biomarker. Bacterial marker were further divided into (d) Gram-positive and (e) Gram-negative PLFAs in ambient controls (black circles) and drought-history plots (grey circles); (means; $n=3$, $\pm\text{SE}$). Nonlinear regressions (dashed lines) describe the ^{13}C dynamics in ambient controls (black) and drought-history plots (grey). Effects of drought-history and time after labelling was assessed by two-way repeated measures ANOVA; Asterisks mark significant differences at single labelling points computed by paired t-tests (Bonferroni corrected; $^{\circ}p<0.1$; $*p<0.05$, $**p<0.01$; $***p<0.001$). See Table 4 and Table S1 in the supplementary information for detailed statistical information and model fittings, respectively.

Table 1: Microclimatic conditions during ^{13}C pulse-labelling. *Date* and time of the day (*Time*) of $^{13}\text{CO}_2$ pulse-labelling for *control* and *post-drought* plots, respectively. $[\text{CO}_2]_{\text{start}}$ and $[\text{CO}_2]_{\text{labelling}}$ show the CO_2 -concentrations in ppm in the labelling-chamber before, and during labelling. $^{13}\text{CO}_2$ describes the average isotopic composition in atom% during labelling analysed by Quantum cascade laser (QCL, Aerodyne Research Inc., Billerica, MA, USA); the average amount of $^{13}\text{CO}_2$ added is given in ml min^{-1} . Photosynthetic active radiation (PAR in $\mu\text{mol m}^{-2}\text{s}^{-1}$) during labelling as well as air temperature inside (T_{in}) and outside (T_{out}) the chambers are displayed (minimum – maximum temperature, in $^{\circ}\text{C}$). CO_2 concentration in the labelling chamber was monitored using an infrared-gas-analyser (EGM-4, PP Systems, Hitchin, UK).

Date		Time	$[\text{CO}_2]_{\text{start}}$	$[\text{CO}_2]_{\text{label}}$	$^{13}\text{CO}_2$	$^{13}\text{CO}_2_{\text{add}}$	PAR	T_{out}	T_{in}
23.08.2011	<i>drought-hist.</i>	11:25	280	491 ± 31	23.5 ± 1.7	30.3	1539–1820	18.8–24.2	22.8–33.2
	<i>ambient</i>	11:55	216	554 ± 28	22.9 ± 1.9	43.2	1539–1820	20.5–24.4	24.9–30.1
24.08.2011	<i>drought-hist.</i>	10:32	237	499 ± 39	18.4 ± 2.7	13.5	1500–1730	26.0–30.9	26.7–37.7
	<i>ambient</i>	11:09	248	556 ± 5	23.2 ± 1.3	37.9	1500–1730	26.0–28.8	28.8–39.9
25.08.2011	<i>drought-hist.</i>	10:24	253	509 ± 18	22.6 ± 1.4	23.1	1320–1670	15.7–24.1	15.7–24.1
	<i>ambient</i>	10:54	256	496 ± 28	19.0 ± 1.9	21.4	1320–1730	14.9–25.8	14.9–25.8

Table 2: Average plant biomass and total carbon and nitrogen contents of plant biomass, as well as different soil C and N pools, and soil microbial biomass assessed by phospholipid fatty acid (PLFA) analysis at the time of pulse labelling in undisturbed controls and post-drought treated plots. Differences between treatments were determined by two-way ANOVA (ns, not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). SWC, gravimetric soil water content upper 10 cm; T_{soil} , soil temperature at 10 cm soil depth; BM, Biomass; C_{tot} , N_{tot} , total C, total N content; EOC, extractable organic C; EON extractable organic N; NH_4^+ , Ammonium; NO_3^- , Nitrate; PLFA_{tot} , total phospholipid fatty acids; $\text{Fungi}_{\text{tot}}$, total fungal PLFA markers; $\text{Bacteria}_{\text{tot}}$, total bacterial PLFA markers; Gram-positive, Gram-negative, gram positive or gram negative bacterial PLFA markers; Fungi:Bacteria, the ratio of total fungal to bacterial PLFA markers.

Plant parameters	<i>(n=21)</i>	Drought-history		Moisture-level		Interaction	
		F(1)	p	F(1)	p	F(1,1)	p
Shoot BM	g dw m ⁻²	1.0	ns	0.8	ns	0.1	ns
Fine root BM	g dw m ⁻²	0.1	ns	5.9	*	0.1	ns
Shoot C_{tot}	mmol C g ⁻¹ dw	1.9	ns	7.4	**	0.1	ns
Shoot N_{tot}	mmol N g ⁻¹ dw	19.1	***	0.5	ns	1.9	ns
Shoot $\delta^{15}\text{N}$	‰	14.1	***	1.1	ns	0.5	ns
Shoot C:N	ratio	60.4	**	4.7	*	1.9	ns
Fine root C	mmol C g ⁻¹ dw	2.4	ns	0.4	ns	0.9	ns
Fine root N	mmol N g ⁻¹ dw	15.5	***	0.1	ns	1.1	ns
Fine root $\delta^{15}\text{N}$	‰	1.2	ns	0.1	ns	0.1	ns
Fine root C:N	ratio	10.7	***	0.4	ns	1.4	ns
Fine Root Resp.	μmol C g ⁻¹ dw h ⁻¹	23.6	***	2.8	°	1.3	ns
Soil parameters	<i>(n=24)</i>	F(1)	p	F(2)	p	F(1,2)	p
SWC	%	79.4	***	92.1	***	10.0	***
T_{soil}	°C	23.6	***	59.9	***	0.9	ns
Soil C_{tot}	%	0.1	ns	0.5	ns	0.1	ns
Soil N_{tot}	%	0.3	ns	0.5	ns	0.1	ns
EOC	μmol C g ⁻¹ dw soil	9.6	**	1.8	ns	1.5	ns
EON	μmol N g ⁻¹ dw soil	2.6	ns	3.9	*	2.1	ns
NH_4^+	μmol N g ⁻¹ dw soil	0.1	ns	15.4	***	0.2	ns
NO_3^-	μmol N g ⁻¹ dw soil	7.6	**	0.2	ns	1.0	ns
PLFA_{tot}	μmol C g ⁻¹ dw soil	1.4	ns	13.3	***	1.6	ns
$\text{Fungi}_{\text{tot}}$	μmol C g ⁻¹ dw soil	0.9	ns	12.2	***	0.2	ns
$\text{Bacteria}_{\text{tot}}$	μmol C g ⁻¹ dw soil	1.6	ns	6.1	**	1.4	ns
Gram-positive	μmol C g ⁻¹ dw soil	0.9	ns	0.5	ns	1.6	ns
Gram-negative	μmol C g ⁻¹ dw soil	1.3	ns	12.0	***	1.3	ns
Fungi:Bacteria	ratio	0.1	ns	16.9	***	10.1	***

Table 3: Species and constrained score distributions for CCA axis 1 and CCA axis 2 were evaluated by two-way ANOVA using drought-history (drought-history and ambient controls) and moisture level (dry, wet) as grouping factors. Asterisks mark level of significance: ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	Species scores				Constrained scores			
	CCA1		CCA2		CCA1		CCA2	
	F	p	F (1)	p	F	p	F	p
Drought-history	0.27	ns	0.25	ns	1.62	ns	6.231	*
Moisture-level	23.84	***	11.16	*	24.06	***	26.84	***
Interaction	0.41	ns	3.40	*	0.19	ns	2.25	ns

	Two-way repeated measures ANOVA						Ambient Controls ¹³ C dynamics						Drought-history ¹³ C dynamics					
	Treatment F(1)	p	Time F(5)	p	Interaction F(1,5)	p	Amount at ¹³ C peak		Peak time (h)	Model type	Rate constant k	MRT (1/k) (h)	Amount at ¹³ C peak		Peak time (h)	Model type	Rate Constant k	MRT (1/k) (h)
							Mean	±SE					Mean	±SE				
Shoot BM	14.24	***	5.12	**	0.27	ns	400.3	122.8	3.5	exp	0.0143	69.9	199.0	86.2	6.5	exp	0.0147	69.8
Fine root BM	76.22	***	1.60	ns	2.47	ns	32.0	9.2	48.0	exp-lin	0.1017	9.8	11.6	5.5	3.5	exp-lin	0.0087	33.7
Fine Root Resp.	209.88	***	8.21	***	2.39	ns	0.4	0.2	3.5	exp-lin	0.0023	434.8				na		
EOC	29.83	***	12.16	***	2.37	ns	6.9	3.7	48.0	exp-lin	0.0081	123.5	2.3	1.1	6.5	exp-lin	0.0072	138.9
PLFA _{tot}	0.95	ns	2.50	*	4.35	**	5.4	0.7	25.5	exp-lin	0.0409	24.5	12.6	4.9	144	exp	0.0240	na
Fungi _{tot}	2.00	ns	3.68	**	4.17	**	1.5	0.8	25.5	exp-lin	0.0150	66.7	3.0	1.2	144	exp	0.0208	na
Bacteria _{tot}	0.31	ns	2.03	ns	4.32	**	1.2	0.5	48.0	exp-lin	0.0086	116.3	4.9	2.1	144	exp	0.0426	na
Gram-positive	0.70	ns	1.58	ns	3.33	*	0.4	0.2	3.5	exp-lin	0.0031	332.6	1.6	0.7	144	exp	0.0479	na
Gram-negative	0.42	ns	2.38	ns	4.47	**	0.7	0.5	25.5	exp-lin	0.0085	117.6	2.9	1.2	144	exp	0.0416	na

Table 4: Effects of drought-history and Time after labelling on ¹³C dynamics in plant, soil and soil microbial carbon pools. Differences between drought-history and ambient control plots were assessed by two-way repeated-measures ANOVA, using drought treatment and time as factors and plot numbers as within-factor ($n=3$). Significant effects are marked by asterisks (ns, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$). For both ambient controls and drought-history plots the time-point and amount of ¹³C excess peak after pulse-labelling are given for plant shoots and fine roots (in $\mu\text{mol } ^{13}\text{C g}^{-1} \text{ dm}$), fine root respiration ($\mu\text{mol } ^{13}\text{C g}^{-1} \text{ dm h}^{-1}$), extractable organic carbon (EOC) and phospholipid fatty acids (PLFA_{tot} both in $\text{nmol } ^{13}\text{C g}^{-1} \text{ dm}$); Total PLFAs were separated to fungal (Fungi_{tot}) and bacterial (Bacteria_{tot}), which were further divided into Gram-positive and Gram negative bacterial markers. Rate constant of ¹³C turnover (k) and mean residence times (1/k; given in h) of ¹³C were determined by nonlinear regressions (*exp*: exponential decay/increase; *exp-lin*: combination of exponential and linear regression; see Table S1 in the supplementary information for a more detailed description of the different models).

Supplementary information:**Calculation of ^{13}C turnover times and description of the different models:**

Plant shoots are the primary compartment to take up ^{13}C , thus after a labelling pulse they follow a typical exponential decay curve dominated by net ^{13}C export (Epron et al., 2012; Studer et al., 2014), as ^{13}C can be transferred to other plant compartments or is lost through respiration:

$$N(t) = N_0 * e^{(-k*t)} \quad (\text{Exponential decay}) \quad (1)$$

with N_0 is the amount of label at the ^{13}C peak 1.5 hours after labelling, k is decay constant and t is time after the labelling pulse.

The other compartments (plant roots, root respiration, EOC, and microbial marker) follow a different pattern, which is more similar to label dynamics during continuous labelling (Studer et al. 2014). After the labelling pulse the ^{13}C accumulates after a certain lag-phase either exponentially, or follows a sigmoidal curve to a peak or a plateau and then decreases either exponentially or linearly (Epron et al. 2012; Studer et al. 2014):

$$N(t) = N_0 + a * e^{(-k_1*t)} + k_2 * t \quad (\text{Exponential increase with linear decrease}) \quad (2)$$

with N_0 is the ^{13}C pool at time 0, a is the peak amount of ^{13}C , k_1 is an accumulation constant during the accumulation phase, k_2 is the decay constant after the ^{13}C label peak, and t is the time after the labelling pulse. To determine the mean residence time we only considered the decay constant k_2 . As in drought-history plots the microbial PLFA markers were still in the accumulation period we alternatively used an exponential increase curve to determine the mean accumulation time:

$$N(t) = N_0 * e^{(k*t)} \quad (\text{Exponential increase}) \quad (3)$$

with N_0 describing the initial amount of ^{13}C , k is the accumulation constant, t is the time after labelling. As the peak was not yet reached no mean residence time was calculated.

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Table S1: Nonlinear regressions describing the turnover of ^{13}C through the plant-soil system that were used for the determination of mean residence times of ^{13}C in sampled compartments. Asterisks mark levels of significance ($^{\circ}\text{p}<0.0.1$; $^*\text{p}<0.05$; $^{**}\text{p}<0.01$; $^{***}\text{p}<0.001$).

	Ambient controls				Drought-history			
	function	R ²	F	p	function	R ²	F	p
Shoots	$N(t)=381.7*\exp^{(-0.014*t)}$	0.44	15.4	***	$N(t)=170.9*\exp^{(-0.015*t)}$	0.30	9.7	**
Fine roots	$N(t)=38.4-31.7*\exp^{(-0.115*t)}-0.1017*t$	0.34	3.4	*	$N(t)=8.3-8.3*\exp^{(-1.21*t)}-0.0087*t$	0.16	1.4	ns
Root Resp.	$N(t)=0.4-0.4*\exp^{(-1.175*t)}-0.0023*t$	0.36	3.9	*	na			
EOC	$N(t)=2.9-3.0*\exp^{(-0.204*t)}-0.0081*t$	0.45	5.4	**	$N(t)=2.0-2.2*\exp^{(-0.314*t)}-0.0072*t$	0.20	1.78	ns
PLFA _{tot}	$N(t)=6.4-6.6*\exp^{(-0.209*t)}-0.041*t$	0.29	2.8	ns	$N(t)=0.38*\exp^{(0.024*t)}$	0.69	48.5	***
Fungi _{tot}	$N(t)=2.3-2.4*\exp^{(-0.155*t)}-0.015*t$	0.41	4.6	*	$N(t)=0.15*\exp^{(0.021*t)}$	0.66	42.2	***
Bacteria _{tot}	$N(t)=1.5-1.4*\exp^{(-0.270*t)}-0.009*t$	0.19	1.7	ns	$N(t)=0.01*\exp^{(0.043*t)}$	0.66	43.0	***
Gram-pos.	$N(t)=0.5-0.5*\exp^{(-0.228*t)}-0.003*t$	0.22	1.9	ns	$N(t)=0.002*\exp^{(0.048*t)}$	0.63	38.0	***
Gram-neg.	$N(t)=1.3-1.2*\exp^{(-0.144*t)}-0.009*t$	0.29	2.7	ns	$N(t)=0.007*\exp^{(0.041*t)}$	0.67	45.5	***

Chapter 3

“Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland”

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Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland

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Abstract. Future climate scenarios suggest an increased frequency of summer drought periods in the European Alpine Region. Drought can affect soil nitrogen (N) cycling, by altering N transformation rates, as well as the abundances of ammonia-oxidizing bacteria and archaea. However, the extent to which drought affects N cycling under in situ conditions is still controversial. The goal of this study was to analyse effects of drought on soil N turnover and ammonia-oxidizer abundances in soil without drought history. To this end we conducted rain-exclusion experiments at two differently managed mountain grassland sites, an annually mown and occasionally fertilized meadow and an abandoned grassland. Soils were sampled before, during and after drought and were analysed for potential gross rates of N mineralization, microbial uptake of inorganic N, nitrification, and the abundances of bacterial and archaeal ammonia-oxidizers based on gene copy numbers of the *amoA* gene (AOB and AOA, respectively).

Drought induced different responses at the two studied sites. At the managed meadow drought increased NH_4^+ immobilization rates and NH_4^+ concentrations in the soil water solution, but led to a reduction of AOA abundance compared to controls. At the abandoned site gross nitrification and NO_3^- immobilization rates decreased during drought, while AOB and AOA abundances remained stable. Rewetting had only minor, short-term effects on the parameters

that had been affected by drought. Seven weeks after the end of drought no differences to control plots could be detected. Thus, our findings demonstrated that in mountain grasslands drought had distinct transient effects on soil nitrogen cycling and ammonia-oxidizers, which could have been related to a niche differentiation of AOB and AOA with increasing NH_4^+ levels. However, the effect strength of drought was modulated by grassland management.

1 Introduction

Soil water availability is a key factor for physiological processes determining plant productivity and the activity of soil microorganisms (Knapp et al., 2002; Moyano et al., 2013; Stark and Firestone, 1995). Increasing frequencies of drought periods and heavy rainfall events, as predicted for the European Alpine Regions (Gobiet et al., 2014; IPCC, 2007, 2012; Schär et al., 2004; Seneviratne et al., 2006), will lead to strong soil water imbalances and can therefore affect ecosystem nutrient cycling, such as the nitrogen (N) turnover in the soil. Nitrogen is the major growth-limiting nutrient in most non-fertilized terrestrial ecosystems (LeBauer and Treseder, 2008), and its turnover in soils is mainly controlled by microbial processing, such as fixation of atmospheric N, decomposition of organic N sources, as well as uptake and release of

ammonium (NH_4^+) and nitrate (NO_3^-) during mineralization and nitrification processes (Booth et al., 2005; Schimel and Bennett, 2004). These individual steps in soil N cycling occur on different temporal and spatial scales (Schimel and Bennett, 2004) and have been shown to strongly differ in their response, both direction and magnitude, to drought (e.g. Aueyung et al., 2012; Chen et al., 2011; Emmett et al., 2004; Gleeson et al., 2010; Stark and Firestone, 1996).

Nitrogen mineralization and microbial immobilization of NH_4^+ and NO_3^- can be termed as “broad” processes combining multiple distinct pathways performed by a wide range of microorganisms covering a large spectrum of ecophysiological optima (Schimel and Schaeffer, 2012). “Broad” processes could therefore be more stable to drought than “narrow” ones, such as nitrification (Allison and Martiny, 2008; Schimel and Schaeffer, 2012). Nitrification, the oxidation of ammonia (NH_3) via nitrite (NO_2^-) to nitrate (NO_3^-), has long been expected to be solely performed by a highly specialized group of autotrophic bacteria (Kowalchuk and Stephen, 2001; Schimel et al., 1989), but also archaea of the phylum thaumarchaeota conduct the first step of nitrification, the conversion of NH_3 to hydroxylamine (NH_2OH) (Könneke et al., 2005). This step is catalyzed by the enzyme ammonia-monooxygenase (AMO), of which the subunit A (amoA) is highly conserved encoded in ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Treich et al., 2005). Although both AOB and AOA can contribute to ammonia-oxidation, it is unclear to what extent (Prosser and Nicol, 2012) they can be considered as potentially, functionally redundant (Leininger et al., 2006; Schauss et al., 2009). They have however been shown to differ in physiology and ecology (Fergüder et al., 2009). In many soils archaeal amoA genes are more abundant than their bacterial counterparts (Alves et al., 2013; Leininger et al., 2006; Prosser and Nicol, 2008), but AOB seem to outcompete AOA and dominate nitrification in agricultural soils (Jia and Conrad, 2009). N-rich grasslands (Di et al., 2009), and at high levels of NH_4^+ (Brankatschk et al., 2010; Di et al., 2010; Verhamme et al., 2011). Archaeal AMO, in turn, appears to have a higher affinity, and lower inhibition constant for ammonia (Martens-Habben et al., 2009; Prosser and Nicol, 2012), which could be advantageous at low ammonia concentrations.

Whereas the dynamics of AOB and AOA to ammonia are well studied, much less is known about responses of ammonia-oxidizers, specifically of AOA, to drought and rewetting under in situ conditions. Generally, AOB and AOA feature different physiological prerequisites (Schauss et al., 2009), presumably also leading to different responses of AOB and AOA to soil drying and rewetting. During drought periods nutrient concentrations in the soil solution increase, forcing microbes to balance the increasing osmotic potential by accumulating or producing osmolytes (Roeßler and Müller, 2001), which could lead to large amounts of N tied up in the microbial biomass (Schimel et al., 2007). Rainfall events in turn, introduce a water pulse causing a sud-

den decrease of the osmotic soil potential; microbes release accumulated osmolytes to avoid lysis (Schimel et al., 2007; Roeßler and Müller, 2001) and are triggered back from low activity or dormant states (Barnard et al., 2013; Placella and Firestone, 2013) resulting in peaks of carbon and N mineralization (Birch, 1958; Fierer and Schimel, 2002; Evans and Wallenstein, 2012), and of nitrification (Fierer and Schimel, 2002). Thus, both drying and rewetting could lead to high ammonium concentrations in the soil solution which has been shown to adversely affect AOA, but not AOB abundances in non-drought adapted grassland soils, likely related to a niche differentiation of AOB and AOA (Thion and Prosser, 2014).

Most studies on the effects of drought and rewetting on grassland N-cycling and on ammonia-oxidizer abundances originated either from seasonal dry sites (e.g. Fierer and Schimel, 2002; Gleeson et al., 2010; Placella et al., 2013) or from soil mesocosm incubation studies (Thion and Prosser, 2014). However, well water-supplied ecosystems, such as many grasslands in mountainous areas, will be experiencing a higher frequency of drought and heavy rainfall events (Gobiet et al., 2013; IPCC, 2012). These grasslands, which are often in transitions from land-management to abandonment, play a pivotal role in nutrient retention and erosion protection with repercussions on densely populated watersheds downstream. Thus, there is an urgent need study possible effects of such climate extremes on soil N cycling in situ.

The aim of this study therefore was to investigate effects of drought and subsequent rewetting on gross N mineralization, gross nitrification, and the abundances of ammonia-oxidizers of mountain meadows under different land-management levels, an extensively managed meadow and an abandoned grassland site in the Austrian Central Alps. At both sites we conducted rain-exclusion experiments to simulate drought and subsequent rewetting, which allowed us to examine the effects of drought in soils with different preconditions, such as soil organic matter content (Meyer et al., 2012), organic and inorganic N availability (specifically NH_4^+), and with potentially different abundances of bacterial and archaeal ammonia-oxidizers. We hypothesized that the phylogenetically “broad” process of N mineralization is less affected by drought than the more “narrow” process of nitrification. We expected AOB and AOA to respond differentially to drought – more specifically, that archaeal amoA abundances would decrease as a consequence of rising ammonium concentrations in the soil solution. Additionally, we hypothesized that the impact of drought on N-turnover and ammonia-oxidizer abundances would be stronger on the managed meadow than on the abandoned site, as the higher soil organic matter content at the abandoned site could act as a buffer against soil drying (Brady and Weil, 2002; Franzluebbers et al., 2002).

Table 1. Soil parameters, nitrogen pools ($\mu\text{g N g}^{-1}\text{ DW soil}$ and in $\mu\text{g N g}^{-1}\text{ soil water: water sol.}$), nitrogen turnover rates ($\mu\text{g N g}^{-1}\text{ DW soil d}^{-1}$), mean residence times (MRT) of ammonium and nitrate (h), as well as microbial abundances (gene copies $\text{g}^{-1}\text{ DW soil}$) in soils of the meadow and the abandoned site ($n = 28$; means $\pm$ standard error). Effects of site and sampling time, as well as their interaction were assessed by repeated-measures ANOVA for non-drought-treated controls. Asterisks mark levels of significance: $^{\circ} p < 0.1$; $^* p < 0.05$; $^{**} p < 0.01$; $^{***} p < 0.001$ (differences between sites for WHC_{max} ; $n = 16$, and SOM content $n = 12$ were analysed by t -tests).

	Meadow	Abandoned site	Site		Time		Site $\times$ time	
	Mean ($\pm$ SE)	Mean ($\pm$ SE)	$F(1)$	p	$F(6)$	p	$F(1,6)$	p
SOM content (%)	13.3 (± 0.8)	22.5 (± 1.5)		*** 1				
$\text{WHC}_{\text{max}}(\text{g H}_2\text{O g}^{-1}\text{ DW})$	1.5 (± 0.1)	2.0 (± 0.3)		*** 1				
$\text{SWC}(\% \text{ of } \text{WHC}_{\text{max}})$	44.2 (± 1.7)	45.4 (± 1.8)	0.8		5.2	***	2.6	*
$\text{C}_{\text{tot}}(\%)$	7.0 (± 0.2)	11.2 (± 0.7)	33.0	***	1.5		0.9	
$\text{N}_{\text{tot}}(\%)$	0.7 (± 0.1)	0.9 (± 0.1)	15.2	***	1.3		0.8	
$\text{C}_{\text{tot}}:\text{N}_{\text{tot}}$	10.1 (± 0.1)	12.0 (± 0.2)	73.7	***	1.3		0.2	
$\delta^{13}\text{C}(\text{‰})$ bulk soil	-26.5 (± 0.2)	-25.6 (± 0.1)	44.1	***	0.9		1.4	
$\delta^{15}\text{N}(\text{‰})$ bulk soil	4.9 (± 0.2)	4.1 (± 0.2)	11.2	**	1.4		0.9	
EON	31.4 (± 1.5)	55.6 (± 5.5)	33.5	***	2.1		2.9	*
NH_4^+	5.7 (± 0.6)	11.1 (± 0.9)	42.7	***	2.1		2.8	*
NO_3^-	2.2 (± 0.6)	0.4 (± 0.1)	14.2	***	1.1		3.6	**
$\text{MRT}_{\text{NH}_4^+}$	27.4 (± 6.8)	33.3 (± 6.1)	1.7		2.2	$^{\circ}$	1.3	
$\text{MRT}_{\text{NO}_3^-}$	30.3 (± 10.0)	2.2 (± 0.7)	29.4	***	1.8		2.5	*
EON water sol.	51.3 (± 3.4)	60.6 (± 4.6)	3.8	$^{\circ}$	3.9	*	2.5	*
NH_4^+ water sol.	9.2 (± 0.8)	12.0 (± 0.7)	12.0	**	2.3	$^{\circ}$	2.0	$^{\circ}$
NO_3^- water sol.	3.3 (± 0.9)	0.5 (± 0.1)	20.1	***	0.7		4.0	**
Gross N Min	8.5 (± 1.0)	11.1 (± 2.0)	0.1		0.9		1.4	
Gross NH_4^+ Immo	8.1 (± 0.8)	13.4 (± 1.7)	1.1		0.5		1.4	
Gross Nit	5.9 (± 0.7)	11.1 (± 1.4)	12.4	**	2.9	*	1.7	
Gross NO_3^- Immo	4.6 (± 0.9)	12.4 (± 1.1)	47.4	***	4.7	***	2.9	
AOA	1.79×10^6	1.82×10^6	0.0		0.5		0.5	
AOB	8.68×10^5	2.25×10^5	37.2	***	1.3		0.4	
AOA:AOB ratio	2.8 (± 0.6)	26.3 (± 13.2)	27.0	***	0.4		0.7	

2 Material and methods

2.1 Study sites

The two studied grasslands are located in the Austrian Central Alps near Neustift, Stubai Valley ($47^{\circ}07'\text{N}$, $11^{\circ}19'\text{E}$) and are characterized by a mean annual temperature of 3°C and a mean annual precipitation of about 1100 mm. Drought simulations were conducted on a typical extensively managed mountain meadow (to be referred to as “meadow”, 1850 m a.s.l.) and on an abandoned meadow site (to be referred to as “abandoned site” 1900 m a.s.l.). At both sites, soils were characterized as dystric cambisols (FAO classification) with a pH in the uppermost 10 cm soil depth of 5.5 (determined in CaCl_2). At the meadow total aboveground plant biomass is cut and harvested once a year; parts of the meadow are slightly grazed by cattle in spring and autumn, and fertilized with manure every two to three years (Bahn et al., 2006). The meadow is characterized by high plant primary production (Schmitt et al., 2010), by higher soil respiration rates (Bahn et al., 2008), as well as by lower soil organic matter (SOM), total carbon (C_{tot}) and nitrogen (N_{tot}) con-

tents compared to the abandoned site (Table 1, and Meyer et al., 2012). At the abandoned site all management activities were terminated in 1983. The dominant plant community was determined as *Trisetetum flavescentis* at the meadow, and as *Seslerio-Cariacetum* at the abandoned site (Schmitt et al., 2010).

2.2 Experimental set-up and soil sampling

At both the meadow and the abandoned site, four drought and corresponding control plots were established in spring 2011 in a paired plot design. Drought was simulated by installing rain-out shelters, covering an area of $3.0 \times 3.5\text{ m}$, for 10 weeks starting on 31 May 2011. The shelters were equipped with light- and UV-B-permeable plastic foil (UV-B Window, Foiltec GmbH, Germany, light-permeability ca. 95 %, UV-B permeability $> 70\%$) to exclude any precipitation. To maintain the traditional land-management, the total area of the meadow was mown (i.e. aboveground biomass was cut and removed) during the drought period (2 August 2011), while there was no land-management activity at the abandoned site. After 10 weeks of drought (10 August 2011) all plots

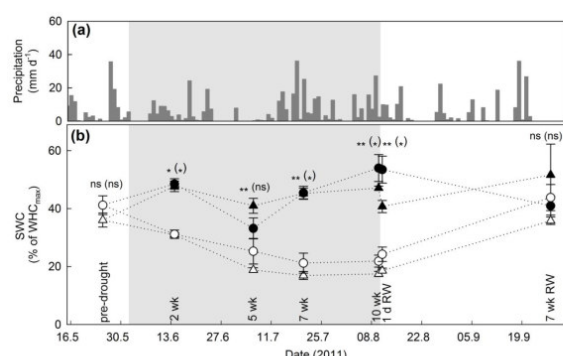


Figure 1. Precipitation (a) and soil water content (b, SWC) calculated as percentage of the respective WHC_{max} . Grey bars indicate precipitation (in $mm\ d^{-1}$). Filled symbols represent controls of the meadow (triangles) and abandoned site (circles), open symbols show the respective drought-treated plots (error bars indicate standard error, $n = 4$). Asterisks indicate differences between controls and drought treatments at single sampling points (levels of significance Bonferroni-corrected; ns, not significant; * $p < 0.05$; ** $p < 0.01$, symbols without parentheses refer to the meadow; symbols in parentheses to the abandoned site). The period of drought treatment is marked as grey background.

received previously collected rainwater over a time period of 3 min to simulate a short heavy rainfall event of 20 mm; subsequently rain-out shelters were removed and all plots were again exposed to natural precipitation.

Soils were sampled 1 week before the onset of drought simulation, then every 2 to 3 weeks during drought, 1 day and 7 weeks after rewetting (Fig. 1). For each sample two soil collars (5×7 cm, 10 cm depth) were pooled after the uppermost litter layer had been removed. Then soil collars were homogenized and sieved to 2 mm. Aliquots of soil were immediately frozen at -80°C for molecular analyses; the remaining samples were stored at 4°C until further processing.

2.3 Soil parameters and N pools

For both sites maximum soil water holding capacity (WHC_{max}) was determined by adding excess amounts of deionized water to aliquots of soil samples and leaving them for 48 h over water to allow maximum saturation. Then 2 g of water-saturated soils were dried for 48 h at 60°C . Soil water content (SWC) was measured gravimetrically by drying of 5 g of fresh soil for two days at 60°C in a drying oven and calculated relative to WHC_{max} . Total carbon (C_{tot}) and N (N_{tot}) content were determined from dried and ground soil samples by EA-IRMS (EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). Total extractable N was determined from K_2SO_4 extracts (2 g of soil were extracted with 20 mL $0.5\text{ M } K_2SO_4$) using a TOC/TN anal-

yser (TOC-V CPH E200V/TNM-122V; Shimadzu, Austria). NH_4^+ was measured photometrically from K_2SO_4 extracts using a modified indophenol reaction method (Kandeler and Gerber, 1988). NO_3^- was determined from water extracts (2 g of fresh soil were extracted with 20 mL of MilliQ water) by chemically suppressed ion-chromatography (DX500, Dionex, Austria) on a Dionex AS11 column. Extractable organic nitrogen (EON) was calculated by subtracting inorganic (NH_4^+ and NO_3^-) from total extractable N.

2.4 Potential gross N transformation rates

Potential gross NH_4^+ and NO_3^- transformation rates were determined using ^{15}N pool dilution techniques described by Kaiser et al. (2011). For determination of microbial gross N mineralization and NH_4^+ immobilization, fresh aliquots of soil (2 g in duplicates) received 0.5 mL $(NH_4)_2SO_4$ (0.125 mM; 10 atom % ^{15}N). For determination of gross nitrification and microbial NO_3^- uptake (NO_3^- immobilization), 2 g of fresh soil samples (in duplicates) received 0.5 mL KNO_3 (0.25 mM, 10 atom % ^{15}N). Labelled samples were incubated for 4 and 24 h at room temperature and finally extracted with 20 mL 2 M KCl. Both control and drought-treated soils received solute ^{15}N label, which could have altered the conditions, specifically in drought treated soils (Chen et al., 2011). Therefore, the reported rates should be considered as the N-turnover potential of the soil microbial community.

Potential gross N mineralization and NH_4^+ immobilization rates were determined by microdiffusion of NH_3 from KCl-extracts into acid traps, which were analysed for nitrogen concentrations and atom-percent excess of ^{15}N by EA-IRMS (EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). For potential gross nitrification and NO_3^- immobilization rates NH_3 was removed from the extracts, before converting NO_3^- to NH_3 by adding Devarda's Alloy. Again, NH_3 was trapped and analysed for N concentration and atom-percent excess of ^{15}N . Potential gross N mineralization, gross NH_4^+ immobilization, as well as potential gross nitrification and gross NO_3^- immobilization rates were calculated as described in detail by Wanek et al. (2010).

2.5 Nucleic acid extraction

DNA was extracted from 0.35 g of soil using the FastDNA[®] SPIN Kit for Soil (MP Biomedicals, CA, USA) and the Precellys24 Instrument (Bertin Technologies, France). After extraction, the DNA was tested in quantity and quality with a spectrophotometer (Nanodrop, PeqLab, Germany) and stored at -20°C until further processing.

2.6 Quantitative Real-Time PCR

The abundances of the bacterial and archaeal ammonia-monooxygenase gene (*amoA*) served as proxy for

Table 2. Thermal profiles and primers used for real-time PCR quantification of functional genes of ammonia-oxidation.

Target gene	Thermal profile	No. of cycles	Primer	Source of standard
amoA AOA	95°C – 10 min 94°C – 45 s/ 55°C – 45 s/72°C – 45 s	40	amo19F, CrenamoA16r48x Leininger et al. (2006), Schauss et al. (2009)	Fosmid clone 54d9
amoA AOB	95°C – 10 min 94°C – 60 s/ 58°C – 60 s/72°C – 60 s	40	amoA1F, amoA2R Rothauwe et al. (1997)	Nitrosomonas sp.

ammonia-oxidizers and were detected by quantitative Real-Time PCR, which was carried out on a 7300 Real-Time PCR System (Applied Biosystems, Germany) using SYBR green as fluorescent dye. The PCR was performed in 96-well plates (Applied Biosystems, Germany) for all investigated genes. The reaction mixes were performed according to Töwe et al. (2010); the thermal profiles of the PCRs are given in Table 2. In a preliminary test dilution series of the DNA extracts were tested to avoid inhibition of PCR, resulting in an optimal dilution of 1 : 128 for all samples (2.5 ng DNA μL^{-1} per sample). Serial plasmid dilutions of the respective functional genes ranging from 10^6 to 10^1 gene copies μL^{-1} were used as standards for the determination of the gene abundances of each sample (Table 2). To confirm the specificity of the amplicons after each PCR run, a melting curve and a 2 % agarose gel stained with ethidium bromide were conducted. The efficiencies (Eff) of the amplification were calculated from the standard curve with the formula $\text{Eff} = [10^{(-1/\text{slope})} - 1] \times 100\%$ and resulted in the following values: amoA of ammonia-oxidizing archaea (AOA) 83.4 % to 91.8 % ($R^2 = 0.997$) and amoA of ammonia-oxidizing bacteria (AOB) 94.8 to 95.4 % ($R^2 = 0.999$).

2.7 Statistics

To determine site-specific differences, all available parameters were compared by repeated-measures ANOVA using only the controls of both sites. As we found significant differences between sites, we subsequently tested for effects of drought by comparing control samples from each study site with drought plots over the course of the experiment by repeated-measures ANOVA, using the plot number as within-factor. To test for differences between drought and control samples at single sampling time points we used paired *t*-tests with Bonferroni-adjusted levels of significance. All data were tested for normality by Shapiro–Wilk test and for homoscedasticity by Levene's test. If data did not meet ANOVA assumptions they were log-transformed or rank-normalized. All data, including SWC, C_{tot} , N_{tot} , all N pools and potential N-turnover data, as well as ammonia-oxidizer gene abundances were subjected to principal component analyses (PCA), after they were transformed to meet PCA assumptions. To determine differences between sites and drought on the distribution of the samplings along the PC-axes, we conducted a two-way ANOVA. All statistical analyses were performed using R 2.15.2 (R Core Team, 2012).

3 Results

3.1 Effects of drought on soil parameters and soil N pools

During drought simulation, in total 358 mm of precipitation were excluded, equalling one-third of mean annual precipitation. This led to a significant decrease of SWC to 16.9 % ($\pm 1.4\%$) at the meadow, and to 21.2 % ($\pm 3.5\%$) at the abandoned site (Table 1, Fig. 1). However, the initial WHC_{max} was significantly lower at the meadow than at the abandoned site (Table 1), thus the amount of water remaining in meadow soils was lower during drought as compared to the abandoned site. At both sites, rewetting had no immediate effects on the SWC in drought-treated plots, but 7 weeks after the end of drought the SWC levels had approximated those of controls (Fig. 1).

At both sites drought had no significant effect on the N pools (Table 2, Fig. 2) when concentrations were calculated per gram dry soil, although the meadow was characterized by significantly lower EON and NH_4^+ , but higher NO_3^- concentrations compared to the abandoned site (Table 1, Fig. 2). However, when N concentrations were calculated per g soil water after 5 weeks of drought, EON and NH_4^+ concentrations were significantly increased at both sites, and remained on a higher level until 1 day after rewetting (Table 1, Fig. 3). In particular at the meadow, drought induced fivefold increases of NH_4^+ concentrations, while at the abandoned site NH_4^+ concentrations at most tripled. In contrast, NO_3^- concentrations in the soil solution were not affected by drought, but strongly increased 1 day after rewetting at the meadow, while at the abandoned site no clear effect of drought was detected (Table 1, Fig. 3). At both sites 7 weeks after the end of drought EON and NH_4^+ concentrations were similar in drought and control plots – only in soils of the meadow were NO_3^- concentrations in drought-treated soils significantly increased (Table 1, Fig. 3).

3.2 Effects of drought on microbial N transformation rates

Potential gross rates of microbial N mineralization and NH_4^+ immobilization were similar at both sites (Table 1, Fig. 4). However, only at the meadow drought significantly increased potential gross NH_4^+ immobilization rates, which consequently decreased the mean residence time (MRT) of NH_4^+

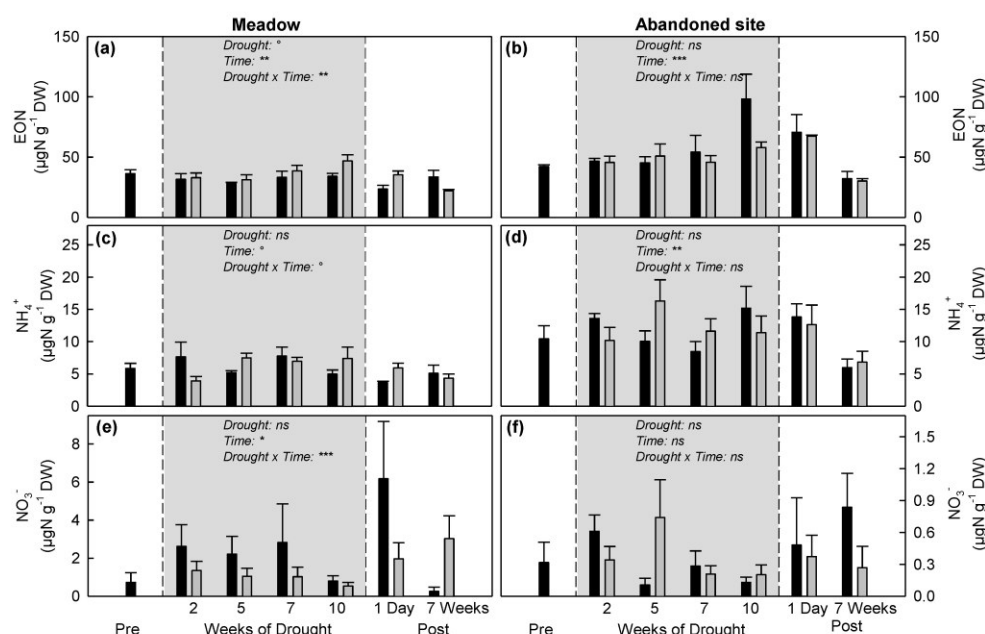


Figure 2. Soil N concentrations per g^{-1} dry soil over the course of the experiment at the meadow (left panels) and the abandoned site (right panels) in controls (black bars) and drought-treated plots (grey bars). (a, b) Extractable organic N (EON), (c, d) ammonium (NH_4^+) and (e, f) nitrate (NO_3^- , note the different scaling!). The grey background indicates the period of drought treatment. Differences between control and drought at single sampling points were assessed by *t*-tests with Bonferroni-corrected levels of significance ($n = 4$, respectively, bars show means; error bars indicate standard error). Effects of drought and sampling time were assessed by two-way repeated-measures ANOVA; for further details see Table 3.

(per g dw) from 27.4 ± 6.8 h to 14.4 ± 1.3 h. At the abandoned site, in contrast, potential gross N mineralization and immobilization, as well as the MRT of NH_4^+ (50.8 ± 10.5 h) were not affected by drought (Table 3, Fig. 4). At neither site potential N mineralization, nor NH_4^+ immobilization responded to rewetting.

Potential gross rates of nitrification and microbial NO_3^- immobilization were significantly lower and the NO_3^- pool turned over much slower (30.3 ± 9.9 h) at the meadow than at the abandoned site (2.2 ± 0.7 h Table 1, Supplement Fig. S1). At both sites potential nitrification and microbial NO_3^- immobilization rates were influenced by sampling time, indicating a strong seasonal variability (Table 1, Fig. 4). At the meadow drought affected neither potential nitrification, nor potential NO_3^- immobilization rates. At the abandoned site, in contrast, drought significantly altered both potential nitrification and NO_3^- immobilization rates, which showed less variance compared to controls (Table 3, Fig. 4). One day after rewetting at both sites potential nitrification and NO_3^- immobilization rates slightly increased in drought-treated plots, but seven weeks after rewetting, the rates were again similar to the controls (Fig. 4).

3.3 Effects of drought on the abundance of ammonia-oxidizers

The abundance of archaeal amoA genes (as a proxy for AOA) was similar at both sites, ranging from 8.1 ± 10^5 to 3.2 ± 10^6 copies g^{-1} DW soil at the meadow and from 5.2 ± 10^5 to 3.7 ± 10^6 copies g^{-1} DW soil at the abandoned site. At the meadow bacterial amoA gene copies (as a proxy for AOB), ranging from 2.5 ± 10^5 to 1.7 ± 10^6 copies g^{-1} DW soil, were as abundant as archaeal amoA gene copies, thus AOA:AOB ratios ranged between 1 and 10. At the abandoned site, in contrast, AOB numbers ranged from 8.0 ± 10^4 to 7.8 ± 10^5 copies g^{-1} DW soil, which was significantly lower, consequently AOA:AOB ratios were significantly higher (ranging between 10 and 100) than at the meadow (Table 1, Fig. 5).

At the meadow drought significantly decreased archaeal amoA gene copy numbers, while the abundance of bacterial amoA remained unaffected, thus the ratio of AOA:AOB significantly decreased compared to the controls (Table 3, Fig. 5). At the abandoned site, in contrast, drought affected neither AOA, nor AOB abundances.

Table 3. Effects of the drought simulation and sampling time on all measured soil parameters and microbial abundances for the meadow and the abandoned site assessed by repeated-measures ANOVA ($n = 28$); within-factor was plot identity ($n = 4$); asterisks mark levels of significance: $^{\circ} = p < 0.1$; $* = p < 0.05$; $** = p < 0.01$; $*** = p < 0.001$.

	Meadow						Abandoned site					
	Drought		Time		Drought $\times$ Time		Drought		Time		Drought $\times$ Time	
	$F(1)$	p	$F(6)$	p	$F(1, 6)$	p	$F(1)$	p	$F(6)$	p	$F(1, 6)$	p
SWC	175.2	***	5.5	***	7.7	***	69.2	***	4.4	**	8.5	***
C_{tot}	3.3		1.4		1.0		2.2		3.8	**	2.1	$^{\circ}$
N_{tot}	2.7		1.3		1.0		1.8		3.5	**	1.9	$^{\circ}$
$C_{\text{tot}}:N_{\text{tot}}$	2.2		1.5		0.6		6.4	*	6.2	***	1.9	
$\delta^{13}\text{C}$ (‰) bulk soil	3.6	$^{\circ}$	1.1		0.7		3.9	$^{\circ}$	1.9		0.3	
$\delta^{15}\text{N}$ (‰) bulk soil	14.9	***	3.6	**	1.8		< 0.1		2.8	*	1.0	
EON	2.9	$^{\circ}$	4.4	**	3.4	**	1.1		11.3	***	1.1	
NH_4^+	0.4		2.0	$^{\circ}$	2.3	$^{\circ}$	< 0.1		4.0	**	1.3	
NO_3^-	0.2		2.8	*	3.6	**	0.1		0.6		1.2	
$\text{MRT}_{\text{NH}_4}^+$	3.7	$^{\circ}$	1.4		1.6		1.8		2.0		2.8	
$\text{MRT}_{\text{NO}_3}^-$	< 0.1		1.0		2.3		0.6		1.3		0.4	
EON water sol.	82.2	***	5.1	***	6.0	***	66.1	***	15.0	***	4.0	**
NH_4^+ water sol.	47.6	***	4.3	**	4.7	**	42.2	**	5.7	***	3.2	*
NO_3^- water sol.	4.2	*	2.2	**	1.8		4.0		0.8	$^{\circ}$	2.7	*
Gross N Min	1.9		3.6	**	1.2		< 0.1		2.8	*	0.9	
Gross NH_4^+ Immo	4.5	*	2.8	*	1.5		< 0.1		0.3		0.5	
Gross Nit	0.1		1.2		0.6		4.9	***	3.6	$^{\circ}$	4.1	**
Gross NO_3^- Immo	1.1		1.5		3.3	**	7.5	**	8.6	***	2.2	$^{\circ}$
AOA	10.8	**	0.8		0.7		0.1		1.1		0.4	
AOB	2.4		1.6		0.8		0.1		0.5		1.2	
AOA : AOB	19.9	***	1.1		1.0		2.5		1.1		0.4	

3.4 Drought effects in relation to site-specific differences

To summarize and illustrate drought effects in relation to site-specific differences, a PCA was conducted, including all studied parameters. In total, three factors with eigenvalues > 1 were identified and accounted for 68.9 % of the total variance. In a biplot showing the first two factors (PC1 and PC2, accounting in total for 55.1 % of the variance) on PC1 samples were separated (34.9 %) according to sampling site (two-way ANOVA with factor loadings: ($F(1) = 102.4$; $p < 0.001$) and drought treatment ($F(1) = 5.9$; $p < 0.05$, interaction: ns). PC2 explained 20.1 % and primarily displayed drought effects ($F(1) = 9.1$; $p < 0.01$), but was not affected by site ($F(1) = 0.9$; $p = \text{ns}$; interaction: ns). The main parameters responsible for the separation along PC1 were higher EON and NH_4^+ concentrations, as well as higher potential gross nitrification and gross NO_3^- immobilization rates at the abandoned site, which were in contrast to higher abundances of AOB and NO_3^- concentrations that were dominating at the meadow (Fig. 6). This pattern was supported by positive correlations between EON and NH_4^+ concentrations in controls of both the meadow ($R = 0.59$, $p < 0.001$) and the abandoned site ($R = 0.57$, $p < 0.01$). Moreover, in controls of the meadow NO_3^- correlated negatively with EON

concentrations ($R = -0.67$, $p < 0.001$), but positively with AOB abundances ($R = 0.42$, $p < 0.05$). Only in controls of the abandoned site EON correlated with gross N mineralization ($R = 0.60$, $p < 0.001$) and with SWC ($R = 0.60$, $p < 0.001$). PC2 separated the drought treatment from the controls, although this effect was stronger for the meadow than for the abandoned site. SWC was distributed along PC2 opposing the vectors for potential gross N mineralization and gross NH_4^+ immobilization (Fig. 6). Specifically in soil samples from the meadow subjected to drought the potential gross NH_4^+ immobilization rates correlated negatively with SWC ($R = -0.62$, $p < 0.001$).

4 Discussion

4.1 Effects of drought on soil N dynamics and ammonia-oxidizer abundances

In our study the potential of the microbial community to mineralize organic nitrogen to ammonium was not affected by drought irrespective of site-specific differences related to land management. Thus, this comparably “broad” soil process remained stable and could show to indicate that N mineralization may have been dominated by drought-tolerant

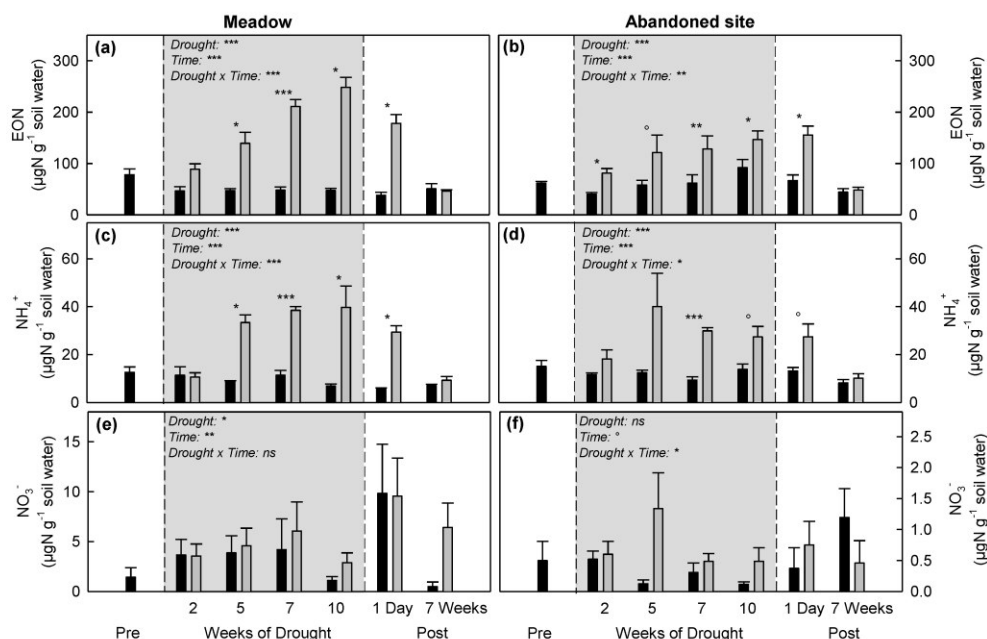


Figure 3. Soil N concentrations per g^{-1} soil water over the course of the experiment at the meadow (left panels) and the abandoned site (right panels) in controls (black bars) and drought-treated plots (grey bars). (a, b) Extractable organic N (EON), (c, d) ammonium (NH_4^+) and (e, f) nitrate (NO_3^- , note the different scaling!). The grey background indicates the period of drought treatment. Differences between control and drought at single sampling points were assessed by *t*-tests with Bonferroni-corrected levels of significance; ° $p < 0.1$; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, ($n = 4$, respectively, bars show means; error bars indicate standard error). Effects of drought and sampling time were assessed by two-way repeated-measures ANOVA; for further details see Table 3.

microbial generalists like fungi (Allison and Martiny, 2008; Schimel and Schaeffer, 2012). Only in soils of the managed meadow did potential gross rates of microbial NH_4^+ immobilization and therefore NH_4^+ turnover increase during drought. This points to a potentially increased microbial N demand to facilitate the synthesis of nitrogenous osmolytes to balance the osmotic potential between the soil solution and microbial cells (Schimel et al., 2007). At the abandoned site, however, the microbial NH_4^+ uptake potential remained unaffected by drought, which suggests a high stability of this process, or a microbial community that might use other than N-containing substrates for osmolyte production (Schimel et al., 2007).

As microbial nitrification appears to be a more “narrow” process than microbial N mineralization (Schimel and Schaeffer, 2012), we expected nitrification rates to respond sensitively to dry conditions. In the presented study, however, responses of potential nitrification to drought were diverging between the studied sites. In accordance with other studies, where grassland and heathland sites had been subjected to drought (Hartmann et al., 2013; Larsen et al., 2011), the microbial nitrification potential was not affected by drought at the managed meadow. At the abandoned site, in turn, drought reduced the dynamics of potential nitrification and

NO_3^- immobilization, which was also shown, e.g. for forest soils (Chen et al., 2011), or grassland under oak forest (Stark and Firestone, 1996). Thus, responses of potential nitrification rates to drought seem to be strongly context dependent and may not be generalized.

Drought distinctly affected bacterial and archaeal amoA gene copy numbers, which were used as proxy for the abundance of AOB and AOA. As expected, AOB were stable and not affected by drought at both sites. The observed decrease of AOA in response to drought at the managed meadow in our study was similar to results reported by Thion and Prosser (2014), where the observed decrease of archaeal amoA abundance during drought was likely related to strong increases of NH_4^+ during drought. In our study soil NH_4^+ concentrations calculated per gram dry soil were not affected during drought at either site, similar to the study of Hartmann et al. (2013) in subalpine grassland soils. When NH_4^+ concentrations were calculated per g soil water, however, five-fold increases at the meadow and up to threefold increases at the abandoned site were observed. Thus, it is likely that the stronger increase of NH_4^+ in the soil solution of the meadow could have induced the decrease of archaeal amoA genes. Thus, our findings corroborate that responses of archaeal

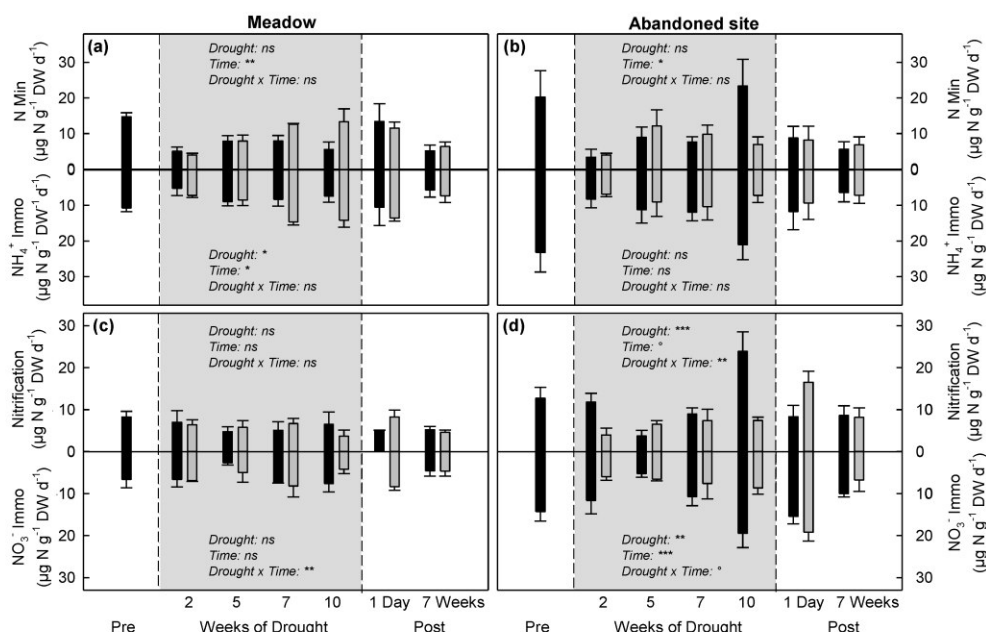


Figure 4. N transformation rates over the course of the experiment at the meadow (left panel) and the abandoned site (right panel) in control (black bars) and drought-treated plots (grey bars). (a, b) Gross N mineralization (upper part) and gross microbial NH_4^+ immobilization rates (lower part); (c, d) gross nitrification (upper part) and gross microbial NO_3^- immobilization rates (lower part). The grey background indicates the period of drought treatment. Differences between control and drought-treated plots at single sampling points assessed by *t*-tests with Bonferroni-corrected levels of significance ($n = 4$; error bars indicate standard error). Effects of drought and sampling time were assessed by two-way repeated-measures ANOVA; for further details see Table 3.

amoA abundances to drought could be driven by changes in soil NH_4^+ concentrations as a consequence of decreasing water content rather than by decreasing soil water content alone (Thion and Prosser, 2014; Delgado-Bazquerizo et al., 2013).

Overall, these findings partly support the hypothesis that the impact of drought on the abandoned site could be stronger at the meadow than at the abandoned site. In contrast to the managed meadow, where aboveground plant biomass is annually removed and harvested, at the abandoned site a thicker litter layer (Meyer et al., 2012), and higher SOM content were accumulated, which might have protected the soil from drying out (Brady and Weil, 2002; Franzluebbers, 2002; Knapp et al., 2008).

4.2 Effects of rewetting and recovery dynamics

Rewetting after drought has been shown to induce short-term increases of N mineralization in soil, which are likely caused by microbial release of accumulated osmolytes to avoid lysis, but also by re-connecting soil pores and increasing the nutrient availability for microbes (Evans and Wallenstein, 2012; Fierer and Schimel, 2002; Saetre and Stark, 2005). We therefore expected a strong increase of N mineralization rates in the soils after rewetting, but at both sites one day after rewet-

ting neither gross N mineralization, nor NH_4^+ immobilization rates responded to water addition, which is similar to findings by Chen et al. (2011). Due to the described short-term character of N mineralization peaks after rewetting we might have missed a possible increase.

The potential rates of nitrification, as well as of NO_3^- immobilization were increased at both sites one day after rewetting, while neither AOB, nor AOA gene copy numbers were affected. Placella and Firestone (2013) detected strong increases in the transcript abundances of both bacterial and archaeal *amoA* within two hours after rewetting at constant gene copy numbers, accompanied by increases in nitrification rates, which suggests an activity pulse of ammonia-oxidizing organisms (Fierer and Schimel, 2002). In non-drought-adapted grassland soils, however, AOB were found to have benefited from the flush of NH_4^+ after rewetting and to be more tolerant and resilient after drought than AOA (Thion and Prosser, 2014). Nonetheless, rewetting after long dry periods may initially rather stimulate microbial activity, and DNA-based methods could underestimate potential dynamics (Barnard et al., 2013).

In spite of the fact that effects of drought were very diverse and divergent between sites, 7 weeks after termination

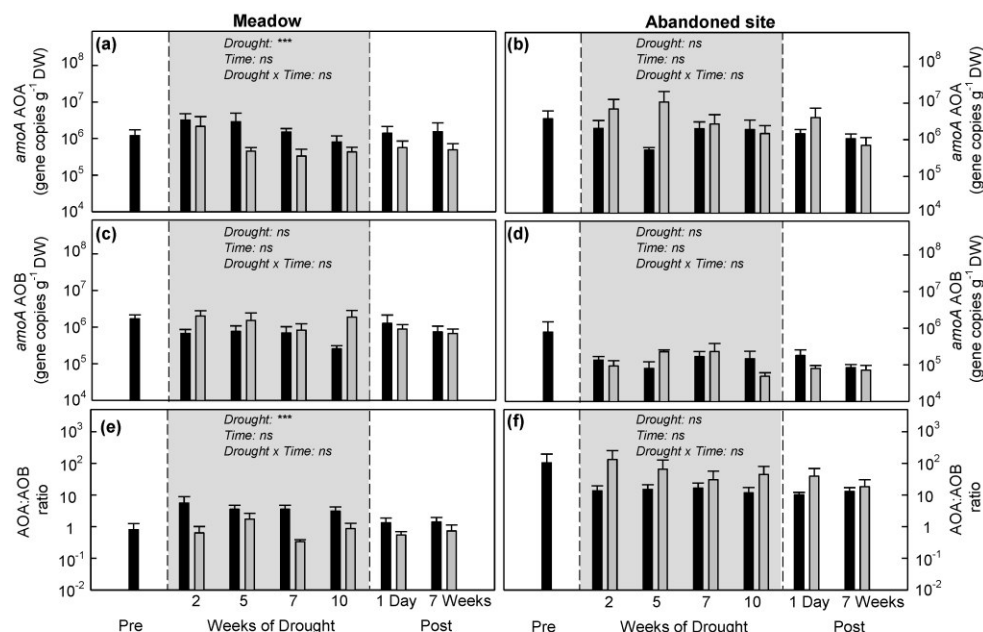


Figure 5. Gene copy numbers of ammonia-oxidizers at the meadow (left panel) and abandoned site (right panel) over the course of the experiment: (a, b) display archaeal (amoA AOA) and (c, d) bacterial (amoA AOB) amoA gene copy numbers, and (e, f) the ratio of AOA : AOB gene copy numbers. Black bars show controls, grey bars drought treatments ($n = 4$, error bars indicate standard error). The grey background indicates the period of drought treatment. Differences between control and drought-treated plots at single sampling points assessed by t -tests with Bonferroni-corrected levels of significance. Effects of drought and sampling time were assessed by two-way repeated-measures ANOVA; for further details see Table 3.

of drought all determined parameters, (except NO_3^- concentrations at the meadow), were similar to undisturbed controls, which indicates a high resilience of the studied grasslands after drought. Moreover, we detected a strong temporal variability of organic and inorganic N concentrations in soils at both sites that might be related to overall seasonal microclimatic fluctuations, and indirectly to altered levels of plant N demand and plant phenology (see e.g. Kaiser et al., 2011).

5 Summary and conclusions

Our study showed that experimental drought under in situ conditions distinctly affected N cycling and gene abundances of ammonia-oxidizers in soils of differently managed mountain grassland sites. Potential N mineralization was less affected by drought than the nitrification potential. However, we could not detect a link between the potential nitrification rates and abundances of bacterial and archaeal ammonia-oxidizer, as was reported by Meyer et al. (2013) for intensively managed agricultural soils. This lack of correlation could indicate that the ammonia-oxidizers may not have fully exploited their nitrification potential (Prosser and Nicol, 2012). Alternatively, they may have down-regulated their

transcriptional activity (Barnard et al., 2013; Placella et al., 2013) or heterotrophic nitrification by bacteria or fungi could have contributed to NO_3^- production (e.g. Pedersen et al., 1990). It might also hint at functional and structural differences of AOA (Alves et al., 2013) and AOB populations between the studied sites (Gleeson et al., 2010).

The distinct responses of ammonia-oxidizer in the studied grasslands, specifically of AOA, could likely be related to increases of NH_4^+ in the soil solution rather than to decreases of soil water content per se, but our results only partially confirmed this idea. Generally, impacts of drought were more pronounced at the managed as compared to the abandoned grassland, which could have been caused by beneficial effects of a thicker litter layer and higher soil organic matter content on soil moisture at the abandoned grassland. In addition, differences found in the prevalent N-pools, nitrification rates, as well as abundances of ammonia-oxidizing organisms between the two grasslands, suggest that effects of drought on soil N dynamics could have been modulated by the level of land-use.

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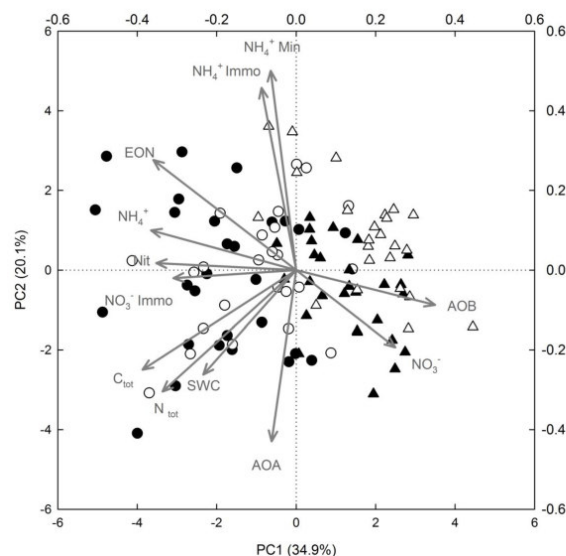


Figure 6. Biplot, displaying PC1 and PC2 derived from principal component analysis. Filled symbols represent control plots at the meadow (triangles) and the abandoned site (circles), open symbols show the drought treatment of the respective sites. Vectors display the variables contributing to PCA (C_{tot} = total carbon, N_{tot} = total nitrogen; for further abbreviations see Sect. 3).

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Appendix:

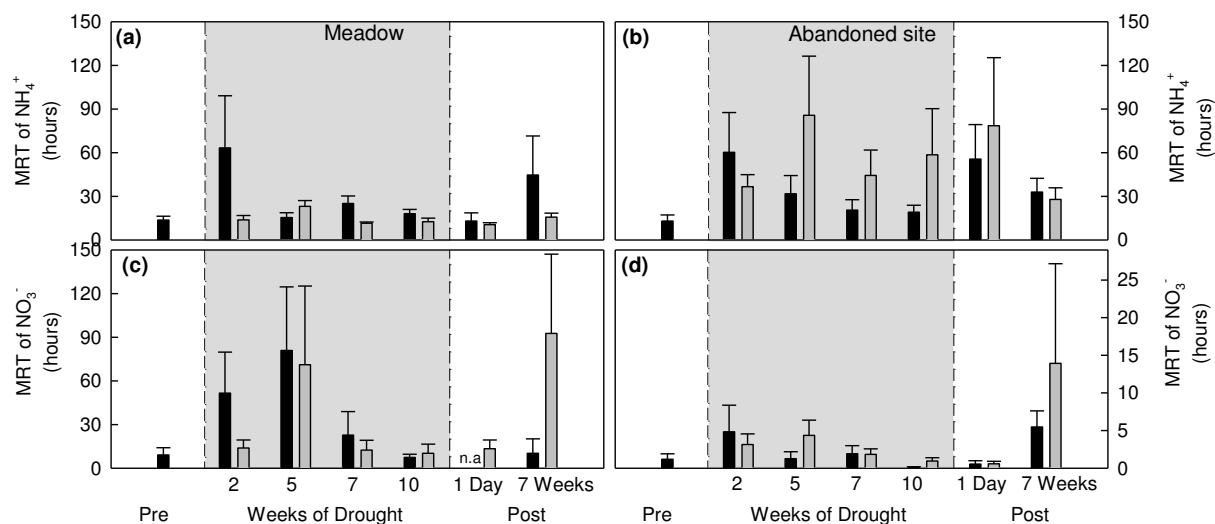
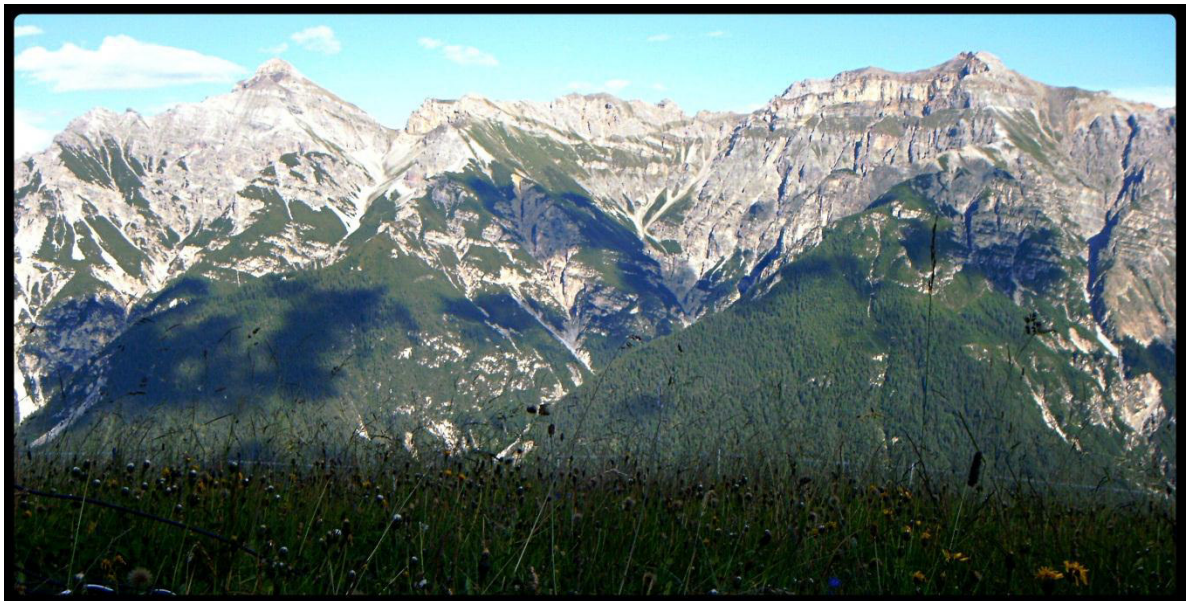


Figure A1. Mean residence times (MRT) of (a, b) NH_4^+ and (c, d) NO_3^- given in hours in soils of the meadow (left panel) and abandoned site (right panel) in control (black bars) and drought plots (grey bars). MRT was calculated by dividing the NH_4^+ and NO_3^- concentration by the respective gross immobilisation rate. The grey background indicates the period of drought treatment. Asterisks mark significant differences between control and drought at single sampling points assessed by t-tests ($n=4$, error bars indicate standard error). Note the different scaling of the y-axis in d.

Synthesis



Synthesis

In the preceding chapters I analysed and discussed the effects of drought – under *in situ* conditions – on plant and microbial carbon and nitrogen dynamics in mountain grasslands. Under ambient conditions the assimilated carbon was found to be rapidly allocated belowground, and quickly incorporated into microbial biomass (Figure 1 A, see also Bahn *et al.* (2013)). Although drought could lead to a higher root to shoot ratio in plants (Figure 1 B, Chaves *et al.* (2003)), in our study drought had no significant effect on plant biomass distribution, or on bulk plant carbon content. However, it induced alterations of the plant carbon turnover, as indicated by decreased uptake and belowground allocation of ^{13}C in drought treated plants compared to ambient controls (see Chapter 1, Fuchslueger *et al.* (2014)), while the proportion of newly assimilated carbon allocated to fine roots remained constant (Hasibeder *et al.*, 2014). The increase of non-structural carbohydrate pools in drought-treated plants, which can be interpreted as an osmotic adaptation, apparently occurred at the cost of root respiration, which was to a large extent fuelled by older carbon reserves. Thus, our study showed that water limitation during drought has the potential to induce shifts in the pattern of plant carbon allocation (Hasibeder *et al.*, 2014) and that these changes, in contrast to responses to shading

(Bahn *et al.*, 2013), are rather driven by altered sink demands in fine roots.

Moreover, plants with a drought-history significantly decreased uptake and belowground allocation of carbon when they were exposed to a further short-term dry period compared to their counterparts that grew under ambient conditions (see Chapter 2). Root respiration rates in plants with drought-history were strongly decreased and almost exclusively fuelled by older carbon reserves, while in ambient plots even during the dry period fresh carbon was metabolized. Thus, recurring drought periods appeared to be strongly imprinted in the plant's physiology, and plants with drought-history responded faster and stronger to dry conditions than plants without drought history. Nonetheless, timing of drought seems to be an important factor determining drought responses (Craine *et al.*, 2012). In our study, plants that grew under ambient soil moisture conditions exposed to drought showed no alterations in nitrogen content, while plants that re-grew during drought showed lower shoot and higher fine root nitrogen content. This could be either driven by a re-distribution of nitrogen within plants, i.e. from shoots to fine roots, or could be a result of lower nitrogen uptake due to reduced diffusivity and a reduced transfer to aboveground plant parts, or likely reflecting a shift

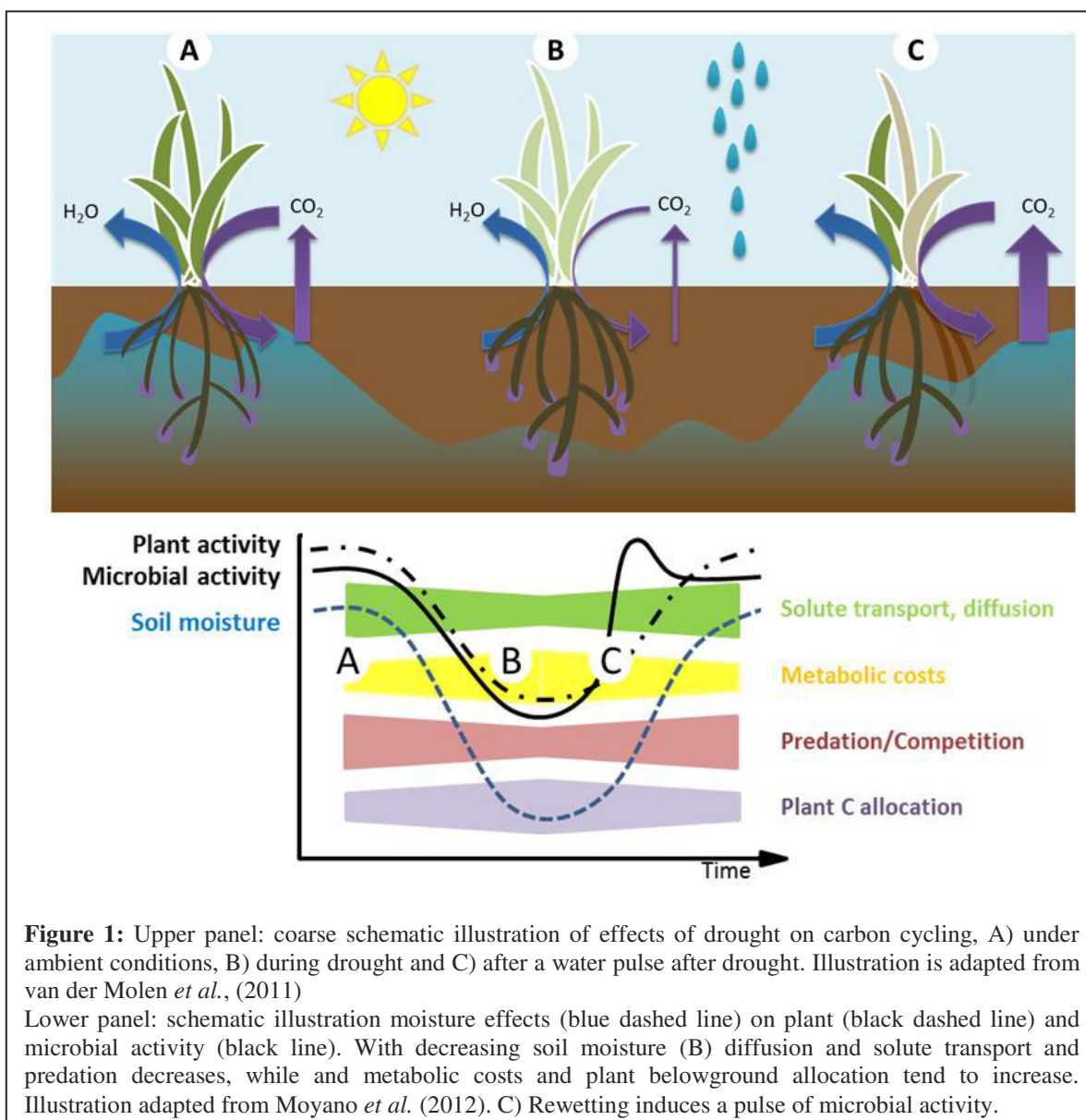


Figure 1: Upper panel: coarse schematic illustration of effects of drought on carbon cycling, A) under ambient conditions, B) during drought and C) after a water pulse after drought. Illustration is adapted from van der Molen *et al.*, (2011)

Lower panel: schematic illustration moisture effects (blue dashed line) on plant (black dashed line) and microbial activity (black line). With decreasing soil moisture (B) diffusion and solute transport and predation decreases, while metabolic costs and plant belowground allocation tend to increase. Illustration adapted from Moyano *et al.* (2012). C) Rewetting induces a pulse of microbial activity.

in plant community composition (e.g. Lambers *et al.*; Chaves *et al.*, 2003).

In soils, drought weakened the link between plant exudates and microbial uptake, leading to an accumulation of plant-derived ¹³C in the extractable organic carbon pool, rather than to an incorporation into microbial PLFAs (see Chapter 1, Fuchslueger *et al.* 2014a). Contrary to our initial hypothesis, that microbial biomass decreases during drought, the PLFA content was significantly higher in drought-treated soils than under ambient conditions. Specifically PFLA markers for gram-positive bacteria, which are thought to endure comparable lower water potential (Csonka, 1989; Lennon *et al.*, 2012), were increased. As summarized in Figure 1 (B), drought could have reduced competition of plant roots with

microbial populations. In addition, the predation pressure may have been lower, although e.g. abundances of collembola and mites were not significantly affected by drought (Seeber *et al.*, 2012). Alternatively, microbes could have increasingly switched to dormant states and could have produced additional phospholipid membranes during sporulation or changing to cysts (Csonka, 1989; Schimel *et al.*, 2007; Lennon & Jones, 2011). Any of these adaptation strategies are cost-intensive and could lead to a decreased activity (see Figure 1 B; Schimel *et al.* 2007; Moyano, *et al.* 2013), which in our study was indicated by the lower uptake of recent plant derived ¹³C.

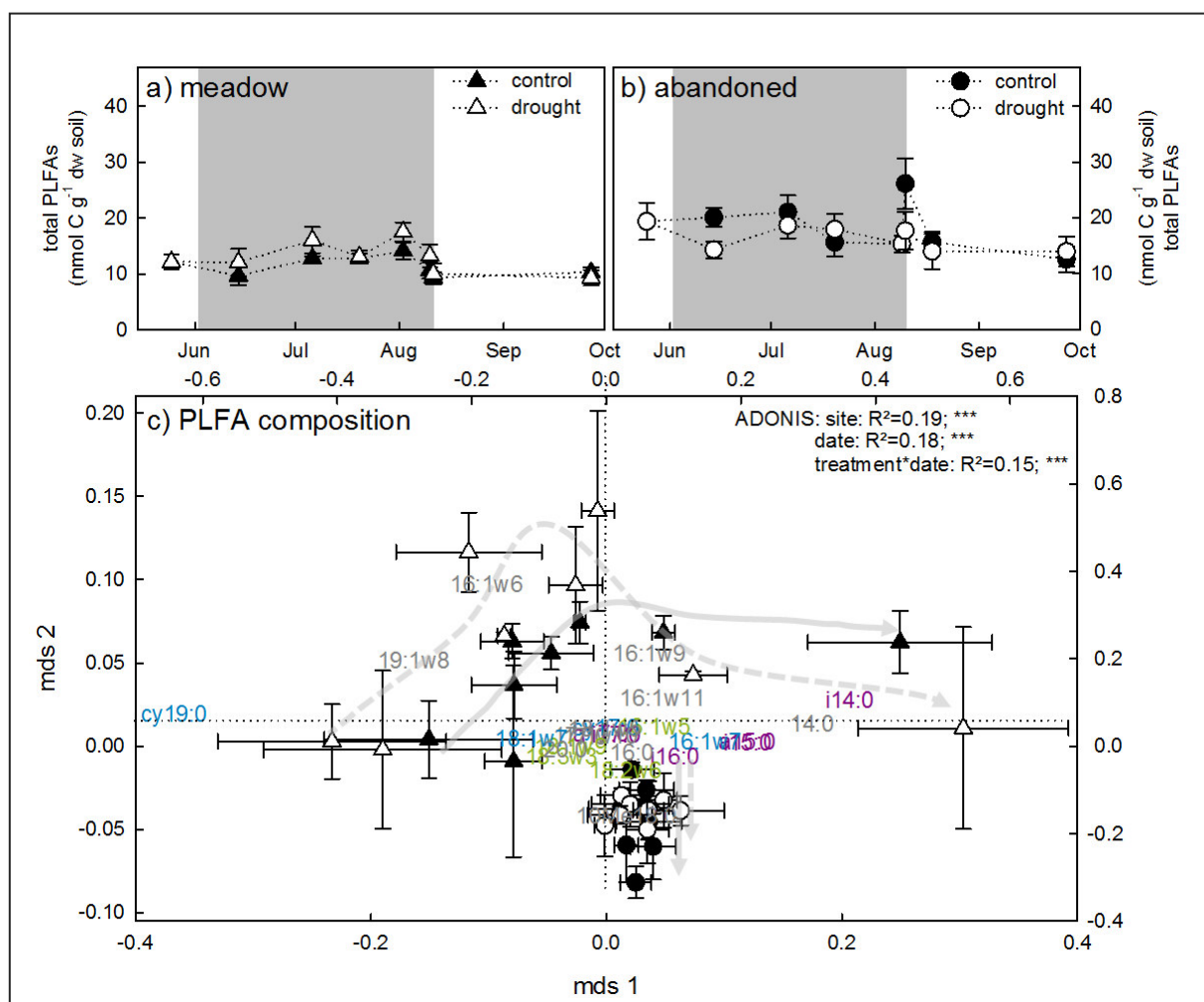
Mowing, i.e. clipping and harvesting of plant shoots, induced a strong transient ¹³C pulse in the extractable organic carbon pool in the drought

treatment, while in controls the ^{13}C in this fraction remained at a constant lower level, and rather increased in microbial PLFAs. Thus, our findings corroborate that plants release a pulse of labile compounds into the soil after mowing, which can stimulate microbial activity, mineralization processes and thereby enhance nutrient availability for plants (Patra *et al.*, 2008; Hamilton *et al.*, 2008). This carbon pulse may not have been available for microbial metabolism during drought. After mowing the PLFA content (and after rewetting also the PLFA composition) became similar in drought treated plots and ambient controls. This indicates that the microbes in the studied mountain grassland are highly resilient even after an extreme drought period and inactive microbes may quickly resuscitate, regrow and recover and disconnected populations re-connect (Figure 1C). Non-surprisingly, the ^{13}C peak in the extractable organic carbon pool in drought plots vanished after rewetting, and the accumulated labile plant carbon could have fuelled a possible CO_2 mineralization peak after rewetting (see Figure 1C, e.g. Birch, 1958) or may have been washed out.

To understand effects of drought on the nitrogen turnover in soils in more detail, we set up an additional drought experiment on two sites (an abandoned and a managed meadow) with different land-management and potentially different nitrogen levels (see Chapter 3, Fuchslueger *et al.*, (2014b); Tasser *et al.*, (2005)). At the abandoned site, where agricultural practices like mowing and grazing have been ceased 30 years ago, organic matter content (Meyer *et al.*, 2012), as well as organic nitrogen and ammonium concentrations were higher, while nitrate levels were much lower compared to the extensively managed meadow. During drought organic nitrogen and NH_4^+ concentrations per gram dry soils were not affected, but when calculated per volume of soil water, they strongly increased particularly in soils of the meadow. However, the potential of microbes to produce and take up NH_4^+ was not altered by drought and by rewetting. Nitrification, in turn showed, less temporal variability particularly in soils of the abandoned site during drought periods compared to ambient controls, while the corresponding nitrate concentrations remained stable. Bacterial *amoA* gene copy numbers (AOB) were lower in soils from the abandoned site than at the meadow, whereas archaeal *amoA* gene copy

numbers (AOA) were similar at either site. Only at the meadow, AOA gene copy numbers decreased during drought. At the abandoned site, in turn, neither AOB, nor AOA responded to drought. The strong increases of NH_4^+ concentrations per unit of soil solution during drought at the meadow could have adversely affected AOA and could further indicate a niche separation of AOA and AOB along NH_4^+ concentrations (Jia & Conrad, 2009; Di *et al.*, 2010; Thion & Prosser, 2014). Moreover, our study indicated that at the abandoned site effects of drought on soil microbes may have been less pronounced than at the meadow, likely due to a larger protective humus layer at the abandoned site (Meyer *et al.*, 2012) that might have buffered water loss during drought simulation (Franzluebbers, 2002; Knapp *et al.*, 2008; Goebel *et al.*, 2011).

The thirty years of abandonment led to a shift in plant species composition compared to the meadow (see Schmitt *et al.*, 2010; Grigulis *et al.*, 2013). Similarly, the different responses of AOA to drought between the abandoned site and managed meadow could have been caused by inherent differences between the microbial (and AOA) community composition (Zeller *et al.*, 2001; Tasser *et al.*, 2005). Similar as the soil organic carbon content, the amount of total PLFAs per dry soil was significantly higher at the abandoned site compared to the meadow (Figure 2a,b), however the two sites strongly differed in PLFA composition, indicating distinct microbial communities (Figure 2c). These distinct communities moreover exhibited strong differences in temporal dynamics: in soils from the meadow, where the plant community apparently is shaped by the annual mowing and harvesting events during the growing season, also PLFA composition showed a strong seasonal dynamic and responded to drought. At the abandoned site, in turn, the vegetative period of plants is not interrupted by agricultural management activities, and also seasonal microbial community variation was minimal and not altered by drought (see Figure 2c). This suggests that, similar as e.g. in a temperate broadleaf forest soil (Kaiser *et al.*, 2011), the possibly more fluctuating plant carbon inputs and phenological dynamics constrained by management at the meadow may exert a stronger control on the microbial community structure than at the abandoned site. Moreover, effects of drought on grassland microbial community appeared to be strongly modulated by land-management intensity.



Nonetheless, the microbial community in the mountain meadow appeared to be highly resilient to drought periods, since even after three years of recurring drought simulations the community returned to a composition similar as under ambient conditions (see Chapter 2). This is comparable to other multi-year irrigation manipulation experiments, where altered moisture regimes did not lead to lasting effects on the microbial community structure (Cruz-Martínez *et al.*, 2009; Landesman & Dighton, 2010), while it has also been shown that after several drying-rewetting cycles higher abundances of drought-tolerant taxa were detectable (Fierer *et al.*, 2003; Evans & Wallenstein, 2014).

Figure 2: PLFA dynamics during the sampling season in 2011 at the a) meadow and the b) abandoned site. The grey background indicates the period of rain exclusion. c) PLFA composition displayed as non-metric multidimensional scaling plot based on a Bray-Curtis-Similarity matrix using relative abundances of PLFAs. Triangles indicate samples from the meadow, circles indicate the abandoned site. Filled symbols represent ambient controls, open symbols drought treatment. Fungal markers are marked in green, gram-positive bacterial markers in purple, gram-negative bacterial markers in blue, general biomass markers are shown in grey. Effects of drought and sampling date were assessed by ADONIS. The light grey arrows indicate a temporal dynamic in control (full line) and drought-treated (dashed line) plots.

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Appendix

Zusammenfassung

In vielen Regionen Europas werden, als Folge globaler Erwärmung, stärkere Anstiege von Extremwetterereignissen, so wie Dürreperioden und Starkregenereignissen prognostiziert. Vor allem die geringe Wasserverfügbarkeit während Dürreperioden kann sowohl Pflanzenproduktivität, als auch mikrobiell gesteuerten Kohlenstoff (C) und Stickstoffkreisläufe (N) im Boden beeinflussen. Es ist allerdings nicht klar, wie sich Dürre indirekt, also auf die Verfügbarkeit von labilem C, der von Pflanzen an den Boden und an Bodenmikroorganismen abgegeben wird, auswirken könnte, und ob Veränderungen auch noch nach dem Ende einer Dürreperiode weiterwirken. Des Weiteren ist noch unklar, wie sich extreme Dürreperioden auf spezialisierte Gruppen von Mikroorganismen, wie Ammonium-oxidierende Bakterien (AOB) und Archaeen (AOA), die den limitierenden Schritt in Nitrifizierungsprozessen katalysieren, auswirken. Der Schwerpunkt dieser Arbeit lag darin, diese Unsicherheiten in einem Freiland-Dürre Experiment, mit Hilfe von ‚state-of-the-art‘ Techniken zu untersuchen.

Das erste Kapitel dieser Dissertation behandelt Auswirkungen einer Dürreperiode auf die Verteilung von ^{13}C markiertem, frisch-assimiliertem C innerhalb der Pflanzen und dessen Transfer zu mikrobiellen Fettsäure-Biomarkern (PLFAs) im Boden. Sowohl die Aufnahme, als auch der Transport von ^{13}C zur Wurzelbiomasse war deutlich geringer unter Dürre als unter Kontrollbedingungen. Die Menge an mikrobiellen PLFAs, speziell von Gram-positiven Bakterien war deutlich höher als in den Kontrollen; die Aufnahme von AOA stark zurückgingen, signifikant allerdings nur auf der Mähwiese, wo die Ammonium Konzentrationen in der Bodenwasserlösung während der Dürre stark anstiegen. Diese differentiellen Reaktionen von Ammoniak-oxidierenden Organismen könnte daher eher auf eine starke Nischenteilung von Bakterien und Archaeen entlang Ammonium Konzentrationen zurückzuführen sein, als auf unterschiedliche Reaktionen auf den Rückgang von Bodenwasser.

Zusammenfassend konnte gezeigt werden, dass sich extreme Trockenereignisse auf die

des von Pflanzen stammenden ^{13}C war jedoch deutlich niedriger. Da ^{13}C gleichzeitig in der extrahierbaren organischen C-Fraktion im Boden deutlich anstieg, könnte der Transfer von Pflanzen zu Mikroorganismen durch den niedrigen Wassergehalt während der Dürreperiode unterbrochen worden sein.

Im zweiten Kapitel wird untersucht, ob wiederholte extreme Dürreperioden sich auf die C-Dynamik zwischen Pflanzen und Mikroorganismen während einer weiteren, natürlich vorkommenden Trockenperiode auswirken. Pflanzen mit Dürre-Vergangenheit zeigten deutlich niedrigere Stickstoffgehalte in der oberirdischen Biomasse und reduzierten während der Trockenperiode stark die C-Aufnahme. Die Menge, als auch die Zusammensetzung von mikrobiellen PLFAs reagierten jedoch – unabhängig ihrer Dürre-Vergangenheit – stärker auf die kurze Trockenperiode. Wiederholte Dürreperioden beeinflussen also sowohl Pflanzen, als auch Mikroorganismen, aber auf unterschiedlichen Zeitskalen.

Das dritte Kapitel dieser Dissertation fokussiert auf Effekte von Dürreperioden auf den N-Kreislauf und auf Ammoniak-oxidierende Bakterien und Archaeen in einer extensiv-bewirtschafteten Mähwiese und einer Brachfläche. Physiologisch ‚breite‘ Prozesse, so wie N-Mineralisierung, reagierten kaum auf Dürre; es gab jedoch Änderungen im vergleichsweise spezialisierten Prozess der Nitrifizierung, allerdings nur auf einer der untersuchten Flächen. Die Gen-Abundanzen (*amoA*) von AOB waren während der Dürre stabil, während die Abundanzen Aufnahme und Allokation von C zu Wurzeln, und auf die Inkorporation in mikrobielle PLFAs auswirken, und eher trockentolerante mikrobielle Gruppen fördern. Wiederkehrende Dürren beeinflussten den N-Gehalt in Pflanzen, und führten – im Vergleich zu ungestörten Kontrollen – zu einer Verlangsamung des C-Umsatzes. Mikroorganismen, andererseits zeigten sich stark resilient und reagierten eher auf kurzfristige Änderungen des Wasserhaushalts. Es zeigte sich außerdem, dass die Effektstärke von Dürreperioden stark von der Bewirtschaftungsform beeinflusst werden kann.

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L. Fuchslueger, M. Bahn, K. Fritz, R. Hasibeder, S. Kienzl, M. Schmitt, M. Watzka, A. Richter; Resilience of belowground carbon allocation dynamics after drought in a mountain grassland, European Geosciences Union General Assembly, Vienna, Austria, May 2014

L. Fuchslueger, M. Bahn, K. Fritz, R. Hasibeder, A. Richter; Drought induced changes of plant C turnover affect the soil microbial community in a subalpine meadow; American Geophysical Union Fall Meeting, San Francisco, USA, December 2012

L. Fuchslueger, K. Fritz, R. Hasibeder, M. Bahn, A. Richter; Tracing the effect of summer drought on the plant-soil carbon transfer in a mountain meadow; Annual Conference of the Ecological Society for Germany, Austria and Switzerland; Oldenburg, Germany, September 2011

Poster presentations

L. Fuchslueger, M. Bahn, R. Hasibeder, S. Kienzl, K. Fritz, M. Schmitt, M. Watzka, A. Richter; Post-drought effects on the plant-microbial carbon transfer in a mountain meadow; Annual Meeting of the Austrian Soil Science Society, Vienna, Austria, September 2014

L. Fuchslueger, S. Kienzl, M. Bahn, A. Richter; Low land-use intensity mitigates drought-induced changes on microbial community functioning in mountain grassland; Open Science Conference on Climate extremes and biogeochemical cycles; Seefeld, Austria April 2013

L. Fuchslueger, C. Kaiser, M. Koranda, A. Richter; Natural abundance of ^{13}C in a long-term tree girdling experiment; Stable isotope network Austria (SINA) - meeting, Seibersdorf, Austria, Nov. 2010

Participation in workshops

SIBAE Training School on Stable Isotopes and Sampling Issues; University of Lisbon, granted by the COST Action ES0806. November 2011

List of Publications

[13] **L. Fuchslueger**, E.-M. Kastl, F. Bauer, S. Kienzl, R. Hasibeder, T. Ladreiter-Knauss, M. Schmitt, M. Bahn, M. Schlöter, A. Richter, U. Szukics; Effects of drought on nitrogen turnover and ammonia-oxidizer abundances depend on land-management in mountain grasslands; *Biogeosciences*; 11 (2014), 6003-6015; [ISI impact factor: 3.753, Q1 (29/140 Ecology)].

[12] **L. Fuchslueger**, M. Bahn, K. Fritz, R. Hasibeder, A. Richter; Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow; *New Phytologist* 201 (2014), 916-927; [ISI impact factor: 6.373, Q1 (9/196 Plant Sciences)].

[11] R. Hasibeder, **L. Fuchslueger**, K. Fritz, A. Richter, M. Bahn; Summer drought alters dynamics of carbon allocation to roots and root respiration in mountain grassland; *New Phytologist*; published online (2014); [ISI impact factor: 6.373, Q1 (9/196 Plant Sciences)].

[10] C. Kaiser, M. Kilburn, P. Clode, **L. Fuchslueger**, M. Koranda, J. Cliff, Z. Solaiman, D. Murphy; Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway versus direct root exudations; *New Phytologist*; published online (2014); [ISI impact factor: 6.373, Q1 (9/196 Plant Sciences)].

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Further skills and qualifications

Technical expertise

- Planning and coordination of samplings and handling of large sample numbers
- Application of compound specific stable isotope analysis of plant biomass (carbohydrates, organic acids) and soil samples (phospholipid fatty acids, dissolved organic carbon) by LC-Isolink-IRMS and GC-Isolink-IRMS
- Chemical analysis using chromatographical (GC-FID, HPLC) and photometrical assays
- Determination and interpretation of soil extracellular soil enzymes using fluoro- and photometrical assays
- Analysing microbial N turnover rates in soils using ^{15}N pool dilution assays
- Gas flux measurements in incubation and field studies using Infrared Gas Analysers (IRGA)

Driving License B

Languages

German: fluently writing and speaking
English: fluently writing and speaking
Basics in French and Spanish

Computing

Excellent skills in MS-Office, R, Sigma plot, Statistica, Statgraphics, Chromeleon, IsoDat.

Reviewing activities

Plant and Soil, Microbial Ecology, Annals of Forest Science, Science of the Total Environment

Memberships

European Geosciences Union, Austrian Soil Science Society, Stable Isotope Network Austria (SINA)
