Mechanisms of Resistance and Resilience in the Plant-Soil System of Mountain Grassland Communities

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CHAPTER 1 – Introduction

1.1 Background

Terrestrial ecosystems account for the largest fluxes in the global carbon (C) cycle, driven by the exchange of carbon dioxide (CO_2) between atmosphere and biosphere (Canadell et al., 2000; IPCC, 2007). In times of global change and rising atmospheric CO_2 concentrations, it becomes more and more important to study the mechanisms behind the terrestrial C cycle, in order to better estimate future CO_2 fluxes and eventually find climate mitigation strategies. Extreme climatic events, predicted to occur more frequently with increasing atmospheric temperatures (IPCC, 2013), are known to strongly affect terrestrial C cycling and have the potential to turn the biosphere in a net CO_2 source (Canadell et al., 2007; Ciais et al., 2005). Furthermore, in the terrestrial biosphere land use change has been recognised as main component of global change, altering ecosystem structure and functioning, and ultimately biogeochemical cycling (Chapin et al., 2000; Walker and Steffen, 1997).

A major part of the terrestrial biosphere is filled by grassland ecosystems, which cover about 25% of the total land surface (IPCC, 2013). Besides their importance for fodder production in many areas worldwide, grasslands are one of the largest contributors to terrestrial C storage, primary by sequestering C in soil (White et al., 2000). Regarding climate change, extreme drought has been found to be a major threat for grassland C cycling (Reichstein et al., 2013), while the effects of land-use change are particularly pronounced in mountain grasslands (Huber et al., 2005). The species composition and functioning of grassland ecosystems strongly depends on the land use type (Laliberté and Tylianakis, 2012; Socher et al., 2013; Tilman et al., 1997). By favouring particular plant species with varying growth and regeneration strategies, land use can alter the stress response of grassland communities (Lavorel et al., 1998; Lavorel and Grigulis, 2012). To date, there is little known about how different plant strategies affect the drought response of ecosystem C allocation. In addition, varying grassland management also changes the soil microbial community and thus plant-microbial interactions (de Vries et al., 2013).

The interaction of plants and soil microorganisms is a key process connecting C and nutrient fluxes in terrestrial ecosystems (Wardle et al., 2004). Moreover, the soil microbial community strongly depends on the belowground C allocation (BCA) by plants and is very responsive to changes in the C supply (Bardgett et al., 2005). An example for the connection of C and nitrogen (N) fluxes in grassland ecosystems is provided in Box 1. The transfer of plant-derived C to the rhizosphere fuels the microbial activity in soils (Gleixner, 2013), and indirectly supports the degradation of dead soil organic matter (SOM). This is associated with the mineralisation of nutrients like N (Kuzyakov et al., 2000). Mineralised N is available for plant uptake and important for plant productivity. So far, it is unclear how the drought response of such plant-soil feedbacks is modified by shifts in the grassland community and what the underlying mechanisms are. Yet, plant functional composition (Bahn et al., 2014) and plant-microbial interactions (Bardgett et al., 2009) have the potential to strongly alter ecosystem resistance and resilience to climate extremes.

Box 1: Example for the link of carbon and nitrogen fluxes

Plant-soil interactions play a crucial role in the terrestrial C cycle and link plant photosynthesis with the activity of soil microorganisms, which in turn substantially determines C and N cycling in soils (Chapin et al., 2009; Ostle et al., 2009). A large fraction of the assimilated plant carbon is rapidly allocated belowground and transferred to the rhizosphere as root exudates or via mycorrhizal interactions (Brüggemann et al., 2011). Overall, it is estimated that the use of recently plant assimilated C accounts for approximately half of the heterotrophic respiration, i.e. CO_2 release, from soils (Högberg and Read, 2006). In grassland ecosystems, arbuscular mycorrhiza (AM) fungi are the primary consumer of fresh plant-derived C, followed by saprotrophic fungi and bacteria in the rhiszosphere (e.g. de Deyn et al., 2011; Denef et al., 2009; Mellado-Vázquez et al., 2016). Symbiotic interactions with AM fungi, which are directly linked to root cortex cells, increase the access of roots to resources from soil through a wide network of very thin hyphae (Lambers et al., 2008; Rillig, 2004). Exudates from roots and hyphae are accessible to non-mycorrhizal saprotrophic fungi and bacteria (Drigo et al., 2010; Paterson et al., 2016), which are able to degrade soil organic matter (SOM) that mainly consists of polymeric residues from dead organisms (Lehmann and Kleber, 2015). In consequence, a higher supply with fresh plant-derived C can increase the microbial activity in soils and enhance the depolymerisation of SOM, which is associated with the release of plant-accessible N that is needed for plant growth (Cheng et al., 2012).

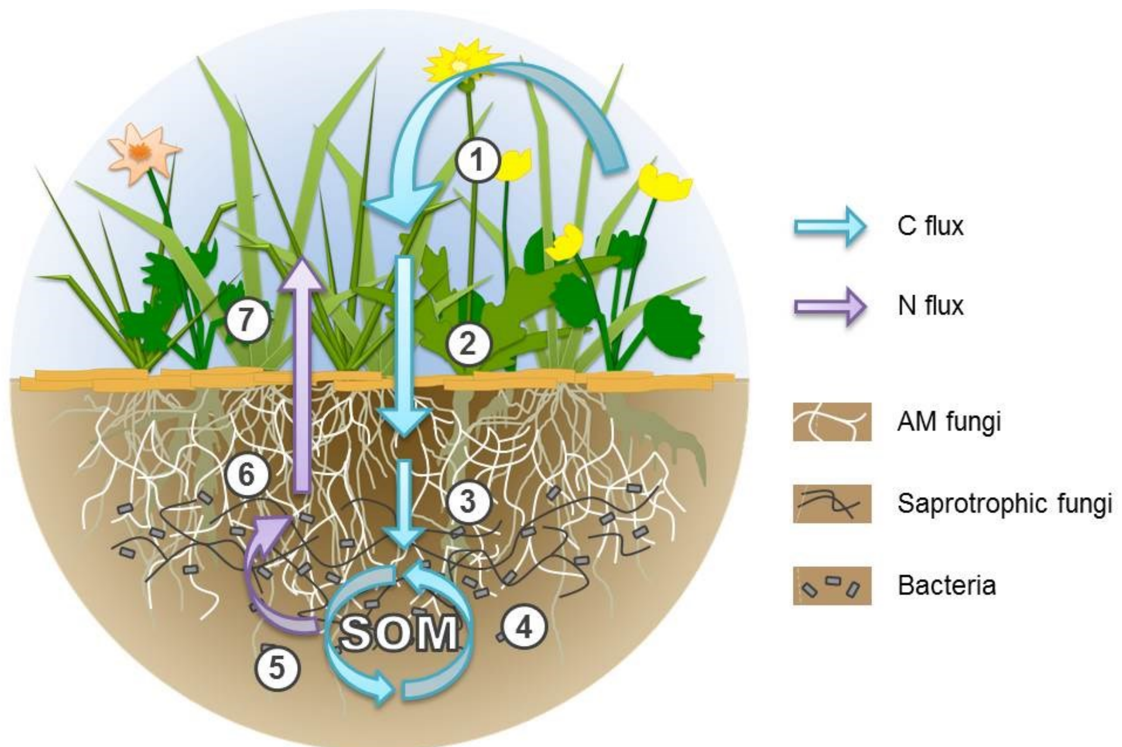

Figure 1: Plant-microbial interaction and the link of carbon (C) and nitrogen (N) fluxes in the plant-soil continuum of grassland ecosystems. 1, Photosynthesis; 2, Belowground C allocation; 3, C transfer to microorganisms in the rhizosphere; 4, Soil microbial C cycling associated with decomposition of soil organic matter (SOM); 5, N mineralisation from SOM; 6, root N uptake; 7, N allocation to shoots and use for growth.

This thesis aims at improving our knowledge about the mechanisms that provide terrestrial ecosystems with resistance and resilience to climate extremes. It focusses on the drought and recovery responses of C and N cycling in the plant-soil system and how they are modified by land-use, using the example of different mountain grassland communities. Because of the complexity of such element fluxes in terrestrial ecosystems and the diversity of influeincing ecological factors, the following parts of the introduction provide a comprehensive overview of the current state of research.

1.2 Carbon and nitrogen cycling in terrestrial ecosystems

The contribution of the terrestrial biosphere to the global C cycle mainly consists of the CO_2 exchange with the atmosphere. This includes the photosynthetic assimilation of CO_2 from the atmosphere with c. 120 Pg (1 Pg = 10^{15} g) C per year, which is nearly counterbalanced by the release of CO_2 to the atmosphere through plant and soil respiration (Canadell et al., 2000). The plant biomass pool holds c. 550 Pg C, while the majority of assimilated C, i.e. up to 2400 Pg C, is stored in soils (IPCC, 2013). From the assimilated C a large fraction is directly used by plants for growth and maintenance (Chapin et al., 1990). These processes involve CO_2 losses to the atmosphere through respiration (Trumbore, 2006). The unused plant C can either be stored in non-structural carbohydrate pools or can be further transported to the soil (Brüggemann et al., 2011). Soil microorganisms play a key role in belowground C cycling and process most of the C that enters soil (Gleixner, 2013), from which a part is stored in the microbial biomass and another part is respired as CO_2 during microbial C decomposition. Residues of dead soil microorganisms that are not directly reused by the soil community are stabilised and stored as SOM (Trumbore, 2006). Changed environmental conditions can affect terrestrial C pools by altering the ratio of CO_2 assimilation and respiration (Arnone et al., 2008; Chapin et al., 2006), with ecosystems that are a net sink for atmospheric CO_2 turning into a net source and vice versa. Shifts in net CO_2 fluxes between biosphere and atmosphere can have feed-backs on climate, because increasing atmospheric CO_2 concentrations, among other greenhouse gases, induce a global temperature increase (IPCC, 2013, 2007). On the other hand, the climate also influences C cycling in terrestrial ecosystems, as the C turnover depends on temperature and precipitation (Carvalhais et al., 2014). In particular, the decomposition of SOM by microorganisms is sensitive to temperature changes (Davidson and Janssens, 2006; Frey et al., 2013), and the soil microbial activity follows an optimal curve of soil moisture (Moyano et al., 2013; Skopp et al., 1990).

In terrestrial ecosystems, N is often the most limiting nutrient for plant growth (Vitousek and Howarth, 1991). All organisms need N for biomass production, especially for the biosynthesis of proteins that are needed for cell structure and metabolic processes. In plants, large amounts of N are allocated to the enzyme Ribulose 1-5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) (Evans and Seemann, 1989; Spreitzer and Salvucci, 2002), which plays a central role in photosynthesis. In consequence, leaf nitrogen concentrations typically correlate with

photosynthetic activity (Milcu et al., 2014; Walker et al., 2014; Wright et al., 2001). In contrast to CO_2, plants are not able to fix molecular nitrogen (N_2) from the atmosphere, and thus need to rely on the N supply by microorganisms. Hence, some plants, i.e. legumes, developed direct symbiotic interactions with bacteria that are able to fix N_2 inside root nodules. However, nitrogen fixation requires high amounts of energy (Vitousek and Howarth, 1991), thus most plants primarily gain N through the uptake of small N-containing molecules from soil. The bottleneck in soil nitrogen cycling is the depolymerisation of SOM by extracellular enzymes (Schimel and Bennett, 2004). At this process, termed 'mineralisation' in the broader sense (cf. Figure 1 in Box 1), soil microorganisms break down larger molecules to amino acids, amino sugars, peptides, ammonium or nitrate. Plants actively compete with microorganisms for these nutrients but can also profit from interactions with mycorrhiza fungi that have increased access to soil pores through their hyphae (Hodge et al., 2000). Whether the primary N source for plants is ammonium or nitrate depends on the activity of nitrifying bacteria ('nitrification'), which can oxidise the ammonium released during SOM decomposition to nitrate (Schimel and Bennett, 2004). Plant species can differ in their preference to take up one form of N, likely depending on the prevailing soil N conditions at their habitat (Lambers et al., 2008).

1.3 Food webs in soil

Soils are complicated subparts of terrestrial ecosystems that provide, due to their structural heterogeneity, diverse niches and habitats for numerous organisms (Or et al., 2007). The variable pore and aggregate sizes together with preferential flow paths provide a suite of conditions, harbouring organisms with very different demands. For example, generally oxic soils can contain anoxic micro-niches in their aggregates that allow for the growth of anaerobic bacteria (Blagodatsky and Smith, 2012), which have a distinct metabolism that can catalyse other biochemical reactions (e.g. denitrification) compared to aerobic bacteria (e.g. nitrifying bacteria). Moreover, complex food-webs evolve from the input of fresh plant material into soil and the presence of large amounts of dead SOM. Most of the plant inputs are processed by soil microorganisms (Berg and Laskowski, 2005; Gleixner, 2013), i.e. bacteria and fungi, which generally have the highest abundance in top soil (Salomé et al., 2010), where aboveground litter is entering the soil system and where fine root biomass is large. The number of bacteria per gram organic soil has been estimated in the order of 10^9 cells, including thousands of different species (Berg and Laskowski, 2005), while in the same gram of soil up to 100 meters of hyphae from more than 200 different fungal species can exist (Bardgett and van der Putten, 2014). In addition, there is also a considerable diversity of soil animals, particularly small invertebrates, including a variety of trophic levels from plant (litter)-feeders (primary decomposers) to bacterial- and fungal feeders (secondary decomposers) up to predators (Bardgett and van der Putten, 2014; Scheunemann et al., 2016). The soil fauna contributes to a lesser extent to the input of plant material but more importantly ensures the mixing of soil (Berg and Laskowski, 2005). Eventually, dead SOM is formed by the residues of dead cells from all taxa and consists of a variety of different

compound classes (Lehmann and Kleber, 2015). The chemically more inert (recalcitrant) compounds in SOM are typically decomposed more slowly (Gleixner, 2013) and are only accessible to bacteria and fungi, which can break down larger molecules by the secretion of exoenzymes into the soil matrix (Blagodatskaya and Kuzyakov, 2008; Schimel and Bennett, 2004). In consequence, the microbial activity is of uttermost importance for the cycling of C and related nutrients in soil.

In general, two different soil food webs can be defined, first the root exudate-based food web, and second the detritus-based food web that includes the decomposition of SOM (Buscot and Varma, 2005). Plants invest up to 40% of the net fixed C in root exudates (Brüggemann et al., 2011) that consist of low molecular weight compounds, such as sugars, organic acids and amino acids. These compounds are either actively released by fine roots and associated mycorrhizal fungi (Cheng et al., 2012) or can stem from lysed cells or border and root cap cells sloughed off during root growth (Dennis et al., 2010). Especially at the root tips short-chain polysaccharides are released to from mucilage that reduces the friction resistance when roots “explore” the various soil niches. The labile root exudates are the basis for a ‘fast energy channel’ (Buscot and Varma, 2005) that stimulates the microbial activity and leads to ‘priming effects’ in the rhizosphere (Kuzyakov and Cheng, 2001). If the priming effect is positive, the decomposition of older and more stable SOM is increased (cf. Box 1), whereas a negative priming effect leads to reduced SOM turnover when microorganisms are oversaturated by labile substrates (Blagodatskaya and Kuzyakov, 2008). In the classical view the primary consumers of the fast energy channel are fast-growing bacteria with low nutrient use efficiency (Buscot and Varma, 2005), so-called “r-strategists” or “copiotrophs” (de Vries and Shade, 2013; Griffiths and Philippot, 2013). However, based on results from preceding experimental studies (e.g. Denef et al., 2007; Scheunemann et al., 2016), showing that saprotrophic fungi take up large amounts of root exudates in the rhizosphere, Ballhausen and de Boer (2016) recently proposed a framework with saprotrophic fungi as primary consumers and fungus-feeding bacteria as secondary consumers of plant-derived C. Saprotrophic fungi are non-mycorrhizal fungi that can use exoenzymes to decompose polymeric organic material (Buscot and Varma, 2005), and thus also play an important role in the detritus-based soil food web. This food web can again be divided into a fungal and a bacterial energy channel, corresponding to the primary consumers of plant debris and SOM. In this case, the primary consumers in the bacterial channel consist of rather slow-growing bacteria with high nutrient use efficiency, referred to as “K-strategists” or “oligotrophs” (de Vries and Shade, 2013). Which energy channel is dominant in the detritus-based food web particularly depends on the C to N ratio (C/N) and the quality of substrates. High C/N values and the predominance of more recalcitrant material like complex polyaromatic coumpounds (e.g. lignin or humic acids) typically favour the fungal channel (Buscot and Varma, 2005).

1.4 Ecological stability and disturbance responses

Ecosystems vary in their response to changed environmental conditions,

i.e. their stability following a disturbance. Because of the high complexity of natural ecosystems, there is a multitude of possible response variables. Ecological studies of the terrestrial biosphere often investigate the effects of varying land management or plant biodiversity, i.e. the number and abundance of different species, on ecosystem functioning and its stability. In order to make this possible, a wide range of variables can be measured, including for example: Biomass production (e.g. Cardinale et al., 2007; Hoover et al., 2014; Isbell et al., 2015; Tilman et al., 2006, 1997), plant functional traits (e.g. Fontana et al., 2017; Legay et al., 2014; Lienin and Kleyer, 2012; Quétier et al., 2007; Ravenek et al., 2014), CO_2 exchange (e.g. Arnone et al., 2008; Barthel et al., 2011; Burri et al., 2014; Hagedorn et al., 2016; Roy et al., 2016), plant C allocation (e.g. Galiano et al., 2017; Hasibeder et al., 2015; Palta and Gregory, 1997; Ruehr et al., 2009; Sanaullah et al., 2012), microbial C cycling (e.g. de Deyn et al., 2011; Denef et al., 2009; Fuchslueger et al., 2014a; Lange et al., 2015; Mellado-Vázquez et al., 2016) and related N dynamics (e.g. Canarini and Dijkstra, 2015; de Deyn et al., 2009; Fuchslueger et al., 2014b; Robson et al., 2010; Zeller et al., 2000). The ecological stability can be described for each variable by several characteristics in the course of the (eco-) system's stress response. Since there are different terminologies for these characteristics, they need to be clearly defined in the frame of each study that aims to assess ecosystem stability (see recent discussions by Hodgson et al., 2015; Ingrisch and Bahn, 2018; Nimmo et al., 2015; Yeung and Richardson, 2016).

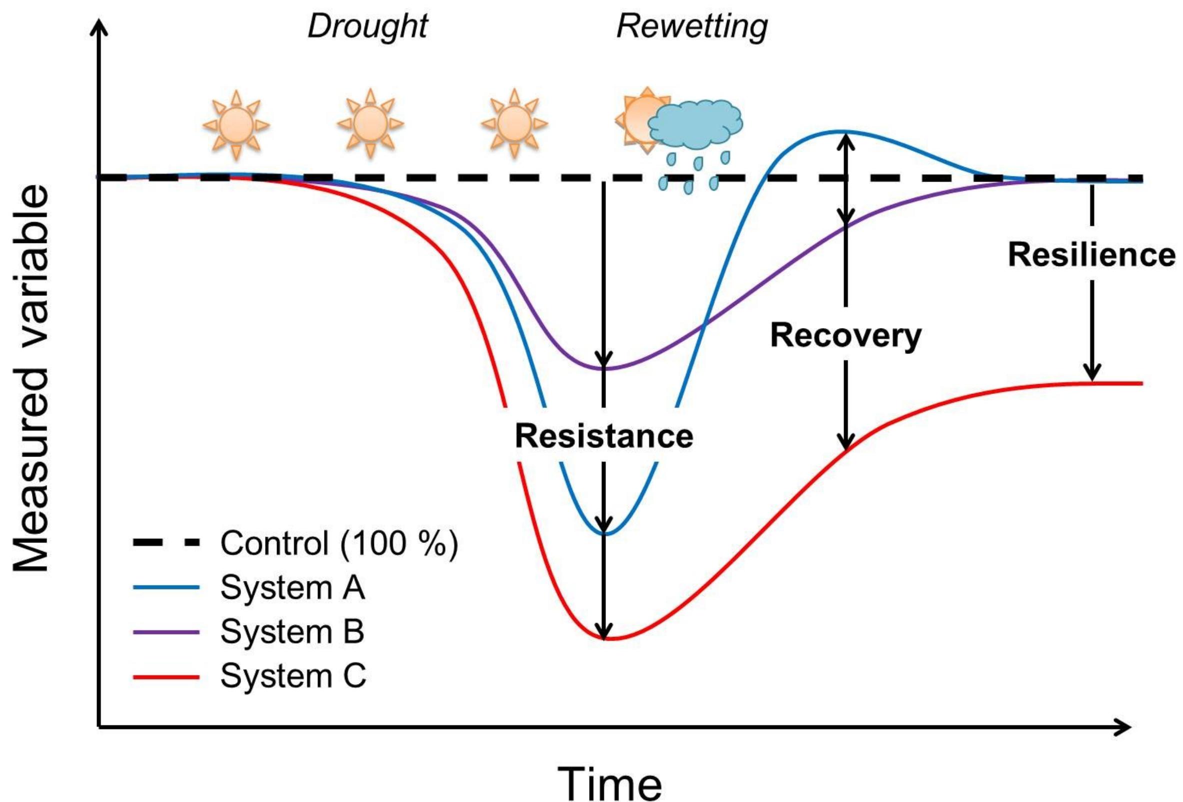

Figure 2: Possible response curves of a measured variable in three different systems (A-C) experiencing the same severe drought stress and subsequent rewetting.

Figure 2 depicts three possible ecosystem responses to a disturbance, using the example of severe drought and subsequent rewetting. First, biological systems can differ in their 'persistence', i.e. the time after an exogenous disturbance until a measured variable shows a response (Pimm, 1984). Subsequently, the 'resistance' indicates how close the variable remains near its initial value at maximum stress, contrariwise to the 'sensitivity', which describes the magnitude of change (Shade et al., 2012). After the disturbance has ended, systems can differ in their 'recovery', i.e. the endogenous processes that bring a measured variable back towards its initial value, with the rate of recovery being the 'elasticity' (Hodgson et al., 2015). Though for variables with high elasticity it is possible that a temporal increase above the initial value occurs during the recovery phase (e.g. for the microbial activity of dried soils after rewetting; Birch, 1958; Fierer and Schimel, 2003). The last characteristic of a system's stability is its 'resilience', i.e. the intensity of disturbance that a system can absorb without shifting to an alternative state (Gunderson, 2000; Holling, 1973). In the latter case the system reaches a tipping point during the disturbance, at which it cannot recover to the original equilibrium and thus is not resilient anymore.

1.5 Drought and rewetting in terrestrial ecosystems

1.5.1 Hydrologic effects of precipitation scarcity in plants and soil

Most plants in terrestrial ecosystems depend on the water supply from soil or groundwater, while the access to latter is more limited for herbaceous species compared to trees. Plants use a decreasing gradient between soil water potential and leaf water potential to transport nutrients that were taken up by roots through their xylem to aboveground parts (Blum, 2011; McDowell et al., 2008). This gradient is maintained by the opening of stomata in leaves, which allows for the exchange of gases, i.e. the transpiration of water and the diffusive inflow of CO_2. Plants can buffer fluctuations in soil water potential by regulating stomatal conductance, adjusting root structure to increase water scavenge or by increasing the solute concentration to maintain lower water potentials compared to soil (Chaves et al., 2003; McDowell et al., 2008). If the water deficit becomes more severe, plants can also shed older leaves to decrease the water demand in the canopy and reallocate their resources to younger leaves (Chaves et al., 2003). However, when the drought stress more and more increases plants reach a point, where the water retention of the soil matrix is too strong to maintain water uptake by plants. This point is called 'permanent wilting point' and depends on both, vegetation and soil type (Lambers et al., 2008). In general, drought responses of plants, from stomatal adjustment to reduced growth to leaf senescence and wilting, are highly regulated processes that are controlled by phytohormones like abscisic acid (Blum, 2011; Lambers et al., 2008).

During sustained drought, the water losses from plant transpiration and evaporation at the soil surface exceed water inputs, resulting in a continuous decrease of soil moisture. Soils do not dry out uniformly, as they have a complex structure of differently sized solid aggregates with contorted pore spaces (Or et al., 2007). Besides the establishment of preferential flow paths, the heterogeneity in the spatial arrangement of soil aggregates also leads to the inhomogenous distribution of

nutrients and the forming of diverse niches and biological ‘hotspots’ (Bundt et al., 2001). The flow paths channel the water flow and by association the diffusion of soluble substances as well as the transfer of particulate matter. In consequence, drying of soils takes place sequentially from macropores (>75 μm) to mesopores (30-75 μm) to micropores (<30 μm), eventually disconnecting the water film around individual soil aggregates from each other (Moyano et al., 2013). This strongly reduces the diffusion of solutes in soil, and thus limits the access of plants to nutrients but also the access of soil microorganisms to their C substrates (Skopp et al., 1990). Conversely, the diffusivity of gases increases trough the dried soil pores, leading to more aerobic conditions. Besides the effects on the metabolism of aerobic and anaerobic microorganisms, this can also change the predation pressure by soil animals (Or et al., 2007), e.g. microbial grazers like nematodes. Compared to drying, rewetting more suddenly changes the conditions in soil. The increased water availability is often associated with an initial pulse of high solute concentrations from material that was immobilised during drought (Canarini et al., 2017). In general, the time needed for a full rewetting depends on how long the soil was dry before. This time can be modified by factors like sand/clay content, soil pH and the content as well as hydrophobicity of SOM (Goebel et al., 2011).

1.5.2 Alterations in plant carbon allocation

Drought not only affects the water budget of plants but also has the potential to strongly alter their C budget due to reductions in photosynthesis (Lawlor and Cornic, 2002). Besides the reduced C uptake through closed stomata, photosynthesis can further be limited by the lower root N uptake during drought (Lambers et al., 2008), decreasing the production of photosynthetic enzymes like RuBisCO. Consequently, in addition to the hydrologic collapse or ‘hydraulic failure’ plants may also die due to C starvation (McDowell et al., 2008). In this context, plants are divided into ‘isohydric’, i.e. plants that reduce stomatal conductance to maintain relatively constant leaf water potentials during drought, and ‘anisohydric’, i.e. plants that allow the leaf water potential to decline according to the soil water potential during drought. It is assumed that the anisohydric strategy increases the risk that the evapotranspiration exceeds a critical value after which hydraulic failure occurs, whereas isohydric plants rather suffer from C starvation as a consequence of the reduced photosynthesis when stomata are closed during drought (McDowell et al., 2008). Drought-induced decreases in the photosynthetic activity certainly reduce the amount of C that is available for plant organs that act as C sink. Nonetheless, this is not the limiting factor for plant growth, which is resulting from the biochemical downregulation of more sensitive processes like the elongation of leaf cells and the biosynthesis of proteins (Lambers et al., 2008). On the other side, the reduced C supply is affecting C pools related to other functions like inter-compartmental transport (Brüggemann et al., 2011), osmoregulation (Chaves et al., 2003) and storage (McDowell et al., 2008). These functions are largely fulfilled by non-structural carbohydrates (NSCs), e.g. sucrose, fructan and starch.

The disaccharide sucrose consists of each one glucose and fructose monomer

and is the main product of the photosynthetic pathway. It primarily serves as transport sugar for the exchange of C between different plant organs (Lambers et al., 2008), especially from aboveground sources (leaves) to belowground sinks (roots). When sucrose arrives in sink tissues it can be cleaved to its monomers or it is used as storage, like in the taproot of sugar beet (Lambers et al., 2008). Glucose and fructose molecules from cleaved sucrose can be either used as energy source in the metabolism, for the building of structural compounds, for the formation of storage NSCs or as root exudates (Brüggemann et al., 2011). Storage NSCs like starch or fructan can be utilised when the C supply through photosynthetic assimilation is limited, e.g. under environmental stress conditions or during (re-)sprouting in spring. Starch is a polysaccharide that is built from hundreds to thousands of glucose monomers. It is ubiquitous in the plant kingdom and serves as transitory storage for excess photosynthates in leaves as well as long-term storage in stems and roots (Lambers et al., 2008). Fructan is built from a variable number of fructose monomers and one initial glucose monomer. It can be found as oligosaccharide (three to ten fructose units) or polysaccharide and is present in a number of grass and forb species, particularly in the stem (Janeček et al., 2011; Pollock, 1986). In addition to storage function, fructan accumulation was also found to play a role in freezing and desiccation tolerance (Van den Ende, 2013; Vijn and Smeekens, 1999).

Various studies reported a preferential use of freshly assimilated C for BCA under drought conditions (Barthel et al., 2011; Burri et al., 2014; Hagedorn et al., 2016; Hasibeder et al., 2015; Huang and Fu, 2000; Palta and Gregory, 1997; Sanaullah et al., 2012), which is likely at the expense of aboveground storage formation under reduced C supply (Bahn et al., 2013). In combination with reduced turnover of root sucrose during drought (Hasibeder et al., 2015), the preference for BCA can lead to the accumulation of water-soluble sugars, especially sucrose, in roots (Hagedorn et al., 2016; Sicher et al., 2012). Water-soluble sugars are often used for osmoregulation (Chaves et al., 2003; Chen and Jiang, 2010), as higher concentrations increase the osmotic pressure of cells, i.e. tendency of water to diffuse into the cells. Other explanations for root sugar accumulation during drought include a decreased C need for metabolic activity or storage formation (Hasibeder et al., 2015), potentially simultaneously occurring with osmotic adjustment. In consequence, the maintenance of BCA during drought may be important for plants in order to sustain the functioning of roots, i.e. water and nutrient uptake (Skinner and Comas, 2010); or to preserve C resources in roots that are less susceptible to drought, because of a water potential more close to the soil, than shoots (Blum, 2011). The C preserved in roots may be used later to initiate the regeneration of aboveground biomass after rewetting or to fuel the microbial activity in the rhizosphere in order to increase the mineralization of N, which is needed to increase the photosynthetic capacity during regrowth. However, the drought response of plant C allocation can vary, depending on the plant species, functional type or community composition. In tree species, increased residence time of recent C in leaves (Ruehr et al., 2009) and a general increase of NSCs in aboveground and belowground plant organs (Galiano et al., 2017) have been observed. For different mixtures and monocultures of grassland species, Sanaullah et al. (2012) also found that the

preference for C allocation to shoots or roots can vary. Thus, further research about the response of BCA to drought and how it is modified by varying plant (functional) composition may proof as helpful to improve C cycle predictions in the context of global change.

1.5.3 Responses of the soil microbial community

In soils only a minor part of the present microorganisms is metabolically active at the same time, typically yielding a number between 4% and 40% of the total microbial community (Lennon and Jones, 2011). Three factors mainly determine the microbial activity in soils: 1) substrate availability, 2) temperature and 3) soil moisture (Moyano et al., 2013; Or et al., 2007; Schimel et al., 2007; Skopp et al., 1990). Depending on the combination of these factors, the soil microbial activity steers the degradation of SOM (Carvalhais et al., 2014; Davidson and Janssens, 2006) and the release of greenhouse gases like CO_2, methane (CH_4) and nitrous oxide (N_2O) (Blagodatsky and Smith, 2012). Extreme drought events affect soil microbial activity especially by the physical effects of reduced soil moisture and by shifts in the substrate availability. As soil moisture levels broadly vary over time and between different locations, soil microorganisms have adapted to different moisture niches and associated changes in oxygen and substrate diffusion (Borken and Matzner, 2009; Lennon et al., 2012). Thus, severe drought unequally affects functionally distinct parts of the soil microbial community.

The cellular water potential of soil microorganisms is closely coupled to the soil water potential due to the semipermeable nature of cell membranes (Schimel et al., 2007). Extreme water deficit can lead to death of active microbial cells, while some inactive forms like spores can also tolerate complete dehydration and become active when water conditions improve (Potts, 1994). The reduction of metabolic activity or complete dormancy is a phylogenetically wide-spread trait of microorganisms that helps to survive adverse environmental conditions (Lennon and Jones, 2011). Dormant microorganisms can for example physically differentiate to resting structures like spores or cysts, change their cell structure, reduce their cell size or their RNA and DNA content, or alter the composition and quantity of lipids and fatty acids. When the conditions become more favourable, dormant cells can resuscitate and again become active (Lennon and Jones, 2011). Similar to plants, microorganisms can also counteract desiccation by increasing the intracellular concentrations of osmotically active compounds (Potts, 1994; Schimel et al., 2007). Bacteria mainly use N-containing osmolytes, i.e. amino acids (e.g. proline and glutamine) or derivatives of these (e.g. glycine betaine). In contrast, fungi typically use N-free polyols (e.g. erythritol, glycerol and mannitol), which reduces the N costs for osmotic adjustment. Another adjustment to the changed physical conditions in dry soil is the secretion of protective compounds, i.e. extracellular polysaccharides (Roberson and Firestone, 1992). Latter, amongst other exopolymeric substances (e.g. DNA, proteins and lipids), are the primary component of biofilms that commonly embed microbial cells in their colonies and form the interface to the surrounding matrix (Donlan, 2002). The viscosity of biofilms can be adjusted to retain water and to provide more

favourable mechanical properties in drying soil (Or et al., 2007). In general, adjustments to changes in the hydration status strongly alter C resource allocation in the cell metabolism and are associated with high energy costs (Schimel et al., 2007).

Drought typically leads to a decoupling of plant photosynthesis and belowground processes, which can be assessed by soil respiration (Barthel et al., 2011; Burri et al., 2014; Hagedorn et al., 2016; Ruehr et al., 2009). Heterotrophic microbial processes are a major contributor to soil respiration (Trumbore, 2006) and, furthermore, drought has been found to decrease the amount of recent plant assimilates recovered in the microbial biomass (Fuchslueger et al., 2016, 2014a). Shifts in the C supply are known to strongly affect the soil microbial community, which can respond very quickly in in a non-linear way (Bardgett et al., 2005). Changes in C availability either result from the reduced mobility of substrates and microorganisms in dry soil (Moyano et al., 2013; Skopp et al., 1990) or from alterations in plant BCA and root exudation (Brüggemann et al., 2011; Dennis et al., 2010). Latter may be temporarily increased to fuel nutrient mineralisation in the rhizosphere, to enhance access to water via mycorrhizal interactions or to increase the viscosity of mucilage at the root tips in order to protect against the higher friction resistance of drying soil during root growth. In these cases, rhizospheric microorganisms could profit from the higher substrate availability and respond with enhanced growth rates, if the water deficit is not too limiting. Indeed, using nitrifying bacteria as model organisms Stark and Firestone (1995) found that during drying of soil the bacterial activity is first reduced by substrate limitation, until a critical threshold is reached when cell dehydration becomes the more limiting factor. Another example are arbuscular mycorrhiza (AM) fungi that interact with most land plant species (Délano-Frier and Tejeda-Sartorius, 2008; Rillig, 2004) and have been found to extend their hyphal network during drought, thereby indirectly increasing root access to water from finer soil pores (Allen, 2007). On the other side, plants may also reduce root exudation during drought as a consequence of osmotic adjustment or to preserve the limited C resources. If the supply of labile plant-derived C is reduced, SOM degrading microorganisms, i.e. saprotrophic fungi and slow-growing oligotrophic bacteria, can profit from the reduced competition with fast-growing copiotrophic bacteria (de Vries and Shade, 2013; Schimel et al., 2007). A simple functional classification of bacteria can be obtained from the structure of their cell wall, which is visible under a microscope after 'Gram'-staining of pedptidoglycans, polymeric compounds built-up from sugars and amino acids (Buscot and Varma, 2005). Gram-positive (G+) bacteria have a thicker peptidoglycan layer in their cell wall than Gram-negative (G-) bacteria, and thus are considered to be more resistant to drying and rewetting (Barnard et al., 2013; Schimel et al., 2007). In addition, G+ bacteria were typically found to prefer C sources related to SOM or detritus, in contrast to G- bacteria that were more strongly linked to the rhizosphere (e.g. Bahn et al., 2013; Bai et al., 2016; Denef et al., 2009; Kramer and Gleixner, 2008; Mellado-Vázquez et al., 2016). This also translates into the classification of G+ bacteria as K-strategists/oligotrophs and G- bacteria as r-strategists/copiotrophs (Philippot et al., 2013). Similar to G+ bacteria, fungi are perceived as inherently more resistant to desiccation, as they can tolerate lower water potentials and have a higher spatial

access to resources than bacteria (Buscot and Varma, 2005; Schimel et al., 2007). In consequence, severe drought events can induce shifts to a microbial community that is more dominated by fungi and G+ bacteria (Fuchslueger et al., 2014a; Schimel et al., 2007).

The rewetting of soil after severe drought events brings a sudden shift in physical conditions for soil microorganisms. To avoid the bursting of cells, microorganisms need to quickly release the osmolytes they stored during drought (Schimel et al., 2007). Solutes that accumulated in soil due to adjustments in the microbial metabolism (Canarini et al., 2017; Warren, 2014) and the break-down of larger soil aggregates during drought (Denef et al., 2001; Schimel et al., 2011) may notably increase the substrate availability after rewetting. As a result, rewetting events are typically followed by an immediate pulse of dissolved organic carbon (DOC) in soil (Canarini et al., 2017) and a subsequent peak in microbial C mineralisation, called 'Birch-effect' after its discoverer (Birch, 1958). The Birch-effect is measurable as increased soil respiration rate after a few hours up to a few days from rewetting (Canarini et al., 2017), which is also linked to increased rates of nitrogen mineralisation (Birch, 1958; Borken and Matzner, 2009; Canarini and Dijkstra, 2015; Fierer and Schimel, 2002). So far, the exact C source of the Birch-effect has not been revealed (Borken and Matzner, 2009; Canarini et al., 2017; Moyano et al., 2013), and thus a contribution of recently assimilated plant-derived C (e.g. released osmolytes or fresh root exudates) cannot be excluded. At least a part of the mineralised C seems to stem from the microbial biomass itself (Fierer and Schimel, 2003). In any case, the microbial biomass usually increases after the respiration pulse (Canarini et al., 2017; Fierer and Schimel, 2002), allowing for increased SOM decomposition that also provides nutrients for plants. In particular, bacteria were found to have a high recovery rate (Barnard et al., 2013; de Vries et al., 2012; Meisner et al., 2013), indicating that fast-growing species like G- bacteria can profit the increased substrate availability. Based on their cell structure with two cell membranes including the intermembrane space, G- bacteria are also able to perform more complex or specialised metabolic functions (Schimel et al., 2007), e.g. nitrification. In consequence, the increased microbial activity following drought-rewetting events can support the recovery of plants by providing nutrients (especially N) that are necessary to rebuild the photosynthetic apparatus and aboveground biomass. Although the rewetting pulse may not completely compensate the decreased C and N turnover during drought, high uncertainties remain concerning the impact of land use on the response of microbial N cycling (Borken and Matzner, 2009).

1.6 Combined effects of climate and land use change

1.6.1 Global change and local impacts – The Alps as an example

Mountain regions provide many resources and services that are also important for surrounding lowlands (EEA, 2010; Huber et al., 2005). These include biodiversity, food production, recreation and water storage. For example the European Alps are considered as "water towers for Europe" (EEA, 2009). However, this region has

already experienced a temperature warming twice as fast as the northern hemisphere on average (Auer et al., 2007; Beniston, 2005). The trend is predicted to continue and may result in a temperature increase of up to +4 °C by the end of the 21st century (Gobiet et al., 2014), yielding in higher rates of warming than in other regions. The faster warming goes hand in hand with more irregular precipitation patterns, increasing the probability and frequency of severe drought periods and heavy rainfall events in European mountain regions (Beniston, 2005; Gobiet et al., 2014; IPCC, 2012, 2007).

Mountain areas are strongly affected by land-use change (Huber et al., 2005; MacDonald et al., 2000; Spehn and Körner, 2005), as the use of modern agricultural technologies is limited due to the demanding terrain, restricting the usage of heavy machines. Furthermore, in some regions like the European Alps, economical shifts to more profitable domains like tourism have been observed (Schermer et al., 2016). This already led to large-scale shifts in grassland management practices, such as the abandonment of former hay meadows or pastures (MacDonald et al., 2000). Such land use changes are accompanied by shifts in the plant functional composition and the soil microbial community, altering C and N cycling in the ecosystem (Grigulis et al., 2013; Legay et al., 2014). For the plant community the ‘leaf economics spectrum’ or ‘resource use strategy’ has been found to strongly co-vary with ecosystem functioning in mountain grasslands (Grigulis et al., 2013; Lavorel and Grigulis, 2012).

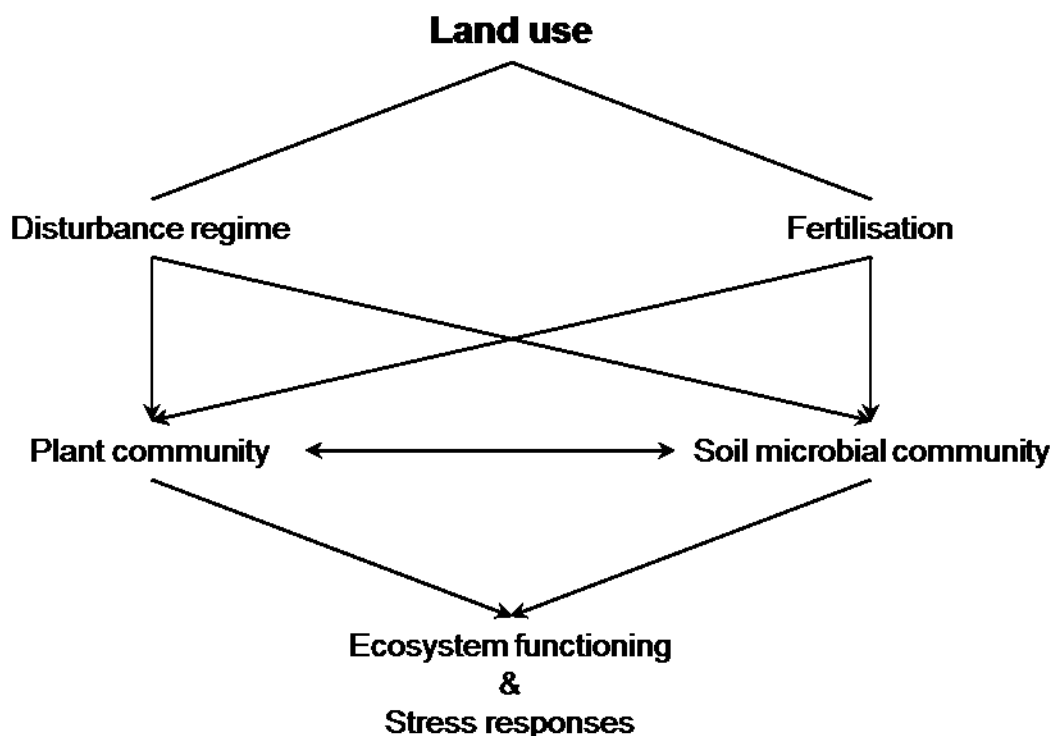

Figure 3: Conceptual flow chart of how land use alters ecosystem functioning and ecosystem stress responses.

Abandonment of mountain grasslands increases the dominance of slow-growing ‘conservative’ plant species with low nutrient demands (Fontana et al., 2017;

Quétier et al., 2007; Wohlfahrt et al., 1999). On the contrary, grassland management suppresses the dominance of certain species through the constant removal of aboveground biomass by cutting or grazing, and favours fast-growing 'exploitative' species with high nutrient uptake capacity due to regular fertilisation. In consequence, abandoned mountain grasslands are less diverse (Dullinger et al., 2003; Niedrist et al., 2009) and less productive (Schmitt et al., 2010) than hay meadows. The shifts in plant composition are coupled to higher fungi to bacteria ratios in abandoned grasslands (Zeller et al., 2001, 2000), which might be related to a higher importance of interactions between plant species and arbuscular mycorrhiza (AM) fungi (Gross et al., 2010). In summary, land use influences the structure and functioning of the plant and soil microbial communities as well as their interactions through variable disturbance regimes and the degree of fertilisation (Figure 3). The plant and soil communities in turn strongly determine overall ecosystem functioning and the response to extreme stress events.

1.6.2 Consequences of land use change for grassland resilience

Biodiversity is well known to alter the ability of ecosystems to resist and recover from a disturbance (Chapin et al., 2000; Hooper et al., 2005; Isbell et al., 2015). In general, higher species richness is associated with a higher functional redundancy, i.e. there is a higher probability that there are several species with a trait that is essential for proper ecosystem functioning. If the functioning of one of these species is impaired by a disturbance, the other species can still ensure the overall ecosystem functioning ('insurance hypothesis'; Loreau, 2000). On the other hand, in terrestrial ecosystems land use changes are known to alter the relative abundance of plant species, their 'evenness' more quickly than species richness (Chapin et al., 2000; Hillebrand et al., 2008). Changes in plant evenness can alter the interaction within and between distinct plant species, i.e. the intra-specific and inter-specific competition or facilitation for resources, and how these interactions respond to a disturbance (Hillebrand et al., 2008). A number of studies on grasslands found that plant species evenness is closely coupled to ecosystem functioning (e.g. Assaf et al., 2011; Kirwan et al., 2007; Lamb et al., 2011; Orwin et al., 2014; Wilsey and Potvin, 2000). In this sense, alterations in species evenness introduced by land-use change may lead to the prevalence of different plant functional traits, which may in turn affect the ecosystem response to a disturbance (Díaz et al., 2007).

For example, in mountain grasslands species with more conservative resource use traits might be more resistant to drought, as they typically have thicker and more N-poor leaves than exploitative species (Lavorel and Grigulis, 2012), making them less susceptible to desiccation and decreased N mineralisation in soil during drought. In addition, conservative plant species can have strong interactions with AM fungi (Gross et al., 2010; Legay et al., 2016), which potentially further increase their resistance by an increased access to water and nutrients from soil. Consequently, in abandoned grasslands, dominated by conservative species and a fungal-based soil food web (Grigulis et al., 2013; Quétier et al., 2007; Zeller et al., 2001), the nutrient supply might not be a limiting factor for plant functioning during drought. In contrast,

in fertilised meadows with more exploitative species, which are used to high nutrient availability, nutrient limitation may become a problem during drought. Together with the higher productivity of exploitative species, which requires broader and thinner leaves (Lambers et al., 2008), and the prevalence of the bacterial food web (Grigulis et al., 2013), this likely decreases the resistance of rather exploitative meadow communities. To determine a plant's resource use strategy, functional traits like the specific leaf area (SLA; in [m^2/kg]), the leaf nitrogen content (LNC) and the leaf dry matter content (LDMC) can be used as proxies (Díaz et al., 2004; Garnier et al., 2004; Laliberté and Tylianakis, 2012; Quétier et al., 2007; Wright et al., 2004). These traits, related to the leaf economics, have been found to strongly correlate with the productivity, i.e. the rate of CO_2 assimilation and biomass production, of plants. Exploitative species typically have a higher SLA and LNC, but a lower LDMC than conservative species. In addition to the resource use strategy, the functional type (grass/monocotyledon or forb/dicotyledon) has also been found to affect the resistance of herbaceous species to drought (Bollig and Feller, 2014; Gilgen et al., 2010; Gilgen and Buchmann, 2009; Zwicke et al., 2013). In temperate regions grasslands mainly consist of C3 species, which are named according to the product of the first step of their photosynthesis (3-phosphoglycerate, a compound with three C atoms). The stomatal opening of C3 grasses has been found to be less responsive to drought compared to C3 forbs (Bollig and Feller, 2014). This can lower the resistance of C3 grasses to drought, as the later closing of stomata can lead to higher water losses. In contrast, C4 grasses that dominate in warmer and more arid regions (Lambers et al., 2008) can be more resistant than C3 forbs (Hoover et al., 2014), as C4 plants developed an alternative carbon fixation pathway (using oxaloacetic acid with four C atoms as first photosynthetic product), which reduces the time needed for stomatal opening.

The plant composition of course influences the composition and the functioning of the soil microbial community (Bardgett et al., 2009, 2005; Philippot et al., 2013; Wardle et al., 2004). On the other side, the soil community exerts strong feedbacks on plants (Bardgett et al., 2009; de Vries et al., 2013), influencing the structure and functioning of the aboveground community, and largely affects the response of terrestrial ecosystems to environmental change (Bardgett and van der Putten, 2014). In the rhizosphere, root exudates can attract beneficial microorganisms and stimulate their growth (Bardgett et al., 2014; Philippot et al., 2013), whereas pathogens are repelled by secondary metabolites like salicylic acid (Dennis et al., 2010). For example, in more diverse grasslands higher amounts and higher diversity of root exudates were found to increase the microbial biomass (Eisenhauer et al., 2017), with the root exudate diversity being able to alter the microbial community composition (Steinauer et al., 2016). Despite higher microbial activity, increased rhizospheric carbon inputs were found to be positively related to soil carbon storage (Lange et al., 2015), possibly because higher plant diversity mainly increases the access of root-associated AM fungi and G- bacteria to recently assimilated plant-derived carbon (Mellado-Vázquez et al., 2016). Similarly, the quality and quantity of plant litter is known to have strong influences on the soil microbial community (Wardle et al., 2004), including large shifts between growing and non-

growing seasons (Bardgett et al., 2005). Nitrogen-rich litter from fast growing plants, as found in managed grasslands, leads to bacterial-dominated food webs. Nitrogen-poor and phenolic-rich litter from slow growing plants, which dominate in the late succession of abandoned grasslands, promotes fungal-dominated food webs. In general, according to the differences in cell structure and growth rate (cf. section 1.5.3), there is evidence that bacterial-dominated food webs are less resistant to drought but in turn recover more quickly than fungal dominated-food webs (de Vries et al., 2013, 2012). In consequence, land use alters grassland resilience by changes in the composition of both, the plant and the soil microbial community. Additionally, land use implies regular fluctuations in environmental conditions that create legacy effects, which can modify soil microbial functioning throughout a disturbance (Hawkes and Keitt, 2015). For example, the regular removal of biomass and occasional fertilisation in managed grasslands may lead to an acclimatisation of certain taxa to variable C and N supplies, facilitating high resource use and quick recovery after drought, whereas the more stable conditions in unmanaged grasslands constrain this effect.

1.7 Tracing ecosystem carbon and nitrogen fluxes

Whenever element fluxes from one source to multiple possible targets are studied, stable isotope analysis provides a useful and harmless tool to discern between different fluxes (Bahn et al., 2012). Most elements in the periodic system form stable isotopes, i.e. non-radioactive atoms with the same number of protons but varying numbers of neutrons, which naturally occur in element-specific narrow ranges (Brand and Coplen, 2012). Except for the mono-isotopic phosphorus, all major elements found in organic compounds (hydrogen, carbon, nitrogen, oxygen and sulfur) consist of one main stable isotope and one or two rarer and heavier stable isotopes. As the heavy isotopes form stronger atom bonds than their lighter analogues, enzymatic reactions typically discriminate against heavy isotopes, yielding a higher depletion the more metabolic processes are involved (Brand and Coplen, 2012). This process, called ‘isotopic fractionation’, can vary between different (organism-specific) enzyme reactions and also depends on the current environmental conditions. In consequence, the natural abundance of stable isotopes in a certain compound, compartment or organism can already serve as a proxy for abiotic and/or biotic environmental conditions (e.g. Flanagan and Farquhar, 2014; Guenther et al., 2013; Hobbie and Högberg, 2012; Scheidegger et al., 2000). However, this natural variability brings along a high uncertainty and does not allow for measuring distinct flux rates. Therefore, labelling experiments with high amounts of the naturally rare heavy stable isotopes are the method of choice when element fluxes in the biosphere need to be determined.

In case of C, two stable isotopes exist, namely ^{12}C and ^{13}C, which naturally occur in a ratio of approximately 99:1 ($^{12}C/^{13}C$). In order to assess the short-term C flux in the plant-soil system, a pulse labelling with ^{13}C-enriched CO_2 (e.g. $^{12}C/^{13}C$ = 1:1) can be applied to the plant canopy and the allocation to different targets can be traced by taking samples and analysing their ^{13}C content

(Brüggemann et al., 2011; Epron et al., 2011; Leake et al., 2006). This allows distinguishing how much of the photosynthesised ^{13}C was allocated to different plant and soil pools at a given time after the pulse labelling. Plant BCA can be assessed by measuring the ^{13}C content of bulk shoot and bulk root material, while the shoot/root-internal allocation to storage or soluble sugars can be determined by compound specific ^{13}C isotope analysis on NSCs (Bahn et al., 2013; Hasibeder et al., 2015). To estimate the overall transfer of recently photosynthesised C to the rhizosphere and its uptake by soil microorganisms, aqueous extracts from soil without (soil extractable organic C) and with chloroform fumigation (microbial biomass C) can be analysed for their bulk ^{13}C content (Fuchslueger et al., 2014a; Malik et al., 2013, 2015). The ^{13}C uptake through different groups of soil microorganisms is definable by performing compound-specific ^{13}C isotope analysis on microbial biomarkers, such as phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) (Bai et al., 2016; Denef et al., 2009; Fuchslueger et al., 2014a; Kramer and Gleixner, 2006; Malik et al., 2015; Mellado-Vázquez et al., 2016). PLFAs contain specific markers for G- bacteria, G+ (actino)-bacteria and saprotrophic fungi, and NLFAs include a specific marker for AM fungi (Frostegård et al., 2011; Ruess and Chamberlain, 2010).

For N also two stable isotopes exist, i.e. ^{14}N and ^{15}N, which are found in nature with a ratio of approximately 99.6:0.4 (^{14}N/ ^{15}N). In principle, the mineralisation of nitrogen from polymeric SOM can be studied by amending soils with ^{15}N-labelled plant litter (Herman et al., 2012; Nuccio et al., 2013). However, this would induce a disturbance and may lead to additional fertilisation, both not desired when studying the response of plant-soil interactions to environmental change. Nevertheless, variations in plant N uptake can be determined by adding small amounts of highly ^{15}N-enriched water-dissolved N (e.g. nitrate or ammonium) to the soil and measuring the ^{15}N content of plant material after a certain time (Avice et al., 1996; Dijkstra et al., 2015; Thuille et al., 2015). This indirectly allows concluding about how well plants are able to use the excess N, resulting from the pulse of soil microbial activity after rewetting dry soil.

1.8 Aim and outline of this thesis

The general aim of this thesis is to identify mechanisms of how terrestrial ecosystems respond to the combined effects of global climate and societal change. This is done on the example of severe drought events in mountain grassland ecosystems that are strongly subjected to land-use changes. The focus of this work is on C allocation in the plant-soil continuum and plant-microbial interactions as ecosystem key functions. More specific, the objectives of this thesis are:

1) Determine how drought and rewetting affect C allocation in the plant soil continuum and identify the underlying mechanisms.
2) Assess the contribution of plant-microbial interactions to overcome the effects of extreme drought in grassland ecosystems.

3) Study how the drought responses of C allocation and plant-microbial interactions in mountain grasslands are affected by land-use change, especially through shifts in plant functional composition.

Chapter two is based on a common garden experiment on a mountain meadow site in the Austrian Alps, where intact vegetation-soil monoliths from two differently managed grassland sites were used to study how land use alters the drought response of C allocation and plant-microbial interactions. The monoliths were taken from an abandoned site that remained completely unmanaged for more than 30 years and a traditionally managed hay meadow that is cut once per year, fertilised every two or three years and occasionally grazed. Both land use types were treated simultaneously with artificial drought by using rain-out shelters to exclude precipitation, and then ^{13}C pulse labelling was used at peak drought and shortly after rewetting to determine how the C fluxes in the plant-soil system are related to drought resistance and recovery, respectively. To assess potential benefits for plant recovery from interactions with soil microorganisms a ^{15}N labelling was applied at the rewetting and plant N uptake was determined at the recovery.

Chapter three examines more closely how drought alters the link between plants and soil microorganisms, and aims at explaining the mechanisms behind the previously observed disconnection between plant and soil processes during drought. The study was conducted at the same location as in chapter two but used a mesocosm setup, where plastic pots were installed in the soil at the study site, filled with soil from the meadow and planted with six local species in variable mixtures. Drought simulation and ^{13}C pulse labelling were performed according to chapter two and the C fluxes from plants to the rhizosphere and its inhabiting microorganisms were determined in more detail.

In chapter four the species compositions of the mesocosms from chapter three were used to asses effects of plant functional composition on the drought response, independent from other management-related factors. In particular, the chapter deals with how plant resource use strategy and grass to forb ratio alter C allocation in the plant-soil system at peak drought and after rewetting. Similar to chapter two, results from a ^{15}N labelling at the rewetting were used to determine differences in plant N uptake during recovery.

Finally, chapter five discusses the main findings of this thesis and provides an outlook for future research that could further strengthen our knowledge on the contribution of plant-microbial interactions to ecosystem stability in a changing world.

This thesis is based on the following manuscripts:

Chapter 2 – Manuscript 1

Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions

Stefan Karlowsky, Angela Augusti, Johannes Ingrisch, Roland Hasibeder, Markus Lange, Sandra Lavorel, Michael Bahn, Gerd Gleixner

Published in: *Journal of Ecology, 106: 1230–1243, 2018*

In this study we used a common garden experiment on a mountain site with intact vegetation-soil monoliths from a traditionally managed hay meadow and abandoned grassland, which is completely unmanaged since more than 30 years. We simulated drought by excluding precipitation with rain-out shelters and conducted two ^{13}C pulse labelling campaigns, to study how land use modifies the response of C allocation in the plant-soil continuum at peak drought and shortly after rewetting. We found that grassland management affected the responses of plant carbon allocation and plant-microbial interactions to both, drought and rewetting. Drought induced a shift to BCA, especially in the managed meadow, and increased the abundance of AM fungal markers, particularly in the more resistant abandoned grassland. After rewetting strong plant-bacterial interactions and increased nitrogen uptake were associated with a quick recovery of the meadow. We conclude that land use can alter the resilience of grassland ecosystems and that there is a trade-off between high resistance and quick recovery.

Conceived the ideas:	Michael Bahn, Sandra Lavorel and Gerd Gleixner
Designed the experiments:	Stefan Karlowsky (50 %), Angela Augusti, Johannes Ingrisch, Roland Hasibeder, Michael Bahn and Gerd Gleixner
Performed the experiments:	Stefan Karlowsky (70 %), Angela Augusti, Johannes Ingrisch, Roland Hasibeder and Gerd Gleixner
Analysed the data:	Stefan Karlowsky (70 %), Angela Augusti and Markus Lange
Wrote the paper:	Stefan Karlowsky (80 %) and Gerd Gleixner

Chapter 3 – Manuscript 2

Drought-Induced Accumulation of Root Exudates Supports post-drought Recovery of Microbes in mountain Grassland

Stefan Karlowsky, Angela Augusti, Johannes Ingrisch, Mohammad Kamal Uddin Akanda, Michael Bahn, Gerd Gleixner

Published in: *Frontiers in Plant Science, 9:1593, 2018*

For this study on a mountain meadow we planted mesocosms, filled with soil from the meadow site, with six local grassland species in randomised compositions. We simulated drought by excluding precipitation with rain-out shelters and performed two ^{13}C pulse labelling campaigns, to determine how drought and rewetting affect the link between plant photosynthesis and soil microbial processes. We found that during drought plants, despite investing more C resources into the osmotic adjustment of roots, continued transferring recent assimilates to the rhizosphere. This led to the accumulation of ^{13}C tracer in the non-microbial fraction of soil extracts, while the uptake of ^{13}C tracer into the microbial fraction was strongly reduced. Furthermore, from the reduced microbial ^{13}C uptake a smaller fraction was invested into marker lipids related to growth or energy storage. The connection of plant photosynthesis and soil microbial C cycling was, however, rapidly restored after rewetting and the C that accumulated in roots and the rhizosphere during drought disappeared. We conclude that the disconnection of plant and soil processes during drought is a result of substrate diffusion limitation followed by a slowdown of microbial processes in dry soils. Moreover, our data suggests that the continuous plant exudation during drought primes the activity of rhizospheric microorganisms after rewetting.

Conceived the ideas:	Michael Bahn and Gerd Gleixner
Designed the experiments:	Stefan Karlowsky (60 %), Angela Augusti, Johannes Ingrisch, Michael Bahn and Gerd Gleixner
Performed the experiments:	Stefan Karlowsky, (60 %) Angela Augusti, Johannes Ingrisch, Mohammad Kamal Uddin Akanda and Gerd Gleixner
Analysed the data:	Stefan Karlowsky (70 %), Angela Augusti and Mohammad Kamal Uddin Akanda
Wrote the paper:	Stefan Karlowsky (80 %) and Gerd Gleixner

Chapter 4 – Manuscript 3

Plant evenness and functional composition affect belowground carbon allocation in mountain grassland and alter ecosystem stress tolerance

Stefan Karlowsky, Angela Augusti, Johannes Ingrisch, Michael Bahn, Gerd Gleixner

In preparation for: *Agriculture, Ecosystems & Environment*

In this study on a mountain meadow we investigated how the plant composition from a randomised mesocosm experiment affects the response of BCA to drought and rewetting as well as the plant N uptake during recovery. We used plant species evenness, i.e. the relative abundance of each of the six mesocosm species, grass to forb ratio (Gr:Fo) and plant resource use strategy, i.e. community-weighted mean specific leaf area (CWM_SLA) and the ratio of exploitative to conservative species (Ex:Co), to describe differences in the plant communities. Drought was simulated by using rain-out shelters, BCA was assessed by ^{13}C pulse labelling and plant N uptake was determined after adding a ^{15}N label to soil during rewetting. We found that plant species evenness had no effects on the response to drought and rewetting. In general, Gr:Fo and Ex:Co had overlapping but variably strong effects. During drought, plant C allocation mainly depended on Ex:Co, with higher reductions of ^{13}C tracer contents in more exploitative communities. In contrast, the C transfer to soil microorganisms was more strongly affected by Gr:Fo, with a higher microbial ^{13}C tracer uptake in more grass-dominated mesocosms. During recovery root ^{15}N tracer concentrations correlated with CWM_SLA, indicating that more exploitative species increased their N uptake after rewetting. We conclude that both, plant functional type (Gr:Fo) and resource use strategy (Ex:Co and CWM_SLA), alter the response of grassland C and N cycling to drought-rewetting events. However, they seem to operate at different levels in the plant-soil continuum, suggesting that grassland stress responses depend on several functional characteristics of the plant community.

Conceived the ideas:	Michael Bahn and Gerd Gleixner
Designed the experiments:	Stefan Karlowsky (60 %), Angela Augusti, Johannes Ingrisch, Michael Bahn and Gerd Gleixner
Performed the experiments:	Stefan Karlowsky (60 %), Angela Augusti, Johannes Ingrisch, Mohammad Kamal Uddin Akanda and Gerd Gleixner
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CHAPTER 2 – Manuscript 1

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RESEARCH ARTICLE

Journal of Ecology

Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions

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Abstract

1. Mountain grasslands have recently been exposed to substantial changes in land use and climate and in the near future will likely face an increased frequency of extreme droughts. To date, how the drought responses of carbon (C) allocation, a key process in the C cycle, are affected by land-use changes in mountain grassland is not known.
2. We performed an experimental summer drought on an abandoned grassland and a traditionally managed hay meadow and traced the fate of recent assimilates through the plant–soil continuum. We applied two $^{13}CO_2$ pulses, at peak drought and in the recovery phase shortly after rewetting.
3. Drought decreased total C uptake in both grassland types and led to a loss of above-ground carbohydrate storage pools. The below-ground C allocation to root sucrose was enhanced by drought, especially in the meadow, which also held larger root carbohydrate storage pools.
4. The microbial community of the abandoned grassland comprised more saprotrophic fungal and Gram(+) bacterial markers compared to the meadow. Drought increased the newly introduced AM and saprotrophic (A+S) fungi:bacteria ratio in both grassland types. At peak drought, the ^{13}C transfer into AM and saprotrophic fungi, and Gram(−) bacteria was more strongly reduced in the meadow than in the abandoned grassland, which contrasted the patterns of the root carbohydrate pools.
5. In both grassland types, the C allocation largely recovered after rewetting. Slowest recovery was found for AM fungi and their ^{13}C uptake. In contrast, all bacterial markers quickly recovered C uptake. In the meadow, where plant nitrate uptake was enhanced after drought, C uptake was even higher than in control plots.
6. *Synthesis*. Our results suggest that resistance and resilience (i.e. recovery) of plant C dynamics and plant-microbial interactions are negatively related, that is, high resistance is followed by slow recovery and vice versa. The abandoned grassland was more resistant to drought than the meadow and possibly had a stronger link to AM fungi that could have provided better access to water through the hyphal network. In contrast, meadow communities strongly reduced C allocation to storage and C transfer to the microbial community in the drought phase, but in the recovery phase invested C resources in the bacterial communities to gain more nutrients for

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regrowth. We conclude that the management of mountain grasslands increases their resilience to drought.

KEYWORDS
^{13}C pulse labelling, below-ground carbon allocation, carbohydrates, land abandonment, nitrogen uptake, NLFA, PLFA, resilience, resistance, stress tolerance

1 | INTRODUCTION

Extreme drought events may be the biggest climate change-related threat for the global carbon cycle (Reichstein et al., 2013), and their impacts on mountain ecosystems are highly uncertain (IPCC, 2007, 2012, 2013). In the European Alps, temperature increased twice as fast during the last century than in the remaining northern hemisphere (Auer et al., 2007). Moreover, regional climate models project additional temperature increases that are accompanied by lower precipitation during summer (Gobiet et al., 2014). Therefore, further research to understand the impact of extreme droughts on mountain ecosystems is needed.

Mountain ecosystems are also impacted by socioeconomic changes, which typically lead to changes in land management intensity and land-use change (MacDonald et al., 2000; Spehn & Körner, 2005; Tasser & Tappeiner, 2002; Vittoz, Randin, Dutoit, Bonnet, & Hegg, 2009). The abandonment of marginal grasslands changes the composition of plant communities and their likely response to environmental factors. Abandonment also leads to (1) changes in the C dynamics, like lower plant productivity (Schmitt, Bahn, Wohlfahrt, Tappeiner, & Cernusca, 2010), (2) shifts from root to shoot litter inputs (Meyer, Leifeld, Bahn, & Fuhrer, 2012), (3) more fungal-dominated soil communities (Zeller, Bardgett, & Tappeiner, 2001) and (4) changes in nutrient dynamics, like slower nitrogen (N) cycling in soil (Robson, Lavorel, Clement, & Roux, 2007; Zeller, Bahn, Aichner, & Tappeiner, 2000). Currently, it remains unclear how these altered ecosystems respond to climatic extremes (Bahn, Reichstein, Dukes, Smith, & McDowell, 2014).

To investigate the response of ecosystems to disturbances, such as climate extremes, we have to consider two different factors. On the one hand, the capacity of a system to resist to disturbances, that is, the ability to maintain ecosystem functioning during a perturbation, and on the other hand, its "resilience," that is, the ability to return to initial ecosystem functioning after a perturbation (Nimmo, Mac Nally, Cunningham, Haslem, & Bennett, 2015; Pimm, 1984). The resistance of a system can be measured directly at maximum stress in comparison with a control (Nimmo et al., 2015). Resilience can be measured only after the stress is released, either as time till the functioning is fully recovered or at a given time point quantifying the remaining stress response (Hodgson, McDonald, & Hosken, 2015; Yeung & Richardson, 2016). Currently, it remains unclear if high resistance that keeps a function active will also lead to faster recovery of this function.

Below-ground C allocation (BCA) is a key process of the carbon cycle that influences the residence time of C in ecosystems and promotes the ability of plants to recover from disturbances (Brüggemann et al., 2011; Chapin, Schulze, & Mooney, 1990). However, so far the response of BCA to drought is variable. Sometimes BCA decreases (Ruehr et al., 2009), sometimes it remains unchanged (Hasibeder, Fuchslueger, Richter, & Bahn, 2015) and sometimes BCA increases during drought (Barthel et al., 2011; Burri, Sturm, Prechsl, Knohl, & Buchmann, 2014; Huang & Fu, 2000; Palta & Gregory, 1997). It is very likely that drought increases the need of recent assimilates in the roots for maintenance respiration (Barthel et al., 2011), for growth (Burri et al., 2014; Huang & Fu, 2000) and for osmotic adjustment (Hasibeder et al., 2015; Van den Ende, 2013; Vijn & Smeekens, 1999). Often, the enhanced BCA under stress is maintained at the expense of above-ground C storage (Bahn et al., 2013; Barthel et al., 2011) and either less storage carbohydrates (e.g. starch, fructans) are produced or the storage pools are metabolized to sucrose that is needed for transport and for the formation of below-ground C storages (Benot et al., 2013; Brüggemann et al., 2011). In consequence, compound-specific investigations are needed to better understand the underlying mechanisms.

However, BCA also influences the soil-microbial activity and community structure and their feedbacks to the plant community (Bahn et al., 2013; Bardgett, Bowman, Kaufmann, & Schmidt, 2005; Bardgett, de Deyn, & Ostle, 2009; Chapin et al., 2009; Gleixner, 2013; Kuzyakov, 2010). First of all, the microbial community facilitates plant access to soil-derived nutrients (e.g. nitrogen and phosphorus) that are necessary for plant regrowth after disturbance. However, the role of individual parts of the microbial community has to be differentiated. Arbuscular mycorrhiza (AM) fungi can improve plant water uptake during drought and may consequently contribute to plant resistance to drought (Allen, 2007). Greater fungal biomass, frequently observed in abandoned grasslands compared to managed grasslands (Grigulis et al., 2013; Zeller et al., 2000, 2001), enhances the resistance to drought (de Vries et al., 2012; Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2014a; Schimel, Balser, & Wallenstein, 2007). On the other hand, bacteria-dominated communities may contribute more to the resilience of plant communities because of their faster response time and higher growth rate (de Vries et al., 2012). Gram-negative bacteria are, for example, directly linked to the flow of root exudates (Bahn et al., 2013; Denef, Roobroeck, Manimel Wadu, Lootens, & Boeckx, 2009; Kramer & Gleixner, 2008). In contrast, Gram-positive bacteria, which additionally feed on soil organic matter (Bai, Liang, Bodé, Huygens, & Boeckx, 2016; Kramer & Gleixner, 2008; Mellado-Vázquez et al., 2016), may be more resistant to drought (Lennon, Aanderud, Lehmkuhl, & Schoolmaster, 2012; Schimel et al., 2007) than Gram-negative bacteria and may even benefit from pulses of organic matter

induced by drought (Fuchslueger et al., 2014a). Isotopic pulse-chase experiments provide the experimental platform to determine the interactions between plant and soil-microbial communities (Mellado-Vázquez et al., 2016).

Drought events (Fuchslueger et al., 2014a; Hasibeder et al., 2015) and grassland management (Grigulis et al., 2013; Schmitt et al., 2010), taken independently, affect C and N cycling in mountain grasslands. However, the combined effects of drought and grassland management intensity and how they affect the resistance and resilience of the grassland community are not well known. Here, we experimentally simulated early summer drought for two mountain grassland communities from an abandoned grassland and a managed hay meadow in an common garden experiment and assessed changes in plant C allocation and plant–soil C transfer using a ^{13}C pulse-labelling approach at peak drought (resistance labelling) and in the recovery phase (resilience labelling). The main focus of this study was to understand (1) how drought affects the C partitioning between storage and transport carbohydrates, (2) how BCA and C transfer to the microbial community respond during and after drought and (3) how land use affects C allocation and its resistance and resilience to drought. We hypothesize that BCA in abandoned grasslands will have greater resistance to drought than hay meadows, due to its comparatively lower productivity and its fungal-dominated microbial community. We furthermore hypothesize that abandoned grasslands will have lower resilience than managed grasslands, because managed meadows and their microbial communities are better adapted to recover from disturbance. Thus, we expect that after rewetting plant C transfer to the rhizosphere recovers more quickly in the managed compared to the abandoned grassland.

2 | MATERIALS AND METHODS

2.1 | Site

The study site is located near Neustift in the Stubai valley in the Austrian Central Alps and is described with its different land-use types by Schmitt et al. (2010). Briefly, both grassland types considered here, an abandoned grassland (1,970–2,000 m a.s.l.; 47°07′31″N, 11°17′24″E) and a hay meadow (1,820–1,850 m a.s.l.; 47°07′45″N, 11°18′20″E), are situated at a southeast exposed hillside with similar inclination (19°–20°), average annual temperature (3°C), annual precipitation (1,097 mm) and soil type (dystric cambisol). The abandoned grassland has been unmanaged for more than 30 years and has a *Seslerio-Caricetum* vegetation community, which is invaded by dwarf shrubs (e.g. *Calluna vulgaris* and *Vaccinium myrtillus*). The meadow is cut once per year at peak biomass in early August and manured every 2–3 years and has a *Trisetum flavescentis* vegetation community consisting of perennial grasses and forbs (Bahn, Schmitt, Siegwolf, Richter, & Bruggemann, 2009). Spring biomass is higher in the meadow (190–313 g/m^2) than in the abandoned grassland (106–215 g/m^2), while peak biomass in summer is similar for both grassland types (*c.* 400 g/m^2; Schmitt et al., 2010). Abandoned grassland soil has higher contents of SOM, extractable organic N and NH_4^+ than meadow soil, which instead has a higher NO_3^- content and a lower C:N ratio (Fuchslueger et al., 2014b). Bulk density (Meyer et al., 2012) as well as total C and N contents (Zeller et al., 2001) and root N concentrations (Bahn, Knapp, Garajova, Pfahringer, & Cernusca, 2006) are higher in the meadow than in the abandoned grassland. Higher fungal biomass was reported for the abandoned grassland compared to meadow (Grigulis et al., 2013; Zeller et al., 2001).

2.2 | Experimental setup and labelling

For both sites, abandoned and meadow, intact vegetation-soil monoliths with *c.* 30 cm soil depth and 25 cm diameter were taken in summer 2013. The monoliths were transferred into stainless steel cylinders with collection space for leachates at the bottom (deep seepage collectors, DSCs; Obojes et al., 2015) and were embedded together in the soil at the meadow site (Ingrisch et al., 2017). In this commonly applied approach, the diameter and the depth of the monoliths might exclude some species present at the two sites and might damage roots as well as mycorrhizal networks. To overcome the latter effect, we preincubated the monoliths for 1 year at the experimental site. While the monoliths probably did not cover all plant species present in these very diverse grasslands (Spehn & Körner, 2005), we are confident that we sampled representative subsets of both grassland communities. In spite of the potential drawbacks, this study design allowed us to investigate the drought response of both land-use types at most comparable conditions, using a randomized block design with replicated drought and control treatments for both land-use types (Figure S1).

In total, 24 monoliths were utilized in this study, to perform two labelling campaigns with three replicates for each land-use type and each control/drought treatment ($2 \times 3 \times 2 \times 2$). Monoliths from the abandoned grassland held about 70% grasses, 26% forbs, 1% legumes and 3% dwarf shrubs, while monoliths from the meadow held about 54% grasses, 44% forbs, 2% legumes and no dwarf shrubs. To prevent a possible inflow of runoff water into the monoliths, the surface level of the DSC cylinders was 2 cm elevated relative to the surrounding soil surface. All monoliths were preincubated over winter on-site and the experiment was started on 21 May 2014 by simulating early summer drought. Six rain-out shelters with a base area of 3 × 3.5 m and 2.5 m height, covered by light- and UV-B permeable plastic foil (Lumisol clear AF, Folitec, Westerburg, Germany, light transmittance *c.* 90%), were installed overall monoliths. Air ventilation was facilitated by leaving the shelters open at the bottom (<0.5 m above-ground) and at the top of the face sides. Monoliths of control treatments were watered manually during rain exclusion, exceeding natural precipitation by 35% for the abandoned grassland and by 43% for the meadow. The amount of water added was adjusted according to soil moisture measurements to avoid water limitation for controls and to compensate for the increased evapotranspiration under the rain-out shelters as well as naturally occurring drought (Ingrisch et al., 2017). Soil temperature (S-TMB sensor and HOBO Micro Station H21-002 data logger; Onset Computer Corporation, Bourne, MA, USA) and soil water content (Decagon EC-5, 5TM, 5TE (combined SWC, Temperature), logger Em50; Decagon Devices, Pullmann, WA, USA) were monitored continuously in the

main rooting horizon on subplots for each land-use type and treatment. On 21 June 2014, the first ^{13}C pulse-labelling campaign on 12 monoliths started, and after finishing on 28th June 2014, the drought simulation was stopped exactly after 5.5 weeks. The rain-out shelters were removed and 50 mm of water was added to all monoliths, which was enough to obtain leachates at the bottom of all DSCs. At the end of rewetting, 20 mg of water-dissolved KNO_3 with 10% ^{15}N (2 mg ^{15}N and 100 ml water per monolith) was distributed equally on the soil of the remaining 12 unlabelled monoliths, which were later used for the second ^{13}C pulse-labelling campaign. After a recovery phase of around 2½ weeks, the recovery labelling was started on 16 July 2014.

The ^{13}C pulse labellings were done always on four monoliths per day, representing both land-use types (abandoned grassland/meadow) and both precipitation treatments (control/drought). The resistance labelling was done on three consecutive days (21 till 23 June) with high radiation. Due to weather conditions, this was not possible for recovery labelling, which was conducted on 16, 18 and 19 July. The pulse labelling was performed similarly as described by Bahn et al. (2009, 2013) and Hasibeder et al. (2015). Briefly, a cylindrical and transparent Plexiglas chamber with 25 cm diameter and 50 cm height was placed on the top of the monoliths with a rubber gasket in between the chamber and the DSC. Elastic bands were used to fix the chamber on external anchor points to ensure gas tightness. Fans and tubes connected to a pump that circulated water cooled with ice packs did air circulation and temperature control, respectively. During the pulse labelling, we monitored the internal air temperature (shaded sensor), CO_2 concentration (Licor 840A; Lincoln, NE, USA) and ^{13}C isotope ratio of CO_2 (Picarro G2101i Analyzer; Picarro Inc., Santa Clara, CA, USA). Solar radiation was measured outside the chamber using a PAR quantum sensor (PQS 1; Kipp & Zonen, Delft, the Netherlands). Pulse labelling was done under comparable light conditions on mostly clear days between 9:45 and 14:45 CET. Highly enriched $^{13}CO_2$ (99.27 atom-% ^{13}C; CortecNet, Voisins-Le-Bretonneux, France) was added to achieve *c*. 50 atom-% ^{13}C in chamber CO_2 with a concentration range of 400–800 ppm during a labelling time of 75 min.

2.3 | Sampling

Plant and soil samples were collected 1.5 hr, 5 hr, 1 day, 2 days, 3 days and 5 days after the pulse labelling. Natural abundance samples were collected from separate monoliths on 26th and 27th June, representing each land-use type and treatment (averaged for later analysis). From a surface of around 10 cm^2, shoot material was cut around 0.5 cm above soil, and soil samples from the first 7 cm were taken directly below the cut surface using a stainless steel tube with 3 cm inner diameter. The metabolic activity of fresh shoots was immediately stopped using microwaves (Popp et al., 1996) and the treated shoots were stored on ice packs for transport. Roots were removed from the soil while carefully sieving the soil to 2 mm. Soil for phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analysis was directly frozen in liquid N_2 and stored at −20°C until further preparation. Subsamples of frozen soil were used to determine the soil water content gravimetrically, by weighing the soil before and after drying for 48 hr at 105°C. The soil water content was calculated as average overall sampling times for each monolith. Roots were washed from remaining soil and dead and coarse roots (diameter >2 mm) were removed. Fine root samples were portioned into two subsamples. One subsample was treated in the same way like shoot samples, and the other one was kept moist with wet paper towels until root respiration measurements. If total root biomass was low, no subsample for root respiration measurements was taken. Microwaved shoot and root samples were dried at 60°C for 72 hr on the same day. Root biomass was directly estimated from the dry mass of all root samples from one monolith. For shoot biomass, all monoliths were harvested completely at the end of each sampling campaign and the total dry mass per monolith was determined. All plant material was ball milled for further analyses (MM200; Retsch GmbH, Haan, Germany).

2.4 | Root respiration measurements

Root respiration was measured directly in the field. About 0.2 to 1.2 mg fresh roots were incubated in a 100 ml Erlenmeyer flask at 15 ± 1°C in a water bath (Hasibeder et al., 2015). Five gas samples were collected, one immediately after closing the flask and the other four after 7, 20, 40 and 60 min. The concentration of CO_2 and the ^{13}C isotope composition were analysed by isotope ratio mass spectrometry (IRMS; Delta$^+$ XL; Thermo Fisher Scientific, Bremen, Germany). All gas samples were analysed at the latest 2 weeks after sampling.

2.5 | Isotopic composition of plant samples and carbohydrates

The ^{13}C and ^{15}N contents of plant samples were analysed by elemental analysis (EA)-IRMS (EA 1100, CE Elantech, Milan, Italy; coupled to a Delta+ IRMS; Finnigan MAT, Bremen, Germany). For carbohydrate analysis, 30 mg of plant powder was weighed and water soluble sugars were extracted using the method of Wild, Wanek, Postl, and Richter (2010), as modified by Mellado-Vázquez et al. (2016). In brief, 3 × 1.5 ml of boiling bidistilled water was added to the plant material and extraction was carried out for 3 × 10 min at 85°C at 1,050 rpm in a horizontal shaker (Thermomixer comfort, Eppendorf AG, Hamburg, Germany). The samples were centrifuged and the combined supernatant was filtered with 0.45 μm cellulose membrane filters (MULTOCLEAR 0.45 μm RC 13 mm; CS-Chromatographie Service GmbH, Langerwehe, Germany) and transferred to anion and cation exchange cartridges (Dionex OnGuard II A and H 1.0 cc cartridges; Thermo Scientific, Sunnyvale, CA, USA) to remove ionic components. The neutral fraction was analysed by high-performance liquid chromatography (HPLC)-IRMS (Dionex UltiMate 3000 UHPLC coupled via a LC-IsoLink system to a Delta V Advantage IRMS; Thermo Fisher Scientific) on a NUCLEOGEL SUGAR 810 Ca^{2+} column (Macherey-Nagel GmbH & Co. KG, Düren, Germany) at 80°C with a flow of 0.5 ml/min bidistilled water (Hettmann, Brand, & Gleixner, 2007). Fructans were mostly visible as one large peak at the beginning of the chromatogram (Benot et al., 2013) and their identity was confirmed after hydrolyses with inulinase from *Aspergillus niger* (Sigma-Aldrich

Chemie GmbH, Munich, Germany) using the HPLC-IRMS. Starch was analysed from the remaining pellets of the sugar extraction. The pellet was washed with a methanol:chloroform:water mixture (12:3:5, by volume) to remove potentially remaining sugars and lipids. The starch was digested with heat stable α-amylase (Göttlicher, Knohl, Wanek, Buchmann, & Richter, 2006; Richter et al., 2009) and finally resulting gluco-oligomer solution was measured after drying at 40°C by EA-IRMS (see above).

2.6 | Neutral and phospholipid fatty acid content and C isotope composition

Neutral and PLFAs were extracted from frozen soil samples using the modified method of Bligh and Dyer (1959), according to Kramer and Gleixner (2006). In this study, total lipids were extracted from *c.* 5 g of bulk soil using pressurized solvent extraction (SpeedExtractor E-916; Büchi Labortechnik AG, Flawil, Switzerland) with a mixture of methanol, chloroform and 0.05 M K_2HPO_4 buffer (2:1:0.8, by volume; pH 7.4). The soil samples were mixed with precombusted quartz sand and transferred into 40 ml stainless steel extraction cells, a recovery standard (1,2-Dinonadecanoyl-sn-Glycero-3-Phos phatidylcholine; Larodan Fine Chemicals AB, Malmö, Sweden) was added on top (recovery rate: 93 ± 27%, n = 52) and the extraction was carried out at 70°C and 120 bar for 3 × 10 min. The pressurized solvent extraction yielded similar amounts of PLFAs compared to the established method (Kramer & Gleixner, 2006) if the extraction was done near room temperature at 40°C (Figure S2). Using 70°C, the extraction efficiency was increased by around 50% on average (Table S1). After extraction, the separated chloroform phase was subjected to silica-filled solid-phase extraction (SPE) columns (CHROMABOND SiOH, 2 g, 15 ml; Macherey-Nagel GmbH & Co. KG) to obtain neutral lipid and phospholipid fractions. Both fractions were hydrolysed and methylated with methanolic KOH and resulting fatty acid methyl esters (FAMEs) were further purified using aminopropyl-modified SPE columns (CHROMABOND NH_2, 0.5 g, 3 ml; Macherey-Nagel GmbH & Co. KG). The FAME C13:0 (Sigma-Aldrich Chemie GmbH) was added as internal standard to all samples prior to quantification by gas chromatography-flame ionization detection (GC-FID).

The PLFAs were analysed on a GC-FID 7890B with a programmable temperature vapourisation (PTV) injector (Agilent Technologies, Palo Alto, CA, USA) using a DB-1MS UI column (60 m × 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies) and helium as carrier gas (1.8 ml/min). The temperature programme started at 45°C for 1 min, then increased in a first ramp of 60°C/min to 140°C, held for 0.5 min, followed by a second ramp of 2°C/min until 264°C and a third ramp until 320°C, held for 3 min. Directly after injection, the PTV was heated up from 55°C to 280°C at a rate of 500°C/min.

Neutral lipid fatty acids were quantified on a GC-FID HP6890 (Agilent Technologies) with constant injector temperature (280°C), using a DB-1MS column (50 m × 0.32 mm internal diameter × 0.52 μm film thickness, Agilent Technologies) and helium as carrier gas (2 ml/min). The temperature programme started with 140°C for 1 min, followed by a first ramp of 2°C/min until 270°C, held for 6 min and a second ramp of 30°C/min until 340°C, held for 5 min.

Identification of FAMEs was done by comparison of chromatograms with different known FAME mixtures (Supelco 37 Component FAME Mix; Sigma-Aldrich Chemie GmbH; BR2 and BR4 mixture, Larodan Fine Chemicals AB) and an in house database (Kramer & Gleixner, 2006; Mellado-Vázquez et al., 2016; Thoms, Gattinger, Jacob, Thomas, & Gleixner, 2010).

Compound-specific ^{13}C isotope analysis of NLFAs and PLFAs was done by GC-IRMS (GC 7890A with PTV injector; Agilent Technologies; coupled via a Conflo IV/GC IsoLink to a Delta V Plus IRMS; Thermo Fisher Scientific) using a DB-1MS UI column (60 m × 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies) and helium as carrier gas (1.8 ml/min). Directly after injection, the PTV was heated up from 55°C to 280°C at a rate of 500°C/min. The GC temperature programme started with 45°C for 1 min, then increased in a first ramp of 60°C/min to 140°C (held for 0.5 min), followed by a second ramp of 4°C/min until 283°C (held for 4.9 min) and a third ramp until 320°C (held for 3 min). Concentrations and ^{13}C isotope content of identified FAMEs were corrected for the methyl group introduced during derivatization. We used the sum of the PLFAs i14:0, i15:0, a15:0, i16:0, a17:0, i17:0 and br18:0 for Gram-positive bacteria (Zelles, 1997, 1999); 10Me16:0 and 10Me18:0 for Gram-positive actinobacteria (Lechevalier, De Bievre, & Lechevalier, 1977; Zelles, 1999) and 16:1ω7 and 18:1ω7 for Gram-negative bacteria (Zelles, 1997, 1999). The PLFA 18:2ω6,9c was used as marker for saprotrophic fungi (Frostegård & Bååth, 1996; Zelles, 1997) and the NLFA 16:1ω5 as marker for AM fungi (Olsson, 1999). Despite its uncertainty as predictor for AM fungi biomass, the NLFA 16:1ω5 is supposed to be more indicative for AM fungi than the PLFA 16:1ω5, based on previous findings showing that the PLFA 16:1ω5 is closer related to bacteria (Mellado-Vázquez et al., 2016). Principal component analyses of all PLFA quantified in this study also showed a strong correlation of the PLFA 16:1ω5 with bacterial makers while the supplementary added NLFA 16:1ω5 had an opposite trend, more related to the saprotrophic fungi marker (Figure S3).

2.7 | Calculation of incorporated ^{13}C and ^{15}N

For all plant and soil samples, we expressed the ^{13}C isotope content as incorporated ^{13}C (mg $^{13}C/m^2$, μg $^{13}C/m^2$ or ng $^{13}C/g_{dry\ matter}$), which refers to the total amount of ^{13}C found in a certain C pool:

$$\text{incorporated } ^{13}C = \frac{(\text{atom\%}_{\text{labelled}} - \text{atom\%}_{\text{unlabelled}}) \times C_{\text{pool}}}{100\%}$$

with $\text{atom\%}_{\text{labelled}}$ being the ^{13}C atom% of the labelled samples, $\text{atom\%}_{\text{unlabelled}}$ being the ^{13}C atom% of natural abundance samples and C_{pool} being the respective C pool (mg C/m^2 for bulk and carbohydrate data from shoots and fine roots; μg $^{13}C/m^2$ or ng $C/g_{dry\ matter}$ for NLFAs and PLFAs from soil). Incorporated ^{15}N of plant samples was calculated in a completely analogous fashion. Root respired ^{13}C (μmol $^{13}C\ m^{-2}\ hr^{-1}$), which corresponds to the amount of ^{13}C released

in respired CO_2 from roots during a certain time, was calculated similar to incorporated ^{13}C:

$$\text{root respired}\,^{13}C = \frac{(\text{atom\%}_{\text{labelled}} - \text{atom\%}_{\text{unlabelled}}) \times CO_{2,\text{resp.rate}}}{100\%}$$

with $CO_{2,\text{ resp. rate}}$ being the respiration rate of CO_2 (μmol CO_2 m^{-2} hr^{-1}).

2.8 | Data analyses

For concentration measurements, average values were calculated over the different sampling times after pulse labelling (1.5 hr, 1 day, 3 days and 5 days for carbohydrates and root respired CO_2; 1 day and 3 days for NLFAs and PLFAs). If necessary, the data were corrected for bulk density differences (Meyer et al., 2012).

For soil-microbial community, the (A+S)-fungi:bacteria ratio was calculated by dividing the sum of the AM fungi marker (NLFA 16:1ω5) and the saprotrophic fungi marker (18:2ω6,9) by the sum of all bacterial PLFA markers, similar to the previously used fungi:bacteria ratio (de Vries & Shade, 2013; de Vries et al., 2012; Fuchslueger et al., 2014a).

Total ^{13}C uptake was calculated as sum of bulk shoot and bulk root-incorporated ^{13}C directly after labelling (1.5 hr sampling). Total ^{15}N uptake was calculated as average overall sampling times because the signal was stable over the experimental time.

All statistical analyses were done using the R 3.3.2 software (R Core Team, 2016). The effects of drought treatment, land-use type and their interaction on soil water content, fine root biomass, carbohydrate concentrations, NLFA and PLFA concentrations, (A+S)-fungi:bacteria ratio as well as ^{13}C and ^{15}N tracer uptake were evaluated for each labelling campaign separately using ANOVA from the R base package and permutational ANOVA from the "lmPerm" package (Wheeler & Torchiano, 2016). We used the standard ANOVA to estimate effect sizes based on F-values and the permutational ANOVA to obtain exact p-values. Permutation tests do not require assumptions about the statistical distribution and are more sensitive with small sample sizes (Ernst, 2004). Time series (in hours after pulse labelling) of ^{13}C tracer data were tested for each labelling campaign separately for the effects of drought, land-use type, sampling time and their interaction using linear mixed-effect models from the "lme4" package (Bates, Maechler, Bolker, & Walker, 2015). In the mixed-effects models treatment, land use and sampling time (as factor) were set as fixed effects, while rain-out shelter and monolith were set as random effects. All models were assessed for violations of normality, heteroscedasticity and independency, and if necessary, ^{13}C tracer data were log (+1) or square root (+1) transformed.

3 | RESULTS

3.1 | Drought effects on plant C allocation and recovery

At the resistance labelling, drought reduced the assimilation of ^{13}C in both grassland types (Table 1, Table S2). This reduction was stronger in the meadow than in the abandoned grassland. Simultaneously, the concentrations of storage carbohydrates, that is, fructan and starch, decreased in the shoots of both communities, and led to a strong increase in root sucrose (Table 1, Table S2). This increase was stronger in the meadow than in the abandoned grassland. The root carbohydrate storage was unaffected by drought, but larger storage pools were found in the meadow.

Drought also reduced the ^{13}C tracer dynamics in shoots and roots of both grassland types (Figure 1a–d, Table S3). The observed reductions were larger in the meadow than in the abandoned grassland. In drought treatments, the ^{13}C tracer declined faster with time in the shoots and increased less in the roots. The initial label uptake into shoots mainly reflected the high ^{13}C incorporation into sucrose (Figure 2a), which was not significantly affected by drought in both grassland types (Table S3) and declined exponentially (Figures S4 and S5). After 24 hr, the shoot tracer dynamics reflected mainly the ^{13}C incorporation into shoot storage carbohydrates. The ^{13}C content of starch decreased over time, like sucrose, but increased in fructans suggesting that shoot fructans have a much smaller turnover than starch. Drought strongly reduced ^{13}C incorporation into the shoot carbohydrate storages of both grassland types, but the ^{13}C incorporation into starch of the abandoned grassland was less affected compared to the meadow (Figure 2a, Figures S4 and S5, Table S3), which confirmed the results of the carbohydrate concentrations.

The ^{13}C tracer dynamics of root carbohydrates was only little affected by drought at the resistance labelling (Figure 2b, Table S3, Figures S4 and S5). In the meadow, drought reduced the ^{13}C incorporation into root storage carbohydrates. In contrast, on the abandoned grassland, no effect or even a slight increase in ^{13}C of root starch was observed. Root sucrose had a slower turnover in both grassland types leading to a to higher ^{13}C incorporation after 5 days from labelling (Figures S4 and S5, Table S3). This slowdown of ^{13}C tracer dynamics in root sucrose was confirmed by the mean residence times (Table S4), but the effect was only significant for the abandoned grassland. Remarkably, the relative amount of ^{13}C that was transferred from above- to below-ground, measured by the root to shoot ratio of ^{13}C incorporation, was not reduced by drought in both grassland types (Figure 1). In fact, this ratio increased over time in the meadow under drought (Figure 1e,f) and the proportion of ^{13}C from the labelling pulse that was found in root sucrose was higher than in controls (Figure S6).

At the resilience labelling, the majority of parameters considered in this study completely recovered and the total ^{13}C uptake was already exceeding the control values, especially in the meadow (Table 1, Figures 1 and 2, Tables S2 and S3). The shoot fructan concentrations still not completely recovered for both grassland types. A legacy effect of drought was also visible in root sucrose and root starch. Both carbohydrates were increased in the abandoned grassland and decreased in meadow. Moreover, the previous drought treatment significantly increased the fine root biomass of the abandoned grassland, leading to higher root biomass in comparison with the meadow. The root respiration rate recovered for both grassland types but was generally higher in the meadow. Recovering meadow roots also respired more $^{13}CO_2$ (Figure S7). Most interestingly, the plant ^{15}N label uptake was

TABLE 1 Soil water content, fine root biomass, total ^{13}C and ^{15}N uptake, root respiration rate, concentrations of plant carbohydrates, concentrations of soil-microbial marker lipids and (A+S)-fungi:bacteria ratio for control/drought treatments of abandoned grassland and meadow ($M \pm SE$ of n = 3 monoliths) at the resistance labelling (peak drought) and the resilience labelling (recovery phase)

			Abandoned		Meadow	
Labelling	Parameter	Unit	Control	Drought	Control	Drought
Resistance	*General*					
	SWC	mass-%	38 ± 3	22 ± 1	38 ± 1	14 ± 1
	Fine roots	g/m^2	348 ± 35	352 ± 46	228 ± 42	252 ± 9
	Total ^{13}C uptake	mg/m^2	742 ± 59	632 ± 171	1,165 ± 255	785 ± 129
	Root resp. CO_2	$nmol\ g_{dm}^{-1}\ s^{-1}$	2.38 ± 0.01[a]	1.69 ± 0.09[a]	3.25 ± 0.16[a]	3.34[b]
	Carbohydrates					
	Shoot sucrose	mg_C/g_{dm}	20.9 ± 2.4	22.1 ± 2.4	14.7 ± 1.5	16.8 ± 0.9
	Shoot fructan		38.3 ± 6.2	26.3 ± 3.3	34.7 ± 2.9	30.2 ± 3.9
	Shoot starch		4.5 ± 0.1	4.8 ± 0.2	8.6 ± 1.9	3.2 ± 0.7
	Root sucrose		3.0 ± 0.5	6.2 ± 0.8	5.5 ± 1.0	11.2 ± 1.4
	Root fructan		19.8 ± 1.2	16.5 ± 2.3	29.1 ± 2.4	32.3 ± 2.9
	Root starch		4.3 ± 0.4	6.2 ± 2.4	14.5 ± 3.6	10.1 ± 1.4
	Micro-organisms					
	AM fungi	$mg_C/m^2_{0\text{-}7\ cm}$	670 ± 176	1,040 ± 123	725 ± 366	808 ± 263
	Sapro. fungi		351 ± 60	385 ± 53	224 ± 19	228 ± 8
	Gram(−) bacteria		1,339 ± 193	1,433 ± 108	1,200 ± 238	1,110 ± 58
	Gram(+) bacteria		1,197 ± 188	1,241 ± 97	884 ± 138	863 ± 33
	Actinobacteria		365 ± 55	374 ± 35	400 ± 81	375 ± 9
	(A+S)-F:B	–	0.34 ± 0.03	0.47 ± 0.04	0.35 ± 0.08	0.45 ± 0.11
Resilience	*General*					
	SWC	mass-%	43 ± 5	36 ± 1	37 ± 2	37 ± 1
	Fine roots	g/m^2	264 ± 18	333 ± 13	237 ± 14	219 ± 11
	Total ^{13}C uptake	mg/m^2	1,293 ± 122	1,355 ± 108	998 ± 189	1,381 ± 66
	Root resp. CO_2	$nmol\ g_{dm}^{-1}\ s^{-1}$	2.38 ± 0.38	2.19 ± 0.19	2.90 ± 0.07	2.72 ± 0.40
	Plant ^{15}N uptake[c]	mg/m^2	1.4 ± 0.1	1.6 ± 0.1	1.8 ± 0.3	3.1 ± 0.5
	Carbohydrates					
	Shoot sucrose	mg_C/g_{dm}	16.4 ± 1.9	16.0 ± 2.1	13.3 ± 2.1	10.5 ± 1.8
	Shoot fructan		57.7 ± 2.0	43.8 ± 7.9	45.6 ± 4.5	40.8 ± 4.4
	Shoot starch		4.2 ± 0.5	4.3 ± 0.9	6.1 ± 0.2	7.0 ± 1.3
	Root sucrose		2.8 ± 0.4	5.1 ± 1.6	7.6 ± 1.7	5.5 ± 1.0
	Root fructan		21.1 ± 2.5	18.9 ± 3.8	34.6 ± 1.7	29.1 ± 2.1
	Root starch		2.7 ± 0.1	3.5 ± 0.7	4.8 ± 0.5	3.6 ± 0.7
	Micro-organisms					
	AM fungi	$mg_C/m^2_{0\text{-}7\ cm}$	764 ± 303	369 ± 51	817 ± 467	213 ± 68
	Sapro. fungi		308 ± 42	333 ± 92	202 ± 33	214 ± 15
	G(−) bacteria		1,094 ± 91	1,227 ± 221	1,037 ± 276	1,169 ± 147
	G(+) bacteria		1,079 ± 106	1,099 ± 220	807 ± 186	1,073 ± 174
	Actinobacteria		326 ± 39	328 ± 60	379 ± 106	423 ± 64
	(A+S)-F:B	–	0.43 ± 0.14	0.28 ± 0.03	0.47 ± 0.17	0.16 ± 0.01

(A+S)-F:B, (arbuscular mycorrhiza + saprotrophic) fungi:bacteria ratio; G(-/+), Gram-negative/positive; resp., respired; Sapro., saprotrophic; SWC, soil water content.

[a]Only two replicates could be measured.

[b]Only one replicate could be measured.

[c]The ^{15}N addition was only done on monoliths used for the resilience labelling, plant ^{15}N uptake is the sum of shoot- and root-incorporated ^{15}N.

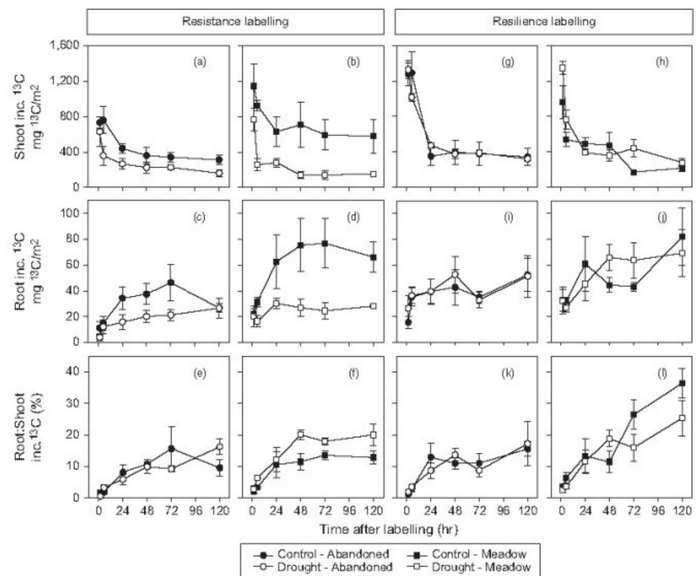

FIGURE 1 ^{13}C tracer dynamics in bulk shoots and roots as well as the root to shoot ^{13}C ratio over time from abandoned grassland (a, c, e, g, i, k/circles) and meadow (b, d, f, h, j, l/squares) control (closed symbols) and drought (open symbols) monoliths; after the resistance (a–f) and the resilience (g–l) ^{13}C pulse labelling. Error bars show ± *SE* (n = 3); inc. ^{13}C, incorporated ^{13}C

increased in the recovery phase, especially in the meadow (Table 1, Table S2).

Furthermore, the ^{13}C tracer dynamics in shoots and roots (Figure 1g–j, Table S3) and the shoot carbohydrate ^{13}C incorporation (Figure 2c, Table S3) recovered completely. Only the mean residence time of shoot sucrose was still lower in previously drought-treated meadow (Table S4, Figure S5). The ^{13}C incorporation in root sucrose of both grassland types responded slightly different at the resilience labelling (Figure 2d). It was increased for drought treatments in the abandoned grassland, while it was decreased in the meadow (Table S3). Consequently, a smaller proportion of ^{13}C from the labelling pulse was found in root sucrose from the recovering meadow community (Figure S6). Overall, at the resilience labelling, BCA was higher in the meadow compared to the abandoned grassland, as more label was found in meadow roots over the course of time (Figure 1i,j, Table S3) and the root:shoot ^{13}C incorporation was higher in the meadow (Figure 1k,l, Table S3), while less label was found in bulk shoots and shoot sucrose (Figures 1g,h and 2, Table S3) from the meadow.

3.2 | Drought effects on C transfer to soil-microbial community and recovery

The abandoned grassland held more saprotrophic fungi and Gram-positive bacteria than the meadow, and this was barely affected by drought (Table 1, Table S2, Figure S3). At the resistance labelling, drought increased the content of AM fungi marker in the abandoned grassland by about 55% on average, but as the variability in this marker is usually high (Olsson, 1999), the effect was insignificant. Nonetheless, the (A+S)-fungi:bacteria ratio was significantly increased by drought in both grassland types (Table 1, Table S2), although the uptake of recent assimilated plant C by AM fungi and saprotrophic fungi was reduced (Figure 3a, Table S3, Figure S8). However, root-associated Gram-negative bacteria received less plant-derived C in both grassland types under drought. The reductions of ^{13}C uptake were consistently stronger in the soil-microbial community of the meadow compared to the abandoned grassland.

At the resilience labelling, all microbial groups had completely recovered from drought, except for the AM fungi, which had significantly reduced marker concentrations in both grassland types (Table 1, Table S2). Correspondingly, the (A+S)-fungi:bacteria ratio was significantly reduced by drought and rewetting. Also, the ^{13}C incorporation into the AM fungi marker was still reduced, whereas the other microbial groups recovered their label uptake (Figure 3, Table S3, Figure S8). Only in the drought-treated meadow, the ^{13}C uptake was strongly increased in Gram-negative bacteria and Gram-positive bacteria including actinobacteria, which was also mirrored by a higher variability in the PLFA composition in the meadow (Figure S3).

4 | DISCUSSION

Our study demonstrates that BCA and plant-microbial interactions of the managed and abandoned grassland differed in their response to drought and rewetting, and thus highlights the important role of land management for the resistance and resilience of marginal grasslands to climate extremes. In addition, our analyses confirmed that the meadow and the abandoned grassland differed in their initial properties (Figure 4, Table 1). The abandoned grassland held more root biomass, similar as observed by (Bahn et al., 2006), and higher shoot sucrose concentrations, whereas the meadow had higher concentrations of root sucrose and the root storage sugars starch and fructan. This suggests that the abandoned grassland invests in root growth to access soil resources, whereas meadows store resources in roots to facilitate regrowth after cutting. The microbial community

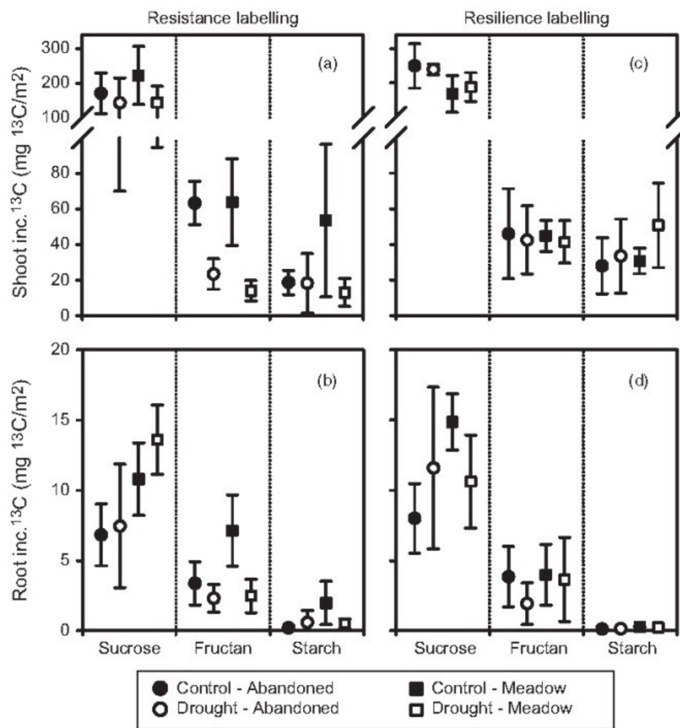

FIGURE 2 Average ^{13}C tracer incorporation into plant shoot (a, c) and root (b, d) carbohydrates of control (closed symbols) and drought (open symbols) monoliths from the abandoned grassland (circles) and the meadow (squares); after the resistance (a, b) and the resilience (c, d) ^{13}C pulse labelling. Dotted lines separate amongst the three investigated carbohydrates (sucrose, fructan and starch). Error bars show ± *SE* ($n = 3$); inc. ^{13}C, incorporated ^{13}C

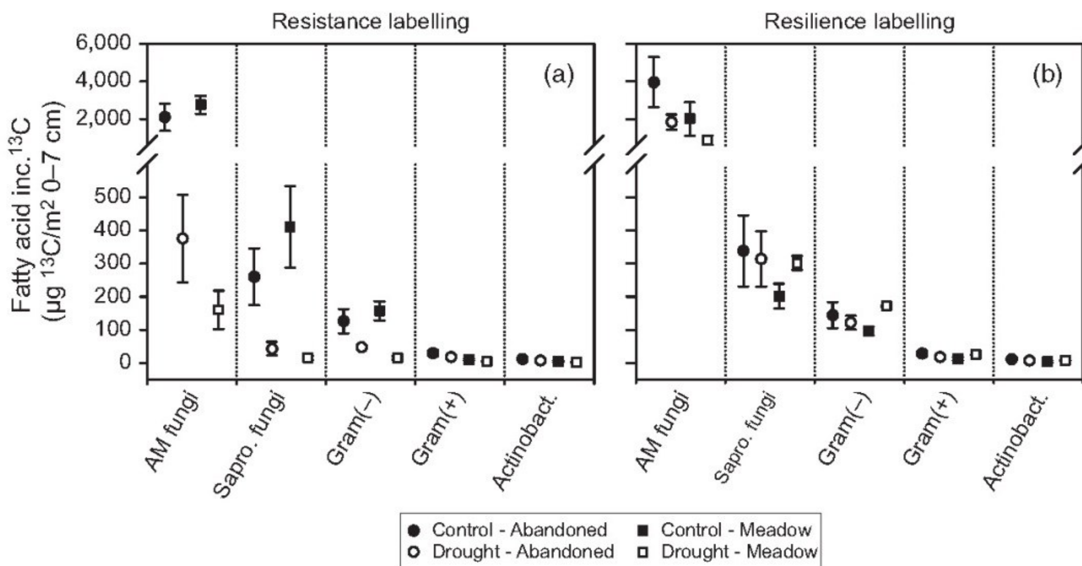

FIGURE 3 Average ^{13}C tracer incorporation in marker fatty acids for arbuscular mycorrhiza fungi (AM fungi), saprotrophic fungi (Sapro. fungi), Gram-negative bacteria (Gram(−)), Gram-positive bacteria (Gram(+)) and actinobacteria (Actinobact.), extracted from soil cores from 0 to 7 cm depth of control (closed symbols) and drought (open symbols) monoliths from the abandoned grassland (circles) and the meadow (squares); after the resistance (a) and the resilience (b) ^{13}C pulse labelling. Dotted lines separate amongst the five different microbial groups. Error bars show ± *SE* ($n = 3$); inc. ^{13}C, incorporated ^{13}C

of the abandoned grassland held more markers of saprotrophic fungi and Gram-positive bacteria, which likely benefit from root turnover (Meyer et al., 2012).

Drought affected both grassland plant communities in a similar way (Figure 4a, Table 1). Above-ground C uptake and storage were reduced and a higher proportion of label was transferred below-ground.

This increase in BCA was stronger in the meadow than in the abandoned grassland. However, recently assimilated C was neither stored in the roots, nor used for growth, nor transferred to the rhizosphere, but remained in the roots as sucrose. As a consequence, the amount of tracer that was transferred to root associated (A+S) fungi and Gram-negative bacteria strongly decreased and led to a decoupling of plant roots and soil micro-organisms. This decoupling was weaker in the fungal-dominated microbial community of the abandoned grassland than in the meadow, although the overall (A+S) fungi:bacteria ratio increased in both grassland types. This suggests that plant communities with conservative species and fungal-dominated microbial communities are less affected by drought than plant communities with exploitative species and bacterial-dominated microbial communities.

Our findings are supported by Bahn et al. (2013), who suggested that under reduced C supply BCA is maintained at the cost of above-ground storage. Unexpectedly, we found that drought-induced reductions in above-ground storage were generally stronger in fructans than in starch pools. Fructans are thought to contribute to drought tolerance (Van den Ende, 2013; Vijn & Smeekens, 1999). Although fructans represented the largest part of water soluble carbohydrates, we did not find a correlation with drought resistance, nor an accumulation of fructans, during drought in our study. We also did not find that the high root sucrose concentrations increased root growth and tracer incorporation into fine roots (Burri et al., 2014; Kahmen, Perner, & Buchmann, 2005), which suggests that the increased BCA is not a result of increased sink demand, but is due to osmotic adjustment of roots (Chaves, Maroco, & Pereira, 2003; Chen & Jiang, 2010; Hasibeder et al., 2015; Sicher, Timlin, & Bailey, 2012). This osmotic role of sucrose is further supported by its low transfer into the rhizospere (Fuchslueger et al., 2014a). The reduced plant-derived C flow also impacts the soil-microbial community (Barnard, Osborne, & Firestone, 2013; Fuchslueger et al., 2014a). The overall microbial community composition generally seems less affected by drought (Canarini, Carrillo, Mariotte, Ingram, & Dijkstra, 2016), but a general increase in fungi:bacteria ratios is often observed, which may

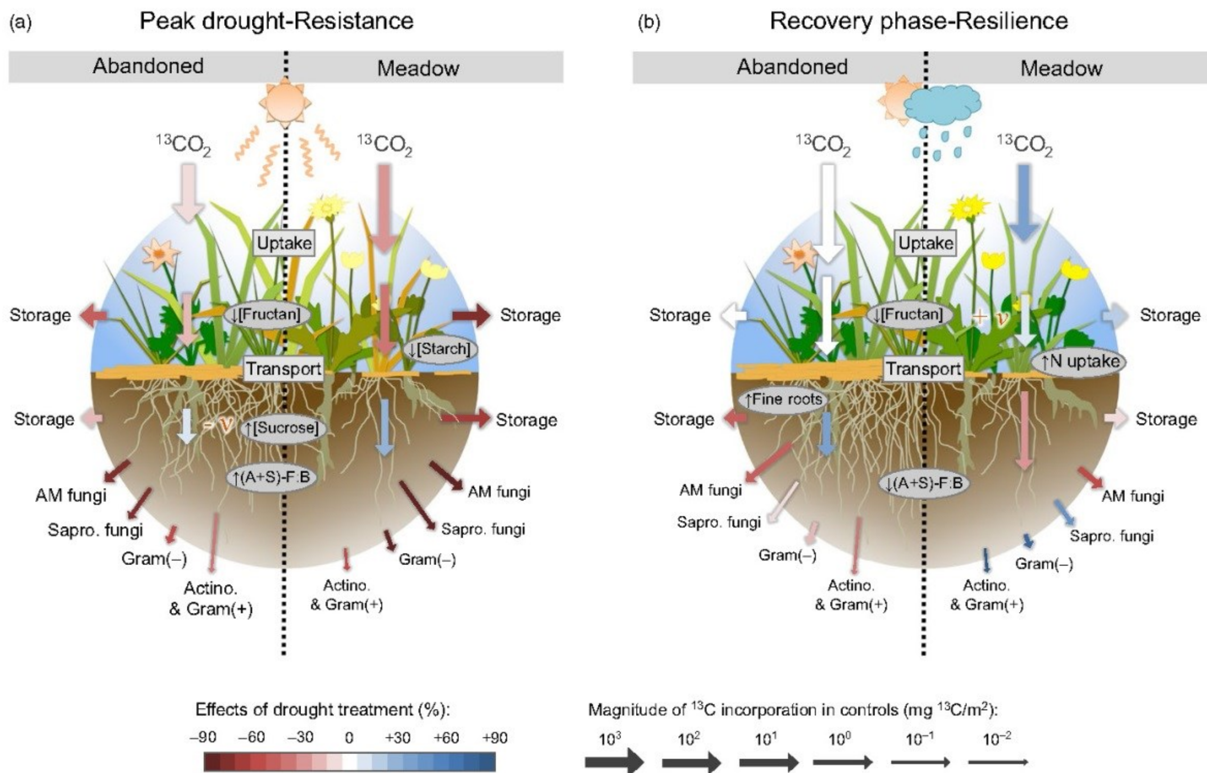

FIGURE 4 Overview of the effects of drought on ^{13}C tracer uptake, allocation in plants and transfer to soil microbes (a) at peak drought (resistance labelling) and (b) in the recovery phase (resilience labelling), in abandoned grassland and meadow. The arrows represent the amount of ^{13}C uptake and ^{13}C incorporation into different pools following the ^{13}C pulse labelling, with the width of the arrow indicating different size classes as determined by the magnitude of ^{13}C incorporation in controls, and the length of the arrow describing the relative differences in controls within each size class, so that the comparison between both land use types and labellings is possible. The effects of the drought treatment are expressed separately by a colour gradient indicating the change relative to the control value (red: reduced ^{13}C incorporation, white: no change, blue: increased ^{13}C incorporation). Shoot and root sucrose pools were used as proxy for transport to the below-ground (central arrows), with $+\nu/-\nu$ indicating higher/lower turnover of ^{13}C tracer in drought monoliths. All arrows for plant carbohydrates and soil-microbial markers represent average values of ^{13}C tracer dynamics. Oval boxes show additional information not related to the ^{13}C tracer flux and drought-related changes in pool sizes or biomasses. Actino., actinobacteria; AM, Arbuscular mycorrhiza; (A+S)-F:B, ratio of AM + saprotrophic fungi to bacteria; Gram(+/−), Gram-positive/negative bacteria

suggest higher resistance of fungal-based food webs (de Vries et al., 2012; Fuchslueger et al., 2014a). In the abandoned grassland, the amount of AM fungal markers increased during drought (Table 1) and the label uptake in the AM fungal markers was less reduced than in the meadow (Figure 3, Table S3), which suggests that mainly AM fungi are relatively resistant to drought. Thereby, AM fungi can support water and nutrient uptake by plants during drought (Allen, 2007; Wardle et al., 2004). Overall, this supports our initial hypothesis that strong plant-fungal, specifically plant-AM fungal, interactions are the basis for the high resistance of the abandoned grassland to drought.

Reduced ^{13}C tracer uptake was also found for the other root associated microbial markers of saprotrophic fungi and Gram-negative bacteria (Bahn et al., 2013; Balasooriya, Denef, Huygens, & Boeckx, 2012; Denef et al., 2009; Kramer & Gleixner, 2008), but not for Gram-positive bacteria including the actinobacteria (Figures 3a and 4a, Table S3). This was especially expected for the Gram-negative bacteria that are directly linked to recent plant C input (Bahn et al., 2013; Bardgett et al., 2005; Mellado-Vázquez et al., 2016), but not for saprotrophic fungi that are generally more resistant to desiccation than Gram-negative bacteria (Lennon et al., 2012; Schimel et al., 2007). The non-significant reduction in label uptake into Gram-positive (actino)-bacterial PLFAs is in line with their overall low ^{13}C uptake compared to root-associated microbes (Figure 3), their delayed label incorporation (Bahn et al., 2013; Fuchslueger et al., 2014a; Malik, Dannert, Griffiths, Thomson, & Gleixner, 2015) and their preference for additional C sources like soil organic matter (Bai et al., 2016; Kramer & Gleixner, 2008; Mellado-Vázquez et al., 2016).

In general, the majority of studied parameters quickly recovered after rewetting, but most interestingly, we also found substantial differences between the two grassland types (Figure 4b, Table 1). The meadow recovered quickly and during recovery from drought, its C uptake was even higher than in controls (see also Ingrisch et al., 2017; for CO_2 fluxes). This C was either allocated to shoot storage or transferred to the rhizosphere. In the abandoned grassland, the C uptake also recovered quickly, but C allocation to storage and transfer to the rhizosphere were still affected by the drought. The higher amount of root sucrose may have facilitated the growth of fine roots (Table 1 and Table S2; Kahmen et al., 2005; Burri et al., 2014). The higher fine root biomass likely increased nutrient and water access after rewetting, possibly because the establishment of new AM fungal-root connections needed more time, that is, was not resilient. In contrast, the meadow obviously restored the above-ground biomass after rewetting, since the total ^{13}C uptake (Table 1) and shoot sucrose turnover (Table S4) were increased without a change in BCA (Figure 1). Simultaneously, root exudation increased in the meadow, as the ^{13}C tracer uptake significantly increased in all bacteria (Table S3, Figure 4b). As a result, the fast regrowth of exploitative meadow plants (Ingrisch et al., 2017) could be supported by the activation of “priming” bacteria (Canarini & Dijkstra, 2015; Kuzyakov, 2010; Roy et al., 2016; Wardle et al., 2004) that led to changes in the microbial community composition (Figure S3b) and likely facilitated a higher N uptake by plants. Overall, the results support our initial hypothesis that the meadow quickly recovers from drought benefiting from strong bacterial interactions, and thus is highly resilient.

Interestingly, our results do not support the hypothesis that in the recovery phase, bacterial communities are favoured over fungal and especially AM fungal communities, as the decreasing (A+S)-fungi:bacteria ratio would suggest (Table 1). This decrease mainly was driven by the significant decreased abundance of AM fungi and less by the insignificant increase in bacteria (Table 1 and Table S2). This is in line with the finding that fungal-based food webs are less resilient than bacterial-based food webs (de Vries et al., 2012; Meisner, Bååth, & Rousk, 2013). Further research is needed to understand the interactions between microbial and plant communities and how they are affected by land use. For example, the rapid recovery of the meadow may result from a history of regular cutting and fertilization, that increased the abundance of “exploitative” species, which can rapidly regrow and effectively gain nutrients (Grassein et al., 2015; Grigulis et al., 2013). This legacy effect of the management could also lead to changes in the soil-microbial community composition and function (Hawkes & Keitt, 2015), which would enable better acclimatization of certain microbial groups to environmental fluctuations and thereby increase their resilience to drought. Conversely, the more stable conditions, like in the abandoned grassland, might constrain microbial responses during recovery, and thus decrease the resilience of certain microbial groups, as suggested by the “historical contingencies” concept of Hawkes and Keitt (2015). Hence, high resilience of marginal grasslands seems to be based on both, adaptations of plant functional traits and microbial processes, confirming the importance of plant-microbial interactions to predicting ecological consequences of climate change.

5 | CONCLUSIONS

Our results highlight that in addition to plant properties, like carbohydrate storage and below-ground carbon allocation, plant-microbial interactions influence the resilience mechanisms of ecosystems. In particular, the role of AM fungi for the resistance of plant communities to drought and the role of bacteria in the recovery phase need further research.

Plant-microbial interactions likely provided better access to resources at different time points, which led to an inverse relationship between resistance and recovery. Resistant communities, which maintain their functioning during drought stress, have fewer nutrient resources available for recovery. Conversely, plant communities that are used to suffer from regular perturbations invest their resources mainly into fast regrowth after disturbance. Both strategies can yield a high overall resilience of ecosystems.

Land use offers the opportunity to manage plant communities and therefore the resilience of ecosystems. Further studies should consequently address the effects of land use on long-term resilience, including multiple stress events, to maintain the functioning of the endangered marginal grassland systems in a changing world.

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AUTHORS' CONTRIBUTIONS

M.B., S.L. and G.G. conceived the ideas; S.K., A.A., J.I., R.H., M.B. and G.G. designed methodology; S.K., A.A., J.I., R.H. and G.G. conducted the experiment and collected the data; S.K., A.A. and M.L. analysed the data; S.K. and G.G. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3s57p (Karlowsky et al., 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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CHAPTER 3 – Manuscript 2

frontiers in Plant Science

ORIGINAL RESEARCH
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Drought-Induced Accumulation of Root Exudates Supports Post-drought Recovery of Microbes in Mountain Grassland

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Droughts strongly affect carbon and nitrogen cycling in grasslands, with consequences for ecosystem productivity. Therefore, we investigated how experimental grassland communities interact with groups of soil microorganisms. In particular, we explored the mechanisms of the drought-induced decoupling of plant photosynthesis and microbial carbon cycling and its recovery after rewetting. Our aim was to better understand how root exudation during drought is linked to pulses of soil microbial activity and changes in plant nitrogen uptake after rewetting. We set up a mesocosm experiment on a meadow site and used shelters to simulate drought. We performed two ^{13}C-CO_2 pulse labelings, the first at peak drought and the second in the recovery phase, and traced the flow of assimilates into the carbohydrates of plants and the water extractable organic carbon and microorganisms from the soil. Total microbial tracer uptake in the main metabolism was estimated by chloroform fumigation extraction, whereas the lipid biomarkers were used to assess differences between the microbial groups. Drought led to a reduction of aboveground versus belowground plant growth and to an increase of ^{13}C tracer contents in the carbohydrates, particularly in the roots. Newly assimilated ^{13}C tracer unexpectedly accumulated in the water-extractable soil organic carbon, indicating that root exudation continued during the drought. In contrast, drought strongly reduced the amount of ^{13}C tracer assimilated into the soil microorganisms. This reduction was more severe in the growth-related lipid biomarkers than in the metabolic compounds, suggesting a slowdown of microbial processes at peak drought. Shortly after rewetting, the tracer accumulation in the belowground plant carbohydrates and in the water-extractable soil organic carbon disappeared. Interestingly, this disappearance was paralleled by a quick recovery of the carbon uptake into metabolic and growth-related compounds from the rhizospheric microorganisms, which was probably related to the higher nitrogen supply to the plant shoots. We conclude that the decoupling of plant photosynthesis and soil microbial carbon cycling during drought is due to reduced

carbon uptake and metabolic turnover of rhizospheric soil microorganisms. Moreover, our study suggests that the maintenance of root exudation during drought is connected to a fast reinitiation of soil microbial activity after rewetting, supporting plant recovery through increased nitrogen availability.

Keywords: plant-soil (belowground) interactions, stress tolerance, mountain grassland, ^{13}C pulse labeling, carbohydrates, NLFA, PLFA, chloroform fumigation extraction

INTRODUCTION

Climate change threatens the functioning of terrestrial ecosystems, which will very likely suffer from more frequent extreme events induced by the ongoing global warming (IPCC, 2012). A large part of the terrestrial biosphere consists of grassland ecosystems that cover approximately 40% of the vegetated land surface and strongly contribute to soil carbon storage (White et al., 2000). The functioning of grasslands and their role in the global carbon cycle are particularly placed at risk by periods of severe drought (Reichstein et al., 2013; Frank et al., 2015). Grasslands in some areas may experience more severe drought effects, such as, for example, in the European Alps, which are affected by faster temperature increases compared to the global average (Beniston, 2005; Auer et al., 2007).

Extreme droughts typically lead to reduced carbon assimilation in plants (Huang and Fu, 2000; Naudts et al., 2011; Roy et al., 2016; Ingrisch et al., 2018) and reduced carbon transfer to the roots and the rhizosphere (Fuchslueger et al., 2014a, 2016; Hasibeder et al., 2015; Karlowsky et al., 2018), resulting in a lower soil CO_2 efflux (Ruehr et al., 2009; Barthel et al., 2011; Burri et al., 2014). Consequently, the reduced belowground carbon allocation (BCA) weakens plant–microbial interactions (Brüggemann et al., 2011). Because soil microorganisms strongly depend on plant-derived carbon inputs (Wardle et al., 2004; Bardgett et al., 2005), important soil functions, such as the microbial mineralization of nitrogen and phosphorous, are limited during drought (Stark and Firestone, 1995; Borken and Matzner, 2009; Delgado-Baquerizo et al., 2013; Fuchslueger et al., 2014b; Canarini and Dijkstra, 2015; Dijkstra et al., 2015). In addition, symbiotic interactions with arbuscular mycorrhizal (AM) fungi, which strongly increase the drought resistance of plants (Allen, 2007), are affected by severe drought (Karlowsky et al., 2018). So far, whether the weakening of the link between plants and soil microorganisms during drought (i.e., the reduced soil microbial usage of recently assimilated plant-derived carbon) is due to (1) the altered carbon allocation of plants leading to reduced root exudation, (2) the limited substrate mobility in the rhizosphere, or (3) a slowdown of soil microbial metabolism is unknown. Possibly, these three mechanisms appear at the same time and interact with each other.

Drought has been shown to induce a shift of carbon allocation from the aboveground to the belowground plant organs (Palta and Gregory, 1997; Huang and Fu, 2000; Burri et al., 2014) and to increase the amounts of soluble sugars in the roots (Hasibeder et al., 2015; Karlowsky et al., 2018). The latter two studies also showed that drought-induced reductions of storage sugar concentrations are more pronounced in shoots than roots. The increase of soluble root sugars has been attributed either to osmotic regulation to support the survival of root biomass (Sicher et al., 2012; Hasibeder et al., 2015) while maintaining the carbon demand for respiration (Barthel et al., 2011) or to increased fine root growth to enhance plant access to deeper soil water resources (Huang and Fu, 2000; Burri et al., 2014). Until now, whether these drought-reduced changes in plant carbon allocation to stored reserve sugars versus soluble root sugars that are linked to exudation are affecting the carbon released into the rhizosphere has been unknown. In a recent meta-analysis of the scarce existing literature, Preece and Peñuelas (2016) found that drought can have variable effects on the rhizospheric carbon release. Strikingly, the authors of this study reported a trend toward increased root exudation per gram of plant biomass (including either root and shoot biomass or shoot biomass only) under moderate drought. However, the root biomass response to drought strongly varies among the different studies (Kreyling et al., 2008 and references therein), potentially affecting the total amount of carbon released to the rhizosphere. For example, Fuchslueger et al. (2014a) found that a slightly increased root to shoot ratio during drought was mirrored by higher amounts of plant-derived carbon in the extractable organic carbon (EOC) of soil.

The drying of soil itself has major impacts on the exudate transfer from the release site to rhizospheric microorganisms, which might increase the competition for substrates between functionally different microbial groups. In contrast to AM fungi, which are directly connected to the root carbohydrate pool, saprotrophic fungi (SF) and bacteria depend on the diffusion of substrates for their nutrition (Manzoni et al., 2012). As the lower water content during drought conditions limits the diffusion of substrates (Skopp et al., 1990), the uptake of nutrients by SF and bacteria is limited. Moreover, experimental results suggest that the microbial activity in the soil depends on the environmental conditions that affect diffusion pathways between substrate sources and microorganisms (Nunan et al., 2017). Consequently, if root exudation is increased along with root growth during drought, plant-derived solutes likely will accumulate in the rhizosphere due to reduced microbial carbon mineralization. Indeed, increased amounts of dissolved organic carbon immediately after the rewetting of dried soils (Canarini et al., 2017) suggest the existence of such accumulations. These additional carbon sources could further contribute to the pulse of soil respiration, which appears after rewetting and is associated with higher soil microbial activity and nitrogen mineralization (Birch, 1958). The so-called 'Birch effect' is present in planted and unplanted soils (Canarini et al., 2017) and has been suggested to primarily originate from osmolytes, which accumulate in

microbial cells during drought conditions (Fierer and Schimel, 2003). As a stress response to desiccation, the synthesis of microbial osmolytes is increased at the expense of membranes for cell growth (Schimel et al., 2007). To prevent the bursting of cells due to excessive water uptake, accumulated osmolytes need to be rapidly metabolized after rewetting (Warren, 2014). The metabolically active microorganisms are probably also able to use excess plant-derived carbon, which could support plant recovery by further increasing the nitrogen mineralization rate in the soil.

Plant carbon allocation is best analyzed by pulse-labeling of the plant canopy with ^{13}C-enriched CO_2 and tracing of the assimilated ^{13}C by compound specific carbon isotope ($^{13}C/^{12}C$) ratios of plant non-structural carbohydrates (NSCs) (Bahn et al., 2013; Karlowsky et al., 2018). Similarly, root exudation and the subsequent microbial carbon uptake can be determined by combining the K_2SO_4 extraction and chloroform fumigation method (Vance et al., 1987) with ^{13}C analysis (Malik et al., 2013). This allows the flow of plant-derived carbon in EOC and microbial biomass carbon (MBC) from soil to be traced. The water-soluble EOC is mainly a proxy for the exuded plant carbon (**Supplementary Figure S1**), with minor contributions of AM fungi exudation (Drigo et al., 2010; Balasooriya et al., 2012; Kaiser et al., 2015), which is also directly linked to the plant-derived carbon (**Supplementary Figure S1**). To determine the uptake of plant-derived carbon by the different soil microbial groups, compound-specific ^{13}C isotope analysis on phospholipid fatty acid (PLFA) markers from soil can be used (Kramer and Gleixner, 2006). A comparison of the ^{13}C incorporation into MBC and into PLFA markers allows distinctions to be made between the growth and maintenance of soil microorganisms (Malik et al., 2015).

To study the rhizospheric processes, we used a common garden experiment on a mountain meadow using species representing the local meadow community. Our main objective was to assess the effects of drought and rewetting on the response of plant–microbial carbon transfer as a fundamental part of ecosystem functioning (Wardle et al., 2004; Bardgett et al., 2005; Schimel et al., 2007; Brüggemann et al., 2011). We performed two ^{13}C pulse chase campaigns, a first at peak drought and second shortly after rewetting, and studied the response of carbon assimilation, allocation and transfer to soil microbial markers.

Specifically, we hypothesized that the weakening of the link between plant and soil processes during drought is mainly due to decreased transfer of microbial carbon substrates in the rhizosphere and osmotic effects and is not due to decreased carbon release from roots increasing the competition for carbon between microorganisms. Furthermore, we expected that drought would lead to an accumulation of root sugars and easily degradable EOC in soil, which are available for priming plant and soil microbial activity after rewetting.

MATERIALS AND METHODS

Experimental Site

The study site is near Neustift in the Stubai Valley in the Austrian Central Alps (1,820–1,850 m a.s.l.; 47°7′45′′N, 11°18′20′′E) and is described in Bahn et al. (2009). Briefly, the average annual temperature is 3°C, the annual precipitation is 1,097 mm, and the soil is a dystric cambisol type. The site is a hay meadow that is cut once per year at peak biomass in early August, is lightly manured every 2–3 years, and has a Trisetum flavescentis vegetation type consisting of perennial grasses and forbs (Schmitt et al., 2010). The meadow soil has a loamy sand texture and a bulk density of 0.7 g cm^{-3} (Meyer et al., 2012a). The total soil carbon content in the uppermost 10 cm is 51 g kg^{-1} (Meyer et al., 2012b).

Establishment of Mesocosms

In 2013, a replicated mesocosm experiment with six blocks and eight mesocosms per block was established on the experimental site. For each mesocosm, two dark plastic pots, 45 cm in diameter and 35 cm in height, one inside the other, were used. The external pot was used as water reservoir and the internal one was used to hold the soil and the plants. Each pot was filled with sieved (<5 mm) subsoil (below 10 cm) from the study site and embedded in the soil on the experimental site. To prevent a possible impact from runoff water on the experiment, the upper edge of the mesocosms were raised by 2 cm relative to the soil surface. A representative selection of plant species from the site was chosen, which consisted of grass, forb and legume species. The individual plants (shoots and roots) were excavated at the experimental site in early July 2013 and were pre-incubated for 6–7 weeks in a greenhouse, in the botanical garden of Innsbruck, Austria. Every mesocosm was planted in late August 2013 with three grasses (*Deschampsia cespitosa*, *Festuca rubra*, and *Dactylis glomerata*), two forbs (*Leontodon hispidus* and *Geranium sylvaticum*) and one legume (*Trifolium repens*). At the time of planting, the plant shoots had a height of 5–15 cm. All mesocosms were planted with 36 individuals and with varying relative abundances of the different grass and forb species (**Supplementary Table S1**). The amount of the legume remained constant to exclude a possible nitrogen fertilization effect. The position of individual plants was randomized on a fixed pattern of locations for each mesocosm. All mesocosms were randomized in the block design. In 2014, the plant community was established on the site, and the biomass was harvested according to the common practice on August 22nd, 2014.

Drought Treatment and Pulse Labeling

The experiment began on the 5th of June 2015 by simulating early summer drought (**Supplementary Figure S2A**), similar to the method described by Ingrisch et al. (2018) and Karlowsky et al. (2018) for a common garden experiment with intact vegetation-soil monoliths. In brief, six rain-out shelters (**Supplementary Figure S2B**), with base areas of 3 m × 3.5 m and 2.5 height, covered by light- and UV-B permeable plastic foil (Lumisol clear AF, Folitec, Westerburg, Germany, light transmittance c. 90%), were installed above the mesocosms. Air ventilation was maintained with an opening the bottom (<0.5 m above ground) and at the top of the sides of the rain-out shelters, thereby preventing the entrance of rain water. On a subset of four to five mesocosms per shelter, soil water content (SWC) and temperature were monitored continuously in the main rooting horizon [5TM sensors (n = 17) for combined SWC and temperature measurement and EC-5 sensors (n = 11)

for SWC measurement, connected to Em50 loggers; Decagon Devices, Pullman, WA, United States]. In addition, the SWC was measured manually for each mesocosm with a PR2 Soil Moisture Profile Probe (Delta-T Devices Ltd., Cambridge, United Kingdom) at depths of 5 cm and 15 cm between the 12th of June and the 10th of August (13 times during drought and four times during recovery).

During rain exclusion, the mesocosms of the control treatments were watered manually to SWCs greater than 19% to avoid water limitation. No water was given to drought-treated mesocosms, yielding SWCs of approximately 6 and 10% at depths of 5 and 15 cm, respectively, at peak drought (**Supplementary Figure S3**). Soil moisture at field capacity was estimated on the 1st of June 2018 on the same mesocosms as 38.6% (SD = 6.7%, n = 27) using data (from 5TM and EC-5 sensors) collected when the soil moisture had stabilized a few days after rain. Four weeks after the drought treatment started, the first ^{13}C pulse labeling (peak drought labeling) started on the 4th of July on a subset of 12 mesocosms (six control and six drought treatments). Drought simulation was stopped on the 14th of July 2015, by removing the rain-out shelters and adding water representing 25 mm of precipitation to all mesocosms (control and drought treatments). Because of a natural dry period, from the 15th to the 22nd of July, another 16 and 36 mm of precipitation equivalents were added in total to the control and drought treatments, respectively. On a subset of another 12 mesocosms, after a recovery phase of 10 days, the second ^{13}C pulse labeling (recovery labeling) began on the 24th of July.

Both labeling campaigns were done on three consecutive days (peak drought from the 4th until the 6th of July; recovery from the 24th until the 26th of July) with high radiation. For each labeling campaign, one control and one drought mesocosm were used in each of the six rain-out shelters (**Supplementary Figure S2C**). The ^{13}C pulse labeling was done on 2–6 mesocosms per day. The labeling was always done in parallel on one drought mesocosm and one control mesocosm, with the starting time shifted by 15 min (randomly started with either control or drought mesocosm). Because the plant growth strongly varied between mesocosms from the same planting scheme, we aimed to visually choose pairs of mesocosms that were as similar as possible. Pulse labeling was performed similarly, as described by Bahn et al. (2009, 2013) and Hasibeder et al. (2015). Briefly, a cylindrical and transparent Plexiglas chamber with 45-cm diameter and 50-cm height was placed on the top of the mesocosms with a rubber gasket between the chamber and the mesocosm (**Supplementary Figure S2D**). Elastic bands were used to fix the chamber on external anchor points in order to ensure gas tightness. Air circulation and temperature control were handled by fans and tubes connected to a pump circulating water cooled with ice packs. During the pulse labeling, we monitored the interior air temperature (shaded sensor), CO_2 concentration (Licor 840A, Lincoln, NE, United States) and ^{13}C isotope ratio of CO_2 (Picarro G2201i Analyzer, Picarro Inc., Santa Clara, CA, United States). Solar radiation was measured outside the chamber using a PAR quantum sensor (PQS 1; Kipp & Zonen, Delft, Netherlands). Pulse labeling was done under comparable light conditions on mostly clear days between 10:00 and 15:00 CET. Highly enriched $^{13}CO_2$ (>99 atom% ^{13}C; Sigma-Aldrich, Taufkirchen, Germany) was added pulse-wise to achieve 30–80 atom% ^{13}C in chamber CO_2 over the complete labeling time of 75 min (peak drought labeling) and 30 min (recovery labeling). The CO_2 concentrations were, on average, 568 ± 99 ppm and 671 ± 98 ppm during the peak drought and the recovery labeling campaigns, with some variation caused by the pulse-wise addition of $^{13}CO_2$ (**Supplementary Table S2**). Potential effects of species-specific differences in isotopic fractionation under slightly elevated CO_2 or drought on recovered amounts of ^{13}C can be excluded due to the significant enrichment of ^{13}C from naturally 1.1 to 30–80 atom% during the labeling campaigns.

Sampling

For each mesocosm, plant and soil samples were collected in a time series after the pulse labeling. The time series included samplings at 15 min, 24, 72, and 120 h after the labeling chamber was removed. Because a minimum distance of ∼5 cm had to be kept to the mesocosm edge, to a soil moisture measurement site and to a centrally located soil respiration measurement chamber, the available area for plant and soil sampling was very limited. The first sampling location was randomly chosen in the available area and further samplings were performed either clockwise or counterclockwise in a distance of ∼5 cm. At each sampling, the shoot material, i.e., the leaves and stems, was cut 1 cm above the soil in two 5 cm × 5 cm squares, which included a random selection of plant species from opposite positions in the mesocosm. The shoot material from both squares was pooled together and separated into biomass and necromass. The biomass was immediately treated by microwave to interrupt any metabolic activity (Popp et al., 1996), stored on ice packs for transport and dried at 60°C for 72 h for later analysis of the sugar content and stable carbon isotope composition. For soil samples, soil cores were collected in or next to plant sampling squares on bare soil spots close to plant cover. Sampling was done using a stainless-steel auger with 1.9 cm inner diameter (Eijkelkamp, Giesbeek, Netherlands). At each sampling, four soil cores (two per shoot sampling square) were taken from a depth of 0–7 cm and pooled in a mixed sample. Mixed soil samples were carefully sieved through a 2-mm mesh, and the roots were removed. Soil for EOC and MBC analysis was transported on ice packs, stored at 4°C and extracted/fumigated by no later than 4 days after sampling. Soil for neutral/phospho-lipid fatty acid (NLFA/PLFA) analysis was directly frozen with dry ice and stored at −18°C until further preparation. Subsamples of frozen soil were used prior to the NLFA/PLFA analysis to determine the soil water content gravimetrically, by weighing the soil before and after drying for 48 h at 105°C. Roots were washed from the remaining soil, and the dead as well as coarse roots (diameter > 2 mm) were removed. The total amount of washed fine root samples was divided into two subsamples. One subsample was treated like shoot samples (microwaved), and the other one (not microwaved) was kept moist with wet paper towels and used as quickly as possible for root respiration measurements in the field.

Microwaved shoot and root samples were completely dried in an oven at 60°C for 72 h, starting on the day of harvest. After its

dry weight had been determined, the plant material was carefully ground to a fine powder using a ball mill (MM200, Retsch GmbH, Haan, Germany). This material was then used to analyze the bulk ^{13}C content, the compound-specific ^{13}C isotope composition and the bulk nitrogen concentration. The aboveground biomass of the mesocosms was harvested completely at the end of each labeling/sampling campaign to determine the community shoot biomass. Community root biomass was directly estimated from the dry mass of all root samples for each individual mesocosm. To obtain samples with natural ^{13}C abundance, on the 14th of July, one soil core was taken from each of four unlabeled control mesocosms, and these cores were pooled together. The same was done for the unlabeled drought mesocosms. Similarly, shoot material was collected from all six species of each mesocosm and pooled together for the four control and four drought mesocosms.

Isotopic Composition of Plant Samples and Carbohydrate Analysis

Ground bulk plant material was used to determine ^{13}C contents ($\delta^{13}C$ vs. VPDB) and nitrogen concentrations of shoots and fine roots by elemental analysis (EA) – isotope ratio mass spectrometry (IRMS) (EA - Model NA 1500, Carlo Erba, Milan, Italy; coupled to an IRMS IsoPrime100, Isoprime Ltd., Cheadle, United Kingdom). NSC analysis was done as described by Karlowsky et al. (2018). Briefly, 30 mg of plant powder was weighed, and water-soluble sugars (fructan, sucrose, glucose, and fructose) were extracted using the method of Wild et al. (2010), as modified by Mellado-Vázquez et al. (2016). Analysis was done by high-performance liquid chromatography (HPLC) – IRMS (Dionex UltiMate 3000 UHPLC coupled via a LC-IsoLink system to a Delta V Advantage IRMS, Thermo Fisher Scientific, Bremen, Germany) in a NUCLEOGEL SUGAR 810 Ca^{2+} column (Macherey & Nagel, Düren, Germany) at 80°C, with 0.5 ml/min of bi-distilled water as eluent (Hettmann et al., 2007). In accordance with previous findings from the same study site (Karlowsky et al., 2018), fructan was assigned to one large peak at the beginning of chromatograms, which likely represented fructans with a high degree of polymerization (Benot et al., 2013). For starch analysis, the remaining pellets from the sugar extraction were washed again with a methanol:chloroform:water mixture (12:3:5, by volume) to remove remaining sugars and then digested with heat stable α-amylase (Göttlicher et al., 2006; Richter et al., 2009). The resulting gluco-oligomers were measured by EA-IRMS (EA 1100, CE Elantech, Milan, Italy; coupled to a Delta + IRMS, Finnigan MAT, Bremen, Germany).

Root Respiration Measurements

A subsample (0.2–1.2 mg) of root material, washed from soil and kept moist, was used for root respiration measurement in the field. Fresh roots were placed in a 100-ml Erlenmeyer flask, sealed by a rubber stopper and incubated at 15 ± 1°C in a water bath. The initial CO_2 concentration in the flask was, on average, 491 ± 12 ppm. Root incubation was performed according to Hasibeder et al. (2015), except for the time collection. Specifically, five gas samples were collected: one immediately after closing the flask and the other four after 7, 20, 40, and 60 min, respectively. Gas sampling was performed with a syringe; each time, 15 ml of gas was collected and transferred completely into pre-evacuated 12 ml vials with a rubber septum, to prevent ambient air from entering the vial. After each sampling, 15 ml CO_2-free air was injected into the Erlenmeyer flasks to replace the gas collected. The CO_2 concentration and the ^{13}C isotope composition were analyzed by IRMS coupled with a Multiflow system (IsoPrime100, Isoprime Ltd., Cheadle, United Kingdom). All gas samples were analyzed as soon as possible after sampling and were stored in the laboratory for a maximum of 4 weeks. Root respiration rate and the $^{13}C/^{12}C$ ratio of the CO_2 respired were calculated according to Hasibeder et al. (2015).

Analysis of Soil-Extractable Organic Carbon and Microbial Biomass Carbon

For the determination of the soil EOC and MBC, the method of Vance et al. (1987) with the modifications of Malik et al. (2013), was used. Soil EOC was extracted from a subsample of approximately 5 g of fresh soil with 25 ml of 0.5 M K_2SO_4 solution (distilled water) in a horizontal shaker with 150 rpm for 30 min. The extract was centrifuged at 12,000 × g for 5 min and coarse particles were removed using pre-washed (0.5 M K_2SO_4 solution) filter papers (Whatman Grade 1, d = 150 mm, 11 μm pore size, GE Healthcare UK Ltd., Buckinghamshire, United Kingdom). The filtrate was frozen and stored at −18°C until further processing for analysis. Total organic carbon (TOC) was extracted and processed in the same way as the EOC, after another subsample of approximately 5 g fresh soil had been fumigated for ≥24 h with chloroform. If necessary, drought-treated soils were rewetted to control levels with distilled water prior to the fumigation to avoid differences in the extraction efficiency (Sparling et al., 1990). For the analysis, ~1 ml each of the EOC and TOC extracts was filtered with pre-washed (~0.5 ml of extract) 0.45 μm cellulose membrane filters (MULTOCLEAR 0.45 μm RC 13 mm, CS-Chromatographie Service GmbH, Langerwehe, Germany). To de-gas the samples of inorganic C, filtered extracts were acidified with phosphoric acid to approximately pH 2 and gas-flushed with N_2 for 15 min. The degassed samples were then analyzed as bulk fraction (no column) on an HPLC-IRMS system (see carbohydrate analysis). Each sample was measured in triplicate. Quality was controlled by repeated measurements of citric acid standards ($\delta^{13}C = -18.58$ ‰ vs. VPDB, Fluka Chemie AG, Buchs, Switzerland; $SD = 0.14$‰, $n = 72$). Quantification was performed using a concentration row of the citric acid standard to calibrate the HPLC-IRMS based on CO_2 peak areas. The results for the EOC and TOC were normalized to the used soil dry mass for each fraction, and the concentration of MBC was calculated from the EOC and TOC by the formula: $[MBC] = ([TOC] - [EOC])/k_{MBC}$. For k_{MBC}, a value of 0.45 was used, which is the typical extraction efficiency of MBC after chloroform fumigation (Vance et al., 1987). The $^{13}C/^{12}C$ ratio (i.e., $\delta^{13}C$ or atom% ^{13}C) of MBC was calculated according

to the isotopic mass balance: $^{13}C/^{12}C_{MBC}$ = ($^{13}C/^{12}C_{TOC}$ * [TOC] – $^{13}C/^{12}C_{EOC}$ * [EOC])/([TOC]-[EOC]).

Analysis of Neutral and Phospholipid Fatty Acids

Neutral and phospholipid fatty acid analysis was done according to the method of Bligh and Dyer (1959), as modified by Karlowsky et al. (2018). Briefly, approximately 5 g of frozen bulk soil was extracted with a mixture of methanol, chloroform and 0.05 M K_2HPO_4 buffer (2:1:0.8, by volume; pH 7.4) using pressurized solvent extraction (SpeedExtractor E-916, Büchi Labortechnik AG, Flawil, Switzerland). A recovery standard (1,2-Dinonadecanoyl-sn-Glycero-3-Phosphatidylcholine; Larodan Fine Chemicals AB, Malmö, Sweden) was added (recovery rate: 62 ± 11%, SD, n = 60) to each sample, and the extraction was carried out at 70°C and 120 bar for 3 min × 10 min. Neutral and phospholipid fractions were separated using silica-filled solid-phase extraction (SPE) columns (CHROMABOND SiOH, 2 g, 15 ml, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Both fractions were hydrolyzed and methylated with methanolic KOH, and the resulting fatty acid methyl esters (FAMEs) were further purified for analysis by using aminopropyl-modified SPE columns (CHROMABOND NH2, 0.5 g, 3 ml, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The FAME C13:0 (Sigma-Aldrich Chemie GmbH, Munich, Germany) was added as the internal standard to all samples, and quantification was done by gas chromatography–flame ionization detection (GC-FID) on a GC-FID 7890B system with a programmable temperature vaporization (PTV) injector (Agilent Technologies, Palo Alto, CA, United States) using a DB-1MS UI column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, United States) and helium as the carrier gas (1.8 ml/min). The temperature program started at 45°C for 1 min, then increased in a first ramp of 60°C/min to 140°C (held for 0.5 min), followed by a second ramp of 2°C/min until 242°C, and finally, by a third ramp to 320°C (held for 3 min). Directly after injection, the PTV was heated up from 55 to 280°C at a rate of 500°C/min. Compound specific ^{13}C isotope analysis of NLFAs and PLFAs was conducted by GC-IRMS (GC 7890A with PTV injector, Agilent Technologies, Palo Alto, CA, United States; coupled via a Conflo IV/GC IsoLink to a Delta V Plus IRMS, Thermo Fisher Scientific, Bremen, Germany) using a DB-1MS UI column (60 m × 0.25 mm internal diameter × 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, United States) and helium as the carrier gas (1.8 ml/min). Directly after injection, the PTV was heated from 55 to 280°C at a rate of 500°C/min. The GC temperature program started with 45°C for 1 min, then increased in a first ramp of 60°C/min to 140°C (held for 0.5 min), followed by a second ramp of 4°C/min until 283°C (held for 4.9 min) and a third ramp until 320°C (held for 3 min). Concentrations and ^{13}C isotope content of identified FAMEs were corrected for the methyl group introduced during derivatization. We used the sum of the PLFAs i14:0, i15:0, a15:0, i16:0, a17:0, i17:0, and br18:0 for Gram-positive bacteria (Zelles, 1997, 1999); 10-Me16:0 and 10-Me18:0 for actinobacteria (Lechevalier et al., 1977; Zelles, 1999); and 16:1ω7 and 18:1ω7 for Gram-negative bacteria (Zelles, 1997, 1999). The PLFA 18:2ω6,9 was used as the marker for saprotrophic fungi (Frostegård and Bååth, 1996; Zelles, 1997) and the NLFA 16:1ω5 as the marker for arbuscular mycorrhizal (AM) fungi (Olsson, 1999). Although the NLFA 16:1ω5 does not correctly estimate the biomass of AM fungal populations, it has been found to be more of a proxy than the PLFA 16:1ω5 (e.g., Ngosong et al., 2012; Mellado-Vázquez et al., 2016; Paterson et al., 2016).

Calculation of ^{13}C Tracer Concentrations

To determine the relative abundance of ^{13}C tracer in labeled samples, we calculated the atom% $^{13}C_{excess}$ as follows:

$$atom\%\ ^{13}C_{excess} = atom\%\ ^{13}C_{labeled} - atom\%\ ^{13}C_{unlabeled}$$

with $atom\%\ ^{13}C_{labeled}$ being the atom% ^{13}C of the labeled samples and $atom\%\ ^{13}C_{unlabeled}$ being the atom% ^{13}C of natural abundance samples from unlabeled mesocosms (mixed samples from shoots of all six species were used as reference for the plant community). Values of atom% $^{13}C_{excess}$ are not presented here but can be found in the **Supplementary Figures S9–S12**.

For all plant and soil samples, we expressed the ^{13}C isotope content as incorporated ^{13}C (mg ^{13}C m^{-2}), which refers to the total amount of ^{13}C found in a certain carbon pool on an area basis, and it was calculated as:

$$incorporated^{13}C = \frac{\text{atom}\%^{13}C_{excess} * C_{pool}}{100\%}$$

with C_{pool} being the respective carbon pool (mg C m^{-2}).

The roots respired ^{13}C (mg ^{13}C m^{-2} h^{-1}), which corresponds to the amount of ^{13}C released in respired CO_2 from roots during a certain time, was calculated similarly to the incorporated ^{13}C as follows:

$$root\ respired^{13}C = \frac{\text{atom}\%^{13}C_{excess} * CO2_{resp.\ rate}}{100\%}$$

with $CO2_{resp.rate}$ being the respiration rate of CO_2 (mg CO_2 m^{-2} h^{-1}).

Data Analyses

For root biomass and concentration data, the average values were calculated over the different sampling times after pulse labeling: 1 and 3 days after labeling for NLFAs and PLFAs and 15 min, 1 day, 3 days, and 5 days after labeling for all others. For the soil samples, a bulk soil density of 0.7 g cm^{-3} (Meyer et al., 2012a) was used for calculating area-based pool sizes. The total ^{13}C uptake was calculated as the sum of the bulk shoot and bulk root incorporated ^{13}C at the first sampling directly after labeling (15 min). The ^{13}C tracer fluxes were analyzed for drought effects considering the different sampling times (same times as for concentration data). After removing negative ^{13}C incorporation values (defined as below detection limit), the relative ^{13}C allocation to the different pools was calculated for each sampling time as the ratio of ^{13}C incorporation to total ^{13}C uptake. Relative ^{13}C allocation to shoot and root storage pools was calculated as the sum of relative ^{13}C allocation to fructan and

starch in the shoots and roots. For an overview of the drought effects on all pools (including NLFAs and PLFAs), the relative ^{13}C allocation was averaged for 1 and 3 days samples, and the drought to control ratio was calculated. In general, at 1 and 3 days after pulse labeling, the drought effects on relative ^{13}C allocation were comparable (**Supplementary Figure S4**) and high ^{13}C tracer enrichment was found in all pools of interest, making these two times suitable to assess the strongest differences in ^{13}C allocation patterns. For the calculation of drought to control ratios, only labelings with data from both treatments (i.e., control and drought mesocosms that were labeled at the same time,) were considered. First, the drought to control ratio of each labeling pair was calculated, and second, the average value was formed.

All statistical analyses were done using the R 3.3.2 software (R Core Team, 2016). Time series (in hours after pulse labeling) of the ^{13}C tracer data were tested separately for each labeling campaign for the effects of drought and sampling time, as well as their interaction, using linear mixed-effects models from the 'lme4' package (Bates et al., 2015). In the mixed-effects model, the treatment and sampling time (as factor) were set as fixed effects, whereas the rain-out shelter and mesocosm were set as random effects. Drought effects on relative ^{13}C allocation were analyzed similarly, using treatment and sampling time (as factors) as fixed effects, and labeling pair (control and drought mesocosms labeled in parallel) and mesocosm as random effects. All mixed-effects models were assessed for violations of normality, heteroscedasticity and independency. If necessary, ^{13}C tracer data were log (+1) or square root (+1) transformed. For all other data (i.e., biomass, total ^{13}C uptake and concentration data), the drought effects were evaluated for each labeling campaign separately using permutational ANOVA from the 'lmPerm' package (Wheeler and Torchiano, 2016), from which exact *P*-values (P_{aovp}) were obtained. Permutation tests do not require assumptions about the statistical distribution and are powerful with small sample sizes (Ernst, 2004).

RESULTS

Peak Drought Labeling

The 4 weeks of severe drought had strong effects on the plant community and its biomass at peak drought (**Table 1**). Drought significantly reduced the shoot biomass but had no distinct effect on the total plant biomass, since a strong increase of fine root biomass occurred. Consequently, drought led to a significant increase in the root to shoot ratio. According to the reduction in shoot biomass, the photosynthetic rate (**Supplementary Figure S5A**) and total plant ^{13}C uptake (**Table 1**) were strongly reduced by drought as well. Drought did not change the proportion of total ^{13}C (relative ^{13}C allocation) that was allocated belowground at 24 and 72 h from labeling (**Figure 1A**), although it was lower at 15 min and higher at 120 h (**Supplementary Figure S4**). The little effect of drought on overall BCA was also expressed by similar reductions of ^{13}C tracer incorporation into shoots and roots over the 120-h sampling period (**Supplementary Figure S6**). However, drought more strongly affected relative ^{13}C allocation to NSCs (**Figure 1A**) and their tracer dynamics (**Supplementary Figures S6B–D,F–H**). Significantly less ^{13}C was allocated to shoot storage (**Figure 1A**), i.e., to compounds such as fructan and starch (**Supplementary Figures S6C,D**), whereas slightly more ^{13}C was retained in shoot sucrose over time (**Figure 1A** and **Supplementary Figure S4**). This retention was reflected in the higher sucrose concentrations and lower

TABLE 1 | Drought effects on biomass, ^{13}C tracer uptake, root respiration and biomass N contents.

Labeling	Parameter	Unit	Control	Drought	D^a
Peak drought	Total biomass	g_{dm} m^{-2}	313 ± 23	353 ± 31	n.s.
	Shoot biomass	g_{dm} m^{-2}	131 ± 12	82 ± 9	***
	Root biomass	g_{dm} m^{-2}	182 ± 16	271 ± 25	**
	Root:Shoot ratio	–	1.45 ± 0.21	3.44 ± 0.37	***
	^{13}C uptake	mg_{13C} m^{-2}	366 ± 32	93 ± 6	***
	Root respiration	μmol_{CO2} m^{-2} s^{-1}	0.82 ± 0.03	0.88 ± 0.09	n.s.
	Shoot N	g_N m^{-2}	1.71 ± 0.16	1.14 ± 0.13	**
	Root N	g_N m^{-2}	1.41 ± 0.10	2.35 ± 0.30	***
	Total N	g_N m^{-2}	3.12 ± 0.22	3.49 ± 0.38	n.s.
Recovery	Total biomass	g_{dm} m^{-2}	295 ± 19	267 ± 12	n.s.
	Shoot biomass	g_{dm} m^{-2}	114 ± 8	102 ± 7	n.s.
	Root biomass	g_{dm} m^{-2}	181 ± 20	165 ± 8	n.s.
	Root:Shoot ratio	–	1.7 ± 0.3	1.6 ± 0.1	n.s.
	^{13}C uptake	mg_{13C} m^{-2}	220 ± 29	231 ± 27	n.s.
	Root respiration	μmol_{CO2} m^{-2} s^{-1}	0.81 ± 0.06	0.94 ± 0.11	n.s.
	Shoot N	g_N m^{-2}	1.34 ± 0.09	1.74 ± 0.19	**
	Root N	g_N m^{-2}	1.46 ± 0.19	1.59 ± 0.03	n.s.
	Total N	g_N m^{-2}	2.80 ± 0.23	3.33 ± 0.19	*

[a]*Levels of significance for drought effects: ***P_{aovp} < 0.001, **P_{aovp} < 0.01, *P_{aovp} < 0.05, (*)P_{aovp} < 0.1; n.s., not significant. Mean values ± SE (n = 6) are shown for control and drought treatments. For root respiration and N concentrations, the data were averaged over the four sampling times for each mesocosm.*

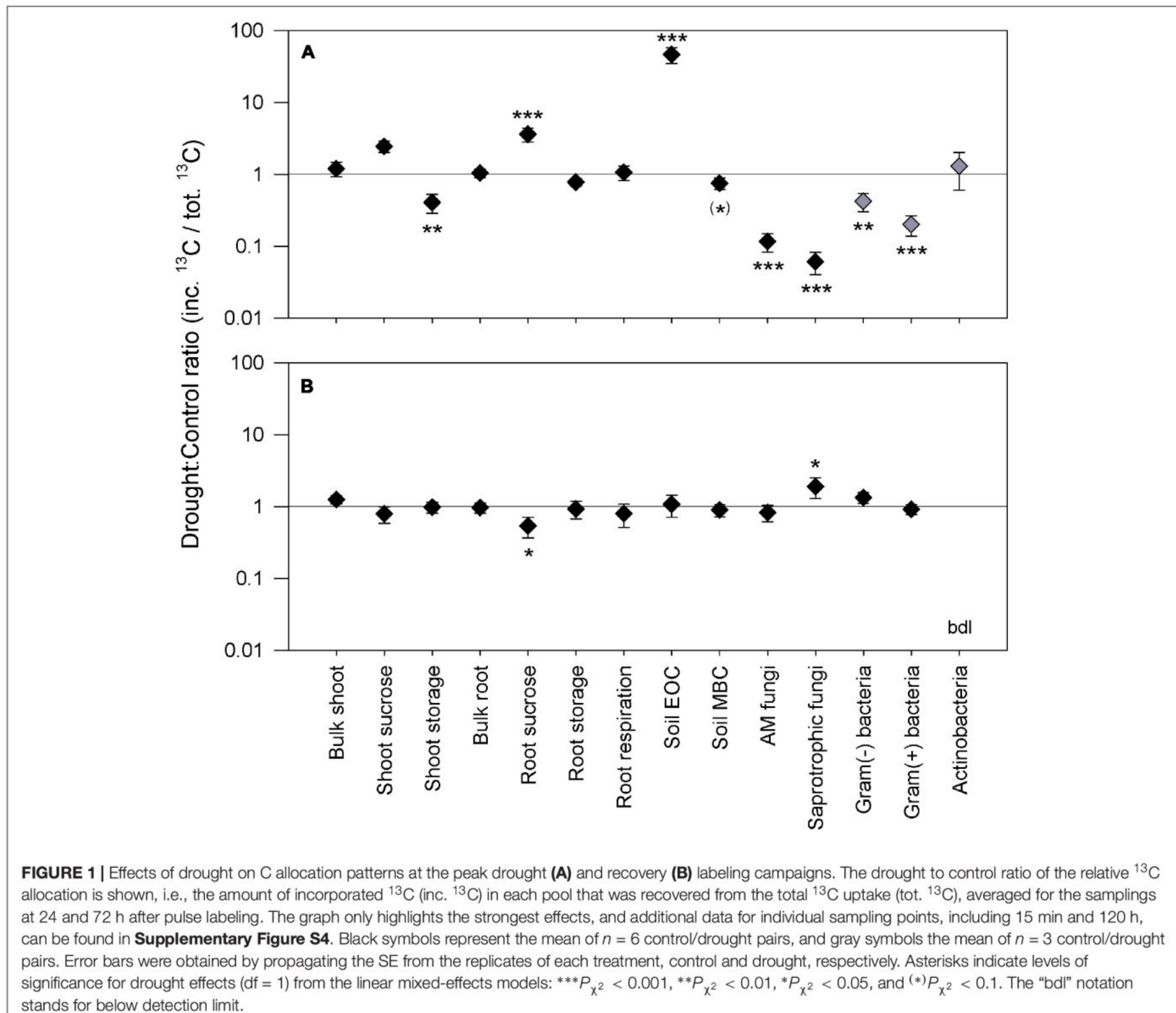

FIGURE 1 | Effects of drought on C allocation patterns at the peak drought **(A)** and recovery **(B)** labeling campaigns. The drought to control ratio of the relative ^{13}C allocation is shown, i.e., the amount of incorporated ^{13}C (inc. ^{13}C) in each pool that was recovered from the total ^{13}C uptake (tot. ^{13}C), averaged for the samplings at 24 and 72 h after pulse labeling. The graph only highlights the strongest effects, and additional data for individual sampling points, including 15 min and 120 h, can be found in **Supplementary Figure S4**. Black symbols represent the mean of n = 6 control/drought pairs, and gray symbols the mean of n = 3 control/drought pairs. Error bars were obtained by propagating the SE from the replicates of each treatment, control and drought, respectively. Asterisks indicate levels of significance for drought effects (df = 1) from the linear mixed-effects models: $^{***}P_{\chi^2} < 0.001$, $^{**}P_{\chi^2} < 0.01$, $^{*}P_{\chi^2} < 0.05$, and $^{(*)}P_{\chi^2} < 0.1$. The "bdl" notation stands for below detection limit.

fructan and starch concentrations in drought shoots compared to controls (**Table 2**). Drought increased the relative ^{13}C allocation to the root sucrose pool (**Figure 1A**), which showed altered tracer dynamics (**Supplementary Figure S6F**), i.e., lower ^{13}C incorporation until 24 h and higher ^{13}C incorporation. Reduced ^{13}C incorporation was found in fructan and starch from roots (**Supplementary Figures S6G,H**), although their concentrations (**Table 2**) were not affected by drought. Indeed, the relative ^{13}C allocation to root storage was on average only little affected by drought (**Figure 1A**), showing a decrease at 24 h and an increase at 120 h (**Supplementary Figure S4**). Apparently, in root fructan, drought mainly led to slower ^{13}C tracer incorporation over time (**Supplementary Figure S6G**). Moreover, considered the higher fine root biomass, the root fructan pool even increased during drought (Control, 6.1 ± 1.3 g_C m^{-2}; Drought, 10.2 ± 1.5 g_C m^{-2}; SE, n = 6; P_{aovp} = 0.009). Similar to root storage, the drought reduced the amount of root-respired ^{13}C but only at the first two sampling points (**Supplementary Figure S7A**). This reduction led to decreased relative ^{13}C allocation to root respiration at 15 min and 24 h; however, it increased at 72 and 120 h (**Supplementary Figure S4**). This effect was not visible on average for 24 and 72 h (**Supplementary Figure S1**). Consequently, the overall respiration rate was not altered by drought (**Table 1**), despite lower respiration rates at the dry mass level (Control, 4.6 ± 0.3 $nmol_{CO2}$ $g^{-1}{}_{dm}$ s^{-1}; Drought, 3.3 ± 0.6 $nmol_{CO2}$ $g^{-1}{}_{dm}$ s^{-1}; $P_{aovp} < 0.001$). Plant nitrogen concentrations were only little affected by drought and tended to be higher in shoots (Control, 1.31 ± 0.04%$_N$; Drought, 1.40 ± 0.06%$_N$; P_{aovp} = 0.076) but not in roots (Control, 0.79 ± 0.05%$_N$; Drought, 0.86 ± 0.06%$_N$; P_{aovp} = 0.206). However, if the differences in biomass were considered, drought led to a reduction of shoot nitrogen content and an increase of root nitrogen content per unit area (**Table 1**).

TABLE 2 | Effects of drought on the sizes of plant bulk and carbohydrate pools for the peak drought and the recovery labeling campaigns.

Labeling	Parameter	C content ($mg_C\ g_{dm}^{-1}$)		
		Control	Drought	D^a
Peak drought	Bulk shoot	422 ± 3	423 ± 3	n.s.
	Shoot sucrose	14 ± 0	16 ± 1	***
	Shoot fructan	57 ± 2	41 ± 3	***
	Shoot starch	8.1 ± 0.6	5.1 ± 1.4	**
	Bulk root	345 ± 15	369 ± 15	(*)
	Root sucrose	4.4 ± 0.4	10.8 ± 0.9	***
	Root fructan	32 ± 2	38 ± 6	n.s.
	Root starch	12 ± 4	16 ± 7	n.s.
Recovery	Bulk shoot	421 ± 4	422 ± 4	n.s.
	Shoot sucrose	12 ± 0	13 ± 1	n.s.
	Shoot fructan	47 ± 4	33 ± 3	**
	Shoot starch	9.0 ± 1.3	8.5 ± 0.8	n.s.
	Bulk root	357 ± 7	379 ± 8	(*)
	Root sucrose	4.4 ± 0.6	2.7 ± 0.1	***
	Root fructan	35 ± 6	29 ± 3	n.s.
	Root starch	21 ± 4	14 ± 4	n.s.

*[a]Levels of significance for drought effects: ***P_{aovp} < 0.001, **P_{aovp} < 0.01, *P_{aovp} < 0.05, (*)P_{aovp} < 0.1; n.s., not significant. Values represent averages among the mesocosms for each treatment (mean ± SE, n = 6), after averaging over the four sampling times for each mesocosm.*

TABLE 3 | Effects of drought on the sizes of soil carbon and microbial marker lipid pools for the peak drought and the recovery labeling campaigns.

Labeling	Parameter	C content ($\mu g_C\ g_{dm}^{-1}$)		
		Control	Drought	D^a
Peak drought	EOC	34 ± 4	102 ± 8	***
	MBC	402 ± 33	429 ± 20	n.s.
	AM fungi	24 ± 3	17 ± 2	*
	Saprotrophic fungi	1.1 ± 0.1	1.2 ± 0.2	n.s.
	Gram (–) bacteria	5.7 ± 0.4	7.1 ± 0.3	**
	Gram (+) bacteria	4.1 ± 0.3	4.8 ± 0.2	*
	Actinobacteria	2.4 ± 0.2	2.9 ± 0.1	*
Recovery	EOC	32 ± 3	32 ± 1	n.s.
	MBC	393 ± 18	393 ± 15	n.s.
	AM fungi	34 ± 2	19 ± 2	***
	Saprotrophic fungi	0.9 ± 0.1	0.9 ± 0.1	n.s.
	Gram (–) bacteria	6.0 ± 0.4	6.6 ± 0.4	n.s.
	Gram (+) bacteria	4.3 ± 0.3	4.6 ± 0.4	n.s.
	Actinobacteria	2.8 ± 0.2	2.9 ± 0.2	n.s.

*[a]Levels of significance for drought effects: ***P_{aovp} < 0.001, **P_{aovp} < 0.01, *P_{aovp} < 0.05, (*)P_{aovp} < 0.1; n.s., not significant. AM, arbuscular mycorrhizal; EOC, extractable organic carbon; MBC microbial biomass carbon. Values represent averages among the mesocosms for each treatment (mean ± SE, n = 6), after averaging over the sampling times (four for EOC and MBC, two for microbial marker lipids) for each mesocosm.*

Regarding the soil, drought led to a threefold increase of water-soluble EOC compared to controls (**Table 3**) but had no effect on the MBC content. Significantly higher relative ^{13}C allocation to the EOC (**Figure 1A** and **Supplementary Figure S4**) resulted from the continuous increase of ^{13}C tracer incorporation into the EOC after the labeling (**Figure 2A**). By contrast, drought consistently reduced the amount of ^{13}C tracer incorporation into MBC over time and delayed the label uptake (**Figure 2B**), leading to lower relative ^{13}C allocation to MBC at 15 min and 24 h (**Supplementary Figure S4**). The reduced microbial ^{13}C incorporation during drought was more pronounced for the individual lipid markers (**Supplementary Figures S8A–D**), yielding significantly decreased relative ^{13}C allocation to AM fungi, saprotrophic fungi, and Gram-negative and Gram-positive bacteria (**Figure 1A**). This effect was not visible for actinobacteria (**Figure 1A**), which, on average, did not incorporate detectable amounts of ^{13}C in control and drought treatments in their lipid markers (**Supplementary Figure S8E**). AM fungi, which took up the largest amount of ^{13}C in the controls, reflected the tracer dynamics of MBC (**Figure 2B** and **Supplementary S8A**). This relation was less pronounced for saprotrophic fungi, whereas bacteria showed a slower label uptake. At the biomass scale, AM fungi were slightly affected by drought, whereas saprotrophic fungi were unaffected, and the bacterial biomass generally increased (**Table 3**).

Recovery Labeling

Ten days after rewetting, drought-treated mesocosms fully recovered their shoot biomass, root:shoot ratio, ^{13}C uptake (**Table 1**), and photosynthetic rate (**Supplementary Figure S5B**). Accordingly, the amount of ^{13}C incorporated in the root and shoot pools mostly recovered (**Supplementary Figures S6I–P**). NSC tracer dynamics partially differed between the control and drought treatments. Drought led to an earlier peak value of ^{13}C incorporation into root sucrose (**Supplementary Figure S6N**) and to faster label decreases in shoot starch and root fructan after peak values were reached (**Supplementary Figures S6L,O**). This also resulted in a lower relative ^{13}C allocation to root sucrose 72 h and 120 h after labeling (**Supplementary Figure S4**), whereas carbon allocation to shoot and root storage was only little affected. Bulk roots mainly reflected the ^{13}C tracer dynamics of root fructan, showing a similar trend over time (**Supplementary Figures S6M,O**), i.e., a decrease of ^{13}C incorporation at 72 h. Despite largely recovered carbon fluxes, the previous drought caused reductions in the concentrations of shoot fructan and root sucrose at the recovery labeling (**Table 2**). The overall root respiration rate was not affected by drought and rewetting (**Table 1**) but was increased at the dry mass level (Control, 4.6 ± 0.8 $nmol_{CO2}\ g^{-1}{}_{dm}\ s^{-1}$; Drought, 5.7 ± 0.6 $nmol_{CO2}\ g^{-1}{}_{dm}\ s^{-1}$; P_{aovp} = 0.039). Furthermore, root respiration had similar ^{13}C tracer dynamics like root sucrose, showing an earlier peak of respired ^{13}C in drought-treated mesocosms (**Supplementary Figure S7B**). Rewetting led to significantly higher nitrogen concentrations in the roots (Control, 0.80 ± 0.05$\%_N$; Drought, 0.98 ± 0.05$\%_N$; P_{aovp} = 0.006) and shoots (Control, 1.18 ± 0.05$\%_N$; Drought, 1.69 ± 0.11$\%_N$; P_{aovp} < 0.001), thereby increasing the shoot and total biomass N content per unit area (**Table 1**).

Overall, plant and soil-related parameters recovered from drought at the recovery labeling. Consistently, the concentrations

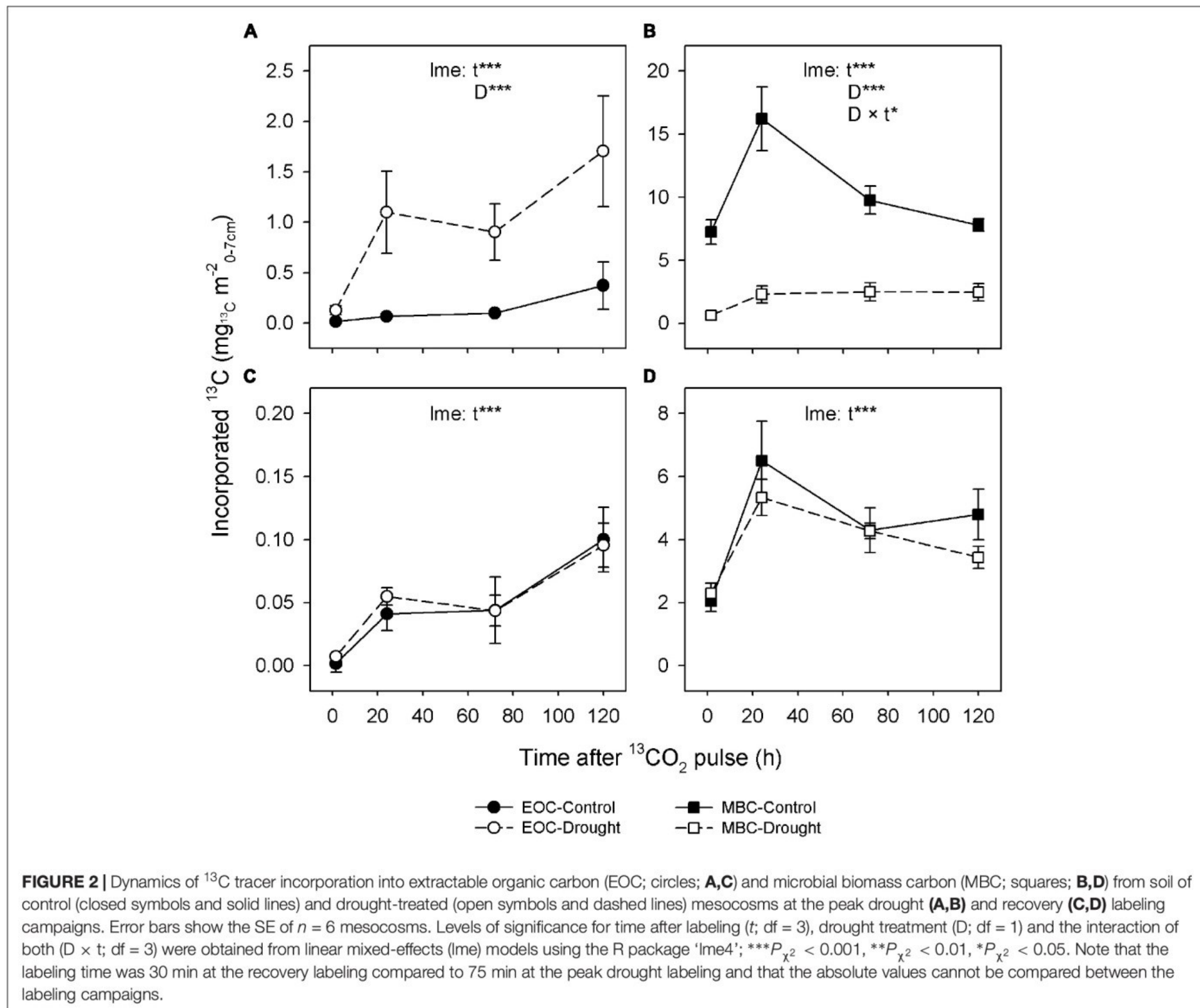

FIGURE 2 | Dynamics of ^{13}C tracer incorporation into extractable organic carbon (EOC; circles; **A,C**) and microbial biomass carbon (MBC; squares; **B,D**) from soil of control (closed symbols and solid lines) and drought-treated (open symbols and dashed lines) mesocosms at the peak drought **(A,B)** and recovery **(C,D)** labeling campaigns. Error bars show the SE of *n* = 6 mesocosms. Levels of significance for time after labeling (*t*; df = 3), drought treatment (D; df = 1) and the interaction of both (D × t; df = 3) were obtained from linear mixed-effects (lme) models using the R package 'lme4'; ***$P_{\chi^2} < 0.001$, **$P_{\chi^2} < 0.01$, *$P_{\chi^2} < 0.05$. Note that the labeling time was 30 min at the recovery labeling compared to 75 min at the peak drought labeling and that the absolute values cannot be compared between the labeling campaigns.

and ^{13}C tracer incorporations of EOC and MBC fully recovered (**Table 3** and **Figures 2C,D**). The ^{13}C uptake in different microbial groups also recovered and showed little variation between the groups (**Supplementary Figures S8F–J**). Only the relative ^{13}C allocation to saprotrophic fungi was significantly increased after rewetting (**Figure 1B**), as visible by the slightly higher ^{13}C incorporation into the saprotrophic fungal marker (**Supplementary Figure S8G**). A similar trend was present for the tracer incorporation into Gram-negative bacterial markers, while no effect was observed on the Gram-positive bacterial markers. In contrast, for the AM fungal marker, a weak trend existed, showing a reduction in the ^{13}C incorporation in drought mesocosms. This trend corresponded to a significantly reduced marker concentration (**Table 3**), which was largely counterbalanced by a higher relative abundance of ^{13}C tracer (atom% $^{13}C_{excess}$) (**Supplementary Figure S9**). For all other microbial groups, the marker concentrations were equal between control and drought treatments.

DISCUSSION

In a previous experiment on intact vegetation-soil monoliths from a managed meadow and an abandoned grassland, we found that drought-induced reductions of plant photosynthetic activity (Ingrisch et al., 2018) were coupled to strong reductions in plant storage NSCs, especially above ground, whereas BCA was maintained at a constant level (abandoned grassland) or even increased (managed meadow) relative to the total carbon uptake (Karlowsky et al., 2018). The carbon allocated to roots was largely recovered in drought-accumulated soluble sugars, whereas the uptake of plant-derived carbon in fatty acid biomarkers of root-associated microorganisms (AM fungi, SF and bacteria) was strongly reduced. Overall, these responses were greater in the managed meadow compared to the abandoned grassland, which likely also profited from enhanced AM fungal growth during drought. Furthermore, we found that after rewetting, the carbon uptake of the SF and bacteria was enhanced in the managed

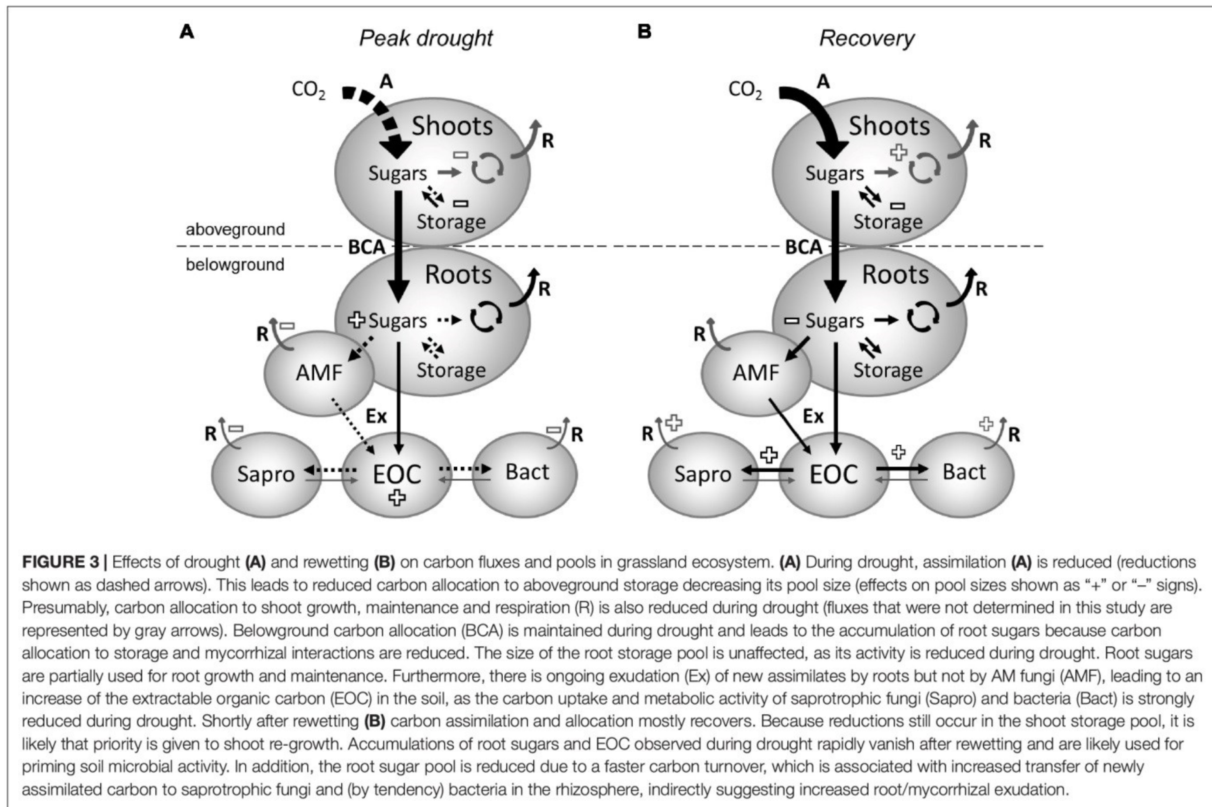

FIGURE 3 | Effects of drought **(A)** and rewetting **(B)** on carbon fluxes and pools in grassland ecosystem. **(A)** During drought, assimilation **(A)** is reduced (reductions shown as dashed arrows). This leads to reduced carbon allocation to aboveground storage decreasing its pool size (effects on pool sizes shown as "+" or "–" signs). Presumably, carbon allocation to shoot growth, maintenance and respiration (R) is also reduced during drought (fluxes that were not determined in this study are represented by gray arrows). Belowground carbon allocation (BCA) is maintained during drought and leads to the accumulation of root sugars because carbon allocation to storage and mycorrhizal interactions are reduced. The size of the root storage pool is unaffected, as its activity is reduced during drought. Root sugars are partially used for root growth and maintenance. Furthermore, there is ongoing exudation (Ex) of new assimilates by roots but not by AM fungi (AMF), leading to an increase of the extractable organic carbon (EOC) in the soil, as the carbon uptake and metabolic activity of saprotrophic fungi (Sapro) and bacteria (Bact) is strongly reduced during drought. Shortly after rewetting **(B)** carbon assimilation and allocation mostly recovers. Because reductions still occur in the shoot storage pool, it is likely that priority is given to shoot re-growth. Accumulations of root sugars and EOC observed during drought rapidly vanish after rewetting and are likely used for priming soil microbial activity. In addition, the root sugar pool is reduced due to a faster carbon turnover, which is associated with increased transfer of newly assimilated carbon to saprotrophic fungi and (by tendency) bacteria in the rhizosphere, indirectly suggesting increased root/mycorrhizal exudation.

meadow (Karlowsky et al., 2018), which was reflected by higher plant nitrogen uptake and a faster recovery of aboveground biomass compared to the abandoned grassland (Ingrisch et al., 2018).

However, we were not able to assess whether the accumulation of root sugars during drought affected the release of carbon to the rhizosphere, nor were we able to determine how the drought-induced shift toward belowground allocation in the meadow might be related to its quick recovery after rewetting. Therefore, the aim of this study was to further elucidate the mechanisms underlying the link between plant photosynthesis and soil microbial carbon cycling during drought and after rewetting.

The Link Between Plant and Soil Microbial Processes at Peak Drought

Surprisingly, drought had no significant effect on the total plant biomass. However, the decrease in shoot biomass and the concurrent increase in fine root biomass indicate that drought led to a shift in plant carbon allocation toward the belowground organs. Similar results have been found before in drought experiments on managed grasslands (Kahmen et al., 2005; Burri et al., 2014) and were attributed by the authors to an adaptation of plants in order to forage the limited water in dry soil. However, the root biomass response to drought can vary (Kahmen et al., 2005) and depends on the severity of the drought (Kreyling et al., 2008). Another root response occurring together with increased BCA is the accumulation of root sugars, especially sucrose (Hasibeder et al., 2015; Karlowsky et al., 2018). Such accumulations of root sugars can indicate an adjustment to dry conditions (Hasibeder et al., 2015) by increasing the concentration of osmolytes that prevent cells from desiccation (Chaves et al., 2003; Chen and Jiang, 2010). In our study, simultaneously increased concentrations of free glucose and fructose in roots (data not shown) further point to osmotic adjustment (Chen and Jiang, 2010).

Independently of its usage, the carbon needed to maintain BCA originates either from recent assimilates or from remobilized aboveground storage compounds. In previous studies, drought increased the proportion of recently assimilated carbon allocated belowground (Palta and Gregory, 1997; Huang and Fu, 2000; Burri et al., 2014; Hasibeder et al., 2015; Karlowsky et al., 2018). Here, we could not identify this effect (**Figure 3A**), suggesting a higher contribution of shoot storage is needed to maintain BCA during drought, as indicated by the depletion of shoot fructan and starch. This might be due to stronger negative effects of drought on carbon assimilation than in the previous studies. Diverging results for the belowground allocation of freshly assimilated carbon have been reported before by Sanaullah et al. (2012) in a lab-based mesocosm experiment with

monocultures and different mixtures of two grasses and one legume, whereas Ruehr et al. (2009) even found that drought increased the residence time of new carbon in leaves from beech trees. Of course, as woody species, trees have additional aboveground storage organs, which likely modify their drought response compared to herbaceous species. As a consequence, the source of the typically observed increase of BCA during drought might vary between fresh assimilates and older reserve carbohydrates, depending on the severity of drought, the timing in the year, as well as the functional composition or type of plants. In general, as previously concluded by Bahn et al. (2013), under reduced carbon supply, BCA in grassland seems to be maintained at the expense of aboveground storage (**Figure 3A**). Furthermore, the increase of nitrogen content in the roots (g_N m^{-2}) of drought-treated plants (**Table 1**) suggests that the disturbance-adapted meadow plants actively preserve their resources belowground during extreme drought, likely to facilitate quick recovery after rewetting (Karlowsky et al., 2018).

Most interestingly, the altered plant resource allocation patterns did not disrupt the release of recently assimilated carbon to the rhizosphere during drought (**Figure 3A**), as visible by the high amount of ^{13}C tracer in the soil EOC fraction, which exceeded control levels shortly after labeling. A similar enrichment of plant-derived carbon in the EOC pool was found by Fuchslueger et al. (2014a) and was attributed by the authors to the role of root exudates in reducing friction resistance in soil and maintaining root-soil connectivity. However, the strong reduction in ^{13}C recovered in the microbial biomass of drought mesocosms points to decreased microbial uptake of recent plant-derived carbon, which probably led to the strong accumulation of carbon in the EOC pool. Nonetheless, increased root exudation during drought, as evidenced by a recent mesocosm study on tree saplings (Preece et al., 2018), could have further contributed to the greater EOC pools in the soil.

Notably, the relative ^{13}C allocation to MBC was much less reduced by drought compared to microbial marker fatty acids (**Figure 1A**). This finding may imply that drought-reduced microbial growth, which can be estimated by the production of new fatty acids, and led to the accumulation of osmotically active compounds in MBC (Schimel et al., 2007). Osmolytes, e.g., amino acids in bacteria and polyols in fungi, are essentially highly water soluble and are more easily recovered than hydrophobic fatty acid-containing lipids in the MBC, which is extracted using aqueous K_2SO_4 solution. Moreover, reduced substrate diffusion, assumed to be the main limiting factor for bacterial activity in dry soil (Skopp et al., 1990; Stark and Firestone, 1995; Nunan et al., 2017), cannot explain the reduced ^{13}C tracer uptake by AM fungi during drought, since mycorrhizal interactions do not depend on substrate diffusion in the soil.

Unexpectedly, bacterial biomass was generally higher in drought-treated mesocosms (**Table 3**). A high resistance to drought was expected for the slow-growing, Gram-positive (actino)bacteria but not for the Gram-negative bacteria with their thin cell wall (Schimel et al., 2007; Lennon et al., 2012). Possibly, Gram-negative bacteria profited from the increased root growth and exudate availability during drought, as the increased amounts of EOC in drought mesocosms at peak drought labeling suggested. If this scenario occurred at earlier stages of drought, when soil moisture conditions were not limiting the bacterial activity, then Gram-negative bacteria could have used the easily consumable carbon from the EOC pool for their growth. Similarly, we did not expect the concentration of AM fungi marker in drought mesocosms to be reduced compared to the controls (**Table 3**). This contrasts previous findings from grassland monoliths (Karlowsky et al., 2018), showing an increase of the (AM + saprotrophic) fungi:bacteria ratio at peak drought. This difference could be due to the use of sieved soil in mesocosms, because the mycorrhizal network strongly interacts with soil structure (Rillig and Mummey, 2006). Other explanations include increased competition for plant carbon between fine roots and AM fungi, or a lower plant dependence on AM fungi due to (a) lower nutrient demand of senescing shoots or (b) higher nutrient availability resulting from decreased competition with soil microorganisms. Additionally, the selected plant species might have interacted differently with AM fungal populations (Legay et al., 2016; Mariotte et al., 2017). Additionally, bacterial foraging of senescing AM fungi structures cannot be excluded and might have contributed to the increase in the Gram-negative bacteria during drought, too.

Carbon Allocation and Plant–Microbial Interactions During Recovery

After rewetting, the mesocosm communities quickly recovered from drought, and both the shoot biomass and the root:shoot ratio were restored to control levels (**Table 1**). The higher fine root growth observed during drought was ceased at recovery labeling, possibly to support the re-growth of shoot biomass. However, the mechanisms behind the change in fine root biomass remain unclear, and thus, we cannot exclude the possibility that this observation was due to initial differences between the mesocosms used for the peak drought labeling and the mesocosms used for the recovery labeling. In general, the root response to drought-rewetting seems to be highly variable because previous studies either found an increase (Fuchslueger et al., 2016; Karlowsky et al., 2018, abandoned grassland) or no change (Karlowsky et al., 2018, managed meadow) in the fine root biomass after rewetting. In the latter study, the root response depended on the land use and was attributed to variable needs of water and nutrient uptake by fine roots, resulting from differences in the recovery of the dominant plant-microbial interactions. On the other side, in this study, the plant ^{13}C tracer uptake and allocation supports the hypothesis that carbon resources are preferentially invested into the regrowth of shoot biomass after rewetting (**Figure 3B**). Despite recovered ^{13}C tracer dynamics, the reduced shoot fructan pool indicates that, during the recovery phase, plants invested more carbon into structural carbohydrates or into respiration (e.g., for repair processes) than in storage. This investment was underpinned by the higher turnover of ^{13}C tracer in shoot starch, which suggests a faster utilization of recent assimilates from transitory

storage (Bahn et al., 2013) in plants recovering from drought. The reduced concentrations of root sucrose after rewetting could also be a result of the preferential use of newly assimilated carbon for shoot regrowth, decreasing the BCA during recovery (Zang et al., 2014). However, since only a marginal effect was observed on the average ^{13}C tracer incorporation in root sucrose and apparently a faster utilization of recent assimilates occurred in roots (**Supplementary Figures S6M–O**), most likely, the reduced sucrose concentrations were a result of increased root-rhizosphere carbon transfer (Hagedorn et al., 2016).

According to a shift in root functioning from resource preservation to nutrient acquisition, the uptake of fresh plant-derived carbon completely recovered for all microbial groups, and the carbon transfer to saprotrophic fungi even increased in the drought mesocosms (**Figure 3B**). These microorganisms were also found to rapidly take up recent plant-derived carbon in grasslands (de Deyn et al., 2011). In contrast to a previous study on the meadow (Karlowsky et al., 2018), we could not find significant excess uptake of ^{13}C tracer in bacteria. However, we cannot exclude that the use of sieved subsoil in this study led to altered microbial responses compared to the undisturbed topsoil in the previous study, as the initial microbial community and its functioning might have differed. Moreover, the rapid uptake of plant-derived carbon by saprotrophic fungi agrees with a recently introduced framework for carbon flow in the rhizosphere by Ballhausen and de Boer (2016), who proposed that a large fraction of the labile carbon from root exudation is primarily taken up by saprotrophic fungi prior to its consumption by fungus-feeding bacteria. As expected, AM fungi generally took up the largest fraction of plant-derived carbon in the soil microbial community (Drigo et al., 2010; Mellado-Vázquez et al., 2016; Karlowsky et al., 2018) but recovered slowly after rewetting the dried soil (de Vries et al., 2012; Meisner et al., 2013; Karlowsky et al., 2018). Interestingly, despite their lower abundance, AM fungi completely recovered their ^{13}C tracer uptake in drought treatments at the recovery labeling, suggesting that the efficiency of plant-mycorrhizal carbon flow increased at this time to support the recovery of the hyphal network.

The recovery of soil microbial growth after drought is typically preceded by a pulse of soil respiration directly after rewetting (Birch, 1958). However, those sources other than the released microbial osmolytes that contribute to the Birch effect are not well known, especially in planted soils (Canarini et al., 2017). Here, we found accumulations of carbon in the root sugar and soil EOC pools during drought, which quickly disappeared after rewetting. This strongly suggests that the release of these easy degradable carbon sources after the end of drought contributes to the acceleration of the soil microbial activity. Data not yet published on soil respiration from the ^{13}C pulse labeling experiment described by Karlowsky et al. (2018) indicate that carbon assimilated during drought contributes to the Birch effect, as ^{13}C applied to the monoliths during peak drought could be recovered in the soil respiration pulse after rewetting. Consequently, this means that the plant-derived carbon, which cannot be used by soil microorganisms during drought, is available for priming the microbial organic matter cycle in soil after rewetting. Such priming effects, e.g., observed after amending soil samples with fresh plant litter (Thiessen et al., 2013), are well-known to support plant growth by increasing nutrient mineralization from soil organic matter. An increase in nitrogen mineralization especially has been reported after rewetting dried soils (Borken and Matzner, 2009; Canarini and Dijkstra, 2015), and this increase probably contributed to the increased root and shoot nitrogen concentrations found at the recovery in this study. Additionally, the transport of preserved nitrogen from roots to shoots could have led to the significantly increased shoot nitrogen concentrations in drought treatments. As the leaf nitrogen concentration typically correlates with the photosynthetic activity (Wright et al., 2001; Milcu et al., 2014), the increased nitrogen uptake likely facilitated the higher assimilation rates needed for recovery (Ingrisch et al., 2018).

CONCLUSION

The results from this study confirm our first hypothesis that the frequently observed weakening of the link between plant photosynthesis and soil microbial carbon cycling during drought is due to reduced microbial uptake rather than to reduced root exudation. Our data from the ^{13}C pulse labeling experiments clearly show that recently assimilated plant carbon accumulates in the rhizosphere in the form of EOC during drought and that this accumulation is linked to reduced microbial uptake of plant-derived carbon. When the soil dries out, the limited diffusion leads to lower accessibility of root exudates for non-mycorrhizal fungi and bacteria. In addition, higher reductions of ^{13}C tracer allocation to growth-related fatty acid markers in comparison to the water-soluble MBC fraction, also in AM fungi, indicate adjustments in microbial metabolic activity; that is, the formation of osmolytes to prevent cell desiccation is favored over growth.

Our second hypothesis that drought leads to the accumulation of root sugars and EOC and that these easy degradable carbon sources are available for priming plant and soil microbial activity after rewetting, is only partially supported by the data. Indeed, we found that carbohydrates accumulated in roots and that the decreased microbial uptake was linked to increased EOC concentrations during drought. However, what causes the depletion of drought-accumulated carbon after rewetting remains unclear. Root sugars could either be used to support the regrowth of shoots or may be invested in plant-microbial interactions to gain more nutrients from soil organic matter decomposition. Drought-accumulated EOC that is not flushed away during the rewetting potentially further fuels the Birch effect, i.e., high microbial carbon and nitrogen mineralization shortly after rewetting. To determine how the preservation of belowground carbon pools during drought is related to microbial activity in the early phase of ecosystem recovery, future studies are needed to trace the flux of ^{13}C label applied at drought in soil after rewetting.

Ultimately, our results indicate that the link between plants and soil microorganisms plays a crucial role in the short-term response of carbon and nitrogen cycling to drought-rewetting events.

DATA ACCESSIBILITY

The datasets analyzed for this study can be found in the figshare repository: https://figshare.com/s/afd9c8f0fab5a572fdb3.

AUTHOR CONTRIBUTIONS

MB and GG conceived the ideas. SK, AA, JI, MB, and GG designed the methodology. SK, AA, JI, MA, and GG conducted the experiments and collected the data. SK, AA, and MA analyzed the data. SK and GG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01593/full#supplementary-material

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CHAPTER 4 – Manuscript 3

Plant evenness and functional composition affect belowground carbon allocation in mountain grassland and alter ecosystem stress tolerance

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Running headline: Plant community effects on grassland drought response

Summary

We investigated the role of plant evenness and functional composition for the resistance and resilience of mountain grassland ecosystems, in order to predict and manage their response to climate change. It is highly uncertain how management-related shifts in species abundances buffer or amplify ecosystem responses to extreme climate events, such as summer drought. In particular, we studied the response of belowground carbon allocation (BCA) in the plant-soil continuum as important ecophysiological process fueling the microbial communities in soil and improving plant access to nutrients from soil.

We set up a common garden mesocosm experiment on a mountain site and manipulated the plant functional composition by varying the evenness of six local grass and forb species with different resource use traits. For the latter we used the specific leaf area (SLA) as proxy and to distinguish between conservative (low SLA) and exploitative (high SLA) species. We calculated plant evenness, grass to forb ratio (Gr:Fo), exploitative to conservative species ratio (Ex:Co) and community-weighted mean SLA (CWM_SLA) as parameters for the plant (functional) composition. We performed two 13-carbon pulse labeling campaigns, first at peak drought and second at the recovery phase, to trace the flow of recent assimilates into plant carbohydrates and soil microbial marker lipids. Additionally, we used a 15-nitrogen label at the rewetting to determine plant nitrogen uptake during recovery.

During drought, SLA significantly decreased in exploitative plants, while SLA remained unchanged or even slightly increased in conservative species. After rewetting, SLA quickly recovered to control levels in most species. Plant evenness had no effect on the response to drought and rewetting. In contrast, plant evenness had general positive but variable effects on plant biomass and plant-mycorrhizal interactions that were associated with increased root nitrogen uptake. At peak drought, Ex:Co had the strongest effects on grassland functioning, which showed negative interaction effects of Ex:Co and drought on carbon uptake, BCA and carbon transfer to microbial biomass. However, these effects were not clearly separated from Gr:Fo, which had in general opposing effects and positively affected the carbon transfer to the soil microbial community during drought. Grassland carbon dynamics quickly recovered after rewetting, and the nitrogen uptake was positively related to community-weighted mean specific leaf area (CWM_SLA).

Our results indicate that plant evenness, resource use strategy (Ex:Co) and functional type (Gr:Fo) affect mountain grassland functioning, and that variations in functional composition alter the response to drought and rewetting. The stronger responses of SLA from exploitative species suggest that trait plasticity is a possibility for less resistant species to adjust their functioning during drought, in order to preserve resources for later recovery. Furthermore, we could show that abundance-based measures of functional composition, i.e. Gr:Fo and Ex:Co, can provide valuable additional information to community-weighted mean traits. In conlusion, the monitoring of multiple parameters for the plant functional composition improves predictions about ecosystem responses to climate change. Furthermore, targeted modifications of the plant functional composition can be used to alter the resistance and resilience of managed ecosystems.

Key words: 13C pulse labeling, 15N labeling, biodiversity, evenness, forbs, grasses, mesocosm experiment, plant strategy, plant-soil (below-ground) interactions, stress tolerance

1 Introduction

Mountain areas are facing the combined effects of climate and societal change, and it is largely unknown how this affects the provision of ecosystem services (Huber et al., 2005). Especially the biodiversity-rich mountain grasslands (Spehn and Körner, 2005) are strongly affected by land use change, altering the composition and functioning of the local grassland community (MacDonald et al., 2000; Tasser and Tappeiner, 2002; Vittoz et al., 2009). On the other side, additional pressure on ecosystem functioning originates from global warming, increasing the probability of more frequently occurring extreme events (IPCC, 2013, 2012, 2007). From such climatic extremes, prolonged drought has been recognized as a major threat for carbon (C) cycling in grassland ecosystems (Ciais et al., 2005; Frank et al., 2015; Gilgen and Buchmann, 2009; Reichstein et al., 2013). So far, there is little knowledge about how differences in plant functional composition and the relative abundance of individual plants, i.e. their evenness, may buffer or amplify the response of mountain grasslands and their C cycling to severe drought.

It is well known that for instance the abandonment of traditional low-moderate intensity management practices, like mowing or grazing, can lead to shifts in the plant community composition by favouring the growth of slow-gowing species with conservative resource use traits (Lavorel et al., 1998; Quétier et al., 2007; Tasser and Tappeiner, 2002). In contrast, higher management intensities generally promote the presence of fast-growing species with exploitative resource use traits. In addition, land-use changes can also be associated with changes in plant species richness (Lavorel et al., 1998; MacDonald et al., 2000; Tasser and Tappeiner, 2002). High species richness is often assumed to promote ecosystem stability by increasing the chance that plants with certain traits are present (‘insurance hypothesis’, (Chapin et al., 2000; Loreau, 2000; Tilman et al., 2006)), which provide the plant either with a high resistance (i.e. the ability to maintain functioning during a disturbance) or a high resilience (i.e. the ability to recover to complete functioning after a disturbance) towards climatic extremes (Nimmo et al., 2015; Pimm, 1984).

However, biodiversity cannot only be altered by the extinction of species, but also by changing the relative abundance of the present plant species, i.e. their ‘evenness’, which is highest if all species are equally abundant (Chalcraft et al., 2009; Hillebrand and Matthiessen, 2009; Wilsey and Potvin, 2000). This becomes particularly important in the context of land-use change, as human impact alters plant evenness more rapidly than plant species richness (Chapin et al., 2000; Hillebrand et al., 2008). Plant species evenness is also closely coupled to ecosystem functioning (e.g. Wilsey & Potvin 2000; Kirwan et al. 2007; Assaf, Beyschlag & Isselstein 2011; Lamb, Kennedy & Siciliano 2011; Orwin et al. 2014). Higher plant evenness typically increases biomass production (Assaf et al., 2011; Brett Mattingly et al., 2007; Kirwan et al., 2007; Orwin et al., 2014; Wilsey and Potvin, 2000) and its temporal stability (Kirwan et al., 2007; Orwin et al., 2014). This is probably based on the complementary use of water and nutrient resources (niche differentiation) or the facilitation between plant species (Hillebrand et al., 2008; Loreau, 2000). While the effects of plant species richness on ecosystem stability have been extensively studied (Isbell et al., 2015;

Otieno et al., 2012; Proulx et al., 2010; Tilman et al., 2006; Van Ruijven and Berendse, 2010; Vogel et al., 2012; Wright et al., 2015), there is a lack of knowledge in how plant evenness can alter ecosystem responses to extreme events like severe drought (Hillebrand and Matthiessen, 2009).

Similar to plant species evenness, it is uncertain how variations in plant functional traits and their spatial distribution affect grassland stress responses. For mountain grasslands, the 'leaf economics spectrum' has been found to strongly co-vary with land management types and ecosystem functioning (Grigulis et al., 2013; Lavorel and Grigulis, 2012). On the one hand, plants can be separated according to their resource use strategy into 'conservative' and 'exploitative' plants (sensu Grime 1977). Exploitative species typically have a higher nutrient acquisition rate, leading to higher leaf nitrogen concentration (LNC) and specific leaf area (SLA) compared to conservative species (e.g. Diaz et al., 2004; Lavorel and Grigulis, 2012; Quétier et al., 2007; Wright et al., 2004). In theory, conservative plants are less affected by extreme drought, since the mineralization and accessibility of nutrients like nitrogen (N) and phosphorous is often limited during drought (Borken and Matzner, 2009; Canarini and Dijkstra, 2015; Delgado-Baquerizo et al., 2013; Dijkstra et al., 2015; Fuchslueger et al., 2014b; Stark and Firestone, 1995). Moreover, conservative species typically have lower growth rates and thicker leaves than exploitative plants (Díaz et al., 2004; Wright et al., 2004), probably increasing their tolerance to drought (Pérez-Ramos et al., 2013; Zwicke et al., 2015). As visible by a decrease of SLA during drought (Poorter et al., 2009; Wright et al., 2004), thicker leaves and a lower growth rate are generally favoured under limiting water conditions. In addition, stronger interactions between arbuscular mycorrhiza (AM) fungi and conservative plant species (Gross et al., 2010; Legay et al., 2016) can further improve their resistance by enhancing water and nutrient supply through the hyphal network (Allen, 2007).

Also the plant functional type may play a critical role in the response to drought. For temperate grasslands in Europe, it has been shown that forb species are often more resistant to drought than grass species (Bollig and Feller, 2014; Gilgen et al., 2010; Gilgen and Buchmann, 2009; Zwicke et al., 2013), which can be explained by less responsive stomatal control of the present C3 grass species (Bollig and Feller, 2014). Though, this relationship may be less pronounced in mountain grasslands (Signarbieux and Feller, 2012).

Unlike the immediate drought response, different mechanisms are thought to be important to facilitate a rapid recovery. Fast growing exploitative species are better able to quickly recover their biomass after a disturbance (Grime, 1977; Gunderson, 2000; Pimm, 1984), and thus may outcompete slow growing species in terms of their resilience. The fast growth of exploitative plants can be further supported by their high nutrient uptake and nutrient use efficiency (Grassein et al., 2015; Grigulis et al., 2013), which in turn increase the capacity for CO2 assimilation and regrowth. Moreover, exploitative plants were found to be more strongly linked to the bacterial community in the rhizosphere (Grigulis et al., 2013; Orwin et al., 2010), which is able to quickly recover its functioning after severe drought (de Vries et al., 2012; Karlowsky et al., 2018a; Schimel et al., 2007), and can further support plant recovery by mineralizing nutrients (especially N) from soil organic matter (Kuzyakov, 2010;

Thuille et al., 2015; Wardle et al., 2004). Such plant-microbial interactions are mainly governed by plant belowground C allocation (BCA) (Brüggemann et al., 2011), since many soil microorganisms strongly depend on plant C inputs and respond quickly and non-linearly to changes in their C supply (Bardgett et al., 2005; Wardle et al., 2004). Consequently, the use of stable isotope tracers to determine C and N fluxes is a powerfull tool to study the response of grassland ecosystems to disturbances (Mellado-Vázquez et al., 2016).

Here we used a common garden mesocosm experiment on a mountain meadow to study how differences in plant functional composition, as predicted by land use change in the Alps (Grigulis et al., 2013; Quétier et al., 2007; Tasser et al., 2005), affect mountain grassland functioning and its response to extreme drought. We manipulated the plant functional composition by varying the relative abundances and evenness of six local meadow species with different resource use strategies. All mesocosms were planted in the same density with three grasses (*Dactylis glomerata*, *Deschampsia cespitosa*, *Festuca rubra*) and three forbs (*Geranium sylvaticum*, *Leontodon hispidus*, *Trifolium repens*), latter including one legume. Our main objective was to assess the effects of plant community composition on the short-term drought-response of BCA and plant-microbial interactions as key ecosystem processes (Bardgett et al., 2005; Brüggemann et al., 2011; Schimel et al., 2007; Wardle et al., 2004). We performed two ^{13}C pulse labeling campaigns, first at peak drought and second shortly after rewetting, to study the resistance and resilience of C assimilation, allocation and transfer to the soil microbial community. In addition, we added a ^{15}N label to the soil at the rewetting to assess the plant N uptake during recovery, in order to estimate potential benefits from shifts in plant-microbial interactions after drought and rewetting (Borken and Matzner, 2009; Canarini and Dijkstra, 2015; Schimel et al., 2007).

Specifically, we hypothesized that: (1) higher plant evenness increases plant productivity, plant-microbial C transfer as well as plant N uptake by increasing species complementary; (2) conservative communities are less productive but more resistant to drought than exploitative communities, due to lower SLA, slower growth and strong interactions with AM fungi; (3) grasses allocate less C to the belowground than forbs and are more vulnerable to drought due to a lower plasticity of their photosynthetic activity; and (4) exploitative communities recover more quickly from drought, based on higher SLA, strong interactions with rhiszospheric microorganisms and high N uptake.

2 Materials and Methods

2.1 Experimental site

The study site is located near Neustift in the Stubai valley in the Austrian Central Alps (1820-1850 m a.s.l.; 47°7'45''N, 11°18'20''E) and is described in Bahn et al. (2006). Briefly, the average annual temperature is 3 °C, the annual precipitation is 1097 mm and the soil is a dystric cambisol. The site is a hay meadow that is cut once per year at peak biomass in early August and manured every 2-3 years, and has a *Trisetum flavescentis* vegetation community consisting of perennial grasses and forbs.

2.2 Set-up of mesocosms

In total 48 mesocosms (Fig. S1a) were installed at the experimental site in summer 2013, from which a subset of 24 mesocosms was used for this study. The mesocosms were arranged in six blocks with eight mesocosms. For each mesocosm, two dark plastic pots, 45 cm in diameter and 35 cm in height, one inside the other, were used. The external pot was used as water reservoir, the internal one was used to hold the soil and the plants. The internal pots were filled with sieved soil (<5 mm) from the study site and embedded into the meadow soil, with the upper edge elevated around 2 cm above soil to prevent a possible impact from runoff water. Plant species were chosen based on the experience from previous mesocosm experiments on the study site, according to their cultivability and known differences in biomass production. The species selection included three grasses (*Dechampsia cespitosa*, *Festuca rubra*, *Dactylis glomerata*), two forbs (*Leontodon hispidus*, *Geranium sylvaticum*) and one legume (*Trifolium repens*). Four different planting schemes (grass-/forb-dominated × high/low productive) with each 12 replicates were applied to the mesocosms by varying the number of individual species, except for the legume (Table S1). For this purpose, single plants were sampled at the experimental site in July 2013 and pre-incubated for 6-7 weeks in a greenhouse, at the botanical garden in Innsbruck, Austria. In August 2013, all mesocosms were planted with 36 individuals according to the four planting schemes. The position of the different plant species was randomized on a fixed pattern (Fig. S1b) and the 48 mesocosms were arranged in a randomized block design (Fig. S1c). In 2014, the plant community was established on the site, and the biomass was harvested according to the common practice on 22nd August.

2.3 Drought treatment and pulse labeling set-up

The experiment was started on 5th June 2015 by simulating early summer drought using rain-out shelters, as previously described (Ingrisch et al., 2018; Karlowsky et al., 2018a,b). Volumetric soil water content (SWC) was monitored as decribed by Karlowsky et al. (2018b). During rain exclusion, mesocosms of control treatments were watered manually to avoid water limitation and SWCs were maintained at 21-42% in 5 cm and 23-49% in 15 cm depth. No water was given to drought treated mesocosms, yielding at peak drought in SWCs of 3-11% in 5 cm and 7-21 % in 15 cm depth.

Four weeks after the beginning of the drought treatment, when SWC reached average values of 7% and 12% in in 5 cm and 15 cm depth, the first ^{13}C pulse labeling campaign (peak drought labeling) started on 4th July on a subset of 12 mesocosms (6 control and 6 drought treated). At that time, drought effects on the vegetation were visible by partial senescense of shoot biomass. Drought simulation was stopped on 14th July 2015, by removing rain-out shelters and adding water according to 25 mm of precipitation to all mesocosms (control and drought treatments). Afterwards SWC was maintained at 16-45% and 29-52% in depths of 5 cm and 15 cm, respectively. Another subset of 12 mesocosms was used for the second ^{13}C pulse labeling campaign (recovery labeling) at the recovery phase. These mesocosms were labeled with ^{15}N on the day of the rewetting by adding 6 mg of ^{15}N (obtained by dissolving 60 mg of KNO_3 with 10 atom% ^{15}N [KNO_3] in 100 ml water) to the irrigation water. After a recovery phase of 10 days, the second ^{13}C recovery labeling started on 24th July. At that time, new leaves were produced and there were almost no differences between drought and control treatments were visible.

Both ^{13}C labeling campaigns were done on each three consecutive days (peak drought: 4th till 6th July, recovery: 24th till 26th July) with high radiation. The ^{13}C pulse labeling was done on 2-6 mesocosms per day, always representing drought and control pairs with a similar vegetation composition, which was estimated visually. Pulse labeling was performed as previously described (Karlowsky et al., 2018b). Briefly, gas-tight and light-permeable chambers were used to label the plant canopy with $^{13}CO_2$ for 75 min (peak drought labeling) or 30 min (revovery labeling). The labeling was done by the pulse-wise addition of highly enriched $^{13}CO_2$ (>99 atom% ^{13}C; Sigma-Aldrich, Taufkirchen, Germany) to the circulated chamber air, yielding in 30–80 atom% ^{13}C in chamber CO_2 during the entire labeling time. This allowed tracing the assimilated ^{13}C label from shoots to roots up to soil and its inhabiting microorganisms.

2.4 Sampling

Plant and soil samples were collected 15 minutes after finishing the ^{13}C pulse labeling. Further harvests were performed 1, 3, and 5 days after the labeling. For each harvest the shoot material was cut 1 cm above soil in two 5 × 5 cm squares with opposite positions in the mesocosm. The position of the two squares was semi-randomly chosen in order to obtain representative samples of the community. The shoot material from both squares was pooled together and separated into biomass and necromass. If available, single leaves from *G. sylvaticum* and *L. hispidus* were separated from the biomass as subsamples for bulk carbon and nitrogen stable isotope analysis. The biomass and leaf subsamples were immediately treated by microwave to interrupt any metabolic activity (Popp et al., 1996), stored on ice packs for transport and stored at -18 °C for later analysis of sugar content and carbon stable isotope composition. For soil samples, soil cores were collected in or next to plant sampling squares (on bare soil spots close to plant cover) and pooled together. Sampling was done using a stainless steel auger with 1.9 mm inner diameter (Eijkelkamp, Giesbeek, Netherlands). At each sampling, four soil cores from 0-7 cm depth were taken and pooled to a mixed sample for each mesocosm. Mixed soil samples were carefully sieved to 2

mm and roots were sorted out. Soil for extractable organic C (EOC) and microbial biomass C (MBC) analysis was transported on ice packs, stored at 4 °C and extracted/fumigated latest four days after sampling. Soil for neutral/phospho-lipid fatty acid (NLFA/PLFA) analysis was directly frozen with dry ice and stored at -18 °C until further preparation. Subsamples of frozen soil were used prior to NLFA/PLFA analysis to determine the soil water content gravimetrically, by weighing the soil before and after drying for 48 h at 105 °C. Roots were washed from remaining soil and dead as well as coarse roots (diameter > 2 mm) were removed. Fine root samples were portioned in two subsamples. One subsample was treated in the same way like shoot samples, and the other one was kept moist with wet paper towels until root respiration measurements that were immediately performed on-site. Microwaved shoot and root samples were completely dried out in an oven at 60 °C for 72 h, starting the same day of harvesting. After dry weight determination, plant material was carefully ground to a fine powder using a ball mill (MM200, Retsch GmbH, Haan, Germany). This material was then used for bulk ^{13}C and ^{15}N as well as compound-specific ^{13}C isotope composition. For determining community shoot biomass, species-specific shoot biomass and specific leaf area (SLA), the mesocosms were harvested completely at the end of each labeling/sampling campaign. Community root biomass was directly estimated from the dry mass of all root samples from one mesocosm. For natural abundance samples, one soil core was taken from each four unlabeled control and drought mesocosms on 14th July, then samples were pooled together to obtain one mixed sample for control and drought treatments. The same procedure was adopted for shoot material collected from all six species.

2.5 Stable isotope analyses on plant material

Ground bulk plant material was used to determine ^{13}C and ^{15}N contents ($\delta^{13}C$ vs. VPDB and $\delta^{15}N$ vs. air) of shoots and fine roots by elemental analysis (EA) – IRMS (EA – Model NA 1500, Carlo Erba, Milan, Italy; coupled to an IsoPrime100 IRMS (Isoprime Ltd., Cheadle, UK). Carbohydrate analysis was done on 30 mg of plant powder as described by Karlowsky et al. (2018a). Firstly, water soluble sugars were extracted with hot water and analyzed by high-performance liquid chromatography (HPLC) – IRMS (Dionex UltiMate 3000 UHPLC coupled via a LC-IsoLink system to a Delta V Advantage IRMS, Thermo Fisher Scientific, Bremen, Germany). Secondly, the remaining sugars were removed from the extracted pellets (methanol:chloroform:water, 12:3:5, by volume) and starch was digested to gluco-oligomers using heat stable α-amylase (Göttlicher et al., 2006; Richter et al., 2009), followed by EA-IRMS analysis (EA 1100, CE Elantech, Milan, Italy; coupled to a Delta+ IRMS, Finnigan MAT, Bremen, Germany).

Root respiration analyses were done as described by Karlowsky et al., (2018b), using the method of Hasibeder et al. (2015). Shortly after sampling, moist root subsamples (0.2 – 1.2 mg) were incubated in 100 ml Erlenmeyer flasks at 15 ± 1 °C. Gas was collected from the flasks at 0, 7, 20, 40 and 60 minutes after closing, and was analyzed by isotope ratio mass spectrometry (IRMS; IsoPrime100, Isoprime Ltd., Cheadle, UK).

2.6 Stable carbon isotope analyses on soil material

Soil extractable organic carbon (EOC) and microbial biomass carbon (MBC) were determined using the chloroform fumigation extraction method of Vance, Brookes & Jenkinson (1987), as modified by Malik, Blagodatskaya & Gleixner (2013). Briefly, for EOC a fresh soil subsample of 5 g was extracted with 0.5 M K_2SO_4 solution (distilled water) by horizontal shaking for 30 minutes. The extract was centrifuged at 12000 *g* for 5 min and filtered (Whatman Grade 1 filter papers, d = 150 mm, 11 µm pore size, GE Healthcare UK ltd., Buckinghamshire, UK) to remove coarse particles. Finer particles were removed with prewashed cellulose membrane filters (MULTOCLEAR 0,45 µm RC 13 mm, CS-Chromatographie Service GmbH, Langerwehe, Germany) and inorganic C was degassed from the solution by acidifing to pH 2 with phosphoric acid and flushing with N_2 for 15 min. Afterwards samples were analyzed as bulk fraction (no column) on a HPLC-IRMS system (see carbohydrate analysis). To assess MBC, another fresh soil subsample of 5 g was incubated for ≥ 24 h with chloroform to extract the total organic carbon (TOC). To avoid differences in the extraction efficiency, drought-treated soils were rewetted to control levels with distilled water prior to the fumigation (Sparling et al., 1990). After complete evaporation of cholorform, samples were processed as described for EOC and TOC was analyzed by HPLC-IRMS. After normalizing EOC and TOC concentrations to soil dry mass, MBC concentrations were calculated from the difference: [MBC] = ([TOC] – [EOC])/k_{MBC}. The factor k_{MBC} corrects for the extraction efficiency of MBC after chloroform fumigation and a value of 0.45 was used (Vance et al., 1987). The $^{13}C/^{12}C$ ratio (i.e. $\delta^{13}C$ or atom% ^{13}C) of MBC was calculated according to the isotopic mass balance:

$$^{13}C/^{12}C_{MBC} = (^{13}C/^{12}C_{TOC} * [TOC] - {}^{13}C/^{12}C_{EOC} * [EOC])/([TOC]-[EOC])$$

Neutral lipid fatty acids (NLFAs) and phospholipid fatty acids (PLFAs) were used as biomarkers for the soil microbial community. Lipid extraction from soil was done using the method Bligh & Dyer (1959), as modified by Kramer & Gleixner (2006) and Karlowsky et al. (2018a). Approximately 5 g of frozen soil were extracted by pressurized solvent extraction (SpeedExtractor E-916, Büchi Labortechnik AG, Flawil, Switzerland) using a mixture of methanol, chloroform and 0.05 M K_2HPO_4 buffer (2:1:0.8, by volume; pH 7.4) at 70 °C and 120 bar for 3 × 10 min. Neutral lipids were separated from phospholipids using silica-filled solid phase extraction (SPE) columns (CHROMABOND SiOH, 2 g, 15 ml, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) and after derivatization with methanolic KOH, the resulting fatty acid methyl esters (FAMEs) were purified for analysis by using aminopropyl-modified SPE columns (CHROMABOND NH2, 0.5 g, 3 ml, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). FAMEs were quantified by gas chromatography – flame ionisation detection (GC-FID) on a GC-FID 7890B system (Agilent Technologies, Palo Alto, USA) using a DB-1MS UI column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness, Agilent Technologies, Palo Alto, USA) and helium as carrier gas (1.8 ml/min). After injection the temperature was held for 1 min at 45 °C, then increased in a first ramp of 60 °C/min to 140 °C (held for 0.5 min), followed by a second ramp of 2 °C/min until 242 °C and a third

ramp until 320 °C (held for 3 min). FAMEs were analyzed for their compound specific ^{13}C isotope composition by GC-IRMS (GC 7890A, Agilent Technologies, Palo Alto, USA; coupled via a Conflo IV/GC IsoLink to a Delta V Plus IRMS, Thermo Fisher Scientific, Bremen, Germany) using a DB-1MS Ultra Inert column (60 m × 0.25 mm internal diameter × 0.25 µm film thickness, Agilent Technologies, Palo Alto, USA) and helium as carrier gas (1.8 ml/min). After injection the GC temperature was held at 45 °C for 1 min, then increased in a first ramp of 60 °C/min to 140 °C (held for 0.5 min), followed by a second ramp of 4 °C/min until 283 °C (held for 4.9 min) and a third ramp until 320 °C (held for 3 min). Concentrations and ^{13}C isotope content of identified NLFAs and PLFAs were corrected for the methyl group introduced during derivatization to FAMEs. The sum of the PLFAs i14:0, i15:0, a15:0, i16:0, a17:0, i17:0 and br18:0 was used to describe Gram-positive bacteria (Zelles, 1997, 1999). The same was done with 10Me16:0 and 10Me18:0 for actinobacteria (Lechevalier et al., 1977; Zelles, 1999), and 16:1ω7 and 18:1ω7 for Gram-negative bacteria (Zelles, 1997, 1999). The PLFA 18:2ω6,9 was used as marker for saprotrophic fungi (Frostegård et al., 2011; Frostegård and Bååth, 1996; Zelles, 1997) and the NLFA 16:1ω5 as marker for arbuscular mycorrhiza fungi (Olsson, 1999).

2.7 Calculation of incorporated ^{13}C and ^{15}N

For all plant and soil samples, we calculated the ^{13}C isotope content as incorporated ^{13}C (mg ^{13}C m^{-2}), which refers to the total amount of ^{13}C found in a certain C pool on an area basis, and was calculated as:

$$incorporated\ ^{13}C = \frac{(atom\%_{labeled} - atom\%_{unlabeled}) * C_{pool}}{100\ \%}$$

with $atom\%_{labeled}$ being the atom% ^{13}C of the labeled samples, $atom\%_{unlabeled}$ being the atom% ^{13}C of natural abundance samples, and C_{pool} being the respective C pool (mg C m^{-2}). Incorporated ^{15}N of plant samples was analogously calculated.

Root respired ^{13}C (mg ^{13}C m^{-2} h^{-1}), which corresponds to the amount of ^{13}C released in respired CO_2 from roots during a certain time, was calculated similar to incorporated ^{13}C:

$$root\ respired\ ^{13}C = \frac{(atom\%_{labeled} - atom\%_{unlabeled}) * CO2_{resp.\ rate}}{100\ \%}$$

with $CO2_{resp.\ rate}$ being the respiration rate of CO_2 (mg CO_2 m^{-2} h^{-1}).

2.8 Data analyses

To study the effects of plant evenness and functional composition on the response of mountain grassland to drought, we calculated different parameters describing the established communities (Table S2). Plant evenness was calculated based on the Shannon index using species-specific shoot biomass (Wilsey and Potvin, 2000). The grass to forb ratio (Gr:Fo) was calculated as the biomass ratio of grass and forb species. The community-weighted mean SLA (CWM_SLA) was used as general indicator for plant resource use strategy, which is closely coupled to productivity (Grigulis et al., 2013). CWM_SLA was

calculated based on species-specific SLA, according to Lavorel et al. (2008):

$$CWM_SLA = \sum_{i=1}^{s} p_i \, SLA_i$$

with *s* being the number of species, p_i the relative abundance (based on biomass) of species *i* and SLA_i the SLA of species *i*. Because SLA responded to the drought treatment, as expected (Poorter et al., 2009; Wright et al., 2001), we used the SLA values of plants from control treatments to seperate the six mesocosm species into two groups. Species with SLA values > 19 m^2/kg were assigned to the exploitative group and species with SLA values < 13 m^2/kg to the conservative group. This grouping was used to calculate the exploitative to conservative ratio (Ex:Co) as the biomass ratio of exploitative and conservative species.

For root biomass, C and N concentrations as well as ^{13}C and ^{15}N tracer incorporation average values were calculated over the different sampling times: 1 d and 3 d after labeling for NLFAs/PLFAs; and 15 min, 1 d, 3 d and 5 d after labeling for all others. For soil samples a bulk soil density of 0.7 g cm^{-3} (Meyer et al., 2012) was used for calculating area-based pool sizes. Total ^{13}C uptake was calculated as sum of bulk shoot and bulk root incorporated ^{13}C at 15 min after labeling. Total ^{15}N uptake was calculated analogously, after averaging over all sampling times from the recovery labeling.

All statistical analyses, except for principal component analysis (PCA), were done using the R 3.3.2 software (R Core Team, 2016). The effects of drought on biomass, SLA, CWM_SLA and N concentrations were evaluated for each labeling campaign separately using permutational ANOVA from the 'lmPerm' package (Wheeler and Torchiano, 2016), from which exact *P* values (P_{aovp}) were obtained. Permutation tests do not require assumptions about the statistical distribution and are powerful with small sample sizes (Ernst, 2004). For comparing the effect of drought on leaf N content (LNC) and ^{15}N tracer incorporation between *G. sylvaticum* and *L. hispidus* at the recovery campaign, Tukey-HSD tests on two-way ANOVAs (including plant identity, water treatment and the interaction of both) were performed using the 'aov' and 'TukeyHSD' functions from the R base package. To compare the effects of drought on ^{13}C tracer dynamics in leaves between *G. sylvaticum* and *L. hispidus*, linear mixed-effect models from the 'lme4' package (Bates et al., 2015) were used for each labeling campaign separately. The mixed-effects models included sampling time (in h after pulse labeling), water treatment, species identity and their interactions as fixed effects, while rain-out shelter and mesocosm identity were set as random effects. All models were assessed for violations of normality, heteroscedasticity and independency. If necessary, ^{13}C tracer data were log, square root or log/square root +1 transformed.

PCAs on species-specific shoot biomass (g $mesocosm^{-1}$) were done for each labeling campaign using the Canoco 4.5 software (Microcomputer Power, Ithaca, USA). If necessary, biomass data were log or square root transformed. The PCA triplots were centered and standardized by species biomass and plant compositional parameters were added as supplement, in order to assess their covariation. For further analyses, shoot and root biomass, ^{13}C allocation in the plant-soil continuum and plant ^{15}N uptake were selected as key variables of ecosystem functioning. To estimate the effects of plant community on the response of ecosystem functioning, regression analyses between plant compositional

parameters and response variables were done separately for control and drought treatments at each labeling campaign. Regression analyses were done based on the Pearson correlation using the 'lm' function in the R base package and coefficients. The combined effects of drought treatment and plant composition on ecosystem functioning were tested using simple linear models from the R base package. Each plant compositional parameter (evenness, Gr:Fo, Ex:Co and CWM_SLA) was evaluated separately. Model evaluation and, if needed data transformation, was done as described above for mixed-effects models.

3 Results

Effects of drought-rewetting on aboveground biomass

The four weeks of drought treatment significantly reduced community aboveground biomass by approx. 40% (P_{aovp} < 0.001) at peak drought. At the species level, shoot biomass was only significantly reduced for *Deschampsia cespitosa* (Fig. 1a), which was the dominant species in most mesocosms (see Table S2 for a list of all mesocosms). A trend to reduced biomass was visible for *Trifolium repens* (Fig. 1a), which was also dominant in several mesocosms (Table S2), while there were no obvious drought effects for *Dactylis glomerata*, *Festuca rubra* *Geranium sylvaticum* and *Leontodon hispidus* (Fig. 1a). In addition to biomass, drought almost significantly reduced CWM_SLA from 16.4 ± 0.7 to 14.9 ± 0.8 $m^2\ kg_{dm}^{-1}$ (mean values ± SE, n = 6, P_{aovp} < 0.001). However, on the species level the effect of drought on SLA was different than the effect on shoot biomass (Fig. 1a-b). There was no change in SLA for *D. cespitosa* and a slight increase for *F. rubra*, while the SLA of the other four species

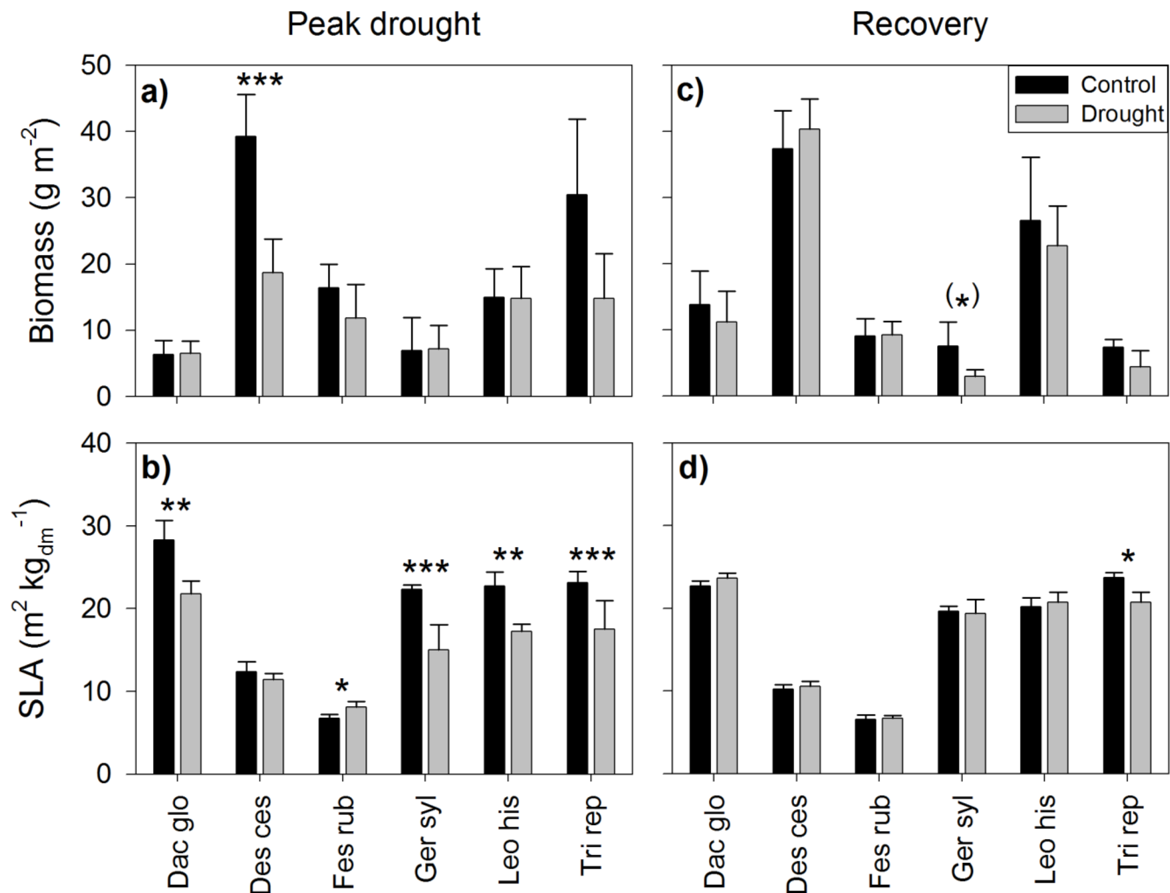

Fig. 1 Aboveground biomass (a, c) and specific leaf area (SLA; b, d) of the six individual species in control and drought-treated mesocosms, harvested at the peak drought (a-b) and the recovery (c-d) labeling campaigns. Bars represent mean values of n = 6 mesocosms and error bars the corresponding SE. Asterisks indicate levels of significance for drought effects determined by permutational ANOVA from the R package 'lmPerm'; ***P_{aovp} < 0.001, **P_{aovp} < 0.01, *P_{aovp} < 0.05, $^{(*)}P_{aovp}$ < 0.1.

consistently decreased. In general, two groups of species could be distinguished in the mesocosm communities: 1) conservative species with low SLA values (< 13 m^2 kg^{-1}) that were relatively unaffected by drought (*D. cespitosa* and *F. rubra*), and 2) exploitative species with high SLA-values in control mesocosms (> 19 m^2 kg^{-1}) that were strongly reduced by drought (*D. glomerata*, *G. sylvaticum*, *L. hispidus* and *T. repens*).

In the recovery phase, ten days after rewetting of the drought-treated mesocosms, community aboveground biomass fully recovered and on the species level only the shoot biomass of *G. sylvaticum* tended to be reduced by drought-rewetting (Fig. 1c). Compared to the peak drought mesocosms, the plant community of the recovery mesocosms differed in the relative abundances of *L. hispidus* and *T. repens* (Fig. 1a,c), with *L. hispidus* being dominant and *T. repens* subdominant in most mesocosms (Table S2). This was due to variable growth dynamics in the different rain-out shelters. Similar to shoot biomass, CWM_SLA fully recovered shortly after the rewetting (control, 15.5 ± 0.8 m^2 kg^{-1}; drought, 14.8 ± 0.4 m^2 kg^{-1}; mean values ± SE, n = 6). On the species level, only the SLA of *T. repens* was significantly, albeit only marginally, reduced (Fig. 1d).

At the community level, shoot N concentrations significantly increased from 1.18 ± 0.05 to 1.69 ± 0.11 $\%_{dm}$ (P_{aovp} < 0.001) at the recovery campaign. This was reflected by increased leaf N concentrations (LNC) in *G. sylvaticum* and *L. hispidus*, with a higher increase in the latter species (Fig. 2a). The analysis of ^{15}N tracer showed that the N uptake from soil was increased after rewetting from drought and that, independent from treatment, the N uptake was higher in *L. hispidus* than in *G. sylvaticum* (Fig. 2b). The higher N uptake of *L. hispidus* was mirrored by a higher recovery of ^{13}C tracer in leaves up to five days after labeling (Fig. S2).

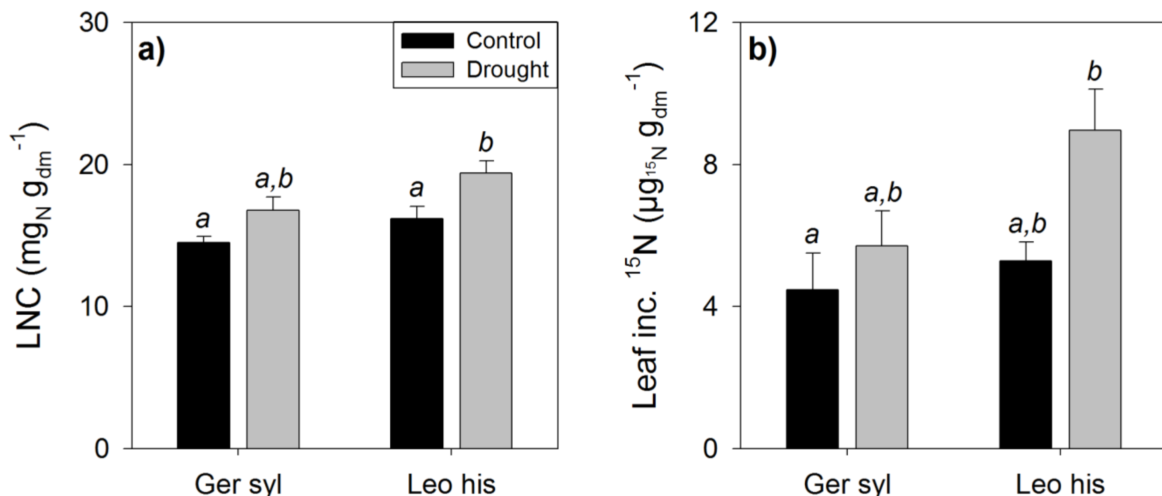

Fig. 2 Leaf nitrogen concentration (LNC; a) and incorporated ^{15}N (inc. ^{15}N; b) of *Geranium sylvaticum* (Ger syl) and *Leontodon hispidus* (Leo his) plants at the recovery labeling campaign. Italic letters show the results from a Tukey-HSD test ($P_{adjusted}$ < 0.05) on a two-way ANOVA (R base package) including the effects of plant identity, water treatment and their interaction.

Mesocosm variability

To assess the differences in mesocosm plant community composition for each labeling campaign, we used principal component (PC) analyses on individual species biomass data and added evenness, Gr:Fo, Ex:Co as well as CWM_SLA as supplementary variables (Fig. 3). At both labeling campaigns, there was a strong covariation between Gr:Fo and Ex:Co, which was based on the generally high abundance of the conservative grass D. cespitosa (Table S2). At the peak drought campaign (Fig. 3a), most of the variability between mesocosms was attributable to differences in the biomass of *T. repens*, *F. rubra*, *G. sylvaticum*, *D. glomerata* and *L. hispidus*, with the first two species showing a clear separation from the latter three species on PC1. To a smaller extend differences in the biomass of *D. cespitosa* contributed to the mesocosm variability, as visible by the good separation of *D. cespitosa* from the other five species on PC2. There was no obvious difference between control and drought treatments, as both had a similar spread over the PC axes, indicating that drought had no significant effect on mesocosm composition. The separation on PC2 was associated with differences in plant evenness and functional composition, which mainly varied along this axis. Gr:Fo was negatively related to evenness and Ex:Co, with the latter two showing covariation on PC2.

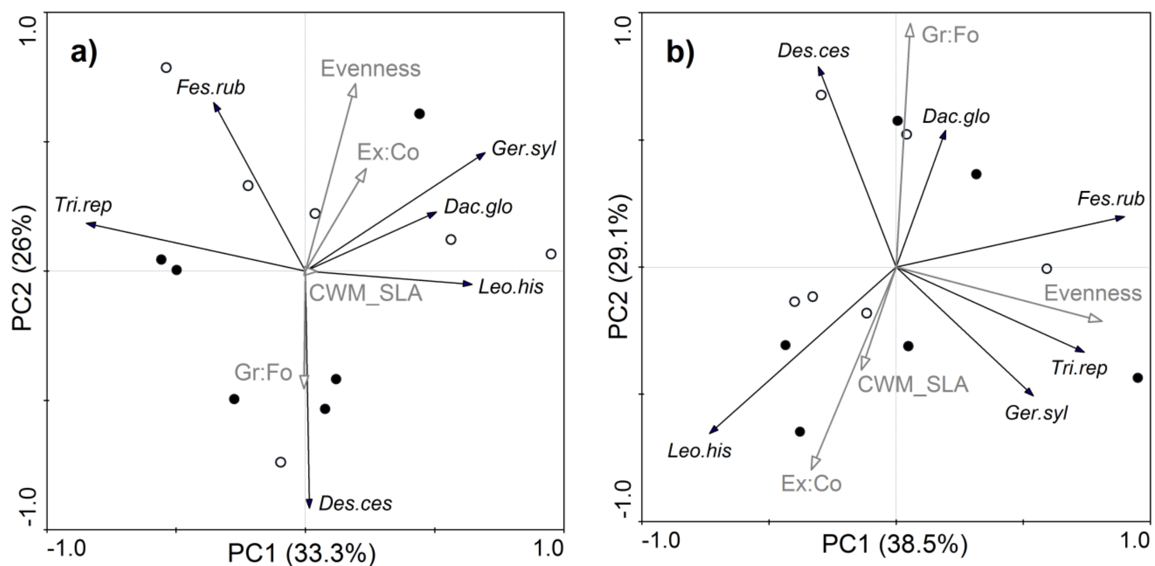

Fig. 3 Triplots from principal component (PC) analyses of species biomass proportions (black arrows) in control (closed circles) and drought (open circles) mesocosms, with plant community parameters added as supplementary variables (grey arrows), at the peak drought (a) and the recovery (b) labeling campaigns. All data were log or square root transformed if needed and standardized prior to PC analyses. Numbers in brackets indicate the explained variance by each PC axis. CWM_SLA, community-weighted mean specific leaf area; Ex:Co, exploitative to conservative ratio; Gr:Fo, grass to forb ratio.

At the recovery campaign, all six species contributed to the variability between mesocosms in a similar way, as indicated by the equal distribution of species biomass on PC1 and PC2 (Fig. 3b). Only *L. hispidus* showed an opposing trend to the other five species, caused by the high abundance of *L. hispidus* in some mesocosms (Table S2). Similar to the peak drought

campaign, there was no obvious difference between control and drought treatments. The separation on PC1 was related to variations in plant evenness, whereas the separation on PC1 was associated with differences in plant functional composition. Gr:Fo was negatively related to Ex:Co and CWM_SLA, with the latter two showing covariation on PC2.

Effects of plant evenness and functional composition on ecosystem functioning and its response to drought

Because of the covariation between the different vegetation parameters (plant evenness and functional composition); we used an explorative approach, i.e. linear regression analyses to test the effects of each plant compositional parameter separately. Table 1 lists the results for control and drought treatments from both labeling campaigns, including selected key parameters (plant biomass, ^{13}C and ^{15}N tracer data). In addition, results from two-way ANOVAs on linear models for the combined effects of drought treatment and each single plant compositional parameter can be found in Table S3 (peak drought campaign) and Table S4 (recovery campaign).

Plant evenness positively correlated with total plant biomass in controls at the peak drought labeling (Table 1). Independent of treatment, higher plant evenness had a positive effect on ^{13}C transfer to AM fungi and root ^{15}N uptake at the recovery labeling (Table S4). For the ^{13}C transfer to AM fungi the effect was particularly strong in drought treatments (Table 1).

Gr:Fo was consistently negatively related to root biomass at both labeling campaigns (Tables S3 & S4), which was particularly pronounced at the recovery labeling (Table 1). At the peak drought labeling, higher Gr:Fo was related to lower ^{13}C uptake, allocation to roots and transfer to soil microbial biomass in controls, while the relation was positive in drought treatments, especially for the ^{13}C transfer to AM fungi. Independent of treatment, at the recovery labeling, higher Gr:Fo was related to reduced ^{13}C allocation to root sucrose, root fructan, soil EOC and soil microbial biomass (Table S4). In previously drought-treated mesocosms, Gr:Fo positively correlated with the ^{13}C flux into root starch, while there was a particularly strong negative relation to the transfer of ^{13}C tracer into microbial biomass, especially to saprotrophic fungi (Table 1). In addition, Gr:Fo negatively correlated with the root ^{15}N uptake in drought mesocosms during recovery.

Ex:Co had strong effects on ^{13}C tracer fluxes in the plant-soil continuum at the peak drought labeling (Table S3), and mostly with an inverse relationship to Gr:Fo (Table 1). In controls, there was significantly more ^{13}C uptake, root allocation and transfer to the microbial biomass in mesocosms dominated by exploitative species. However, the same processes rather negatively correlated with Ex:Co in drought mesocosms. Compared to Gr:Fo, the drought treatment had consistently stronger effects on the relation between Ex:Co and ^{13}C tracer fluxes at the peak drought labeling (Table S3). This was not the case at the reocvery labeling, where the effects of Gr:Fo and Ex:Co were generally less distinct and more

Table 1 Correlations (r-values from ordinary least squares regression) of evenness, grass to forb ratio (Gr:Fo), ratio of exploitative to conservative species (Ex:Co) and community-weighted mean specific leaf area (CWM_SLA) with plant biomass, plant and soil ^{13}C tracer fluxes as well as plant ^{15}N uptake in control (C) and drought (D) treatments at the peak drought and recovery labeling campaigns (n = 6, ^{13}C and ^{15}N data averaged over sampling time).

	Variable	Evenness				Gr:Fo				Ex:Co				CWM_SLA			
		Peak drought		Recovery		Peak drought		Recovery		Peak drought		Recovery		Peak drought		Recovery	
		C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Biomass	Total	0.81*	0.28	-0.35	0.55	-0.50	-0.70	**-0.63**	**-0.61**	0.02	0.58	**0.48**	**0.79°**	-0.31	0.13	0.09	0.90*
	Shoots	0.76°	0.08	-0.29	0.29	-0.30	-0.43	0.32	-0.17	0.12	0.53	0.29	0.44	-0.46	0.35	**0.64**	**0.44**
	Roots	0.58	0.31	-0.22	0.58	**-0.48**	**-0.71**	**-0.74°**	**-0.78°**	-0.07	0.53	0.34	0.83*	-0.09	0.04	-0.18	0.99***
	Root:Shoot	-0.20	0.20	0.08	0.11	-0.17	-0.31	-0.64	-0.39	-0.17	0.06	0.01	0.19	0.30	-0.28	-0.51	0.30
Plant ^{13}C	Total uptake	-0.20	0.24	0.18	-0.31	-0.54	0.73°	-0.35	-0.13	**0.85***	**-0.47**	0.57	-0.17	**0.88***	**0.33**	0.79°	-0.17
	Shoots	0.05	0.26	0.10	-0.32	-0.52	-0.02	-0.12	-0.12	0.73	0.38	0.42	-0.14	**0.61**	**0.78°**	0.72	-0.17
	Roots	-0.03	0.17	0.13	-0.08	-0.53	0.66	**-0.48**	**-0.62**	**0.87***	**-0.55**	**0.53**	**0.45**	**0.93****	**0.03**	**0.63**	**0.57**
	Root:Shoot	-0.36	-0.20	0.46	0.07	-0.10	0.56	-0.62	-0.11	**0.42**	**-0.77°**	0.01	0.15	0.76°	-0.68	-0.28	0.37
	Root respired	-0.67	-0.20	-0.15	-0.34	0.34	-0.28	0.25	0.29	0.02	0.16	0.38	0.19	0.52	-0.19	0.64	0.23
NSC ^{13}C	Shoot sucrose	0.05	-0.46	-0.02	-0.28	-0.45	0.40	0.11	0.07	**0.97****	**-0.16**	0.42	-0.42	0.70	0.12	**0.83***	**-0.24**
	Shoot fructan	-0.40	-0.45	-0.45	0.00	-0.01	0.66	0.39	-0.18	**0.63**	**-0.49**	0.29	0.11	0.69	-0.09	0.53	0.30
	Shoot starch	-0.42	-0.01	0.45	-0.10	0.03	0.13	-0.39	-0.12	0.33	0.16	0.37	-0.29	0.38	0.50	0.66	-0.34
	Root sucrose	0.61	0.18	-0.10	-0.07	**-0.81°**	**0.35**	**-0.68**	**-0.54**	0.46	-0.37	**0.79°**	**0.57**	0.03	-0.09	0.67	0.52
	Root fructan	0.03	-0.56	-0.03	0.04	-0.41	0.22	**-0.63**	**-0.73**	-0.16	-0.05	**0.71**	**0.66**	0.26	0.03	**0.66**	**0.80°**
	Root starch	0.60	0.12	0.42	-0.17	-0.48	-0.42	-0.25	0.75°	0.21	0.56	0.29	-0.39	0.27	0.45	0.64	-0.51
	Suc root:shoot	-0.17	0.22	0.07	0.51	-0.29	0.42	**-0.70**	**-0.47**	-0.17	-0.54	0.11	0.45	0.03	-0.29	-0.45	0.74°
Soil ^{13}C	EOC	-0.36	0.34	-0.54	0.54	0.03	0.58	-0.72	-0.58	-0.31	-0.51	**0.57**	**0.34**	0.26	0.09	-0.01	0.67
	MBC	-0.21	-0.19	0.36	0.29	-0.52	0.70	**-0.54**	**-0.93****	**0.89***	**-0.58**	0.37	0.78°	0.52	-0.11	0.47	0.61
	AM fungi	-0.59	0.26	**0.59**	**0.90***	-0.15	0.77°	-0.12	-0.32	**0.64**	**-0.58**	0.09	0.27	0.63	0.19	0.55	0.41
	Saprotrophic fungi	-0.35	0.45	0.40	0.17	-0.20	0.59	**-0.31**	**-0.77°**	-0.30	-0.42	0.38	0.61	0.03	0.29	**0.71**	**0.30**
	Gram(-) bacteria	-0.55	0.48	0.60	-0.34	-0.24	0.38	-0.16	-0.37	0.03	-0.23	-0.03	0.01	0.15	0.34	0.35	-0.07
	Gram(+) bacteria	-0.31	0.35	0.25	-0.18	0.14	0.18	-0.17	-0.41	0.52	-0.07	-0.21	-0.05	0.65	0.28	-0.17	0.02
Plant ^{15}N	Total	–	–	0.34	0.02	–	–	-0.60	-0.22	–	–	-0.30	-0.05	–	–	-0.12	0.21
	Shoots	–	–	-0.06	-0.11	–	–	-0.70	-0.02	–	–	-0.31	-0.13	–	–	0.17	0.00
	Roots	–	–	**0.72**	**0.56**	–	–	-0.18	-0.86*	–	–	-0.15	0.32	–	–	-0.45	0.91*

Asterisks and circles indicate levels of significance for Pearson correlations from the 'lm' function of the R base package; ** $P_t < 0.01$, * $P_t < 0.05$, ° $P_t < 0.1$

Bold values indicate treatment-independent effects of plant composition and underlined values show interaction effects of drought and plant composition from two-way ANOVA models with $P_F < 0.1$ (see Tables S3 & S4 for details from the peak drought and recovery labeling, respectively)

AM, Arbuscular mycorrhiza; EOC, Extractable organic carbon; MBC, microbial biomass carbon; NSC, non-structural carbohydrate

variable than at the peak drought labeling. Though, Gr:Fo and Ex:Co tended to have inverse effects on plant biomass and 13C tracer fluxes in the plant-soil continuum as well (Table 1). Independent of treatment, Ex:Co positively correlated with total plant biomass, root sucrose ^{13}C and root fructan ^{13}C tracer incorporation at the recovery labeling (Table S4). In drought treatments, there was a higher root biomass and increased ^{13}C transfer to microbial biomass in mesocosms dominated by exploitative plants (Table 1).

The general impacts of CWM_SLA on the measured parameters differed at the peak drought and recovery campaigns. At the peak drought labeling, CWM_SLA correlated with the uptake of ^{13}C tracer and its recovery in shoots and roots. In contrast, at the recovery labeling, CWM_SLA was more related to shoot biomass and BCA, especially to root fructan. At peak drought, higher CWM_SLA was associated with a lower root:shoot ^{13}C ratio in drought treatments due to the lower ^{13}C tracer allocation to roots compared to controls (Table 1). At the recovery, higher CWM_SLA was related to a lower ^{13}C allocation to root starch and a higher root ^{15}N uptake in drought treatments.

4. Discussion

Drought responses of aboveground biomass

Our results suggest that the biomass of the dominant species (*D. cespitosa*) mainly was affected by drought, assuming that the plant communities had a similar variability for both treatments at the peak drought labeling (Fig. 3a). This was possibly due to higher intra-specific competition for resources, which was not present in subdominant species. In consequence, through the lower competiveness of dominant species during drought, subdominant species may profit and are more resistant, as suggested by previous studies (Carlyle et al., 2014; Kardol et al., 2010; Mariotte et al., 2013).

By using SLA as proxy for plant resource use strategy (Díaz et al., 2004; Lavorel and Grigulis, 2012; Quétier et al., 2007; Wright et al., 2004), we were able to classify the mesocosm plants into conservative (*D. cespitosa*, *F. rubra*) and exploitative species (*D. glomerata*, *G. sylvaticum*, *L. hispidus* and *T. repens*). Independent of their functional type (grass or forb), all exploitative species showed a decrease in SLA during drought (Fig 1b). A decrease in SLA is commonly found as adaptation to drought (Poorter et al., 2009; Wright et al., 2004), and can be related to increased water use efficiency (Wright et al., 2001) and/or decreased plant growth rates (Poorter et al., 2009; and references therein). Consequently, through the adjustment of their leaf morphology, the exploitative plants in our study may have preserved more resources, which later contributed to their fast recovery. Similar results were reported by Pérez-Ramos et al. (2013), who associated the progressive leaf scenescence of exploitative grassland species with the protection of meristematic tissues, facilitating regrowth after drought. Contrariwise, in the same study, conservative grassland species promoted their root elongation rates during drought, likely to avoid dehydration. Such an enhanced allocation of resources to the belowground during drought was suggested as one possibility explaining increased SLA values of grasses in a sub-Mediterranean system (Wellstein et al., 2017). This is in line with our study as well, where we observed that drought increased the SLA of the grass *F. rubra* (Fig. 1b) and the community fine root biomass (data not shown).

In consequence, our results underpin the conclusion of Pérez-Ramos et al. (2013), that independent of their resource use strategy, plants can be tolerant to drought. The different drought responses of exploitative and conservative species can both contribute to their resilience, either by increasing the resistance or the recovery (Karlowsky et al., 2018a). However, the different plant strategies can also alter the C and N fluxes in the plant-soil continuum and their response to drought-rewetting, which may affect plant performance on the longer term. By considering such fluxes in the next section, we aimed to gain a more detailed view on how plant functional composition alters grassland resilience to drought.

Effects of plant functional composition on grassland resistance and recovery

The main aim of this study was to determine the effects of plant evenness, plant functional type (Gr:Fo) and plant resource use strategy (CWM_SLA and Ex:Co) on the response of

mountain grassland functioning to drought and rewetting. A straightforward interpretation of our collected data was complicated by the strong co-variation of Gr:Fo and Ex:Co ratio at both labeling campaigns (Fig. 3). Therefore, we used an explorative approach to assess how the plant composition affects selected important ecophysiological variables and their response to drought (Table 1). A summary of drought-rewetting effects on plant biomass, C fluxes in the plant-soil continuum and plant N uptake are depicted in Figure 4, together with an interpretation of how their response is altered by the plant community.

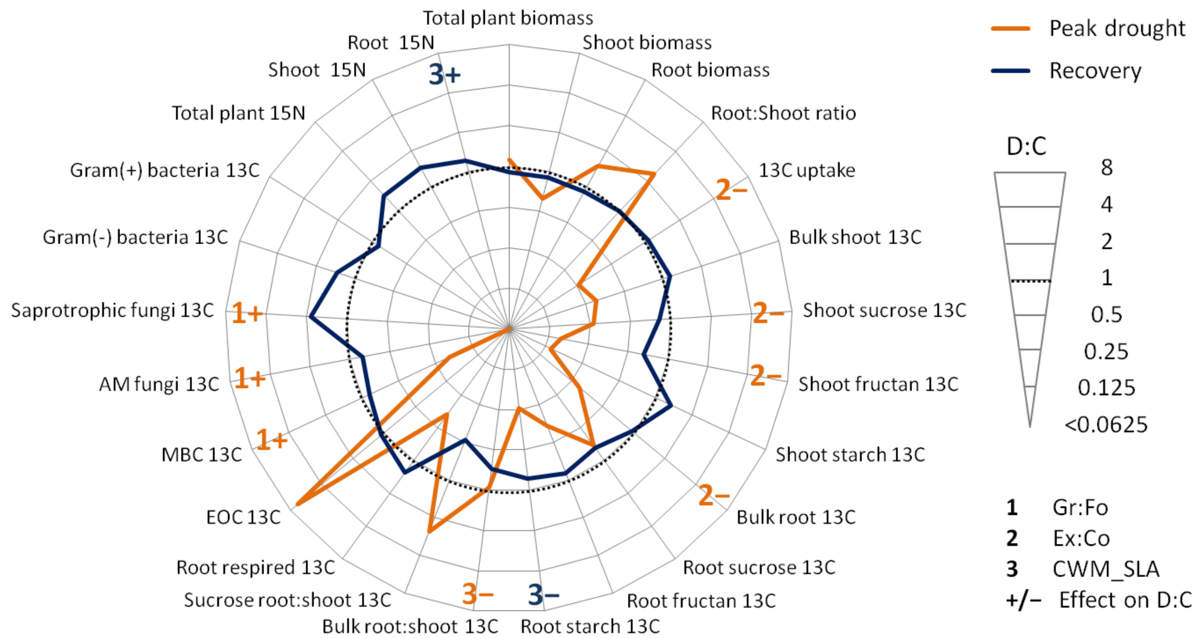

Fig. 4 Relative effect sizes of drought (drought to control ratios, D:C) on plant biomass and ^{13}C tracer fluxes at the peak drought (dark orange line) and recovery (dark blue line) labeling campaigns as well as on plant ^{15}N uptake at the recovery labeling; with positive and negative of grass to forb ratio (Gr:Fo), exploitative to conservative ratio (Ex:Co) and community-weighted mean specific leaf area (CWM_SLA). The dotted black line represents the respective control value (defined as 1) of each variable.

Unsurprisingly, the relatively small spread in plant evenness over the mesocosm communities (Table S2) had only little effects on their functioning and did not alter their response to drought. The positive effect of evenness on total plant biomass of controls at the peak drought labeling is in line with common findings from other studies, reporting that evenness increased biomass production (Assaf et al., 2011; Brett Mattingly et al., 2007; Kirwan et al., 2007; Orwin et al., 2014; Wilsey and Potvin, 2000). The absence of this effect at the recovery labeling is in accordance with the seasonal variability found for evenness effects on biomass (Orwin et al., 2014). The greater C flow to AM fungi with higher evenness at the recovery labeling points to increased competition for soil nutrients (Hartnett et al., 1993; Scheublin et al., 2007) and/or to differences in the mycorrhizal responsiveness (Johnson et al., 1997) between the plant species. Interestingly, this was accompanied by greater root N uptake in mesocosms with higher evenness, suggesting that plants which invested more C into interactions with AM fungi had a competitive advantage for the uptake

of N from soil. Overall, the results support our first hypothesis that plant evenness is positively related to plant productivity and plant-microbial interactions increasing nutrient mining from soil.

In general, Gr:Fo and Ex:Co had the strongest effects on C allocation in the plant-soil continuum and its response to drought-rewetting, albeit with opposing trends for the two ratios. Under control conditions, Gr:Fo seemed to have stronger impacts on C allocation (Table S4), representing known differences for carbohydrate storage pools between grass and forb species (Janeček et al., 2011). However, the interactions of drought with plant C uptake and allocation were stronger for Ex:Co than for Gr:Fo at the peak drought labeling (Tables 1 & S3). This was likely due to a higher drought tolerance of the slow-growing and thick-leaved conservative species (Pérez-Ramos et al., 2013; Zwicke et al., 2015), and is in-line with the notion that plant resource use strategy strongly imapcts ecosystem functioning in mountain grassland (Grigulis et al., 2013). Since the results from this study indicate a stronger C transfer to AM fungi in more conservative communites during drought, this supports our second hypothesis that conservative plants have a higher resistance of C cycling to drought and profit from stronger interactions with AM fungi. Furthermore, this is consistent with a previous study, where we found an increase of AMF markers in a conservative grassland community during drought (Karlowsky et al., 2018a). On the other side, our results contrast the findings from several studies reporting that grass species from temperate regions are typically less resistant to drought than forbs (Bollig and Feller, 2014; Gilgen et al., 2010; Gilgen and Buchmann, 2009; Zwicke et al., 2013), and thus also contradict our third hypothesis. Interestingly, the down-regulated photosynthetic activity during drought in mesocosms with higher Gr:Fo, as expected for the less responsive stomatal control in grasses (Bollig and Feller, 2014), was reflected by a greater C transfer to soil microorganisms, and especially to AM fungi. Notably, the positive effect of Gr:Fo on the C transfer to AM fungi during drought was stronger than the negative effect of Ex:Co (Table S3). This suggests that grasses compensated for the higher stomatal water loss by investing more C into mycorrhizal interactions during drought, possibly to increase their access to soil water through the hyphal network (Allen, 2007). In addition, this might also explain the findings from Signarbieux and Feller (2012), who reported that drought resistance differs for grasses and forbs in lowland grassland but is similar in subalpine and alpine grassland. In conclusion, both plant functional type (Gr:Fo) and resource use strategy (Ex:Co) seem to affect C fluxes in grasslands during drought, and it is obviously difficult to disentangle these effects in complex communities like (semi-)natural grasslands. However, it seems like Ex:Co had stronger effects on plant C uptake and allocation, while Gr:Fo affects the C transfer to AM fungi more strongly during drought. Thus, both ratios probably affect ecosystem functioning at the same time but on different levels. The strong correleations of these abundance-based community indices with grassland C cycling highlight their valuable contribution in addition to the pure measurement of functional traits.

Nonetheless, community-weighted mean traits like CWM_SLA provide a robust method to determine functional diversity (Lavorel et al., 2008), and thus should be considered as well. According to our expectations, similar to Ex:Co, higher CWM_SLA had a positive effect on

productivity-related parameters, i.e. ^{13}C tracer uptake, BCA and shoot biomass. However, under extreme drought SLA can have a high plasticity (Poorter et al., 2009; Wellstein et al., 2017), likely to allow for morphological adaptations to more unfavorable life conditions. This means plants that do not change their SLA during drought are either resistant or will have less resources available during the recovery phase. Because of the drought-induced reduction of SLA in species with normally high SLA values (Fig. 1b), the applicability of CWM_SLA as representative for the plant resource use strategy was restricted at the peak drought labeling. In contrast, CWM_SLA was mostly restored at the recovery labeling, where it was the only significant predictor for the higher plant N uptake in drought treatments compared to controls. This can be related to a high potential of exploitative species to recover, since the photosynthetic capacity is closely coupled to leaf N concentrations (Milcu et al., 2014; Wright et al., 2001) and increased assimilation is needed to restore aboveground biomass after drought. Additionally, the results from leaf-level measurements show that the N uptake during recovery can differ between exploitative species (Fig. 2a-b), possibly because individuals of *L. hispidus* were in a young age state and thus had generally high N demands (Niinemets, 2004). Overall, the results from the ^{15}N labeling support our fourth hypothesis, and the conclusion from a previous study (Karlowsky et al., 2018a), that the excess N uptake in exploitative meadow communities after drought-rewetting is related to their high resilience.

5 Conclusions

Our study suggests that less resistant exploitative plant species can respond to severe drought by adjusting their leaf morphology, which preserves resources and enables a quick recovery after the drought. In contrast, conservative species invest more resources into the maintenance of their functioning during drought, but have fewer resources available for recovery. This supports previous findings, indicating a trade-off between resistance and recovery, depending on the resource use strategy of plants. Since both strategies are suited to ensure the plant survival following extreme drought, further research is needed to assess the effects on the overall resilience.

However, our findings also indicate that various characteristics of the plant community can affect its response to drought. In this study, the biomass-derived effects of plants differing either in resource use strategy (Ex:Co) or functional type (Gr:Fo) were difficult to separate, but tended to affect ecosystem functioning during drought and recovery at distinct levels. There is indication that Ex:Co mainly influences the drought response of plant C allocation, while Gr:Fo has stronger effects on the C transfer to the rhizosphere during drought. The categorization of exploitative and conservative species to calculate Ex:Co proofed as useful, since the use of CWM_SLA as predictor for the predominant plant strategy was restricted during drought, due to the high trait plasticity of the exploitative species. Under the more optimal conditions at the recovery, CWM_SLA was again a good predictor for ecosystem functioning, particularly plant N uptake. In contrast to the functional characteristics, we found that moderate variations in plant evenness, e.g. as occurring in semi-natural grassland, do not affect the response of ecosystem functioning to drought.

In consequence, the plant functional composition determines the response of mountain grassland to drought, but different aspects of functional diversity need to be considered to assess potential consequences for ecosystem functioning.

Author contributions

M.B., S.L. and G.G. conceived the ideas; S.K., A.A., J.I., M.B. and G.G. designed methodology; S.K., A.A., J.I., and G.G. conducted the experiment and collected the data; S.K., A.A. and analyzed the data; S.K. and G.G. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data will be made available via the Publication Repository of the Max Planck Society (MPG.PuRe) or a journal-associated repository.

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CHAPTER 5 – Synthesis

To assess ecosystem resistance and resilience to climate change, it is important to understand the underlying molecular mechanisms. Carbon (C) allocation in the plant-soil continuum largely contributes to the overall functioning of terrestrial ecosystems and is closely coupled to nitrogen (N) dynamics in soil. However, it remains poorly understood how plant-microbial interactions respond to climatic extremes and how these responses are altered by land-use change. Because of the high diversity of terrestrial ecosystems, local case studies are indispensable to improve regional and global biogeochemical models of the C cycle and to provide management recommendations for local stakeholders. In my thesis I therefore use the example of a mountain grassland in the European Alpine Region, which is impacted more rapidly by climate change than other regions (Beniston, 2005; Gobiet et al., 2014) and is subjected to land-use change in wide areas (MacDonald et al., 2000; Schermer et al., 2016). By combining the simulation of extreme drought and rewetting events on grassland monoliths and mesocosms with stable isotope (^{13}C and ^{15}N) labelling, my thesis provides insights into the link of plant and soil microbial processes during peak drought and recovery. Moreover, by using common garden experiments with mesocosms on a mountain meadow the effects of land use and plant functional composition on plant-microbial interactions and their response to drought-rewetting can be assessed. Overall, this allows determining trade-offs between different plant and microbial strategies, affecting ecosystem resistance and resilience to extreme climatic events.

5.1 General Discussion

Photosynthesis and the subsequent allocation of newly assimilated C resources in the plant-soil continuum are a central element of terrestrial ecosystem functioning. In the preceding chapters, I analysed such C fluxes and their response to drought-rewetting in mesocosm setups on a mountain grassland site, by using the results obtained from ^{13}C pulse-chase labelling experiments. Mesocosms have the advantage that different plant-soil communities can be studied under comparable conditions, at the same time and location (Stewart et al., 2013). In chapter two, we used this to compare drought-rewetting responses of mesocosms that consisted of monoliths from a conservative grass-dominated abandoned grassland community and an exploitative meadow community (Fig. 1). In chapter three, we studied the link of plants and soil microorganisms during drought and recovery in greater detail, using mesocosms that were variably planted with six species from the meadow site. In chapter four, we analysed differences in the mesocosm plant functional composition to determine how management-related shifts in the plant community alter the response of grassland C and N dynamics to drought-rewetting. However, such mesocosm experiments also have disadvantages, as they cannot completely reflect the complexity of real-world ecosystems (Stewart et al., 2013), and may be biased through disturbances that were introduced during the experimental setup.

Figure 1: Photographies of the two studied mountain grasslands in the Stubai Valley (Tirol, Austria); left: hay meadow, c. 1850 m a.s.l., cut once per year, occasionally grazed and regularly fertilised (every 2-3 years); right: abandoned grassland, c. 1950 m a.s.l., completely unmanaged for more than 30 years.

The latter we aimed to avoid by pre-incubating monoliths (chapter 2) and planted mesocosms (chapters 3-4; hereafter referred to as 'mesocosms') at the study site for one and two years, respectively, before drought treatments were started. Furthermore, our studies included a randomised block design (Krebs, 1999) with parallelised control and drought treatments, which allowed us to quantify the effects of drought on ^{13}C tracer fluxes, while minimising effects of on-site environmental gradients. By incubating the plant canopy with ^{13}C-enriched CO_2 for a short time, i.e. 30 to 75 minutes, we were able to determine the fate of recent photosynthates from shoots via roots and their rhizosphere through to soil microbial biomass. In chapter two and chapter three, we used multiple sampling times after labelling with ^{13}C to determine the C turnover in different pools (see also appendix for more ^{13}C tracer dynamics). The compound-specific ^{13}C isotope analysis on plant carbohydrates and soil microbial marker lipids allowed us to assess plant physiological processes (e.g. storage formation) and the link between plants and different groups of soil microorganisms. The addition of a ^{15}N label to the water, which was used for rewetting soils after severe drought, allowed us to also study the plant N uptake from soil during recovery (chapter 2 and chapter 4). Through the combined ^{13}C and ^{15}N labellings during recovery, we could indirectly assess if plants can profit from enhanced plant-microbial interactions after rewetting. However, especially for ^{13}C tracer dynamics, there was a high variability between individual biological replicates (chapters 2-3). Factors that may have contributed to this variability include fluctuations in biomass content, species composition, soil moisture, air/soil temperature, cloud cover during the labelling and block-specific growing conditions. Thus, for ^{13}C tracer dynamics, we considered such random effects in statistical models, i.e. linear mixed-effects models (Pinheiro and Bates, 2000), by using block and monolith/mesocosm identity as error terms.

Under non-stress conditions belowground C allocation (BCA) was found to be a quick process, yielding peaks of ^{13}C incorporation into roots and soil microbial biomass starting from one day after labelling (chapters 2-3, see also Bahn et al.,

2013; Fuchslueger et al., 2014; Hasibeder et al., 2015; Malik et al., 2015). In our studies, severe drought consistently reduced the photosynthetic activity of plants, as visible by reduced total uptake of ^{13}C tracer and canopy CO_2 fluxes (see parallel study to chapter 2 by Ingrisch et al., 2018) compared to controls. This was mainly due to decreased stomatal opening (McDowell et al., 2008), since the drought response of aboveground biomass varied, showing reductions in mesocosms and monoliths from the meadow but not in monoliths from the abandoned grassland. From comparing the chapters two to four, I could identify general responses of C allocation during drought and how they are modified by plant functional composition and/or land use (Fig. 2a). The results from chapter two and chapter four indicate that exploitative species more strongly reduced photosynthetic C assimilation than conservative species, according to the suggested differences in desiccation tolerance (Díaz et al., 2004; Lavorel and Grigulis, 2012). Interestingly, drought did not reduce the relative amount of ^{13}C tracer that was allocated from shoots to roots (chapters 2-3), and in case of meadow monoliths the relative BCA was even enhanced compared to controls. However, there is still uncertainty what factor governs the response of plant BCA to drought. Previous studies also reported varying results, from decreased (Ruehr et al., 2009; Sanaullah et al., 2012) to unaltered (Hasibeder et al., 2015) up to increased relative BCA (Barthel et al., 2011; Burri et al., 2014; Huang and Fu, 2000; Palta and Gregory, 1997; Sanaullah et al., 2012). The combined results from these and our studies suggest that the plant functional type, plant resource use strategy or different plant interactions (intra-specific and inter-specific) could affect the drought response of BCA. In addition, there is evidence that the root biomass response depends on the severity, i.e. intensity and duration, of drought (Kreyling et al., 2008). Alterations in root activity, as for example the general increase of osmotically active soluble sugars in roots (especially sucrose, chapters 2-3) or the increased fine root growth in mesocosms (chapter 3) during drought, may also affect BCA through sink control (Farrar and Jones, 2000). Notably, the maintenance of BCA was clearly coupled to reduced ^{13}C allocation to shoot storage carbohydrates (i.e. fructan and starch, chapters 2-3; see also Hasibeder et al., 2015). Together with the consistent decrease of shoot carbohydrate concentrations, this repeatedly supports the conclusion of Bahn et al. (2013) that BCA is maintained at the cost of aboveground storage during reduced C supply. Moreover, our results suggest that the strategy to allocate C resources belowground during drought is more pronounced in exploitative than in conservative species (chapter 2 and chapter 4). Simultaneously, storage carbohydrates were preserved in the roots of meadow species, as the strongly decreased allocation of freshly assimilated C to root storage carbohydrates did not affect their concentrations (chapter 2 and chapter 3). This effect was not explained by differences in plant functional composition (chapter 4). Thus, the strategy of meadow plants to preserve C resources in their roots during drought might be a consequence or legacy of regularly introduced disturbances through land use, since plants have to rely on their belowground resources to facilitate regrowth after the cutting of shoots. Greater root N pools in drought-treated mesocosms (chapter 3) further underpin this conclusion.

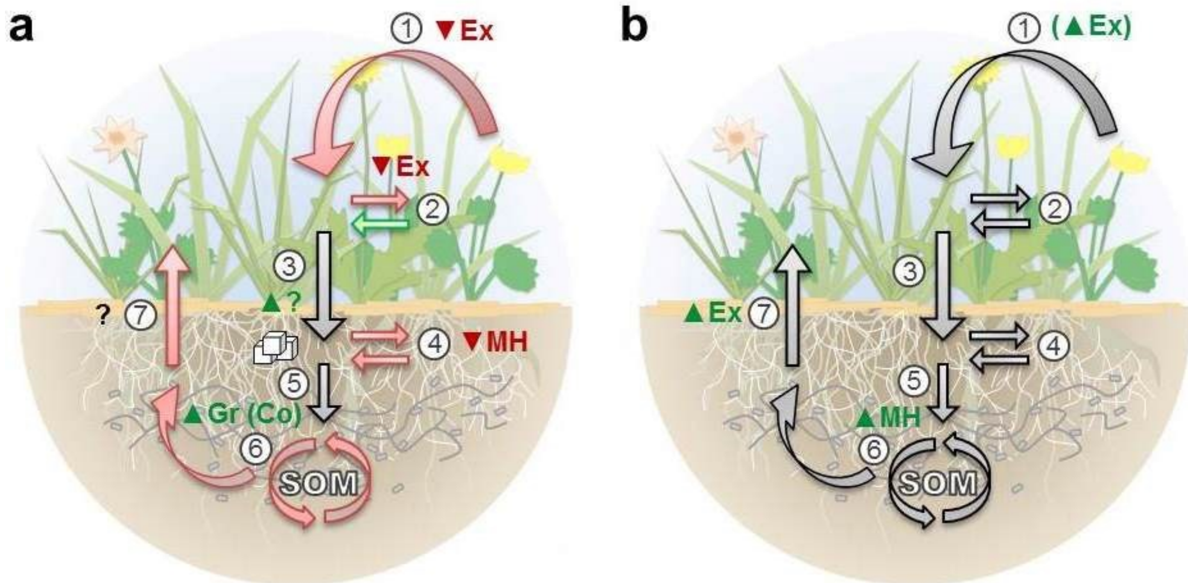

Figure 2: General responses of grassland carbon (C) and nitrogen (N) cycling during peak drought (a) and recovery (b). The colour of arrows shows if fluxes were reduced (red), increased (green) or unaltered (grey) by drought-rewetting. Effects of plant functional composition and management as well as effects with uncertain origin are indicated by next to the arrows, with the colour of text referring to positive (green) or negative effects (red). White cubes stand for the accumulation of soluble sugars. 1, photosynthesis; 2, shoot storage allocation; 3, belowground C allocation; 4, root storage allocation; 5, root exudation; 6, microbial C uptake associated with soil organic matter (SOM) decomposition and N mineralisation; 7, plant N uptake; Co, conservative plants; Ex, exploitative plants; Fo, forbs; MH, management history (i.e. the legacy of management-induced disturbances).

In previous ^{13}C pulse-chase labelling experiments drought has been found to decouple plant photosynthesis and soil processes, i.e. the respiration of freshly assimilated ^{13}C tracer from soil (Barthel et al., 2011; Burri et al., 2014; Hagedorn et al., 2016; Ruehr et al., 2009) and the incorporation of ^{13}C into soil microbial marker lipids (Fuchslueger et al., 2016, 2014a). The latter response we also could find in chapter two, however, the mechanisms behind the decoupling remained unclear. In particular, there was a lack of knowledge, if the link between plants and soil microorganisms is affected due to reduced exudation of labile C by plant roots or if the decoupling mainly is a consequence of the reduced microbial activity in dry soils. Therefore, in chapter three, we used the chloroform fumigation extraction to distinguish between ^{13}C tracer incorporation into extractable organic C (EOC) and microbial biomass C (MBC) from soil (Malik et al., 2013). Assuming that EOC represents the intermediate pool, where root and hyphal exudates pass through prior to their uptake by saprotrophic microorganisms, we could demonstrate that plants continue to transfer recently assimilated C to the rhizosphere during drought (Fig. 2a). Furthermore, the accumulation of ^{13}C tracer in the EOC pool was reflected by strongly decreased ^{13}C incorporation into MBC. Thus, from the results of chapter three we were able to conclude that the disconnection of plant and soil processes during drought is primarily due to reduced microbial activity in dry soils. Moreover, the lower relative ^{13}C allocation to microbial marker lipids compared to MBC suggested

that not only limited substrate diffusion (Moyano et al., 2013; Skopp et al., 1990) but also adjustments in microbial metabolism contribute to the decrease of microbial C cycling during drought (Schimel et al., 2007). An increased use of C resources for the building of osmotically active protective compounds (Potts, 1994) explains why ^{13}C tracer was preferentially allocated to the water-extractable MBC pool instead of membrane lipids. Since the formation of new membrane lipids is especially important for cell growth and division, this also indicates that previously active parts of the soil microbial community transitioned into a dormant state in order to survive the effects of severe drought (Lennon and Jones, 2011).

Interestingly, we could find that abandonment strengthened the link between plants and soil microorganisms during drought (chapter 2). The results from chapter four, showing that a higher grass to forb ratio (Gr:Fo) increased ^{13}C transfer to soil microorganisms during drought, suggest that the dominance of grasses in the abandoned grassland (see also Ingrisch et al., 2018) was responsible for the stronger link. Different root traits (including the link to mycorrhiza) between the present grass and forb species might have contributed to the effect of Gr:Fo on microbial ^{13}C uptake (Bardgett et al., 2014). Furthermore, in contrast to general assumptions about the lower stomatal responsiveness of C3 grasses (Bollig and Feller, 2014; Wellstein et al., 2017), Gr:Fo had no effect on the response of plant biomass and C uptake to drought. This is in line with the findings of Signarbieux and Feller (2012), who observed a difference in the drought response of stomatal conductance only between grasses and forbs in lowland grasslands but not in mountain grasslands. On the other hand, the comparability between drought responses of monoliths (chapter 2) and mesocosms (chapters 3-4) was limited, since the monoliths included established soil plant-soil communities, whereas the sieving of soil and planting of mesocosms likely affected plant-microbial interactions. Such differences, caused by the varying experimental setup, would also explain discrepancies in the soil microbial biomass response to drought, i.e. that drought either increased the abundance of arbuscular mycorrhiza (AM) fungal markers (chapter 2) or Gram-negative (G-) bacterial markers (chapter 3). Thus, the higher plant-soil ^{13}C transfer in the abandoned grassland compared to the meadow could also have been a consequence of stronger interactions of conservative plant species with AM fungi (Gross et al., 2010; Legay et al., 2016), as suggested by the increased amounts of AM fungal markers during drought (chapter 2). In addition, the higher biomass of relatively drought-tolerant saprotrophic fungi and Gram-positive (G+) bacteria (Schimel et al., 2007) may have further increased the microbial use of recently assimilated plant-derived C in the rhizosphere. Such land-use-dependant alterations in soil microbial community were also not reflected in the mesocosm setup, where only the fertilised soil from the meadow was used, because the experiment (chapter 4) focussed on the influence of plant functional composition on grassland resistance and resilience to drought. In general, the effects of Gr:Fo and exploitative to conservative ratio (Ex:Co) on C allocation in the plant-soil system were hardly separable, though Ex:Co and Gr:Fo seemed to act upon different processes. Consequently, land-use change can affect the drought response of ecosystem functioning by shifts in both, plant functional type (Gr:Fo) and plant resource use

strategy (Ex:Co), and more research is needed to disentangle their effects.

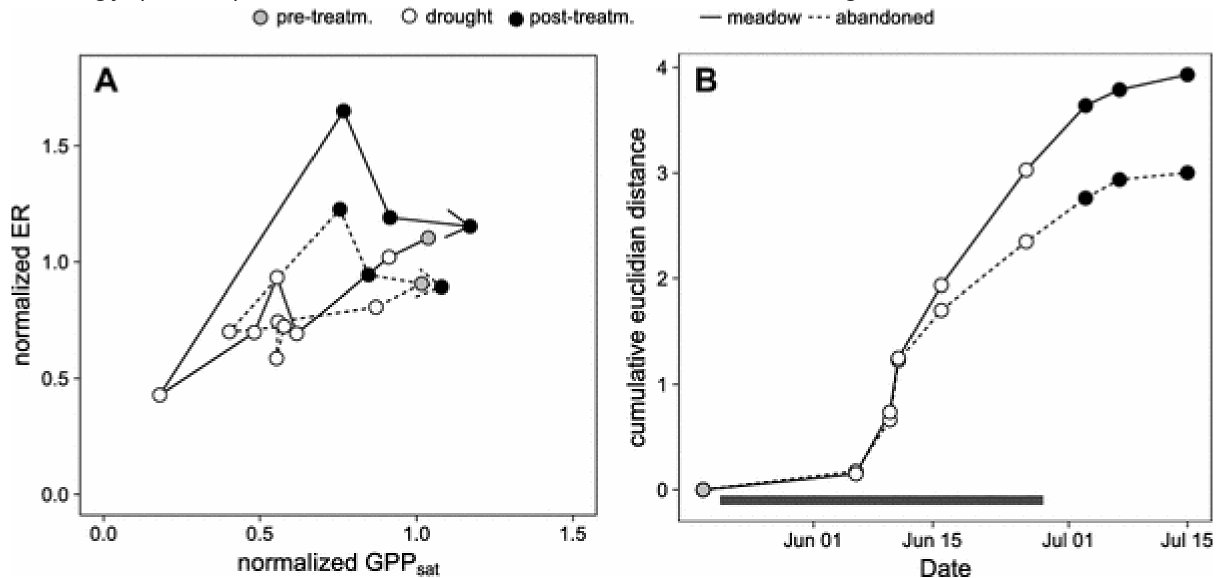

Figure 3: A) The course of normalized light-saturated rates of gross primary productivity (GPPsat) and ecosystem respiration (ER) before (grey points), during (open points) and after (black points) the drought experiment in the meadow (solid line) and the abandoned grassland (dotted line). Normalized fluxes were calculated as the ratio of the flux in drought monoliths to the respective flux in control monoliths. The direction of the path is given by the arrow, symbols denote the periods before (shaded), during (open) and after (closed) drought. B) Cumulative Euclidian distance of the response trajectories of the two grasslands over the course of the drought. The Euclidian distance between two consecutive measurements days is a measure of the system's change in the bivariate flux space. The cumulative Euclidian distance from beginning of the drought (pretreatment) is a measure of the overall perturbation of the grassland. The black horizontal bar indicates period of rain exclusion. Figure and caption are adapted from Ingrisch et al., (2018).

Although management significantly decreased the resistance of mountain grassland to drought, ecosystem functioning completely recovered shortly after rewetting (chapter 2). The higher perturbation of C fluxes in the meadow during drought was followed by a higher rate of recovery compared to the abandoned grassland (Fig. 3), yielding temporary overcompensations of respiration and CO_2 uptake in the meadow. That points to a high physiological plasticity of the meadow species, which is likely due to their exploitative strategy (Guiz et al., 2018). Additionally, by monitoring the specific leaf area (SLA) in chapter four, we were also able to underpin previous findings suggesting that exploitative species have a high morphological plasticity (Pérez-Ramos et al., 2013). Exploitative species with typically high SLA values, which allow for increased photosynthetic capacity (Wright et al., 2001), strongly adjusted their leaf morphology during drought by reducing SLA but were able to quickly restore it to control values after rewetting. This morphological plasticity was related to a complete recovery of C uptake and allocation in the plant-soil system (Fig. 2b). In consequence, the high morphological and physiological plasticity of exploitative plants allowed for the increase of productivity necessary to quickly restore aboveground biomass during recovery. Simultaneously, meadow plants invested more C resources into interactions with rhizospheric microorganisms, such as saprotrohic fungi and bacteria, during recovery (chapters 2-3). Interestingly,

this interaction apparently did not depend on plant functional composition (chapter 4), suggesting that the increased transfer of recent assimilates to the rhizosphere after drought-rewetting is a legacy effect of management in the meadow (Fig. 2b). To facilitate regrowth after management-related disturbances, i.e. grazing or cutting, meadow plants can temporarily increase root exudation to enhance N mineralisation by rhizospheric microorganisms (Hamilton et al., 2008), especially fast-growing bacteria. There is indication that such regular fluctuations in environmental conditions can lead to the acclimatisation of the soil microbial community, altering its response to disturbances compared to microbial communities from more stable environments (Hawkes and Keitt, 2015). Thus, through transferring additional resources to the rhizosphere, meadow plants might be able to further fuel the pulse of microbial activity, which is typically observed after rewetting dry soils and is related to increased C and N mineralisation (Birch, 1958; Borken and Matzner, 2009; Canarini and Dijkstra, 2015). So far, the source of this pulse remained unclear (Canarini et al., 2017; Fierer and Schimel, 2002). However, the results from chapter three strongly suggest that a part of the C substrates used for the priming of soil microbial activity after rewetting comes from the accumulation of root exudates in the soil EOC pool during drought. High concentrations of dissolved organic C (DOC) in soils directly after rewetting prior to the pulse of soil respiration (Canarini et al., 2017) and the disappearing of high EOC concentrations shortly after rewetting (chapter 3) support this conclusion.

Fungal and bacterial activities are known to differ in their dynamics after rewetting dried soils (Barnard et al., 2013; Meisner et al., 2013). The pulse of soil respiration during the first day after rewetting is directly followed by peak of bacterial growth, with a possible lag phase depending on the severity of drought. In contrast, fungal growth starts without a lag phase after rewetting and increases more slowly to peak values after a few days up to one week (Meisner et al., 2013). The results from chapter two and chapter three indicate that especially fast-growing G- bacteria, known to quickly take up readily degradable C in the rhizosphere (e.g. Bahn et al., 2013; Balasooriya et al., 2012; Malik et al., 2015), contributed to the pulse of bacterial activity after rewetting. In addition, G+ bacteria also seemed to profit from the increased substrate availability in the rhizosphere of the meadow, similar to previous findings from an intensively managed lowland grassland (Denef et al., 2009). Notably, the response strongly differed between AM fungi and saprotrophic fungi. While the latter were unaffected in biomass and consistently took up more plant-derived ^{13}C, AM fungal markers were less abundant (chapters 2-3) and incorporated lower amounts of ^{13}C after drought-rewetting (chapter 2). This indicates that plant-mycorrhizal needed a longer time to recover, possibly because plants preferentially invested C resources into root exudation to fuel the decomposition of soil organic matter (SOM) in the rhizosphere. The faster recovery of plant-mycorrhizal interactions in mesocosms is probably due to the higher proportion of AM fungi in microbial biomass (Fig. 4), which might be a result of the loosened soil structure

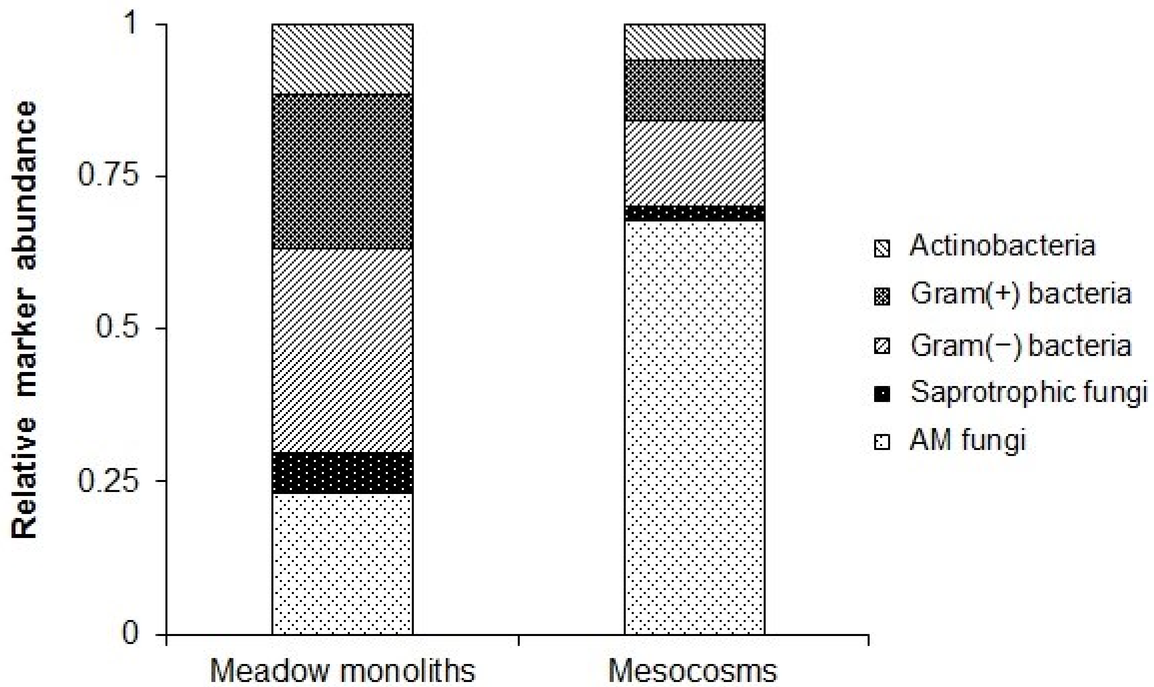

Figure 4: Relative abundance of soil microbial marker lipids (measured as $\mu g_C/g_{dm}$ and normalized to total amount) from the average of control treatments of meadow monoliths (chapter 2) and planted mesocosms (chapters 3-4). AM, arbuscular mycorrhiza.

and lower plant density compared to monoliths. However, it should also be mentioned that the marker used for AM fungi, i.e. the neutral lipid fatty acid 16:1ω5, rather reflects the nutritional status than the biomass of AM fungi (Olsson, 1999). This also could have contributed to difference to saprotrophic fungi, for which the biomass-dependant phospholipid fatty acid 18:2ω6,9 was used (Frostegård et al., 2011). Nevertheless, the increased ^{13}C uptake by saprotrophic fungi during recovery is in line with studies, showing that saprotrophic fungi rapidly take up recently assimilated plant-derived C in the rhiszosphere (Balasooriya et al., 2012; de Deyn et al., 2011; Denef et al., 2007; Scheunemann et al., 2016), and thus likely act as a main consumer of root exudates (Ballhausen and de Boer, 2016). In conclusion, meadow plants enhanced their ability to recover from drought by providing more C substrates to saprotrophic fungi and G-/G+ bacteria, in order to fuel SOM decomposition in the rhizosphere after rewetting. The exploitative strategy of meadow species, in turn, allowed them to take up high amounts of mineralised N for rapid regrowth. This was expressed by the enhanced uptake of ^{15}N label in meadow plants after rewetting (chapter 2), which was significantly correlating with the community-weighted mean SLA (CWM_SLA) of meadow communities (chapter 4). The measurement of community-weighted mean traits is a way to determine functional diversity in ecological studies, which is insensitive of the method used for biomass estimation (Lavorel et al., 2008). In general, high CWM_SLA values have been found to be a good indicator for exploitative plant communities (Garnier et al., 2004; Grigulis et al., 2013). However, since SLA values were affected during drought (chapter 4), the biomass ratio Ex:Co was more appropriate to estimate the effects of

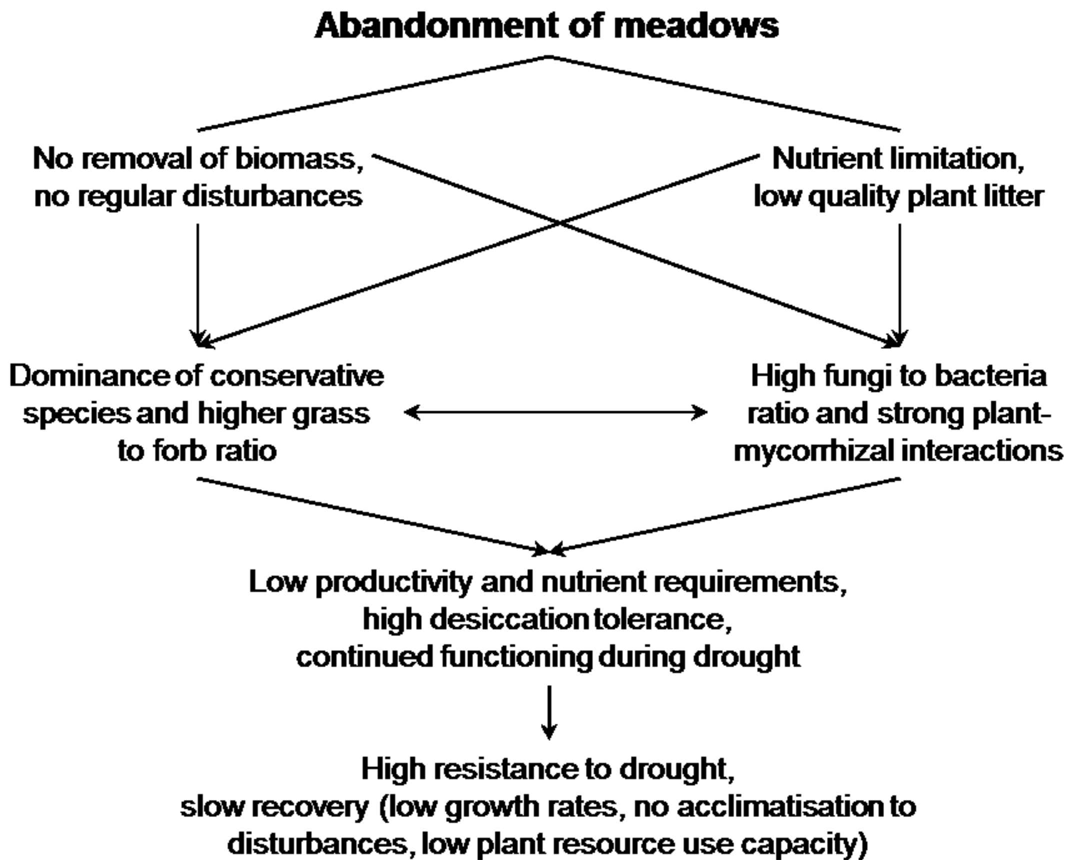

Figure 5: Overview on the effects of abandonment of meadows on the interactions between plant and soil community, ecosystem functioning and ecosystem stress response.

plant resource use strategy on grassland functioning at peak drought. In consequence, both, CWM_SLA and Ex:Co are of value for determining how the plant resource use strategy affects ecosystem responses to disturbances.

Overall, the findings of my thesis indicate that there is a trade-off between resistance and recovery, i.e. high resistance is followed by slow recovery and vice versa. Furthermore the results show that resistance and recovery are underpinned by different mechanisms in the plant-soil system. These mechanisms can be altered by land-use change, as summarised for the example of abandonment of meadows in Figure 5. Although they seem to exclude each other, both, high resistance and quick recovery, were found to provide the studied grassland communities with resilience to climate extremes. Conservative grassland communities were better able to maintain their functioning during severe drought. Their high resistance was based on plant traits related to low productivity, low nutrient demand and high desiccation tolerance but may have also profited from strong interactions with AM fungi. However, the lower resource availability and plant growth rate in conservative communities limited the speed of recovery after rewetting. In contrast, the functioning of more productive exploitative grassland communities was strongly affected by drought but could quickly recover, based on plant traits that supported high nutrient capture and fast re-growth. This effect was apparently enhanced by land use, which provides high nutrient availability through fertilisation and might cause acclimatisation to losses in

aboveground biomass, due to regular cutting and occasional grazing. For example, meadow plants seemed to preserve C resources in their roots during drought, when C supply is limited, in order to support fast re-growth after rewetting. Additionally, meadow soil contained a more bacteria-dominated food web, which is able to quickly recover its activity and is likely also accustomed to regular interruptions of plant C supply due to land use. Our findings indicated that the microbial activity was primed by plant-derived C, which accumulated in the rhizosphere during drought and was accessible after rewetting. Together with the increased transfer of recently assimilated plant C to bacteria and saprotrophic fungi, this probably fuelled the mineralisation of N from SOM in the meadow during recovery. Moreover, the enhanced N uptake of exploitative meadow species after drought-rewetting further supported their recovery by allowing for a higher photosynthetic activity. Ultimately, this leads me to the conclusion that moderately managed mountain grasslands like the studied hay meadow are probably more resilient to climate change than abandoned grasslands. More frequent and severe climate extremes could also yield higher stress intensities for conservative grassland communities, and as regeneration times are limited by the short growing season in mountain regions, the lower recovery rate of abandoned grasslands would have adverse effects for their resilience.

5.2 Outlook

In my thesis I used the example of severe drought events in mountain grassland communities to study 1) the combined effects of climate and land-use change on C fluxes in the plant-soil system and related N fluxes, and 2) the underlying mechanisms of ecosystem resistance and resilience to disturbances. In general, it needs to be tested if the findings from this work are also transferable to other mountain regions (with varying climate, plant composition and soil conditions), to other ecosystem types (e.g. forests and lowland grasslands) as well as to other climate change-related disturbances, such as extreme precipitation events or reduced snow cover during winter in cold regions (Gobiet et al., 2014; IPCC, 2013). Nonetheless, the results from the preceding chapters provide new insights into the basic mechanisms in the pant-soil system, which contribute to the resistance and resilience of terrestrial ecosystems to extreme climatic events.

Our findings from the ^{13}C pulse-chase labelling experiments improved the existing knowledge about the link between plant and soil processes during drought and after recovery (chapters 2-3), and how it is modified by plant functional composition (chapter 4) and land use (chapter 2). In chapter two, we could show that AM fungi might play an important role in providing grassland ecosystems, particularly grass-dominated conservative communities, with resistance to drought. However, there is some remaining uncertainty due to the ambiguous nature of the applied neutral fatty acid biomarker (Olsson 1999). One way to enhance our understanding of the drought response of AM fungi in grasslands would be the *in situ* use of hyphae-ingrowth cores, which allow determining the activity of AM fungi (Cheng et al., 2012; Johnson et al., 2002). Furthermore, by performing a ^{15}N labelling during drought, it may be possible to determine if stronger plant-mycorrhizal interactions also improve

the N uptake under water-limited conditions. In chapter three, we were able to demonstrate that plants continuously exudate C into the rhizosphere during drought, while the uptake and cycling of C by soil microorganisms is limited. This has implications for biogeochemical models dealing with climate change feedbacks on the C cycle, especially as the C that accumulates in the rhizosphere during drought potentially contributes to ecosystem recovery by priming the soil microbial activity. Follow-up studies could gain more insights into this mechanism by using ^{13}C pulse labelling at peak drought and a time series of sampling that starts immediately after rewetting. Moreover, our findings suggest that the link of plant and soil processes during drought and recovery depends on land use (chapter 2), and this dependency needs further elucidation, also in other study areas and ecosystem types.

By using ^{15}N labelling to determine plant N uptake after rewetting, we could show that plants can profit from the increased microbial activity during recovery (chapter 2 and chapter 4). This highlights the importance of plant-microbial interactions during the recovery from extreme events, which was found to be positively affected by grassland management via a quickly responding bacterial community and exploitative plant species with high N uptake. In general, as there is only little literature available, future research should focus on recovery mechanisms in the plant-soil system and their implications for the resilience of terrestrial ecosystems.

The findings from chapter two and chapter four indicate that management has the potential to specifically modify the resistance and resilience of terrestrial ecosystems, especially by adjustments in plant functional composition. However, because ^{13}C labelling experiments are expensive and work-intensive, our studies were only able to reflect a snapshot in time during the recovery. Thus, future studies that include a higher temporal resolution during recovery could allow for a better view on the overall ecosystem resilience, in order to assess differences between various plant-soil communities. Though there were no significant differences in short-term resilience of conservative and exploitative grassland communities, drought events can entail lagged or 'carry-over' effects (van der Molen et al., 2011). Such carry-over effects include for example the incomplete refilling of water and C reserves (Arnone et al., 2008), physical changes of the soil structure, i.e. increased hydrophobicity (Goebel et al., 2011) and the breakup of soil aggregates (Schimel et al., 2011), or reduced seed dispersal (Zeiter et al., 2016). Moreover, severe drought is known to create legacy effects in the response of plants and soil microorganisms during following drought events (e.g. Fuchslueger et al., 2016; Kaisermann et al., 2017; Legay et al., 2018), but there is a lack of knowledge how drought history effects vary between different grassland communities. Thus, by taking into account long-term resilience, varying drought severity and repeated drought events, future research can draw the consequences from the general trade-off between resistance and recovery that was found in this thesis. Finally, more profound estimates of ecosystem resilience can be used to develop sustainable management strategies in the face of climate change, and to better represent the implications of land-use change in C cycle − climate feedback predictions.

SUMMARY

Terrestrial ecosystems form the basis of human life, especially as they contribute to a large extent to food production and have a key position in the global carbon (C) cycle. Yet, heir functioning is put at risk by global societal and climate change. Especially increasing frequencies of extreme climatic events can have devastating consequences for ecosystem functioning. However, there is a lack of knowledge how land-use change, as a consequence of societal transformations, alters ecosystem stress responses. Such alterations can affect ecosystem resistance, i.e. the ability to maintain functioning during a disturbance, and ecosystem resilience, i.e. the ability to recover pre-stress functioning after a disturbance. To improve predictions about ecosystem stress responses and to develop adequate management strategies, the basic mechanisms underlying ecosystem resistance and resilience need to be studied. The C allocation in the plant-soil system is one of the most important processes for the functioning of terrestrial ecosystems. By linking plant and soil processes, belowground C allocation (BCA) also links the C cycle with other biogeochemical element cycles, such as the soil nitrogen (N) cycle. As N is one of the most growth-limiting nutrients, plants that provide C to microorganisms in their rhizosphere can profit from a higher microbial decomposition of soil organic matter (SOM) and the associated release of plant-accessible N. The strength of such beneficial plant-microbial interactions substantially depends on plant functional composition and soil conditions, which in turn can be modified by ecosystem management.

In this thesis I aim to identify mechanisms in the plant-soil system providing terrestrial ecosystems with resistance and resilience to climate change. I use the example of mountain grasslands in the Central Alps, which are subjected to large-scale shifts in land use and experience the effects of climate change more quickly than ecosystems in the surrounding lowlands. Extreme drought events pose a major threat to the functioning of such meadow ecosystems. By using common garden experiments with various mesocosms on a mountain meadow site and *in situ* drought simulation, I study 1) the responses of mountain grassland communities to drought and rewetting and 2) how they are modified by land use and variations in plant functional composition. With the aid of stable C isotope labelling, more specifically ^{13}C pulse-chase labelling, I follow the fate of newly assimilated C in the plant-soil system during drought and subsequent recovery. In particular, I use compound-specific ^{13}C isotope analyses in plant non-structural carbohydrates (NSCs) and in microbial marker lipids (i.e. phospholipid fatty acids and neutral lipid fatty acids). This allows distinguishing different plant C allocation strategies and assessing the link between plants and different groups of soil microorganisms. In addition, I study how plants can profit from interactions with soil microorganisms during recovery by applying a ^{15}N label to soil and determining plant N uptake.

In chapter two, I use a mesocosm experiment with grassland monoliths from a traditionally managed hay meadow and an abandoned grassland. This study focuses on the effects of land use on the stress reactions of C allocation and plant microbial interactions. In addition, the chapter also deals with the basic mechanisms of drought

and recovery responses in the plant-soil system. The results from this study show that drought induces a shift of plant C allocation towards BCA at the expense of aboveground storage NSCs, especially in the managed meadow. This is associated with a lower resistance of the meadow compared to the abandoned grassland, as indicated by higher reductions of aboveground biomass, C uptake and plant-microbial C transfer in the meadow. Because of its conservative plant community the abandoned grassland is less affected by the limited water and nutrient availability during drought. Furthermore, it has a more resistant fungi-dominated soil microbial community and likely profits from stronger interactions with arbuscular mycorrhiza (AM) fungi, as an increase of AM fungi markers during drought suggests. In contrast, the managed meadow is able to recover more quickly from drought, as indicated by a full recovery of C uptake and BCA shortly after rewetting. This is accompanied by a stronger C transfer to the quickly responding bacterial-dominated microbial community in the meadow. Furthermore, the exploitative meadow plant community can profit from the increased soil microbial activity, as shown by an enhanced plant N uptake during recovery. In conclusion, by preserving resources belowground during drought, meadow plants are able to compensate for their lower resistance. By contrast, abandoned grassland plants that continue their functioning during drought as far as possible have fewer resources available for recovery. Ultimately, the results from this study demonstrate that land use can alter the mechanisms underlying ecosystem resilience, and that there is a trade-off between high resistance and quick recovery.

Chapter three focusses on the link between plant and soil processes during drought and after rewetting. Here I use the findings from an experiment with mesocosms that were variably planted with three grass and three forb species, taken from the hay meadow studied in chapter two. Again the results show that drought leads to a preferential use of limited C resources for BCA and the preservation of NSCs in roots from meadow species, partially as soluble sugars to adjust the osmotic balance. Remarkably, this is not associated with a decreased exudation of labile C substrates from roots to the rhizosphere, as highlighted by an accumulation of recently assimilated ^{13}C tracer in the water-extractable organic C (EOC) from soil. Moreover, an increase of EOC concentrations is associated with a strong reduction of ^{13}C tracer incorporation into microbial biomass. Thus, the previously observed disconnection between plant and soil processes during drought is probably due to substrate diffusion limitation, which reduces the microbial access to plant-derived C in the rhizosphere. Furthermore, a stronger reduction of ^{13}C tracer incorporation into microbial marker lipids compared to bulk microbial biomass C suggests that soil microorganisms adjust their metabolism during drought, e.g. by allocating more C resources to the synthesis of protective compounds that increase desiccation tolerance. In addition, the findings from this study indicate that C substrates, which accumulate in the rhizosphere during drought, are rapidly used after rewetting. This likely further fuels the pulse of microbial activity, which is typically observed after rewetting dried soils, and potentially promotes the quick recovery of plant-microbial interactions in the meadow community.

In chapter four, I specifically investigate how management-related alterations

in plant functional composition affect the drought and recovery responses of mountain grassland communities. For this purpose, I use different parameters to describe the plant (functional) composition of the mesocosms from chapter three and study their effects on C and N fluxes in the plant-soil system. The results indicate that the drought response depends on the ratio of exploitative to conservative species (Ex:Co) and the grass to forb ratio (Gr:Fo). The effects of both, Ex:Co and Gr:Fo, are partially overlapping but seem to affect different processes in the plant-soil continuum with varying strength. Higher Ex:Co values are especially associated with stronger reductions of plant ^{13}C tracer uptake, allocation to shoot storage and BCA. On the contrary, higher Gr:Fo values particularly increase the amount of ^{13}C tracer that is transferred to the soil microbial community during drought, especially to AM fungi. However, there is no indication that plant functional composition affects C allocation in the plant-soil system during recovery. However, the specific leaf area (SLA) also shows that exploitative species, which are characterised by high SLA values, have a high morphological plasticity. This likely contributes to the fast recovery of exploitative species despite their lower resistance. Accordingly, the results also show a positive effect of community-weighted mean SLA (CWM_SLA) on plant N uptake. In conclusion, management-related shifts in plant functional composition can alter the response of mountain grassland communities by varying contributions of plant functional type (Gr:Fo) and resource use strategy (Ex:Co, CWM_SLA). Similar to chapter two, the results from this study indicate a trade-off between high resistance and rapid regeneration.

Overall, the findings from my thesis highlight the important role of plant-microbial interactions for providing terrestrial ecosystems with resistance and resilience to extreme climatic events. The results from the combined ^{13}C and ^{15}N labelling experiments improve the existing knowledge about the link between plant and soil processes during drought and subsequent recovery. Furthermore, my thesis demonstrates that the mechanisms underpinning ecosystem resistance and resilience can be altered by land use. In particular, land use has an effect through changes in the plant functional composition, but also through acclimatisation of the plant-soil system to regular management-related disturbances of C allocation. In consequence, by considering plant-microbial interactions and the effects of land use in biogeochemical models predictions about the C cycle and climate change feedbacks can be improved. However, there seems to be a general trade-off between resistance and recovery, i.e. high resistance is followed by a slow recovery and vice versa. The results of this work show that both ways can contribute to short-term ecosystem resilience. Therefore, future studies are needed to assess the long-term effects of land use on resilience and the ecosystem response to more frequent and severe drought events. Ultimately, the results of such studies can help to find appropriate management strategies that reduce the risk of negative climate change impacts on the functioning of ecosystems.

ZUSAMMENFASSUNG

Terrestrische Ökosysteme sind von immenser Bedeutung für die Menschheit, vor allem weil sie zu einem Großteil zur Lebensmittelproduktion beitragen und eine Schlüsselposition im globalen Kohlenstoff(C)-Kreislauf einnehmen. Ihre Funktionsweise wird jedoch durch den globalen Gesellschafts- und Klimawandel aufs Spiel gesetzt. Insbesondere die zunehmende Häufigkeit von Klimaextremen kann verheerende Folgen für Ökosystemfunktionen haben. Allerdings gibt es kaum Wissen dazu, wie durch den Gesellschaftswandel bedingte Landnutzungsänderungen die Stressreaktion von Ökosystemen beeinflussen. Solche Änderungen haben mögliche Auswirkungen auf die Ökosystem-Resistenz, d.h. die Fähigkeit die Funktionsweise unter Stress aufrechtzuerhalten, und die Ökosystem-Resilienz, d.h. die Fähigkeit zur Wiederherstellung der Funktionsweise nach dem Stress. Deshalb müssen die der Resistenz und Resilienz zugrundeliegenden Mechanismen untersucht werden, um Vorhersagen über Ökosystem-Stressreaktionen zu verbessern und angepasste Management-Strategien zu entwickeln. Die C-Allokation im Pflanzen-Boden-System ist einer der wichtigsten Prozesse für die Funktionsweise von terrestrischen Ökosystemen. Durch die Verknüpfung von Pflanzen- und Bodenprozessen verbindet die C-Allokation in den Untergrund (BCA, von engl.: „belowground carbon allocation“) auch den C-Kreislauf mit anderen biogeochemischen Stoffkreisläufen, wie dem Stickstoff(N)-Kreislauf im Boden. N ist einer der am meisten limitierenden Nährstoffe für das Pflanzenwachstum. So können Pflanzen, die den Mikroorganismen in ihrer Rhizosphäre C zur Verfügung stellen, von einem höheren mikrobiellen Abbau der organischen Bodensubstanz und der damit verbundenen Freisetzung von pflanzenverfügbarem N profitieren. Die Stärke solcher nützlichen pflanzlich-mikrobiellen Interaktionen hängt wesentlich von der funktionellen Zusammensetzung der Pflanzen und den Bodenbedingungen ab, welche wiederum durch das Ökosystemmanagement verändert werden können.

In dieser Arbeit verfolge ich das Ziel Mechanismen im Pflanzen-Boden-System zu identifizieren, die terrestrische Ökosysteme mit Resistenz und Resilienz gegenüber Klimawandelfolgen versorgen. Dafür nutze ich das Beispiel von Bergwiesen in den Zentralalpen, welche großflächigen Landnutzungsänderungen ausgesetzt sind und die Auswirkungen des Klimawandels schneller erleben als die Ökosysteme in umliegenden Tiefländern. Extreme Dürre-Ereignisse stellen dabei eine Hauptgefahr für die Funktionsweise solcher Wiesenökosysteme dar. Auf Basis von „Common Garden“-Experimenten mit verschiedenen Pflanze-Boden-Mesokosmen auf einer Bergwiese und *in situ* Dürresimulation untersuche ich 1) die Reaktionen von Bergwiesengemeinschaften auf Trockenheit und Wiederbefeuchtung und 2) wie diese durch Landnutzung und Variationen in der funktionellen Pflanzen-Zusammensetzung verändert werden. Mit Hilfe der stabilen C-Isotopen-Markierung, genauer gesagt der „^{13}C-Pulse-Chase“-Markierung, verfolge ich den Weg von neu assimiliertem C im Pflanzen-Boden-System während Dürre und anschließender Regenerationsphase. Insbesondere verwende ich verbindungsspezifische ^{13}C-Isotopenanalysen in pflanzlichen nicht-strukturellen Kohlenhydraten (NSCs, von engl.: „non-structural carbohydrates“) und in mikrobiellen Marker-Lipiden

(Phospholipid-Fettsäuren und Neutrallipid-Fettsäuren). Diese Analysen ermöglichen es, verschiedene pflanzliche C-Allokationsstrategien zu unterscheiden und die Verbindung zwischen Pflanzen und verschiedenen mikrobiellen Gruppen im Boden zu beurteilen. Darüber hinaus untersuche ich, anhand der Zugabe einer ^{15}N-Markierung zum Boden und der Bestimmung der pflanzlichen N-Aufnahme, welche Pflanzengemeinschaften während der Regenerationsphase von verstärkten Interaktionen mit Bodenmikroorganismen profitieren können.
Im zweiten Kapitel verwende ich ein Mesokosmos-Experiment mit Grünland-Monolithen von einer traditionell bewirtschafteten Mähwiese und einer aufgelassenen Wiese (weiterhin als Brache bezeichnet). In dieser Studie wird vor allem der Einfluss der Landnutzung auf die Stressreaktionen der C-Allokation und der pflanzlich-mikrobiellen Interaktionen untersucht. Daneben befasst sich das Kapitel auch mit den grundlegenden Mechanismen der Dürre- und Regenerationsreaktionen im Pflanzen-Boden-System. Die Ergebnisse dieser Studie zeigen, dass Dürre eine Verschiebung der pflanzlichen C-Allokation in Richtung Untergrund auf Kosten der oberirdischen Speicher-NSCs induziert, vor allem bei der Mähwiese. Das ist mit einer geringeren Resistenz der Mähwiese im Vergleich zur Brache verbunden, was sich in einer höheren Reduktion von oberirdischer Biomasse, C-Aufnahme und pflanzlich-mikrobiellem C-Transfer in der Mähwiese äußert. Durch ihre konservative (Ressourcen-bewahrenden) Pflanzengemeinschaft ist die Brache weniger von der begrenzten Wasser- und Nährstoffverfügbarkeit während Dürre betroffen. Außerdem hat sie eine widerstandsfähigere, von Pilzen dominierte mikrobielle Bodengemeinschaft und profitiert wahrscheinlich von stärkeren Wechselwirkungen mit arbuskulären Mykorrhiza (AM)-Pilzen, wie eine Zunahme der AM-Pilzmarker während Dürre vermuten lässt. Im Gegensatz dazu kann sich die Mähwiese schneller von der Dürre regenerieren, was durch eine vollständige Regeneration von C-Aufnahme und BCA kurz nach der Wiederbefeuchtung angezeigt wird. Damit einher geht ein stärkerer C-Transfer in die schnell reagierende, bakteriell dominierte mikrobielle Gemeinschaft der Mähwiese. Darüber hinaus kann die Ressourcen-ausschöpfende Pflanzengemeinschaft der Mähwiese von der erhöhten mikrobiellen Aktivität im Boden profitieren, wie eine gesteigerte pflanzliche N-Aufnahme während der Regenerationsphase zeigt. Zusammenfassend lässt sich sagen, dass die Pflanzen der Mähwiese durch die Bewahrung von unterirdischen Ressourcen während Dürre ihre geringere Resistenz ausgleichen können. Dahingegen haben die Pflanzen der Brache, die ihre Funktionsweise während Dürre weitestgehend aufrechterhalten, weniger Ressourcen zur Verfügung für die Regeneration. Letztlich zeigen die Ergebnisse dieser Studie, dass Landnutzung die der Ökosystem-Resilienz zugrunde liegenden Mechanismen verändern kann, und dass es dabei einen Trade-off (im Sinne von: „Entweder-oder“) zwischen hoher Resistenz und schneller Regeneration gibt.
Im dritten Kapitel liegt mein Fokus auf dem Zusammenhang von Pflanzen- und Bodenprozessen während Dürre und nach Wiederbefeuchtung. Hier verwende ich die Ergebnisse eines Experiments mit Mesokosmen, die variabel mit drei Gräsern und drei Kräutern bepflanzt wurden, welche von der im zweiten Kapitel untersuchten Mähwiese stammen. Auch hier zeigen die Ergebnisse, dass Dürre zu einer

bevorzugten Nutzung der begrenzten C-Ressourcen für die BCA und zur Bewahrung von NSCs in den Wurzeln der Mähwiesen-Spezies führt. Letzteres geschieht teilweise in Form von löslichen Zuckern, die zur osmotischen Anpassung benutzt werden können. Bemerkenswerterweise ist dies nicht mit einer verminderten Exsudation von labilen C-Substraten aus den Wurzeln in die Rhizosphäre verbunden, wie eine Anhäufung von kürzlich assimiliertem ^{13}C-Tracer im wasserlöslichen organischen C (EOC, von engl.: „extractable organic carbon") vom Boden zeigt. Darüber hinaus ist eine Erhöhung der EOC-Konzentrationen mit einer starken Reduktion des ^{13}C-Tracer-Einbaus in die mikrobielle Biomasse verbunden. So ist die zuvor beobachtete Abkopplung von Pflanzen- und Bodenprozessen während der Dürre wahrscheinlich auf eine limitierte Substratdiffusion zurückzuführen, die den mikrobiellen Zugang zu pflanzlichem C in der Rhizosphäre reduziert. Darüber hinaus deutet eine stärkere Reduktion des ^{13}C-Tracer-Einbaus in den mikrobiellen Marker-Lipiden im Vergleich zum Gesamt-C der mikrobiellen Biomasse darauf hin, dass die Bodenmikroorganismen ihren Stoffwechsel während Dürre anpassen. Zum Beispiel, indem sie mehr C-Ressourcen für die Synthese von Schutzverbindungen bereitstellen, welche die Toleranz gegenüber dem Austrocknen erhöhen. Des Weiteren deuten die Ergebnisse dieser Studie darauf hin, dass die während Dürre in der Rhizosphäre angehäuften C-Substrate nach der Wiederbefeuchtung rasch genutzt werden. Dies treibt wahrscheinlich auch den Puls der mikrobiellen Aktivität, welcher typischerweise nach der Wiederbefeuchtung getrockneter Böden beobachtet wird, weiter an. Damit wird potenziell auch eine schnellere Regeneration der pflanzlich-mikrobiellen Interaktionen in der Wiesengemeinschaft gefördert.

Im vierten Kapitel untersuche ich im Besonderen, wie sich Veränderungen in der funktionellen Pflanzen-Zusammensetzung auf die Dürre- und Regenerationsreaktionen von Bergwiesengemeinschaften auswirken. Dazu verwende ich verschiedene Parameter zur Beschreibung der (funktionellen) Pflanzen-Zusammensetzung der Mesokosmen aus dem dritten Kapitel und untersuche deren Auswirkungen auf die C- und N-Flüsse im Pflanzen-Boden-System. Die Ergebnisse deuten darauf hin, dass die Reaktion auf Dürre vom Verhältnis von Resourcen-ausschöpfenden zu konservativen Arten (Ex:Co, von engl.: „exploitative to conservative ratio") und vom Verhältnis von Gräsern zu Kräutern (Gr:Fo, von engl.: „grass to forb ratio") abhängt. Die Effekte von Ex:Co und Gr:Fo überschneiden sich teilweise, scheinen aber unterschiedliche Prozesse im Pflanzen-Boden-Kontinuum verschieden stark zu beeinflussen. Höhere Ex:Co-Werte sind vor allem mit stärkeren Dürre-Auswirkungen auf die pflanzliche ^{13}C-Tracer-Aufnahme, die C-Allokation zum Spross-Speicher und die BCA verbunden. Im Gegenteil dazu bewirken höhere Gr:Fo-Werte, dass während Dürre größere Mengen an ^{13}C-Tracer zur mikrobiellen Bodengemeinschaft, insbesondere zu AM-Pilzen, weitergegeben werden. Im Unterschied zu den Effekten während Dürre gibt es jedoch keine maßgeblichen Hinweise darauf, dass die funktionelle Pflanzen-Zusammensetzung die C-Allokation im Pflanzen-Boden-System während der Regenerationsphase beeinflusst. Allerdings ist anhand der spezifischen Blattfläche (SLA, von engl.: „specific leaf area") erkennbar, dass Ressourcen-ausschöpfende Spezies (charakterisiert durch hohe SLA-Werte) eine hohe morphologische Plastizität

aufweisen. Diese trägt wahrscheinlich dazu bei, dass sich die Ressourcen-ausschöpfenden Spezies trotz ihrer niedrigeren Resistenz wieder schnell regenerieren. Dementsprechend zeigen die Ergebnisse auch einen positiven Effekt der nach der Pflanzenzusammensetzung gewichteten, mittleren SLA (CWM_SLA, von engl.: „community-weighted mean SLA“) auf die pflanzliche N-Aufnahme. Zusammenfassend lässt sich sagen, dass Veränderungen in der funktionellen Pflanzen-Zusammensetzung die Reaktion von Bergwiesengemeinschaften durch unterschiedliche Beiträge des funktionellen Pflanzentyps (Gr:Fo) und der Ressourcennutzungsstrategie (Ex:Co, CWM_SLA) verändern können. Ähnlich zu Kapitel zwei ist dabei auch ein Trade-off zwischen hoher Resistenz und schneller Regeneration zu beobachten.

Insgesamt heben die Ergebnisse meiner Dissertation die wichtige Rolle von pflanzlich-mikrobiellen Interaktionen für die Resistenz und Resilienz terrestrischer Ökosysteme gegenüber extremen Klimaereignissen hervor. Die Ergebnisse der kombinierten ^{13}C- und ^{15}N-Markierungsexperimente erweitern das vorhandene Wissen über die Verbindung zwischen Pflanzen- und Bodenprozessen während Dürre und anschließender Regeneration. Darüber hinaus zeigt meine Dissertation, dass die Mechanismen, welche der Resistenz und Resilienz von Ökosystemen zugrunde liegen, durch Landnutzung verändert werden können. Landnutzung wirkt dabei insbesondere durch die Veränderung der funktionellen Pflanzen-Zusammensetzung, aber auch durch die Akklimatisierung gegenüber regelmäßigen Störungen der C-Allokation im Pflanzen-Boden-System, die aus der Bewirtschaftung resultieren. Durch die Berücksichtigung von pflanzlich-mikrobiellen Interaktionen und von Landnutzungs-Effekten in biogeochemischen Modellen können daher Vorhersagen über den C-Kreislauf und Klimawandel-Feedbacks verbessert werden. Allerdings scheint es einen generellen Trade-off zwischen Resistenz und Regeneration zu geben, d.h. einer hohen Resistenz folgt eine langsame Regeneration und umgekehrt. Die Ergebnisse dieser Arbeit zeigen, dass beide Wege kurzfristig zur Ökosystem-Resilienz beitragen können. Deshalb sind weitere Studien erforderlich, um zu beurteilen, wie sich Landnutzungseffekte langfristig auf die Resilienz sowie auf die Ökosystem-Reaktion gegenüber häufigeren und schwerwiegenderen Dürreereignisse auswirken. Letztendlich können die Ergebnisse solcher Studien dazu beitragen geeignete Management-Strategien zu finden, welche das Risiko negativer Klimawandel-Auswirkungen auf die Funktionsweise von Ökosystemen verringern.

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APPENDIX

Chapter 2:

TABLE S1 Relative extraction efficiency (mean ± SD) of the new PLFA extraction method using pressurised solvent extraction at 40 °C and 70 °C compared to the established method described by Kramer & Gleixner (2006); measured on the same soil sample from an arable field.

PLFA group	***n*** [a]	**Relative extraction efficiency compared to established method (%)**					
		Pressurised extraction at 40 °C			Pressurised extraction at 70 °C		
Linear saturated	7	132	±	12	170	±	17
Branched saturated	15	118	±	21	165	±	43
Cyclic saturated	2	91	±	8	109	±	8
Monounsaturated	14	94	±	14	116	±	18
Polyunsaturated	1	90	±	–	204	±	–
All PLFAs	39	110	±	22	146	±	40

[a] *n* is the number of PLFAs in each group; for polyunsaturated PLFAs only 18:2ω6c was detected in sufficient amounts.

TABLE S2 Combined effects of drought and land use on soil water content, fine root biomass, total ^{13}C and plant ^{15}N uptake, root respiration rate, concentrations of plant carbohydrates, concentrations of soil microbial marker lipids and (A+S)-fungi:bacteria ratio; at the resistance labelling (peak drought) and the resilience labelling (recovery phase).

Labelling	Parameter	Unit	Drought (df =1)			Land use (df =1)			Drought × Land use (df =1)		
			F^a	P_F^a	P_{exact}^b	F^a	P_F^a	p_{exact}^b	F^a	P_F^a	P_{exact}^b
Resistance	*General*										
	SWC	mass-%	169.0	**<0.001**	**<0.001**	6.2	**0.038**	**0.041**	6.1	**0.039**	**0.042**
	Fine roots	g/m^2	0.1	0.718	0.637	9.4	**0.016**	**<0.001**	0.1	0.783	0.726
	Total ^{13}C uptake	mg/m^2	2.1	0.185	***0.052***	2.9	0.126	**0.011**	0.6	0.448	0.354
	Carbohydrates										
	Shoot sucrose	mg_C/g_{dm}	0.8	0.411	0.300	9.2	**0.016**	**<0.001**	0.1	0.806	0.735
	Shoot fructan		3.7	0.092	**0.008**	0.0	0.975	0.970	0.8	0.408	0.263
	Shoot starch		6.5	**0.034**	**<0.001**	1.6	0.241	0.183	8.7	**0.019**	**<0.001**
	Root sucrose		20.4	**0.002**	**<0.001**	14.3	**0.005**	**<0.001**	1.7	0.229	0.180
	Root fructan		0.0	0.979	0.978	30.7	**<0.001**	**<0.001**	2.0	0.194	0.139
	Root starch		0.3	0.593	0.518	9.5	**0.015**	**<0.001**	1.9	0.203	0.105
	Micro-organisms										
	AM fungi	$mg_C/m^2_{0\text{-}7cm}$	0.8	0.391	0.200	0.1	0.732	0.607	0.3	0.581	0.412
	Sapro. fungi		0.2	0.658	0.576	11.8	**0.009**	**<0.001**	0.1	0.727	0.672
	G(-) bacteria		0.0	0.989	0.981	2.0	0.200	***0.052***	0.3	0.591	0.430
	G(+) bacteria		0.0	0.935	0.910	6.3	**0.036**	**<0.001**	0.1	0.818	0.767
	Actinobacteria		0.0	0.878	0.819	0.1	0.742	0.621	0.1	0.754	0.637
	(A+S)-F:B	-	2.3	0.166	**0.041**	0.0	0.916	0.881	0.1	0.822	0.739
Resilience	*General*										
	SWC	mass-%	1.6	0.237	0.108	1.1	0.328	0.200	1.5	0.252	0.122
	Fine roots	g/m^2	3.1	0.115	0.077	25.0	**0.001**	**<0.001**	9.6	**0.015**	**0.005**
	Total ^{13}C uptake	mg/m^2	3.0	0.123	**0.030**	1.1	0.329	0.196	1.6	0.248	0.118
	Root resp. CO_2	nmol g_{dm}^{-1} s^{-1}	0.4	0.553	0.447	3.2	0.110	**0.047**	0.0	0.982	0.979
	Plant ^{15}N uptake[c]	mg/m^2	6.8	**0.031**	**0.005**	8.9	**0.017**	**<0.001**	3.6	0.096	**0.048**
	Carbohydrates										
	Shoot sucrose	mg_C/g_{dm}	0.6	0.447	0.299	4.7	0.062	**0.009**	0.4	0.568	0.441
	Shoot fructan		3.3	0.105	**0.024**	2.1	0.181	0.073	0.8	0.403	0.264
	Shoot starch		0.3	0.576	0.496	7.3	**0.027**	**<0.001**	0.2	0.689	0.636
	Root sucrose		0.0	0.910	0.885	4.3	0.072	**0.011**	3.0	0.120	**0.031**
	Root fructan		2.1	0.188	0.121	20.2	**0.002**	**<0.001**	0.4	0.545	0.481
	Root starch		0.1	0.752	0.674	3.6	0.095	**0.031**	2.9	0.130	**0.046**
	Micro-organisms										
	AM fungi	$mg_C/m^2_{0\text{-}7cm}$	3.1	0.114	**0.011**	0.0	0.859	0.811	0.1	0.719	0.622
	Sapro. fungi		0.1	0.743	0.675	4.4	0.069	**<0.001**	0.0	0.910	0.873
	G(-) bacteria		0.5	0.521	0.344	0.1	0.778	0.676	0.0	0.998	0.996
	G(+) bacteria		0.7	0.441	0.265	0.7	0.422	0.245	0.5	0.505	0.341
	Actinobacteria		0.1	0.755	0.643	1.1	0.333	0.144	0.1	0.775	0.663
	(A+S)-F:B	-	4.4	0.068	**0.009**	0.1	0.749	0.676	0.5	0.499	0.346

(A+S)-F:B, (arbuscular mycorrhiza + saprotrophic) fungi:bacteria ratio; G(-/+), Gram-negative/positive; resp., respired; Sapro., saprotrophic; SWC, soil water content.

[a]*F*-values and approximate *P*-values from ordinary ANOVA (function 'aov' from the R base package); and [b]exact *P*-values from distribution-independent permutational ANOVA (function 'aovp' from the R package 'lmPerm'); bold values, $P_{F,exact}$ < 0.05 (significant); bold italic values, $P_{F,exact}$ < 0.06 (nearly significant).

[c]The ^{15}N addition was only done on monoliths used for the resilience labelling, plant ^{15}N uptake is the sum of shoot- and root-incorporated ^{15}N.

TABLE S3 Effects of drought and land use on ^{13}C tracer dynamics in above- and belowground plant parts and their carbohydrate pools, and in different soil microbial groups; after the resistance labelling (at peak drought) and after the resilience labelling (at recovery phase).

Labelling	^{13}C incorporation in:	Unit	D			LU			Time			D × LU			D × Time			LU × Time			D × LU × Time		
			df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
Resistance	*Bulk plant material*																						
	Shoot	mg_{13C}/m^2	1	11.9	**<0.001**	1	0.5	0.483	5	61.1	**<0.001**	1	3.1	0.077	5	13.7	**0.017**	5	4.3	0.510	5	7.9	0.161
	Root		1	5.3	**0.022**	1	9.4	**0.002**	5	48.6	**<0.001**	1	0.1	0.747	5	2.2	0.816	5	11.7	**0.039**	5	9.4	0.096
	Root:shoot ratio	$\%_{13C}$	1	0.3	0.562	1	5.1	**0.025**	5	100.3	**<0.001**	1	3.2	0.074	5	10.0	0.076	5	7.9	0.164	5	5.0	0.412
	Carbohydrates																						
	Shoot sucrose	mg_{13C}/m^2	1	0.3	0.588	1	0.1	0.773	3	97.2	**<0.001**	1	1.3	0.260	3	3.0	0.397	3	4.3	0.235	3	6.5	0.091
	Shoot fructan		1	19.9	**<0.001**	1	0.2	0.651	3	11.5	**0.009**	1	0.0	0.920	3	1.6	0.663	3	0.9	0.830	3	1.2	0.752
	Shoot starch		1	8.7	**0.003**	1	1.0	0.321	3	36.9	**<0.001**	1	5.7	**0.017**	3	6.4	0.095	3	0.7	0.864	3	2.6	0.454
	Root sucrose		1	0.6	0.435	1	7.8	**0.005**	3	15.8	**0.001**	1	1.0	0.323	3	8.1	**0.045**	3	16.3	**0.001**	3	3.3	0.353
	Root fructan		1	4.2	**0.039**	1	1.8	0.180	3	62.0	**<0.001**	1	4.1	**0.043**	3	7.7	***0.052***	3	5.6	0.131	3	3.5	0.317
	Root starch		1	3.0	0.081	1	6.8	**0.009**	3	42.8	**<0.001**	1	2.1	0.145	3	4.9	0.177	3	3.0	0.394	3	7.1	0.067
	Micro-organisms																						
	AM fungi	$\mu g_{13C}/m^2$	1	15.6	**<0.001**	1	1.7	0.194	1	0.7	0.389	1	2.8	0.097	1	2.6	0.107	1	1.5	0.223	1	0.6	0.449
	Saprotrophic fungi		1	19.7	**<0.001**	1	0.1	0.770	1	5.0	**0.026**	1	3.3	0.068	1	0.1	0.752	1	4.1	**0.043**	1	2.0	0.159
	Gram(-) bacteria		1	14.3	**<0.001**	1	0.4	0.512	1	0.2	0.629	1	4.7	**0.030**	1	6.1	**0.013**	1	1.7	0.197	1	0.7	0.399
	Gram(+) bacteria		1	1.7	0.196	1	9.7	**0.002**	1	4.2	**0.041**	1	0.4	0.541	1	4.0	**0.046**	1	0.3	0.576	1	0.4	0.542
	Actinobacteria		1	2.0	0.161	1	7.3	**0.007**	1	4.0	**0.046**	1	0.1	0.814	1	0.8	0.359	1	1.6	0.213	1	0.1	0.784
Resilience	*Bulk plant material*																						
	Bulk shoot	mg_{13C}/m^2	1	1.1	0.303	1	1.6	0.200	5	98.8	**<0.001**	1	0.8	0.381	5	7.5	0.189	5	14.4	**0.013**	5	18.7	**0.002**
	Bulk root		1	0.2	0.628	1	4.9	**0.027**	5	30.7	**<0.001**	1	0.3	0.586	5	3.5	0.617	5	5.9	0.319	5	3.4	0.644
	Root:shoot ratio	$\%_{13C}$	1	0.2	0.683	1	4.1	**0.042**	5	103.1	**<0.001**	1	1.2	0.268	5	5.9	0.315	5	6.3	0.278	5	7.0	0.219
	Carbohydrates																						
	Shoot sucrose	mg_{13C}/m^2	1	0.4	0.531	1	1.0	0.311	3	87.9	**<0.001**	1	0.1	0.822	3	4.9	0.177	3	9.0	**0.030**	3	2.8	0.431
	Shoot fructan		1	0.1	0.749	1	0.1	0.779	3	3.4	0.329	1	0.0	0.991	3	4.7	0.194	3	4.9	0.176	3	1.1	0.772
	Shoot starch		1	0.5	0.469	1	0.3	0.571	3	77.3	**<0.001**	1	0.0	0.996	3	1.8	0.609	3	0.2	0.972	3	4.6	0.203
	Root sucrose		1	0.0	0.850	1	0.9	0.349	3	46.8	**<0.001**	1	6.8	**0.009**	3	1.5	0.694	3	2.0	0.573	3	0.4	0.930
	Root fructan		1	0.9	0.342	1	0.8	0.385	3	44.1	**<0.001**	1	0.5	0.464	3	4.8	0.191	3	2.8	0.422	3	1.8	0.611
	Root starch		1	0.0	0.865	1	1.6	0.201	3	37.4	**<0.001**	1	0.5	0.487	3	2.2	0.534	3	0.3	0.957	3	1.4	0.702
	Micro-organisms																						
	AM fungi	$\mu g_{13C}/m^2$	1	3.6	***0.058***	1	0.4	0.549	1	0.8	0.376	1	1.2	0.280	1	0.2	0.648	1	2.0	0.156	1	0.3	0.608
	Saprotrophic fungi		1	0.6	0.449	1	1.2	0.279	1	0.6	0.431	1	1.2	0.267	1	0.9	0.338	1	0.5	0.489	1	0.1	0.734
	Gram(-) bacteria		1	1.3	0.256	1	0.0	0.889	1	0.8	0.386	1	5.5	**0.020**	1	0.2	0.671	1	0.9	0.349	1	0.1	0.816
	Gram(+) bacteria		1	0.0	0.979	1	0.7	0.414	1	1.4	0.244	1	6.4	**0.011**	1	0.0	0.939	1	0.0	0.849	1	0.3	0.587
	Actinobacteria		1	0.1	0.769	1	2.6	0.104	1	6.2	**0.013**	1	3.4	0.066	1	3.0	0.084	1	0.1	0.765	1	0.2	0.617

AM, arbuscular mycorrhiza; D, drought; LU, land use.

Statistics are based on linear mixed-effect models from the R package 'lme4'; bold values, $P < 0.05$ (significant); bold italic values, $P < 0.06$ (nearly significant).

TABLE S4 Mean residence time (MRT) of sucrose from shoots and roots of the abandoned grassland and the meadow, after the resistance and the recovery pulse labelling. MRT was calculated according to Hasibeder *et al.* (2015) and differences between MRT of drought and control treatments were tested using the exact Fisher-Pitman permutation test.

Labelling	C Pool	Land use	Treatment	Exponential fit[a]				MRT (h) mean	SE	P-value of difference in MRT
				Peak (h)	*n*	MRT (h)	SE			
Resistance	Shoot sucrose	Abandoned	Control	1.5	4	17	2	26	10	1.0
				1.5	4	16	2			
				1.5	4	45	4			
			Drought	1.5	4	19	4	24	3	
				1.5	4	28	9			
				1.5	4	26	7			
		Meadow	Control	1.5	4	13	2	23	9	0.7
				24	3	14	1			
				1.5	4	40	8			
			Drought	1.5	4	10	1	17	4	
				1.5	4	18	2			
				1.5	4	24	2			
	Root sucrose	Abandoned	Control	1.5	4	91	50	88	14	0.1
				24	3	111	62			
				24	3	63	35			
			Drought	72	2	–	–	333	0	
				24	3	333	0			
				24	3	333	0			
		Meadow	Control	24	3	63	16	53	5	0.2
				1.5	4	45	10			
				24	3	50	5			
			Drought	24	3	111	12	82	15	
				24	3	59	24			
				1.5	3	77	24			
Resilience	Shoot sucrose	Abandoned	Control	1.5	4	14	6	11.2	1.4	0.7
				1.5	4	11	4			
				1.5	4	9	0			
			Drought	1.5	4	8	1	10.6	1.3	
				1.5	4	11	4			
				1.5	4	13	0			
		Meadow	Control	1.5	4	14	2	14.1	0.5	0.1
				1.5	4	14	6			
				1.5	4	15	1			
			Drought	1.5	4	12	0	11.1	0.3	
				1.5	4	11	1			
				1.5	4	11	2			
	Root sucrose	Abandoned	Control	72	2	–	–	39.3	10.7	0.3
				1.5	4	50	10			
				24	3	29	2			
			Drought	1.5	4	50	3	49.4	2.1	
				1.5	4	53	28			
				24	3	45	2			
		Meadow	Control	24	3	24	14	48.7	21.2	0.7
				1.5	4	31	7			
				1.5	4	91	33			
			Drought	1.5	4	34	2	34.7	8.8	
				1.5	4	20	6			
				24	3	50	0			

[a]Equation $N = N_{Peak}\, e^{-\lambda t}$ fitted to *n* data points using nonlinear least squares regression, Peak refers to the time after labelling at which the ^{13}C label was highest for each monolith, $MRT = \lambda^{-1}$.

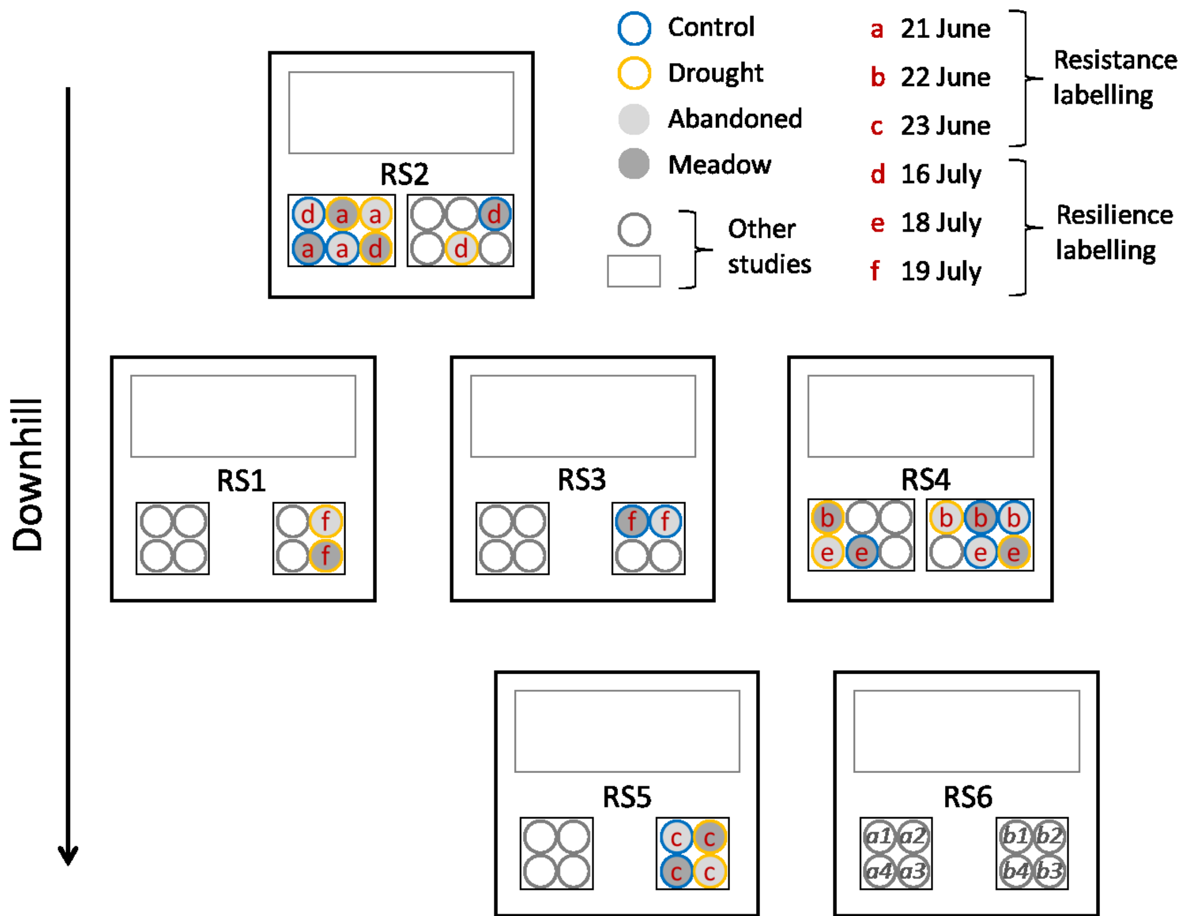

FIGURE S1 Arrangement of monoliths on the experimental field in the Stubai valley on a spot with small inclination; the emphasised monoliths used for ^{13}C pulse labelling in this study represent a subset from a bigger project (see also Ingrisch *et al.* 2017), which was set up in a randomized block design using six rain-out shelters (RS1-6) in total. Numbering of individual monoliths, as found in the data deposited in the Dryad repository (Karlowsky *et al.*, 2017), was done as shown in RS6.

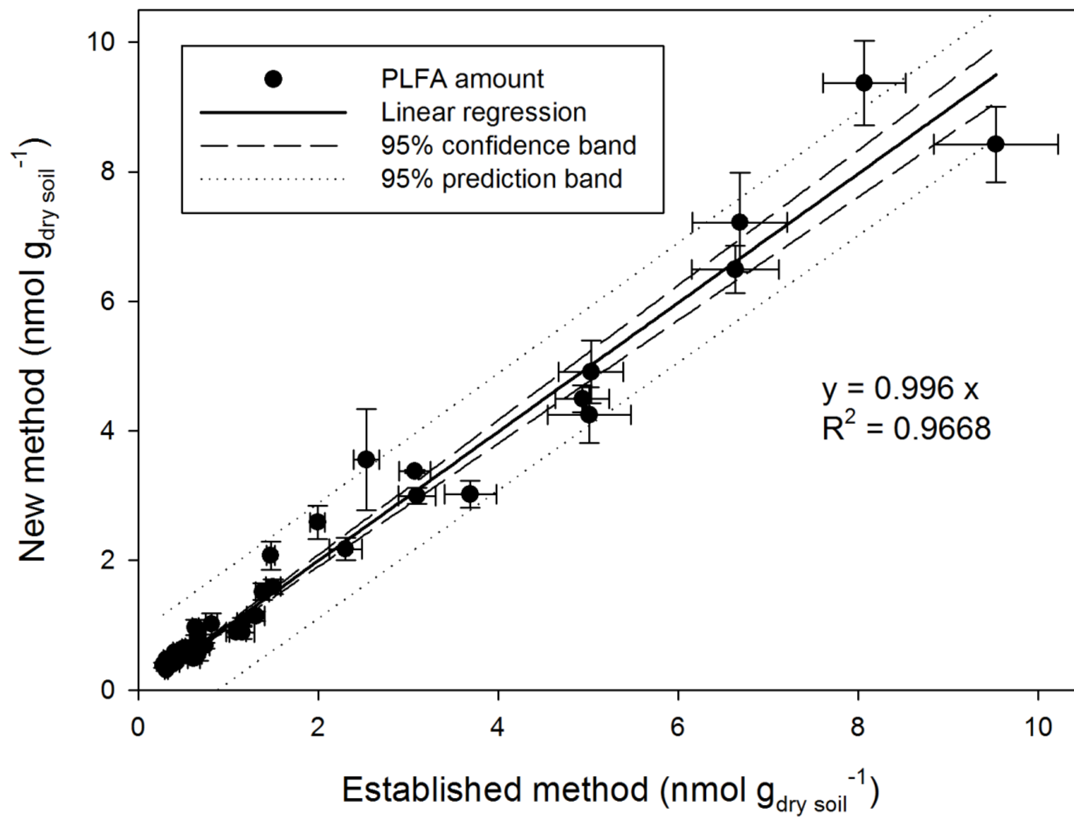

FIGURE S2 Comparison of the extracted PLFA amounts between the new PLFA extraction method (pressurized extraction at 40 °C) and the established extraction method described by Kramer & Gleixner (2006); measured on the same soil sample from an arable field. Error bars show ± SD of n = 3 (new method) or n = 4 (established method) extractions.

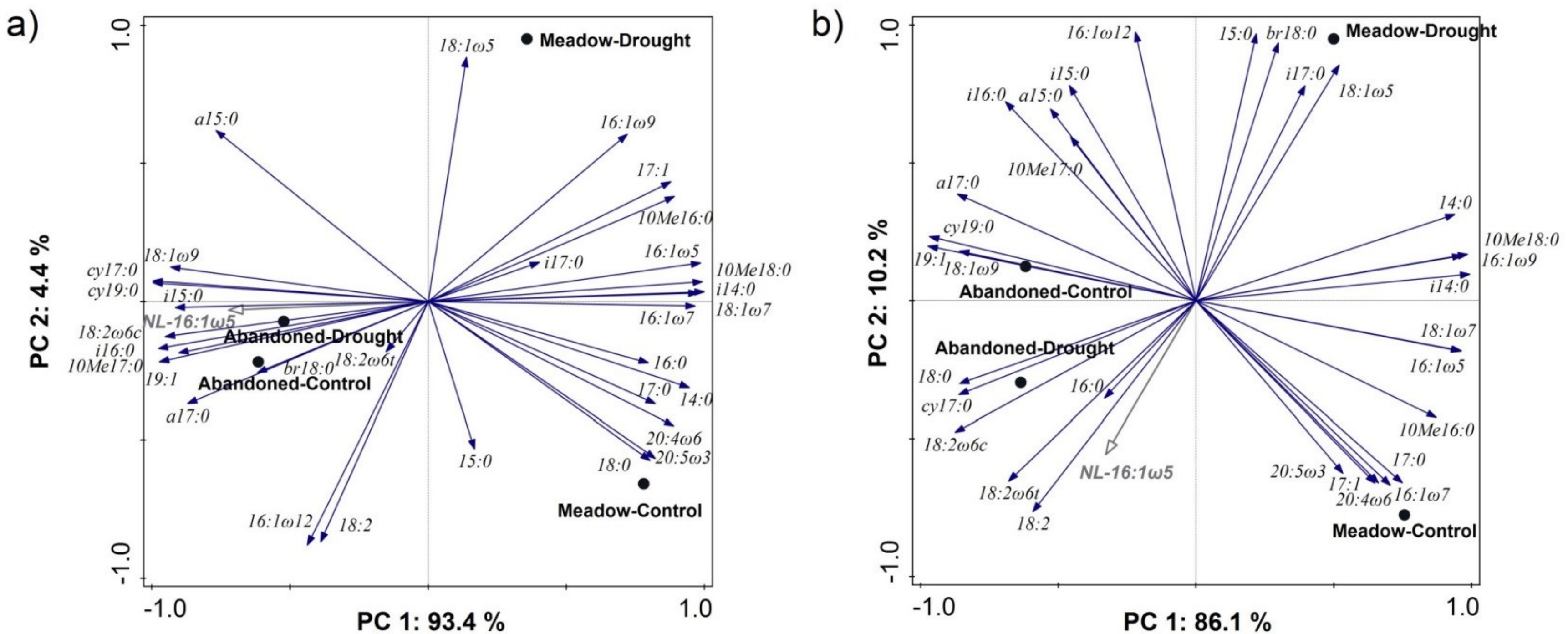

FIGURE S3 Principal component analyses (PCAs) of PLFA abundances in mol-% a) at peak drought (resistance) and b) in the recovery phase (resilience) in control and drought monoliths of abandoned grassland and meadow. The content of the NLFA 16:1ω5 is added as supplementary variable. The PCAs were done using the Canoco 5 software (Microcomputer Power, Ithaca, USA) on log-transformed data (as recommended by the software).

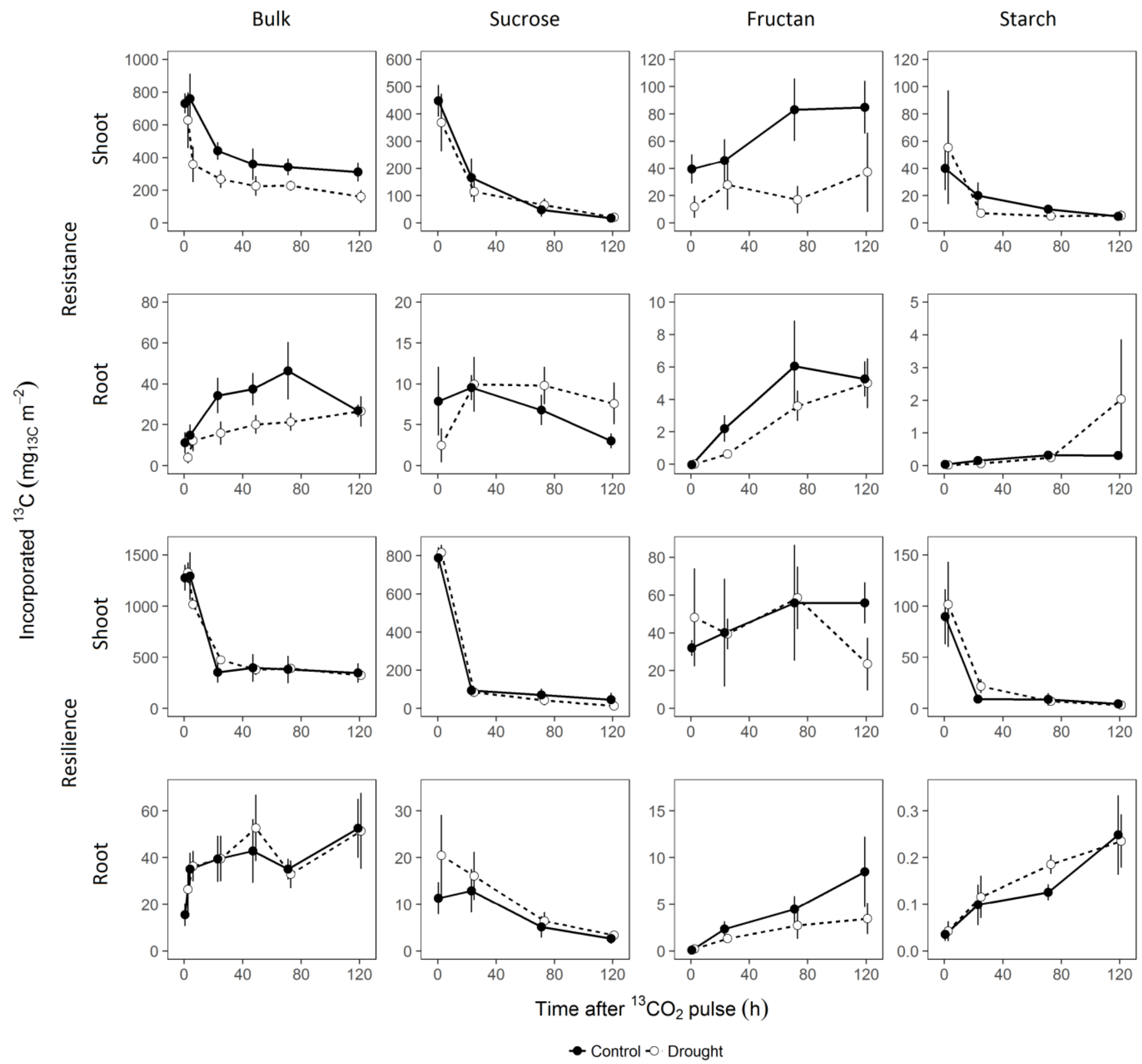

FIGURE S4 Abandoned grassland: ^{13}C tracer dynamics in bulk samples, sucrose, fructan and starch from shoots and roots of control (closed circles, solid line) and drought (open circles, dashed line) monoliths; after ^{13}C pulse labelling during peak drought (Resistance, top) and after rewetting (Resilience, bottom). Error bars show ± SE (n = 3).

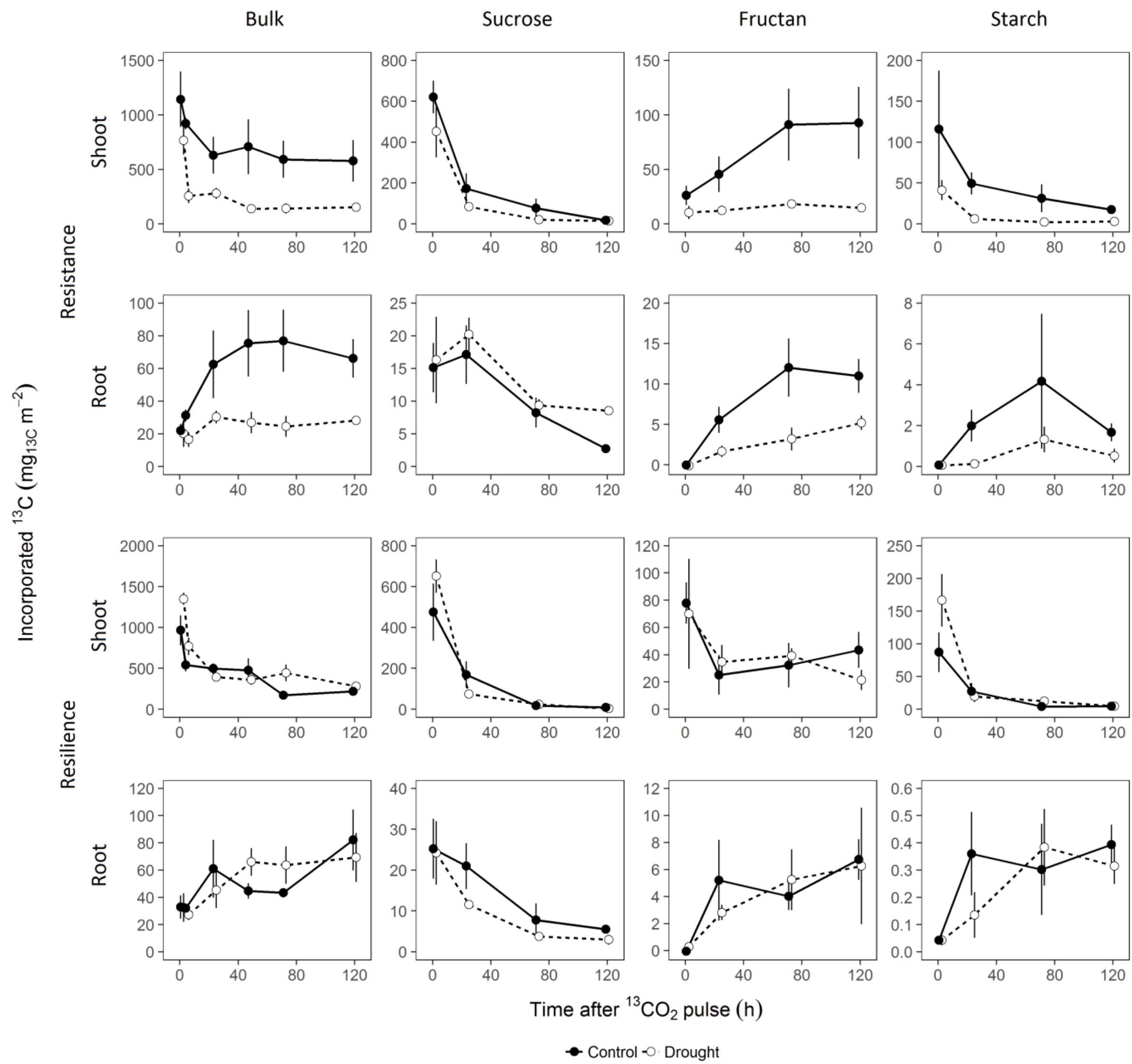

FIGURE S5 Meadow: ^{13}C tracer dynamics in bulk samples, sucrose, fructan and starch from shoots and roots of control (closed circles, solid line) and drought (open circles, dashed line) monoliths; after ^{13}C pulse labelling during peak drought (Resistance, top) and during recovery (Resilience, bottom). Error bars show ± SE (n = 3).

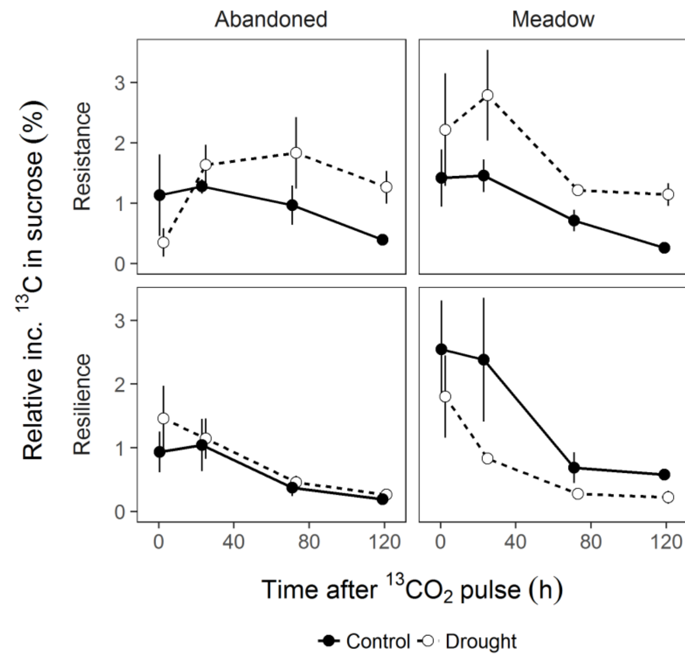

FIGURE S6 Relative incorporated ^{13}C (in % compared to the total ^{13}C uptake in μg_{13C} m^{-2}) in root sucrose of control (closed circles, solid line) and drought (open circles, dashed line) monoliths; from the abandoned grassland (left) and the meadow (right); after ^{13}C pulse labelling during peak drought (Resistance, top) and during recovery (Resilience, bottom). Error bars show ± SE (n = 3).

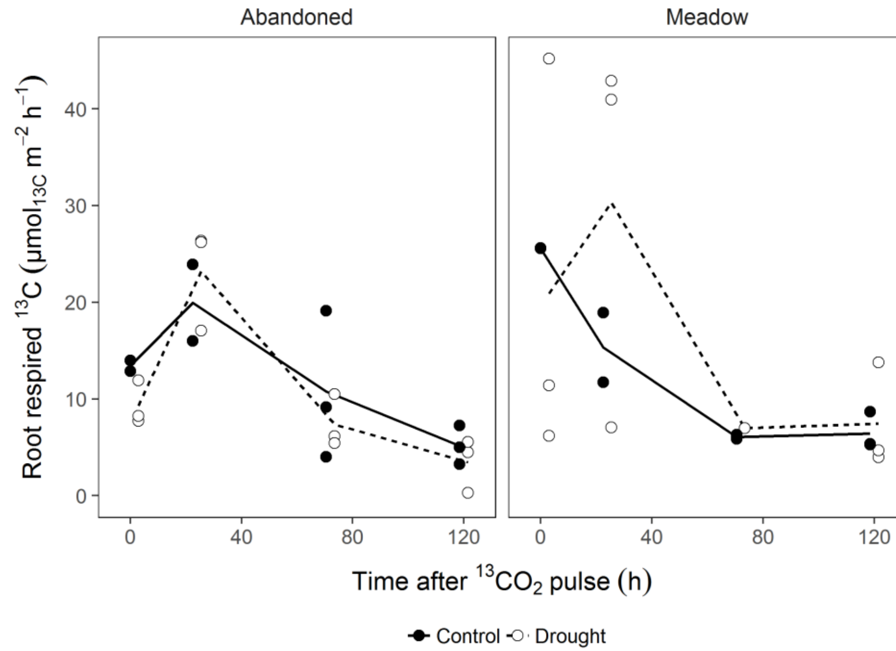

FIGURE S7 ^{13}C tracer dynamics of root respired CO_2 at 15 °C from abandoned grassland (left) and meadow (right) after the resilience pulse labelling (2½ weeks after termination of drought). Depicted are single measurements points as circles and corresponding average values as lines of control (closed circles, solid line) and drought (open circles, dashed line) monoliths.

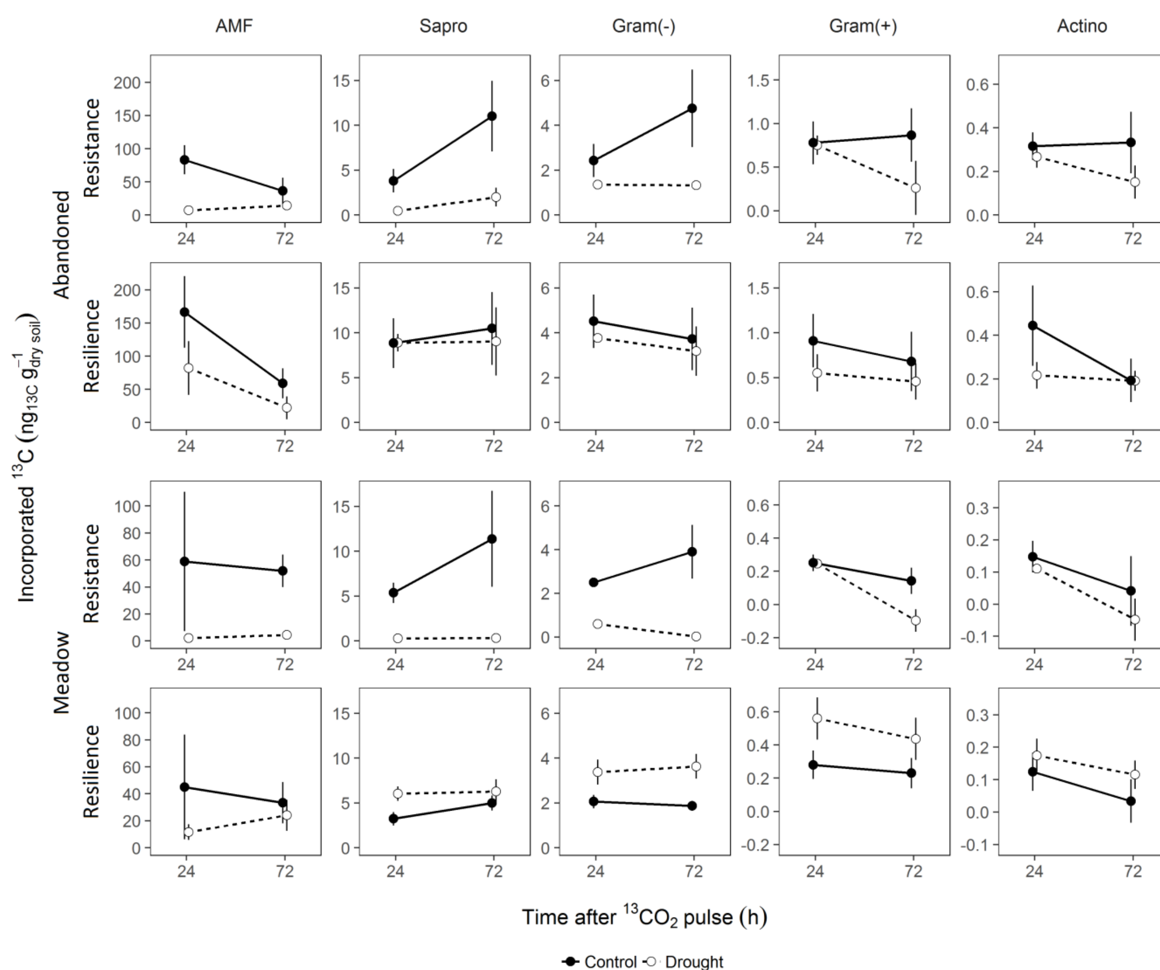

FIGURE S8 ^{13}C tracer dynamics in marker fatty acids for arbuscular mycorrhiza fungi (AMF), saprotrophic fungi (Sapro), Gram-negative bacteria (Gram(-)) and Gram-positive bacteria (Gram(+)), actinobacteria (Actino) of control (closed circles, solid line) and drought (open circles, dashed line) monoliths from abandoned grassland (top) and meadow (bottom); after ^{13}C pulse labelling during peak drought (Resistance) and during recovery (Resilience). Error bars show ± SE (n = 3), except for AMF from meadow control monoliths 24 h after labelling (resistance and resilience) with ± SE (n = 2).

References:

Hasibeder, R., Fuchslueger, L., Richter, A. & Bahn, M. (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytologist*, **205**, 1117–1127.

Ingrisch, J., Karlowsky, S., Anadon-Rosell, A., Hasibeder, R., König, A., Augusti, A., Gleixner, G. & Bahn, M. (2017) Land Use Alters the Drought Responses of Productivity and CO2 Fluxes in Mountain Grassland. *Ecosystems*.

Karlowsky, S., Augusti, A., Ingrisch, J., Hasibeder, R., Lange, M., Lavorel, S., Bahn, M. & Gleixner, G. (2017) Data from: Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. *Dryad Digital Repository*, doi:10.5061/dryad.3s57p.

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Chapter 3:

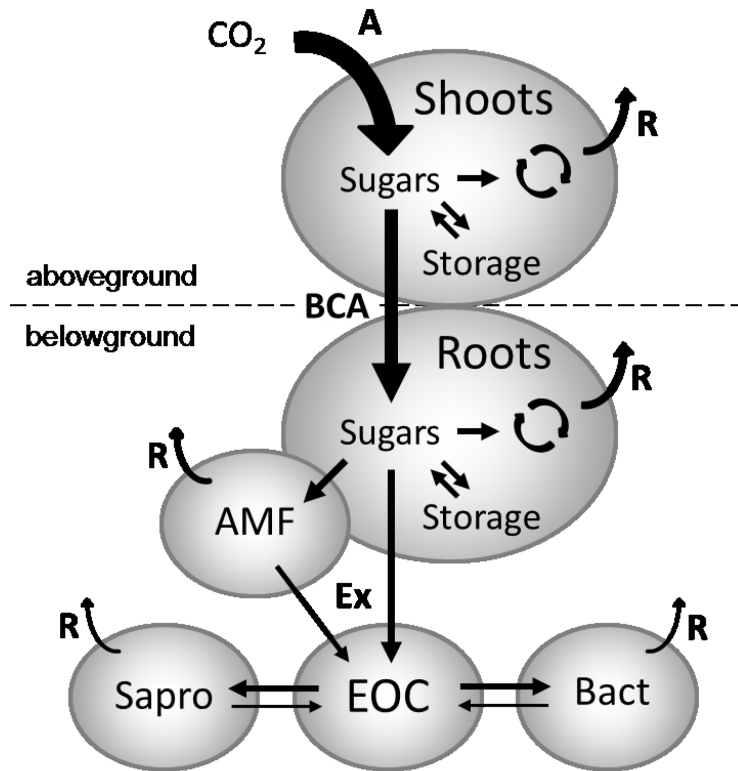
Supplementary Table S1. Planting schemata of mesocosms, with each scheme replicated in six mesocosms.

Species	Scheme 1	Scheme 2	Scheme 3	Scheme 4
Deschampsia cespitosa	6	15	2	5
Festuca rubra	7	7	4	4
Dactylis glomerata	12	3	3	1
Leontodon hispidus	2	5	5	18
Geranium sylvaticum	5	2	18	4
Trifolium repens	4	4	4	4

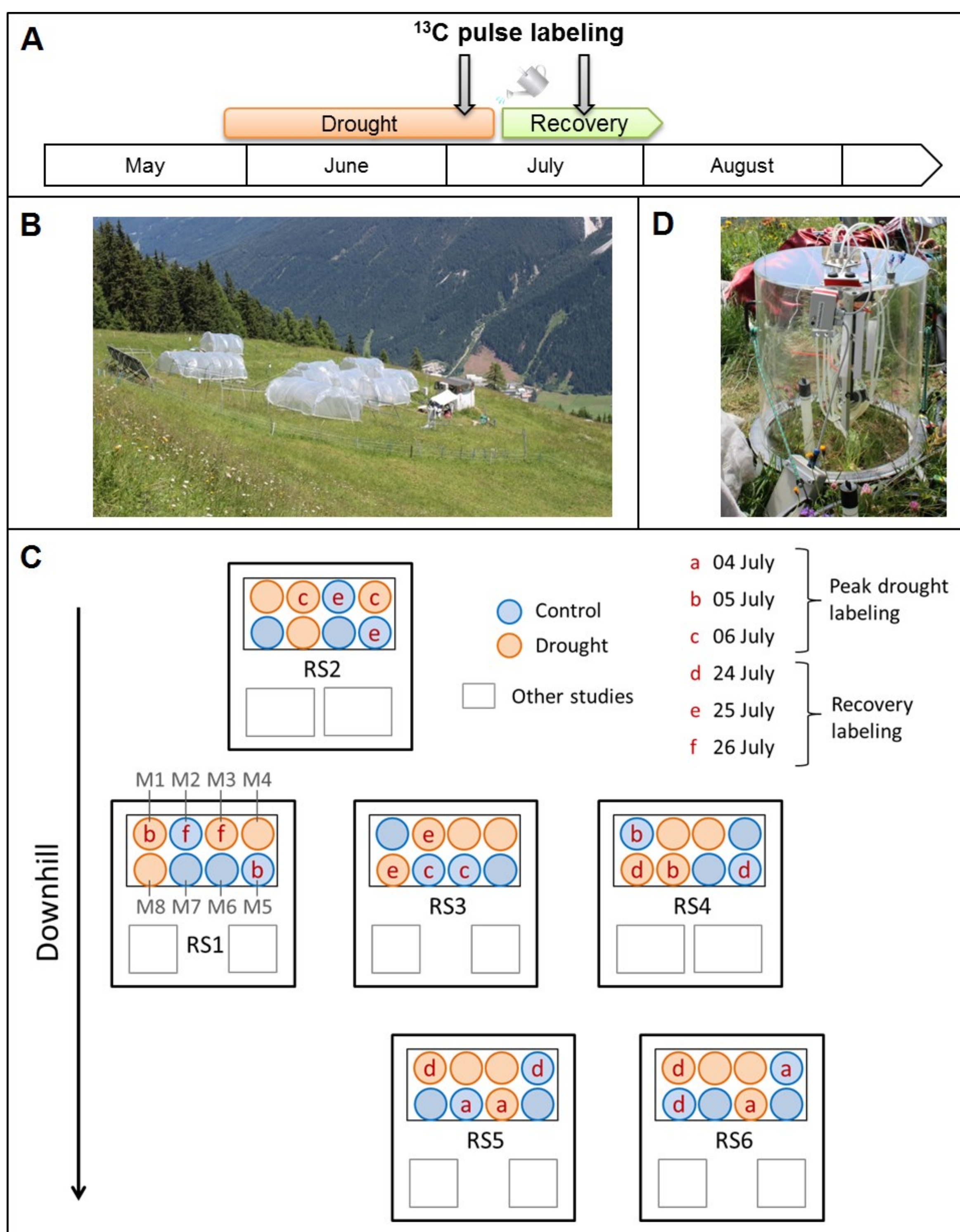
Supplementary Table S2. Concentrations and 13C isotope content of CO2 in the labeling chamber measured on CO2 stable isotope analyzer (Picarro G2201i Analyzer, Picarro Inc., Santa Clara, CA, USA).

Labeling	Mesocosm	n[a]	CO_2 concentration				atom% ^{13}C			
			min	max	mean	SD	min	max	mean	SD
Peak drought	R31.M1	44	470	896	648	117	31	64	44	10
	R31.M5	47	320	927	580	151	56	83	69	8
	R32.M2	51	453	721	569	70	29	64	45	8
	R32.M4	34	405	802	613	95	53	77	64	7
	R33.M6	52	397	755	566	106	44	72	60	7
	R33.M7	61	337	704	516	89	41	75	61	10
	R34.M1	40	355	750	538	109	47	80	65	10
	R34.M7	40	448	730	584	83	36	60	49	7
	R35.M6	46	471	700	600	63	30	57	43	6
	R35.M7	41	355	751	522	112	30	64	46	9
	R36.M4	42	400	727	530	88	45	72	60	7
	R36.M6	46	372	735	548	105	22	70	42	14
Recovery	R31.M2	45	520	802	654	74	59	77	70	5
	R31.M3	32	546	828	691	77	59	77	67	4
	R32.M3	25	465	776	611	92	50	70	59	7
	R32.M5	30	327	744	565	105	46	79	66	8
	R33.M2	32	484	774	604	75	44	64	54	6
	R33.M8	34	530	1046	752	144	55	78	62	6
	R34.M5	10	607	812	696	62	59	67	62	2
	R34.M8	10	629	822	729	74	60	68	64	3
	R35.M1	28	456	760	602	81	48	69	59	6
	R35.M4	19	544	1071	801	170	57	76	68	6
	R36.M1	28	532	910	677	129	55	74	64	6
	R36.M8	20	548	862	668	88	55	72	63	5

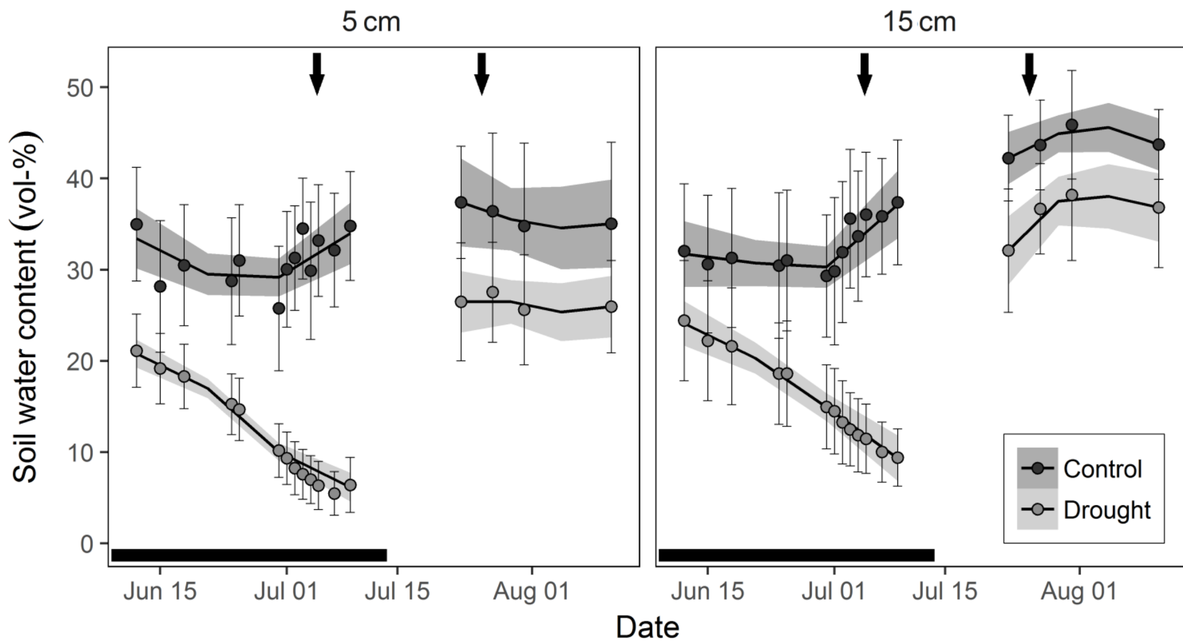
[a] n is the number of sufficient quality measurement points during the labeling period.



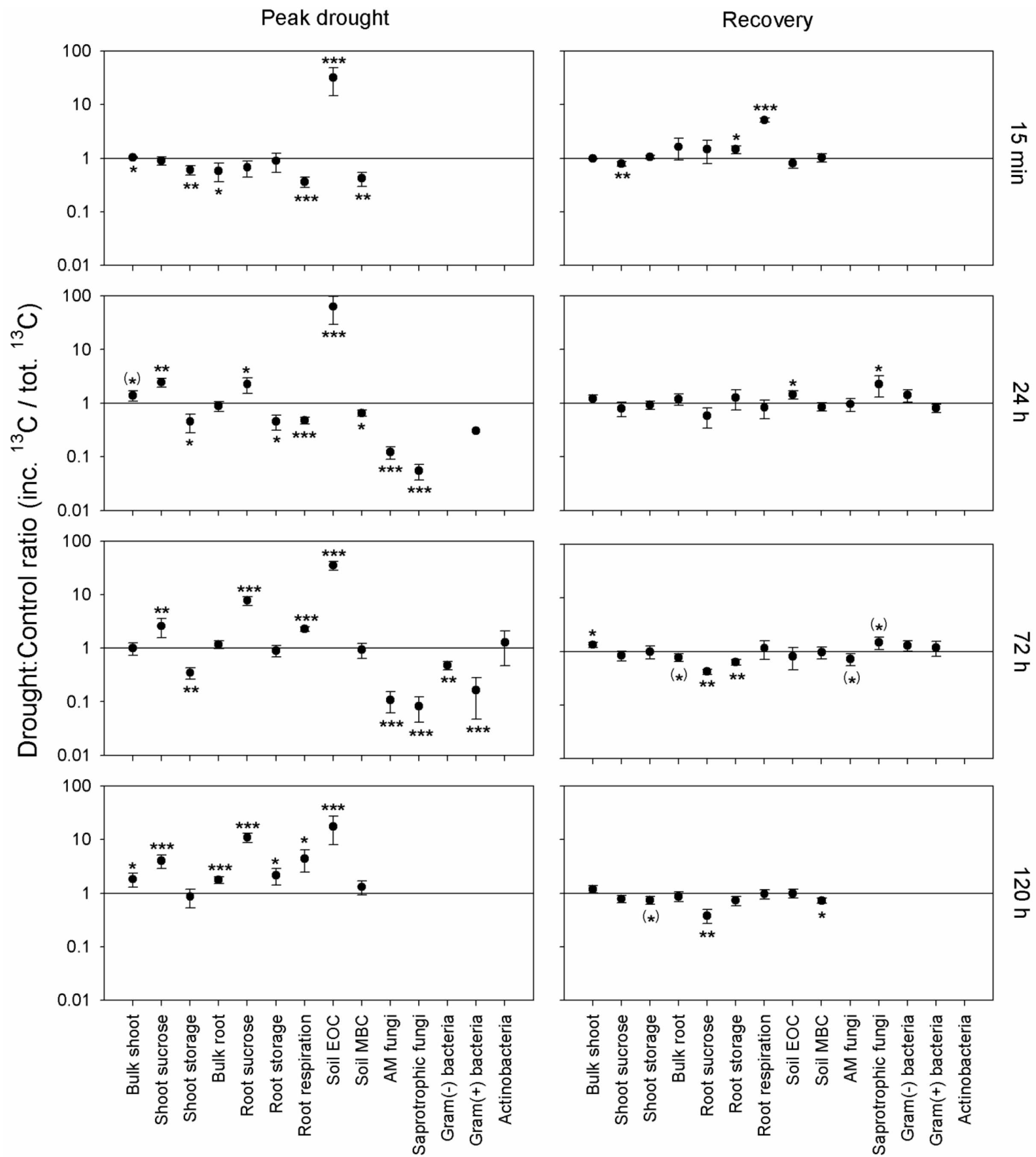
Supplementary Figure S1. Hypothetical pathway of newly assimilated carbon in the plant-rhizosphere system of grasslands (simplified). New carbon from assimilation (A) in shoots is either transformed for storage, used for growth and maintenance, associated with losses by respiration (R), or allocated to roots (belowground carbon allocation, BCA). Similarly, carbon in roots is either stored or used or further transferred to the rhizosphere and its inhabiting microorganisms. Carbon transfer to microbial biomass is possible directly through mycorrhizal interactions with arbuscular mycorrhizal fungi (AMF) or indirectly through exudation (Ex) into the rhizosphere/hyphosphere and uptake by saprotrophic fungi (Sapro) or bacteria (Bact). The extractable organic carbon (EOC) represents an intermediate pool, including exuded compounds as well as residues from dead cells, which can be accessed by saprotrophic fungi and soil bacteria.



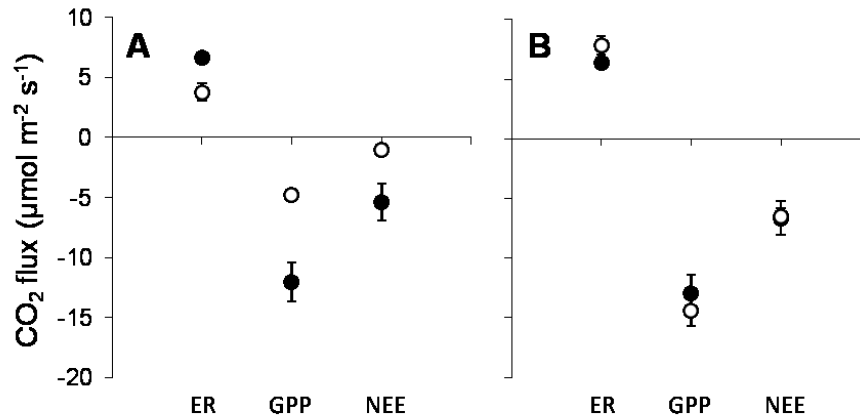
Supplementary Figure S2. Experimental timeline indicating the two ^{13}C labeling campaigns at peak drought and recovery **(A)**; photography of the study site with transparent rainout shelters and a climate chamber used for operating the Picarro $^{13}CO_2$ analyzer **(B)**; scheme of rain-out shelters (RS1-RS6) and mesocosms (M1-M8, see example for RS1), indicated are treatment (blue, control; orange, drought) and labeling dates **(C)**; and plexiglass labeling chamber on top of a mesocosm, sealed by a gas-tight rubber gasket, and cooled with ice water tubes coiled around several fans **(D)**.



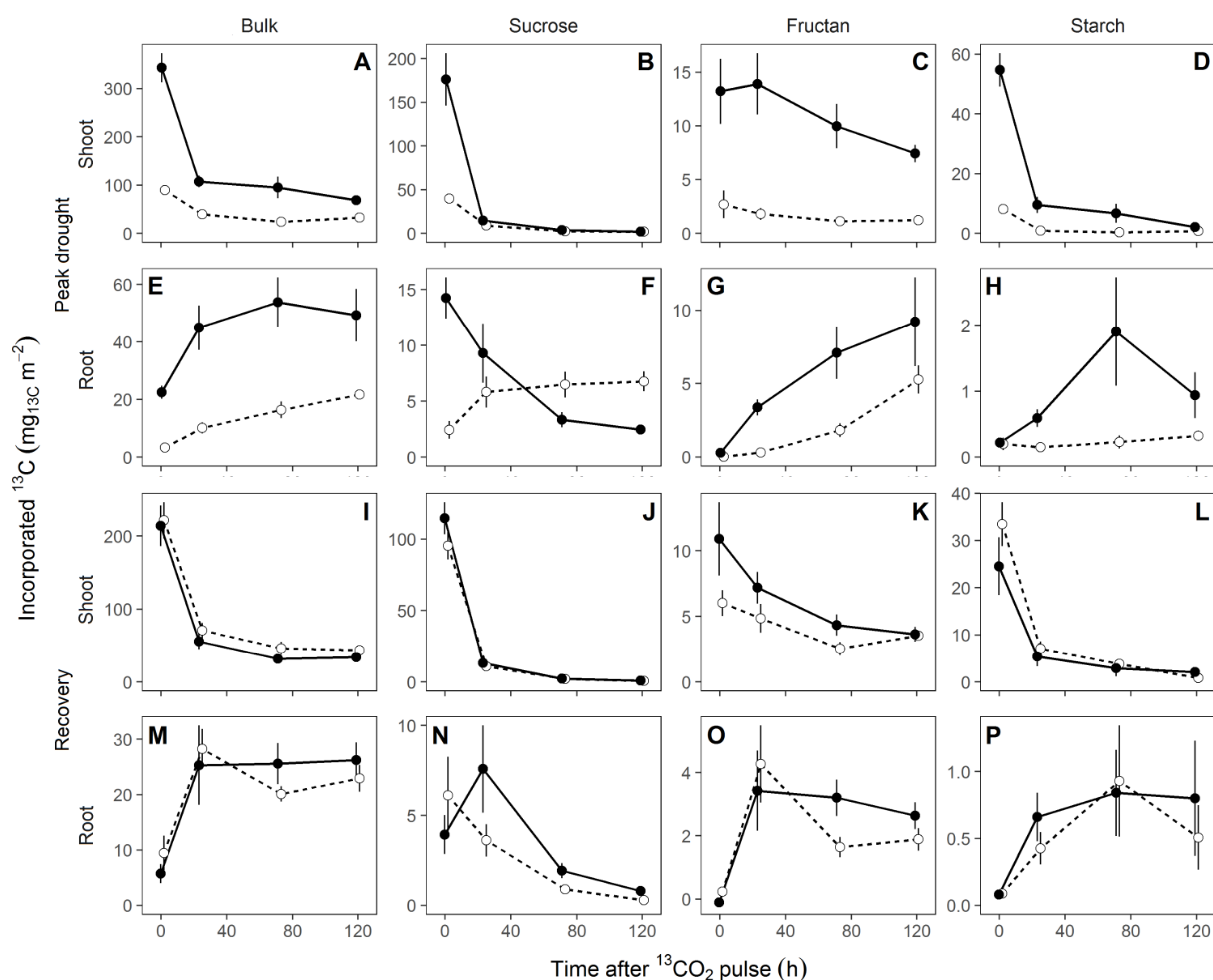
Supplementary Figure S3. Volumetric soil water content at soil depths of 5 cm (left) and 15 cm (right). The black bar above the horizontal axes indicates the time of drought simulation and arrows denote the dates of the two ^{13}C pulse labeling campaigns. Circles show mean values ± SD (n = 12) of control (dark gray) and drought (light gray) treatments. Black lines show the results from local polynomial regression fitting ('loess' function from the R base package, evaluated at n = 4 data points) for each treatment and gray areas the corresponding 95% confidence intervals (control, dark gray; drought, light gray); breaks result from the rewetting event leading to sudden increases in soil water content, which cannot be reflected by the polynomial line fitting.



Supplementary Figure S4. Effects of drought on C allocation patterns at the peak drought and labeling campaigns. Shown is the drought to control ratio of the ^{13}C amount in each pool that was recovered from total ^{13}C uptake (relative ^{13}C allocation) at the four different sampling times after pulse labeling. Black circles represent the mean of n = 1 to n = 6 control/drought pairs. Error bars were obtained by propagating the SE from the replicates of each treatment, control and drought respectively. Asterisks indicate significance levels of drought effects (df = 1) from linear mixed-effects models (R package 'lme4') using treatment as fixed factor and labeling pair and individual mesocosm as random factors; ***$P_{\chi^2} < 0.001$, **$P_{\chi^2} < 0.01$, *$P_{\chi^2} < 0.05$, $^{(*)}P_{\chi^2} < 0.1$.

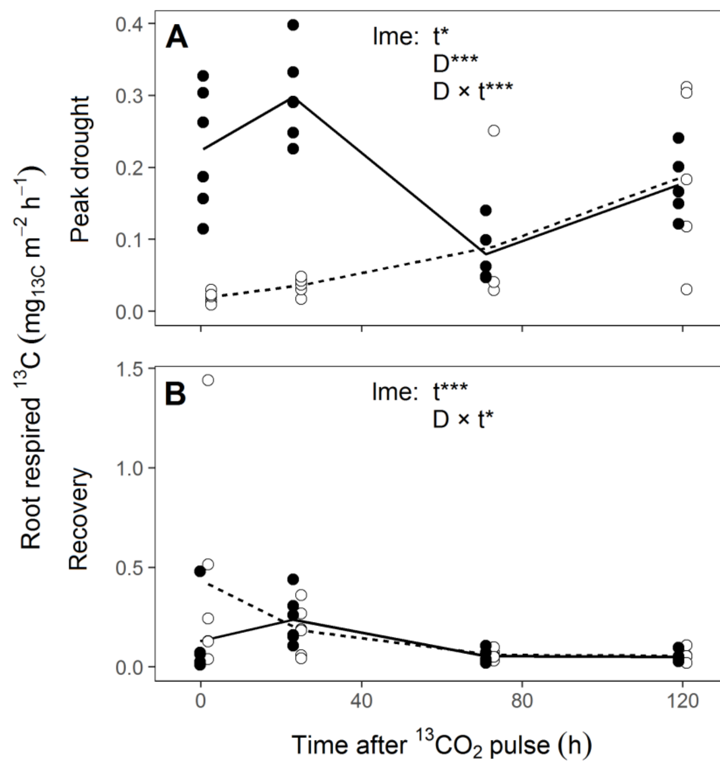


Supplementary Figure S5. Photosynthetic and respiration rates of control (closed circles) and drought (open circles) mesocosms at the peak drought **(A)** and recovery **(B)** labeling campaigns. Error bars represent SE of n = 6 (control mesocosms, peak drought labeling), n = 5 (drought mesocosms, peak drought labeling) or n = 4 (control and drought mesocosms, recovery labeling) mesocosms. Measurements of ecosystem respiration (ER) and net ecosystem exchange (NEE) were done by analyzing changes of chamber CO_2 concentrations in the labeling chamber, under light (NEE) and dark (ER) conditions for a period of 1 minute each, on infrared gas analyzer (Licor 840A, Lincoln, NE, USA). Gross primary productivity (GPP), i.e., the photosynthetic rate, was calculated as: GPP = NEE – ER.

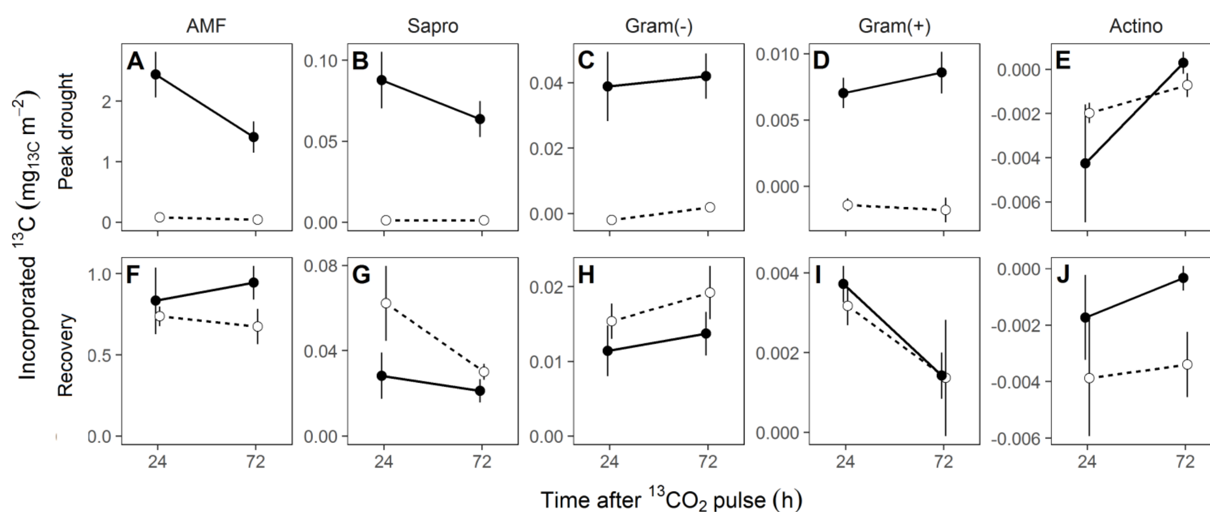


lme:	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
t	***	***	**	***	***	n.s.	***	*	***	***	***	***	***	***	***	***
D	***	**	***	***	***	n.s.	***	**	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.
D × t	(*)	***	n.s.	(*)	n.s.	***	*	n.s.	n.s.	n.s.	n.s.	**	(*)	*	*	n.s.

Supplementary Figure S6. Dynamics of ^{13}C tracer incorporation into bulk shoots and roots as well as their carbohydrates from control (closed circles, solid lines) and drought (open circles, dashed lines) treatments at the peak drought **(A-H)** and the recovery **(I-P)** labeling campaigns. Error bars represent an SE of n = 6 (n = 5 for shoot starch, recovery, drought, 72 h). Levels of significance for time after labeling (t; df = 3), drought treatment (D; df = 1) and the interaction of both (D × t; df = 3) were obtained from linear mixed-effects (lme) models using the R package 'lme4'; ***$P_{\chi^2} < 0.001$, **$P_{\chi^2} < 0.01$, *$P_{\chi^2} < 0.05$ and (*)$P_{\chi^2} < 0.1$. Note that the labeling time was only 30 min at the recovery labeling compared to 75 min at the peak drought labeling and that the absolute values cannot be compared between both labeling campaigns.

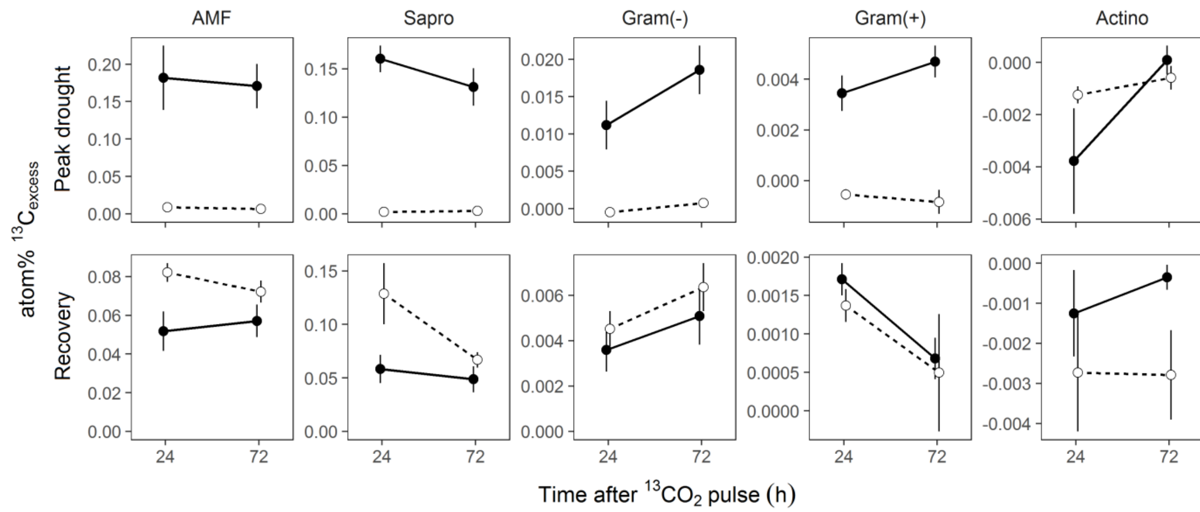


Supplementary Figure S7. Dynamics of ^{13}C tracer incorporation into root respired CO_2 at 15 °C from control (closed circles, solid lines) and drought (open circles, dashed lines) treatments at the peak drought **(A)** and the recovery **(B)** labeling campaigns. Circles show single values for each mesocosm and lines the mean values of n = 4-6 mesocosms at each sampling time. Levels of significance for time after labeling (t; df = 3), drought treatment (D; df = 1) and the interaction of both (D × t; df = 3) were obtained from linear mixed-effects (lme) models using the R package 'lme4'; ***P_{χ^2} < 0.001 and *P_{χ^2} < 0.05. Note that the labeling time was only 30 min at the recovery labeling compared to 75 min at the peak drought labeling and that the absolute values cannot be compared between both labeling campaigns.

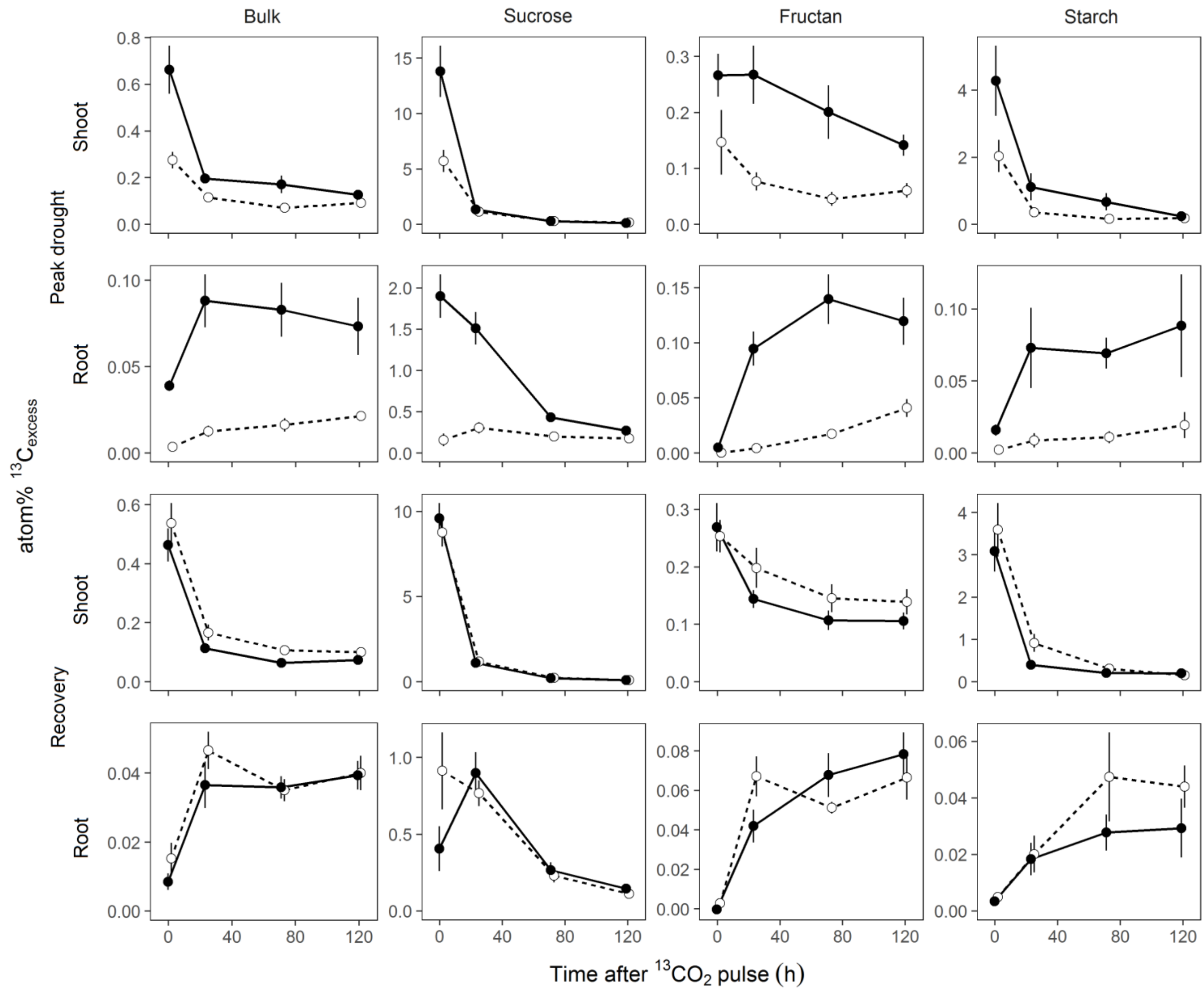


lme:	A	B	C	D	E	F	G	H	I	J
t	**	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	**	n.s.
D	***	***	***	***	n.s.	n.s.	(*)	n.s.	n.s.	*
D × t	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

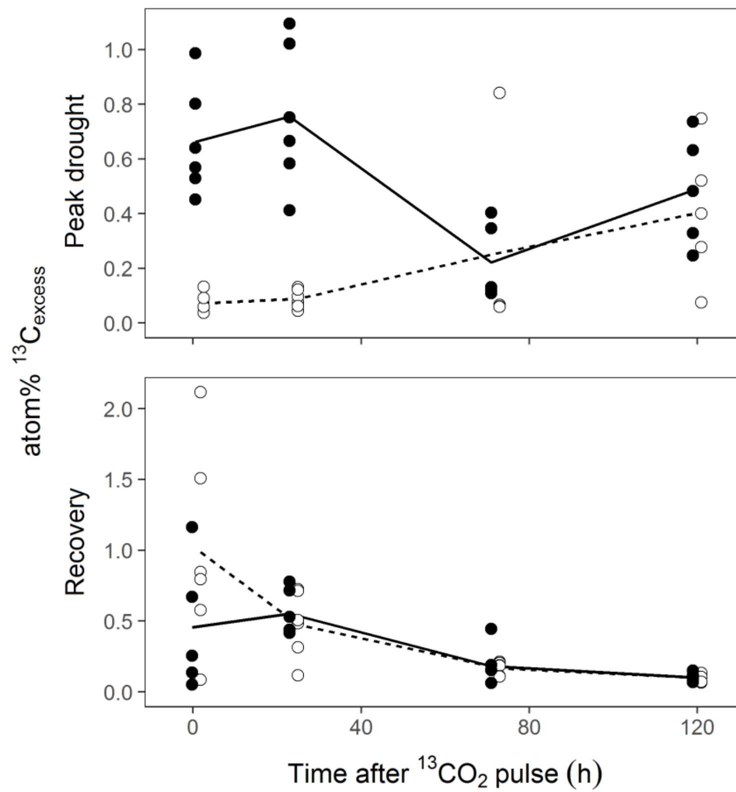
Supplementary Figure S8. Dynamics of ^{13}C tracer incorporation into microbial marker lipids from soil of control (closed circles, solid lines) and drought (open circles, dashed lines) mesocosms at the peak drought **(A-E)** and recovery **(F-J)** labeling campaigns. Error bars represent SE of n = 6. Levels of significance for time after labeling (t; df = 3), drought treatment (D; df = 1) and the interaction of both (D × t; df = 3) were obtained from linear mixed-effects (lme) models using the R package 'lme4'; ***$P_{\chi^2} < 0.001$, **$P_{\chi^2} < 0.01$, *$P_{\chi^2} < 0.05$ and (*)$P_{\chi^2} < 0.1$. Note that the labeling time was only 30 min at the recovery labeling compared to 75 min at the peak drought labeling and that the absolute values cannot be compared between the labeling campaigns. Negative incorporated ^{13}C values result from negligible ^{13}C tracer uptake and natural variations in ^{13}C content between labeled and unlabeled reference samples. Actino, actinobacteria; AMF, arbuscular mycorrhizal fungi; Sapro, saprotrophic fungi; Gram(−)/(+) = Gram-negative/positive bacteria.



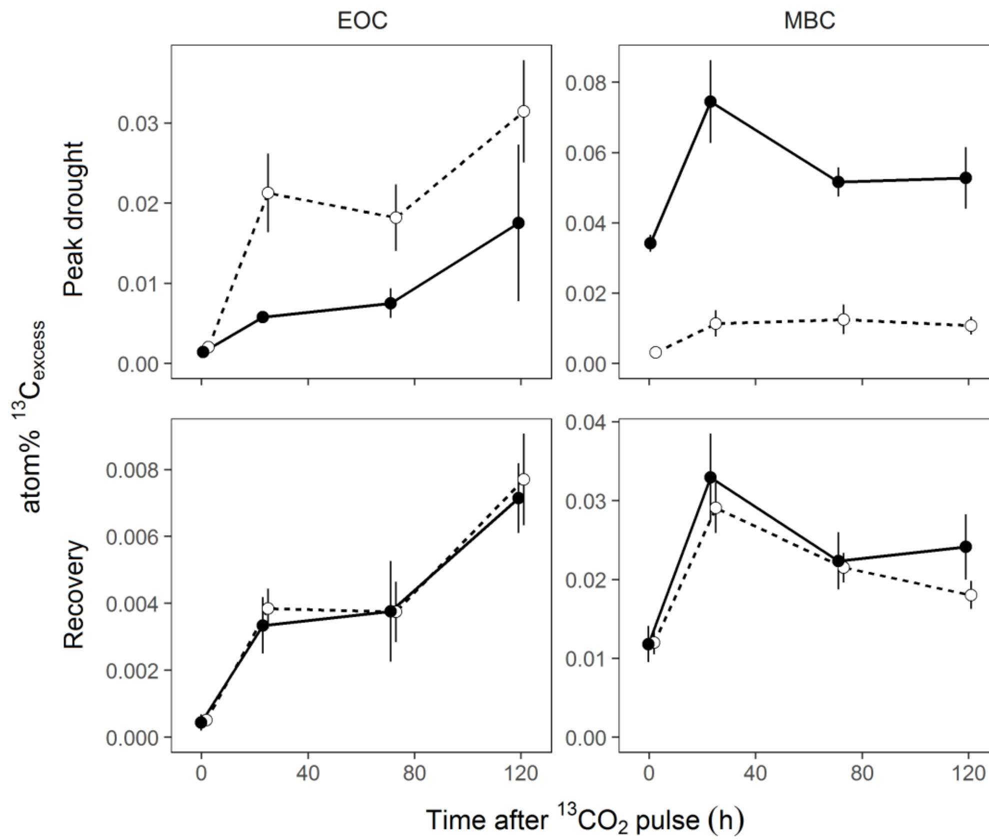
Supplementary Figure S9. Dynamics of atom% $^{13}C_{excess}$ in microbial marker lipids from soil of control (closed circles, solid lines) and drought (open circles, dashed lines) mesocosms at the peak drought and recovery labeling campaigns. AMF: arbuscular mycorrhizal fungi, Sapro: saprotrophic fungi, Gram(−)/(+): Gram-negative/positive bacteria.



Supplementary Figure S10. Dynamics of atom% 13Cexcess in bulk shoots and roots as well as their carbohydrates from control (closed circles, solid lines) and drought (open circles, dashed lines) treatments at the peak drought and the recovery labeling campaigns. Error bars represent SE of n = 6 (n = 5 for shoot starch, recovery, drought, 72 h).



Supplementary Figure S11. Dynamics of atom% $^{13}C_{excess}$ in root respired CO_2 at 15 °C from control (closed circles, solid lines) and drought (open circles, dashed lines) treatments at the peak drought and the recovery labeling campaigns. Circles show single values for each mesocosm and lines the mean values of n = 4-6 mesocosms at each sampling time.



Supplementary Figure S12. Dynamics of atom% $^{13}C_{excess}$ in extractable organic carbon (EOC) and microbial biomass carbon (MBC) from soil of control (closed symbols and solid lines) and drought-treated (open symbols and dashed lines) mesocosms at the peak drought and recovery labeling campaigns. Error bars show SE of n = 6 mesocosms.

Chapter 4:

Table S1 Planting schemata of mesocosms, with each scheme replicated in six mesocosms.

Species	Grass-dominated		Forb-dominated	
	High productivity	Low productivity	High productivity	Low productivity
Deschampsia cespitosa	6	15	2	5
Festuca rubra	7	7	4	4
Dactylis glomerata	12	3	3	1
Leontodon hispidus	2	5	5	18
Geranium sylvaticum	5	2	18	4
Trifolium repens	4	4	4	4

Table S2 Species-specific shoot biomass harvested after each labeling campaign (peak drought/recovery), and plant community parameters for each mesocosm.

Labeling	Treatment	Mesocosm	Species biomass (g mesocosm^{-1})						SDI	Ev[a]	Gr:Fo	CWM-SLA	Ex:Co
			Dac glo	Des ces	Fes pra	Ger syl	Leo his	Tri rep					
Peak drought	Control	R31-M5	0.5	4.5	1.7	0.6	3.4	11.7	1.32	0.74	0.43	18.9	2.62
		R33-M6	0.0	4.7	1.7	0.2	4.5	0.8	1.26	0.70	1.18	17.5	0.85
		R33-M7	2.2	8.7	1.8	0.4	0.2	1.4	1.24	0.69	6.32	15.8	0.40
		R34-M1	0.8	5.5	3.5	0.3	0.6	7.9	1.35	0.76	1.11	16.4	1.08
		R35-M7	0.6	9.9	1.9	0.1	2.4	6.1	1.31	0.73	1.45	13.6	0.78
		R36-M4	1.8	4.1	5.1	5.1	3.1	1.2	1.68	0.94	1.17	16.3	1.22
	Drought	R31-M1	0.8	2.4	1.7	2.0	1.4	6.8	1.54	0.86	0.47	16.9	2.73
		R32-M2	0.6	1.5	1.2	0.6	4.2	0.0	1.32	0.74	0.69	13.4	1.96
		R32-M4	0.2	6.4	1.0	0.1	1.6	1.4	1.23	0.68	2.39	13.5	0.46
		R34-M7	1.4	2.2	1.5	0.4	0.4	1.8	1.63	0.91	1.97	16.7	1.08
		R35-M6	2.3	4.2	0.1	3.6	5.2	0.1	1.41	0.78	0.75	16.6	2.59
		R36-M6	0.8	1.2	5.8	0.1	1.3	4.0	1.37	0.77	1.47	12.3	0.88
	Sign.[a]		n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	°	n.s.
Recovery	Control	R31-M2	0.6	5.8	0.5	0.2	7.1	0.7	1.17	0.65	0.85	13.2	1.37
		R32-M3	4.0	9.2	1.6	0.3	1.5	0.8	1.32	0.74	5.66	14.7	0.62
		R32-M5	2.8	6.3	0.7	3.8	4.4	0.8	1.57	0.88	1.09	18.1	1.69
		R34-M5	0.3	2.6	2.6	2.0	0.7	1.3	1.61	0.90	1.35	13.7	0.84
		R35-M4	0.6	4.6	0.6	0.5	10.0	1.5	1.21	0.67	0.49	17.7	2.42
		R36-M8	4.8	7.1	2.7	0.4	1.5	1.9	1.52	0.85	3.83	15.7	0.88
	Drought	R31-M3	1.5	5.1	2.3	0.4	1.3	2.6	1.58	0.88	2.07	15.2	0.79
		R33-M2	1.2	5.5	1.1	1.2	4.4	0.7	1.49	0.83	1.24	14.4	1.13
		R33-M8	0.1	8.9	1.4	0.0	1.6	0.0	1.06	0.59	6.53	13.0	0.16
		R34-M8	1.1	4.2	0.7	0.3	4.3	0.3	1.34	0.75	1.24	15.6	1.21
		R35-M1	5.3	7.0	2.5	0.3	2.5	0.0	1.40	0.78	5.39	14.8	0.85
		R36-M1	1.6	7.8	0.8	0.6	7.6	0.6	1.30	0.72	1.15	15.9	1.21
	Sign.[a]		n.s.	n.s.	n.s.	°	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	°

[a]Levels of significance for drought effects; statistics were performed using permutational ANOVA from the R package 'lmPerm'; ***: $P_{aovp} < 0.001$, °: $P_{aovp} < 0.1$, n.s.: not significant
CWM-SLA, community weighted mean – specific leaf area; Ev, evenness of plant species; Ex:Co, exploitative to conservative species ratio; Gr:Fo, grass to forb ratio; SDI, Shannon diversity index

Table S3 Peak drought labeling: F-values for the effects of drought treatment (D, df = 1), plant composition (df = 1) and their interaction (df =1) on plant biomass and ^{13}C tracer incorporation in plant and soil pools, based on linear models (R base package) performed separately for each plant compositional parameter.

	Variable	Evenness (Ev)			Grass:Forb (Gr:Fo)			Exploitative:Conservative (Ex:Co)			CWM_SLA (CS)			Legume (Leg)		
		D	Ev	Ev × D	D	Gr:Fo	Gr:Fo × D	D	Ex:Co	Ex:Co × D	D	CS	CS × D	D	Leg	Leg × D
Biomass	Total	1.2	3.0	0.3	1.4	3.0	2.4	1.1	1.4	0.8	0.9	0.0	0.4	1.0	0.0	1.0
	Shoots	14.1**	3.1	1.9	9.9*	0.9	0.2	9.6*	0.8	0.1	10.6*	0.0	1.6	8.9*	0.2	0.0
	Roots	8.3*	2.2	0.0	11.4**	**3.6°**	2.4	8.1*	1.0	0.9	6.6*	0.0	0.1	7.8*	0.1	1.4
	Root:Shoot	18.5**	0.0	0.3	19.3**	0.3	0.5	17.9***	0.0	0.1	22.6**	0.0	0.6	19.0**	0.5	0.1
Plant ^{13}C	Total uptake	98.9***	0.1	0.3	140.6***	1.9	1.9	272.3***	3.3	11.6**	325.3***	**6.9***	3.5	140.2***	1.7	2.2
	Shoots	95.9***	0.3	0.1	103.6***	0.9	0.2	128.9***	2.9	0.5	190.9***	**8.6***	0.1	124.2***	1.6	1.3
	Roots	28.0***	0.0	0.0	39.4***	2.0	1.3	100.8***	**5.1°**	15.8**	135.3***	**7.9***	11.3*	40.6***	1.4	2.2
	Root:Shoot	0.1	0.8	0.0	0.1	0.2	2.3	0.1	1.8	4.8°	0.2	0.3	7.4*	0.1	0.5	0.3
	Root respired	19.5**	0.9	0.2	18.2**	0.1	0.4	17.5**	0.1	0.0	18.5**	0.1	0.5	17.8**	0.2	0.1
Carbohydrate ^{13}C	Shoot sucrose	48.8***	0.1	0.5	56.4***	0.9	1.0	113.1***	**4.1°**	7.7*	66.9***	2.4	1.3	80.6***	1.9	4.2°
	Shoot fructan	24.9**	1.3	0.3	21.6**	0.0	0.3	33.9***	1.0	4.1°	47.7***	1.6	2.0	39.8***	**4.0°**	3.3
	Shoot starch	35.3***	0.9	0.8	29.3***	0.0	0.0	32.8***	0.5	0.5	34.3***	0.8	0.6	63.7***	**4.3°**	5.2°
	Root sucrose	3.5°	2.0	0.7	4.9°	**4.3°**	2.6	3.2	0.0	1.8	0.5	0.0	0.0	2.9	0.2	0.6
	Root fructan	12.1**	0.0	0.8	14.5**	2.0	0.5	11.1*	0.0	0.1	11.5**	0.2	0.1	12.1**	0.7	0.1
	Root starch	12.9**	1.9	0.8	13.7**	3.1	0.3	12.0**	1.9	0.1	11.3**	1.4	0.0	9.8*	0.1	0.1
	Sucroot:shoot	7.2*	0.1	0.2	8.6*	0.0	1.8	9.6*	2.1	0.9	7.5*	0.4	0.3	7.2*	0.1	0.1
Soil ^{13}C	EOC	35.4***	0.2	0.8	34.7***	0.6	0.2	39.0***	1.7	0.2	35.4***	0.1	0.1	49.1***	1.4	0.9
	MBC	86.4***	0.4	0.1	143.0***	0.0	5.9*	140.8***	0.3	5.4*	121.4***	0.3	1.2	138.8***	0.0	5.5*
	AM fungi	48.2***	0.5	0.0	91***	0.8	7.4*	76.5***	2.1	3.5°	118.2***	1.4	1.8	62.9***	0.3	2.9
	Sapro. fungi	496.9***	0.8	0.3	645.2***	0.1	3.7°	542.9***	1.7	0.3	450.8***	0.2	0.1	461.9***	0.5	0.0
	Gram(-) bacteria	194.1***	1.0	3.2	136.9***	0.2	0.4	128.8***	0.0	0.1	132.8***	0.3	0.0	173.0***	1.9	0.9
	Gram(+) bacteria	91.8***	0.0	1.0	84.2***	0.1	0.1	92.0***	0.3	0.7	105.6***	2.0	0.3	95.1***	0.7	0.6

Asterisks and circles indicate levels of significance; *** $P_F < 0.001$, ** $P_F < 0.01$, * $P_F < 0.05$, ° $P_F < 0.1$; bold values highlight treatment-independent effects of plant composition and underlined values highlight interaction effects of drought and plant composition with $P_F < 0.1$

AM, Arbuscular mycorrhiza; EOC, Extractable organic carbon; MBC, microbial biomass carbon; Sapro, saprotrophic

Table S4 Recovery labeling:F-values for the of drought treatment (D, df = 1), plant composition (df = 1) and their interaction (df =1) on plant biomass and ^{13}C tracer incorporation in plant and soil pools as well as ^{15}N tracer incorporation in plant material, based on linear models (R base package) performed separately for each plant compositional parameter.

	Variable	Evenness (Ev)			Grass:Forb (Gr:Fo)			Exploitative:Conservative (Ex:Co)			CWM_SLA (CS)			Legume (Leg)		
		D	Ev	Ev × D	D	Gr:Fo	Gr:Fo × D	D	Ex:Co	Ex:Co × D	D	CS	CS × D	D	Leg	Leg × D
Biomass	Total	1.5	0.0	1.9	2.0	**4.9°**	0.4	1.9	**4.4°**	0.4	1.7	1.0	1.9	1.7	0.2	2.9
	Shoots	0.9	0.0	0.9	0.9	0.0	0.6	1.0	1.1	0.2	1.2	**2.8°**	0.0	1.2	1.1	2.5
	Roots	0.3	0.0	0.8	0.7	**10.3***	2.1	0.4	2.4	0.2	0.3	0.1	1.7	31.0	0.0	0.8
	Root:Shoot	0.0	0.1	0.0	0.0	3.2	1.4	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.5	0.2
Plant ^{13}C	Total uptake	0.1	0.0	0.5	0.1	0.5	0.2	0.1	0.9	1.0	0.1	2.8	1.7	0.1	0.1	0.8
	Shoots	0.5	0.1	0.4	0.4	0.1	0.0	0.5	0.4	0.5	0.6	1.7	1.3	0.5	0.4	1.6
	Roots	0.0	0.0	0.0	0.0	**3.6°**	0.4	0.0	**3.8°**	0.1	0.0	**4.0°**	0.0	0.0	0.3	2.7
	Root:Shoot	3.4	0.7	0.4	4.1°	1.5	1.5	3.0	0.1	0.0	3.2	0.0	0.6	4.1°	1.1	1.7
	Root respired	0.8	0.7	0.5	0.8	0.7	0.0	0.7	0.4	0.0	0.8	1.3	0.0	1.3	**5.2°**	1.7
Carbohydrate ^{13}C	Shoot sucrose	1.2	0.1	0.1	1.2	0.1	0.0	1.4	0.3	1.5	2.1	**4.0°**	2.7	1.6	0.0	2.8
	Shoot fructan	3.9°	0.8	0.7	3.6°	0.2	0.8	3.5°	0.5	0.1	4.2°	2.2	0.1	4.1°	0.1	2.1
	Shoot starch	1.1	0.6	0.9	1.1	0.6	0.4	1.1	0.3	0.8	1.4	2.1	2.1	1.1	0.0	0.9
	Root sucrose	0.1	0.2	0.1	0.3	**6.4***	1.3	0.3	**9.9***	0.1	0.2	2.6	0.0	0.2	0.7	0.8
	Root fructan	0.1	0.0	0.2	0.3	**11.6****	0.0	0.3	**9.9***	0.3	0.2	**6.9***	1.6	0.1	0.0	1.6
	Root starch	0.5	0.0	0.7	0.7	1.9	2.6	0.5	0.0	1.5	0.8	0.3	5.8*	0.6	3.1	0.1
	Sucroot:shoot	3.0	0.4	0.4	6.5*	**8.8***	2.7	3.2	1.4	0.1	3.8°	0.2	2.0	3.3	1.5	0.2
Soil ^{13}C	EOC	0.3	0.9	2.9	0.3	**5.5***	1.3	0.3	**4.2°**	0.3	0.2	0.4	0.6	0.3	0.0	4.5°
	MBC	0.4	0.8	0.1	0.7	**6.7***	0.5	0.5	2.4	0.1	0.5	1.9	0.0	0.4	0.0	0.6
	AM fungi	2.4	**10.6***	0.0	1.1	0.3	0.1	1.1	0.1	0.3	1.4	2.5	0.0	1.3	0.8	1.6
	Sapro. fungi	3.3	0.5	0.1	5.0°	**4.5°**	0.4	4.1°	2.4	0.3	4.7°	**4.1°**	0.0	3.4	0.0	0.6
	Gram(-) bacteria	2.2	0.9	2.6	1.6	0.3	0.2	1.6	0.4	0.2	1.5	0.0	0.0	1.6	0.0	0.3
	Gram(+) bacteria	0.1	0.0	0.3	0.1	1.0	0.3	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.5	0.8
Plant ^{15}N	Total	5.1°	0.3	0.1	6.5*	1.6	1.0	5.2°	0.5	0.0	4.8°	0.0	0.2	6.3*	0.2	2.1
	Shoots	8.7*	0.1	0.0	9.9*	0.6	0.6	9.6*	0.9	0.0	8.7*	0.1	0.0	9.7*	0.1	0.8
	Roots	3.1	**6.5***	0.8	2.0	1.7	0.3	2.3	0.3	3.3	3.0	0.1	6.5*	3.0	**4.0°**	2.8

Asterisks and circles indicate levels of significance; ***P_F < 0.001, **P_F < 0.01, *P_F < 0.05, °P_F < 0.1; bold values highlight treatment-independent effects of plant composition and underlined values highlight interaction effects of drought and plant composition with P_F < 0.1

AM, Arbuscular mycorrhiza; EOC, Extractable organic carbon; MBC, microbial biomass carbon; Sapro, saprotrophic

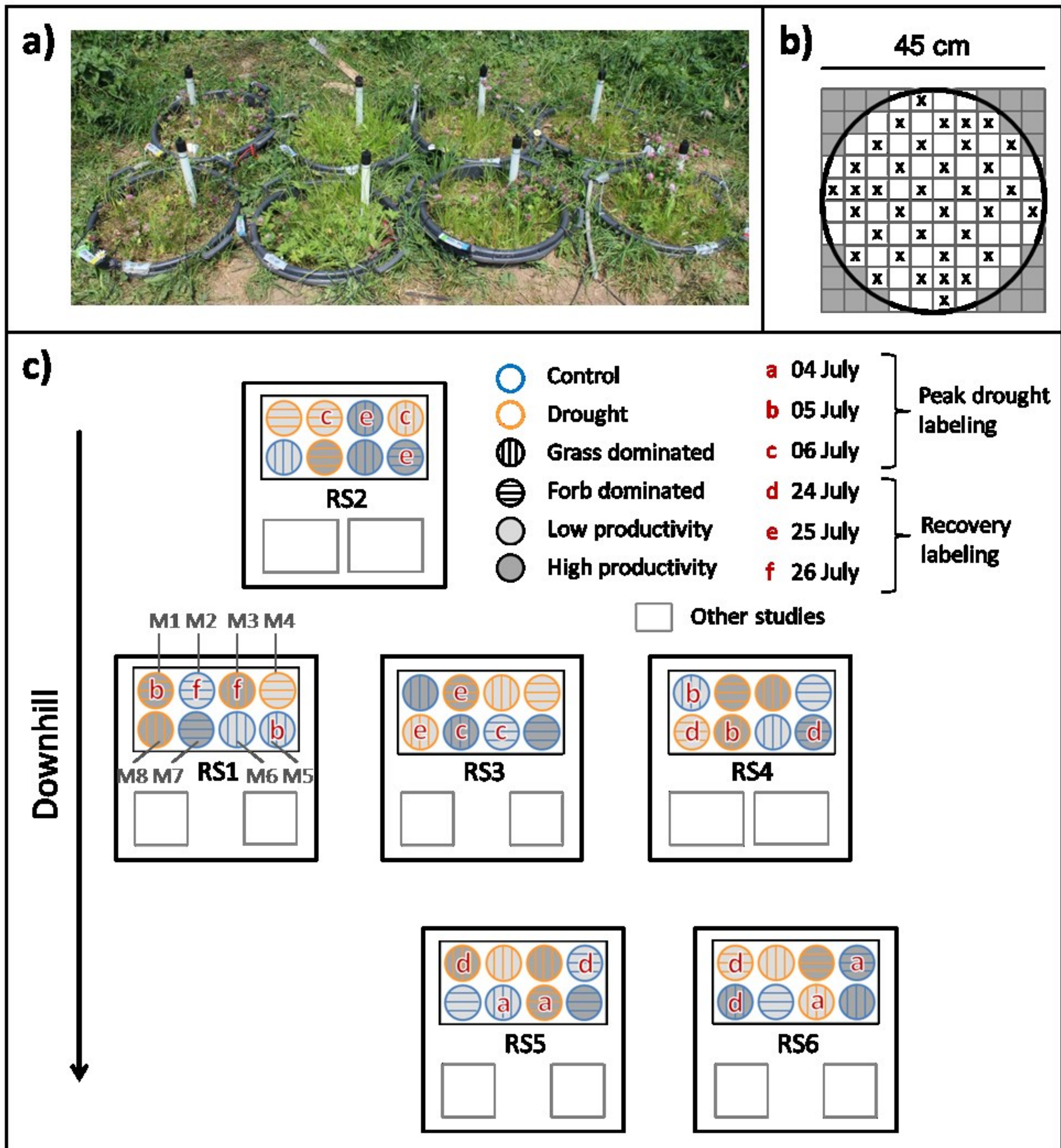


Fig. S1 Photography of mesocosms (with white tubes for soil moisture probes) at the study site in the beginning of July 2015 (a); planting pattern for each mesocosm with fixed positions, which were randomly filled with the six different species using varying relative abundances of conservative and exploitative grasses and forbs (b); local arrangement of the mesocosms in blocks corresponding to the installation of six rainout shelters (RS1-6) with each eight mesocosms (M1-8, see example for RS1).

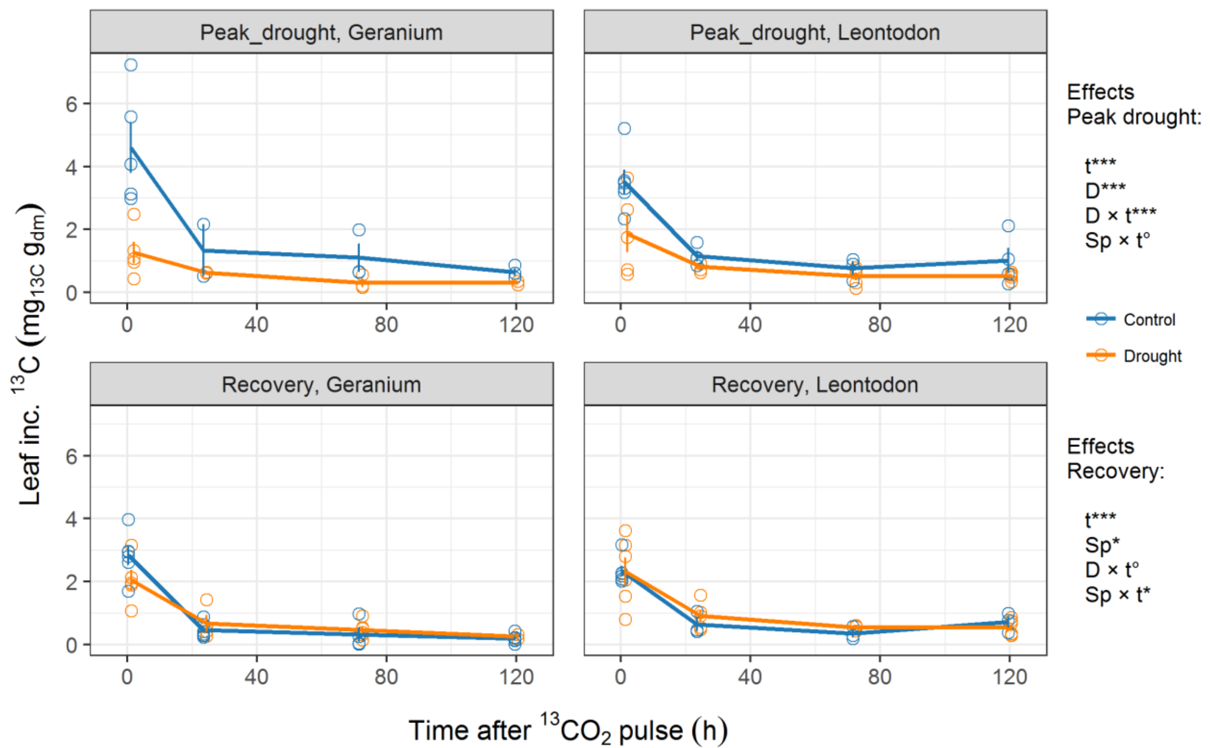


Fig. S2 Dynamics of ^{13}C tracer incorporation (inc. ^{13}C) into control (blue lines and circles) and drought-treated (orange lines and circles) leaves from *Geranium sylvaticum* (left) and *Leontodon hispidus* (right) plants, at the peak drought (top) and the recovery (bottom) labeling campaigns. Lines are based on mean values and error bars represent the corresponding SE of 2 to 6 replicates at each sampling time. Significance levels for the effects of sampling time (t; df = 3), drought (D; df = 1), species identity (Sp; df = 1) and their interactions from linear mixed-effects models (R package 'lme4') are shown for each labeling campaign on the right site; $***P_{\chi^2} < 0.001$, $**P_{\chi^2} < 0.01$, $°P_{\chi^2} < 0.1$.

SELBSTSTÄNDIGKEITSERKLÄRUNG

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Jena, 28. Februar 2019

Stefan Karlowsky

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Roscher C, Karlowsky S, Milcu A, Gessler A, Bachmann D, Jesch A, et al. (2019) Functional composition has stronger impact than species richness on carbon gain and allocation in experimental grasslands. PLoS ONE 14(1): e0204715. https://doi.org/10.1371/journal.pone.0204715

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