

**Processes altering species richness,  
primary production and leaf nutritive  
quality across European grasslands  
exposed to climate change**

Dissertation

zur Erlangung des akademischen Grades

Doctor rerum naturalium (Dr. rer. nat.)

an der Fakultät für Biologie, Chemie und Geowissenschaften

der Universität Bayreuth

vorgelegt von

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Geboren in Stuttgart Bad-Cannstatt, Deutschland

Bayreuth, im Juni 2020



Die vorliegende Arbeit wurde in der Zeit von Juni 2016 bis Juni 2020 in Bayreuth am Lehrstuhl für Störungsökologie unter Betreuung von Frau Professorin Dr. Anke Jentsch angefertigt.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.).

Dissertation eingereicht am: 26.06.2020

Zulassung durch die Promotionskommission: 01.07.2020

Wissenschaftliches Kolloquium: 16.10.2020

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# 1. Summary

## 1.1 Summary

Climate change is altering ecosystems and ecosystem services all over the globe (Parmesan and Yohe, 2003; Cardinale et al., 2012; Sunday, 2020). The severity of climate change, especially warming, increases with latitude and altitude (IPCC, 2013; Pepin et al., 2015), leaving high-elevation and latitude ecosystems especially prone to impacts of climate change. Consequently, grasslands of the northern hemisphere and mountain regions of the world are particularly affected. Grassland ecosystems cover large parts of the world and are often anthropogenically used and maintained. To ensure sustainable use and maintenance of ecosystem services contributing to human well-being of grasslands under future conditions such as warmer and drier climate, it is important to understand the interplay between climate change and land-use intensity on the provisioning of ecosystem functions and services in grasslands, especially in temperature limited mountain grasslands. The impact of the interplay between climate change and land-use intensity on ecosystem functions and services is not yet well understood, hence dissentient evidence exists. Climate change happens globally, however concepts and methods to elucidate generality in patterns and processes are necessary to be able to react to climate change pressures in a timely fashion.

The presented manuscripts in this thesis contribute to i) understanding effects of warming and altered precipitation regimes on plant species richness, community composition, primary production, and leaf nutritive quality in mountain grasslands of central Europe and ii) investigating generality in drivers altering and limiting primary production across European grassland ecosystems. Thus, this thesis improves knowledge on provisioning of ecosystem services in mountain regions, a region with a long history of land-use management exposed to severe climate change. Further, this thesis strengthens the effort to increase compatibility and reproducibility in science to reveal generality in patterns and processes across wide geographical and ecological gradients. To pursue these goals, experimental data from a translocation experiment within the European Alps and two different pan-European coordinated distributed experiments have been used.

Ecosystem services provided by grasslands are threatened by climate change and its potential interaction with existing land-use regimes. To be able to sufficiently and sustainably

maintain biodiversity and provisioning of ecosystem services, understanding the drivers altering biodiversity and, ecosystem functions and services in regions with severe climate change as well as generality of those patterns across larger scales needs to be improved.

i) Mountain grasslands

Species richness of mountain grasslands decreases under warmed conditions, but initial shifts in community composition are non-deterministic (Manuscript 1). Primary production is increasing with warming, though strong interactions with decreasing precipitation, thus plant water availability, are prevalent in natural (Manuscript 1) as well as in managed plant communities (Manuscript 3). Management intensity, including mowing regime and fertilizer quantity, is influencing chemical composition and, thus, the nutritional quality of biomass (Manuscript 2 and 3). Changes in resource allocation under drought conditions support a constant level of production under extensive management, whereas the lack of flexible adjustment of resource allocation reveals a low resistance of productivity under drought conditions under intensive land-use management (Manuscript 3). The local loss of species under warmed conditions (see Manuscript 1) has the potential to further decrease community resistance to dry conditions (see Manuscript 3) as community asynchrony (Blüthgen et al., 2016; Wilcox et al., 2017) and redundancy (Laliberté et al., 2010; Reich et al., 2012) is reduced by the loss of local species richness.

ii) Generality in drivers altering productivity

Productivity as well as its resistance to environmental changes or extreme events are affected by abiotic and biotic conditions of plant communities. The presence of invasive species, able to conquer new habitats due to climate change, can alter the resilience of biomass production (Manuscript 4). Productivity often is limited by belowground resource availability for plants. Unsurprisingly, productivity across Europe is strongly limited by water availability (aridity) (Manuscript 5), which is in alignment with findings on mountain grasslands (Manuscript 1 and 3). What is surprising is that biomass production revealed a strong and climate independent phosphorus limitation, whereas nitrogen limitation was climate dependent (Manuscript 5).

The findings in the manuscripts presented in this thesis underpin the pre-existing evidence of species richness and individual species response to environmental drivers as being

key for provisioning of ecosystem functions. Further, findings of this thesis reveal a low resistance of both natural and managed mountain grassland productivity to altered precipitation regimes, especially drought spells. This might have strong impacts on plant species composition and applied management practice under future climatic conditions. Thus, the natural follow-up to this thesis would be a thorough investigation of how community and species dynamics support ecosystem service provisioning under future climate and land-use scenarios, addressing research question such as:

What are long term effects of warming on community composition of mountain grasslands?

What is the role of single species in the provision of ecosystem services – are there “keystone” species for specific ecosystem services?

How is temporal stability of ecosystem services linked to species composition?



## 1.2 Zusammenfassung

Der Klimawandel verändert Ökosysteme und Ökosystemdienstleistungen weltweit (Parmesan and Yohe, 2003; Cardinale et al., 2012; Sunday, 2020). Die Schwere des Klimawandels, insbesondere Erwärmung, nimmt mit zunehmendem Breitengrad und Höhe über dem Meeresspiegel zu (IPCC, 2013; Pepin et al., 2015), so dass hoch gelegene Ökosysteme in hohen Breitengraden besonders anfällig für die Auswirkungen des Klimawandels sind. Folglich sind Grasländer der nördlichen Hemisphäre und der Gebirgsregionen der Welt besonders betroffen. Graslandökosysteme bedecken weite Teile der Welt und werden oft vom Menschen genutzt und erhalten. Die nachhaltige Nutzung und Erhaltung der von Grasländern bereitgestellten Ökosystemdienstleistungen, die zum menschlichen Wohlergehen beitragen, ist besonders unter künftigen Bedingungen wichtig. Um dies zu gewährleisten muss die Wechselwirkung zwischen Klimawandel und Landnutzung bei der Bereitstellung von Ökosystemdienstleistungen durch Grasländer, insbesondere in Bergregionen, besser verstanden werden. Die Auswirkungen des Zusammenspiels zwischen Klimawandel und Landnutzungsintensität auf die Ökosystemfunktionen und -dienstleistungen sind noch nicht ausreichend verstanden, weshalb es widersprüchliche Aussagen gibt. Der Klimawandel vollzieht sich global, daher sind Konzepte und Methoden zur Untersuchung von grundlegenden Mustern und Prozessen notwendig, um rechtzeitig auf den Druck des Klimawandels reagieren zu können.

Die in dieser Arbeit vorgestellten Manuskripte tragen dazu bei, i) die Auswirkungen der Erwärmung und veränderter Niederschlagsregime auf den Pflanzenartenreichtum, die Zusammensetzung der Pflanzengemeinschaften, die Primärproduktion und die Blattnährstoffqualität in montanen Grasländern zu verstehen und ii) grundlegende, allgemeingültige Faktoren zu untersuchen, die die Primärproduktion in europäischen Graslandökosystemen beeinflussen und beschränken. Somit verbessert diese Arbeit das Wissen über die Bereitstellung von Ökosystemdienstleistungen in Bergregionen, einer Region mit einer langen Geschichte der Landnutzung, die besonders stark dem Klimawandel ausgesetzt ist. Darüber hinaus verstärkt diese Arbeit das Bemühen, die Vergleichbarkeit und Reproduzierbarkeit in der Wissenschaft zu erhöhen, um die Allgemeingültigkeit von Mustern und Prozessen über weite geographische und ökologische Gradienten hinweg aufzuzeigen. Um diese Ziele zu verfolgen, wurden experimentelle Daten aus einem

Translokationsexperiment innerhalb der europäischen Alpen und aus zwei verschiedenen paneuropäisch „*coordinated distributed experiments*“ verwendet.

Die von Grasländern erbrachten Ökosystemdienstleistungen sind durch den Klimawandel und seine potenzielle Wechselwirkung mit bestehenden Landnutzungsregimen bedroht. Um die biologische Vielfalt und die ausreichende und nachhaltige Bereitstellung von Ökosystemdienstleistungen zu erhalten, muss das Verständnis der zu Grunde liegenden treibenden Kräfte in Regionen unter starkem Klimawandeleinfluss, sowie die Allgemeingültigkeit dieser Muster in größerem Maßstab verbessert werden.

#### i) Pflanzengesellschaften in Gebirgsregionen

Der Artenreichtum von Pflanzengesellschaften in Gebirgsregionen nimmt unter erwärmten Bedingungen ab, aber anfängliche Verschiebungen in der Zusammensetzung der Gemeinschaften sind nicht deterministisch (Manuskript 1). Die Primärproduktion nimmt mit der Erwärmung zu, obwohl sowohl in natürlichen (Manuskript 1) als auch in bewirtschafteten Pflanzengemeinschaften (Manuskript 3) starke Wechselwirkungen mit abnehmenden Niederschlägen, folglich pflanzenverfügbares Wasser, vorherrschend sind. Die Intensität der Bewirtschaftung, einschließlich des Mahdregimes und der Düngemittelmenge, beeinflusst die chemische Zusammensetzung und damit die Nährstoffqualität der Biomasse (Manuskript 2 und 3). Unter extensiver Bewirtschaftung führen Veränderungen in der Ressourcenallokation unter Dürrebedingungen zu einem konstanten Produktionsniveau, wohingegen unter intensiver Bewirtschaftung der Mangel an flexibler Anpassung der Ressourcenallokation eine geringe Resistenz der Produktivität unter Dürrebedingungen zeigte (Manuskript 3). Der lokale Artenverlust unter erwärmten Bedingungen (siehe Manuskript 1) hat das Potenzial, die Resistenz der Gemeinschaft gegenüber Dürre (siehe Manuskript 3) weiter zu verringern, da die Asynchronität (Blüthgen et al., 2016; Wilcox et al., 2017) und Redundanz (Laliberté et al., 2010; Reich et al., 2012) der Gemeinschaft durch den Verlust des lokalen Artenreichtums reduziert wird.

#### ii) Grundlegende, produktionsbeeinflussende Faktoren in Grassländern

Sowohl die Produktivität als auch ihre Resistenz gegenüber Umweltveränderungen oder Extremereignissen werden von den abiotischen und biotischen Bedingungen jeder Gemeinschaft beeinflusst. Das Vorhandensein invasiver Arten, die in der Lage sind, aufgrund

des Klimawandels neue Lebensräume zu erobern, kann die Resistenz der Biomasseproduktion verändern (Manuskript 4). Die Produktivität von Pflanzen wird oft durch die Verfügbarkeit von Bodenressourcen begrenzt. Es überrascht nicht, dass die Produktivität in ganz Europa durch die Wasserverfügbarkeit (Trockenheit) stark eingeschränkt wird (Manuskript 5), was im Einklang mit den Erkenntnissen über montane Grasländer steht (Manuskript 1 und 3). Überraschend ist, dass die Produktivität eine starke und klimaunabhängige Phosphorbegrenzung zeigte, während die Stickstoffbegrenzung klimaabhängig war (Manuskript 5).

Die Ergebnisse in den in dieser Arbeit vorgestellten Manuskripten untermauern die bereits vorhandenen Beweise für die Schlüsselrolle von Artenreichtum und der Reaktion einzelner Arten auf Umweltfaktoren zur Bereitstellung von Ökosystemfunktionen. Darüber hinaus zeigen die Ergebnisse dieser Arbeit eine geringe Resistenz der Produktivität von sowohl natürlichen als auch bewirtschafteten Grassländern in Gebirgsregionen bezüglich veränderte Niederschlagsregime, insbesondere Dürreperioden. Dies könnte unter künftigen klimatischen Bedingungen starke Auswirkungen auf die Zusammensetzung der Pflanzengesellschaft und die angewandten Landnutzungsregime haben. Daher wäre die natürliche Fortsetzung dieser Arbeit eine gründliche Untersuchung der Art und Weise, wie die Gemeinschafts- und Artendynamik die Bereitstellung von Ökosystemdienstleistungen unter zukünftigen Klima- und Landnutzungsszenarien unterstützt, wobei unter anderem folgende Forschungsfragen behandelt werden:

Was sind die langfristigen Auswirkungen der Erwärmung auf die Zusammensetzung der montanen Grasland-Gemeinschaften?

Welche Rolle spielen einzelne Arten bei der Bereitstellung von Ökosystemdienstleistungen - gibt es "Schlüsselarten" für bestimmte Ökosystemdienstleistungen?

Wie hängt die zeitliche Stabilität von Ökosystemdienstleistungen mit der Artenzusammensetzung zusammen?

## 2. Glossary

Stability is a concept that is often used in ecology but is rarely clearly defined, thus requiring careful definition in advance. In this thesis “stability” will be used as an overarching, broad concept including its substantial constituents (*sensu* Grimm 1997): “constancy” (staying essentially unchanged), “resilience” (returning to the reference state or dynamic after a temporary disturbance) or “persistence” (persistence through time of an ecological system) or one of the to them related aspects, namely “resistance” (staying essentially unchanged despite the presence of disturbances), “elasticity” (speed of return to the reference state or dynamic after a temporary disturbance) or “domain of attraction” (the whole of states from which the reference state or dynamic can be reached again after a temporary disturbance). Especially the stability property “resistance”, as the magnitude of change of any ecosystem property to altered external conditions, will be used in later chapters.

Other terms to define are:

**Ecosystem service:** a quantifiable entity or property provided by an ecosystem to humankind; can be categorized into *supporting, provisioning, regulating* and *cultural* ecosystem services (*sensu* Millenium Ecosystem Assessment, 2005).

**Ecosystem function:** an emergent property resulting from natural, internal ecosystem processes and the given ecosystem structure. Multiple ecosystem functions can together represent an ecosystem service.

An example for clarity: Leaf chemical composition is an ecosystem function. Leaf chemical composition represents leaf nutritive/forage quality, and together with the amount of production they contribute to a key provisioning ecosystem service, namely forage provision.

**Multifunctionality:** the provisioning of multiple ecosystem functions and services (*sensu* Manning et al., 2018)

## 3. Background of the thesis

In this section, the state-of-the art of climate change research in grasslands is described, leading to the open research frontiers addressed in this thesis. First, climate change with a special focus on mountain regions is lined out. Second, the impacts of climate change on diversity of plant communities (species occurrence and changes in composition and abundance of species), ecosystem functions (focusing on quantity and chemical composition of aboveground production), the link between biodiversity and ecosystem functions, as well as the stability of provisioning of these ecosystem functions and services are described. Third, three roads to increase compatibility and reproducibility in science are highlighted, which ultimately improves scientific understanding in generality in patterns and processes of ecosystems across large ecological scales.

### 3.1 Climate Change with focus on mountain regions

Climate change sits at the forefront of scientific importance and this has become increasingly reflected in society (e.g. Fridays for Future, Plant-for-the-Planet, *atmosfair*). Climate change is multifaceted, reflecting a composite of climatic components including temperature, precipitation, CO<sub>2</sub> concentration and, additionally, the interacting (additive or non-additive) effects of those. These composites are changing in mean values (Easterling et al., 1997; IPCC, 2013), variability (Easterling et al., 2000; Schär et al., 2004) and spatial extent (Williams et al., 2007), which all affect ecosystems with their inherent ecosystem functions and consequently provided ecosystem services.

Global mean temperature has already risen in the last century by +0.74 °C (Hulme, 2005; Trenberth et al., 2007) and is predicted to rise further in the future (IPCC, 2013, 2018). Predictions of future conditions range between 1 and 6 °C depending on varying emission scenarios (IPCC, 2013; Xu et al., 2018). The rate of mean temperature increase was also speeding up in the last century, almost doubling when comparing 1900-1950 to 1950-2000 (Trenberth et al., 2007). The severity of climate warming increases in high latitudes and high altitudes (Böhm et al., 2001; Pepin et al., 2015) and is most pronounced in high latitude mountain systems e.g. European Alps or Pyrenees (Diaz and Bradley, 1997). The minimum temperatures are increasing even faster than the maximum in those northern mountain

ecosystems (Diaz and Bradley, 1997). Such temperature changes (increasingly stronger as a function of elevation; elevation dependant warming *sensu* Pepin et al., 2015) might have severe effects on mountain ecosystems and their adjacent lowlands in the future (Viviroli et al., 2007; Loarie et al., 2009).

The mean temperature in the European Alps has increased by 1.1 °C during the last century (1890-2000) nearly a doubling compared to the rise of mean temperature in the nearby low land of 0.55 °C (Böhm et al., 2001); in some high-elevation areas this is even a threefold increase to 1.5 °C (e.g. Säntis 2500 m a.s.l., Beniston, 2006). Especially since the 1980s, spring and winter temperatures show the strongest increase in this region (Böhm et al., 2001) causing an earlier snowmelt (Inouye and Wielgolaski, 2003; Klein et al., 2016; Asam et al., 2017) and a reduced insulating snow cover (Gobiet et al., 2014). Especially regions below ~1500 m a.s.l. show a reduction in total amount of snow as well as snow cover period during the winter month since 1985 (Steger et al., 2013). These changes in winter climate can, depending on timing, have drastic ecological effects on cold-temperature limited ecosystems, i.e. accelerate the rate of local species loss (Niittynen et al., 2018) and turn cold-temperature limited ecosystems from carbon sinks to sources, ultimately fuelling a biosphere-atmosphere feedback (Kreyling, 2019).

Alongside increasing temperatures, precipitation regimes are predicted to shift (IPCC, 2013; Giorgi et al., 2019). An overall reduction of precipitation within the growing season (April to September) of 20 to 30% is projected for the European Alps (Beniston, 2006; Calanca, 2007). This reduction is in large part due to a predicted decrease in the number of wet days (days with rainfall events) of up to ~20% during the summer months, causing longer periods without precipitation between each event (Calanca, 2007; Gobiet et al., 2014). Changes in precipitation show negative elevation dependency with the strongest relative drying in lowlands of the European Alps (Gobiet et al., 2014), whereas temperature's elevation dependency is positive, mostly coupled to snow-albedo effects (Gobiet et al., 2014; Pepin et al., 2015). Coupling these shifts in precipitation regimes with the projected increase in temperature and occurrence of heatwaves (Schär et al., 2004), alongside higher evapotranspiration and water demand (Briffa et al., 2009), drought frequency and severity is also increasing. For example the heat wave and drought of 2003, which had a 15% chance of occurrence in recent history would increase to a 50% chance of occurrence under future conditions, thus becoming

normal rather than exceptional (Calanca, 2007). In general, the occurrence of drought events will increase from 18% to 57% and the severity of drought events will double until the end of the 22<sup>nd</sup> century. This increase in drought severity also introduces drought extremes which never occurred before in the alps, thus imposing extreme challenges to land-use and society (Calanca, 2007).

All this evidence can be visualized via the metaphor developed by Hansen *et al.* (1988) of the “climate dice” with the sides representing normal, cold or warm extreme climatic conditions being equally loaded. During the last 30 years this dice has become loaded, heavily favouring warm extremes (Hansen *et al.*, 2012) and is likely to change vegetation and ecosystem dynamics around the globe (Parmesan and Yohe, 2003) and especially in mountain regions (Ernakovich *et al.*, 2014; Schirpke *et al.*, 2017).

## 3.2 Effects of climate change on plant communities and ecosystem functions

### Climate change effects on plant species richness and community composition

Species use and compete for available resources (Tilman, 1977). All available resources in space and time within an ecosystem define the multidimensional niche space available (Hutchinson, 1957, 1978). Different types of resource acquisition, allocation, use-efficiency and requirements (in most cases minima) determine species occurrence and co-existence, as species use and fill the available niche space and compete for limiting resources (Tilman, 1982; Harpole *et al.*, 2016). For plants, resources can be broadly split into belowground (e.g. nutrients and water) and aboveground (e.g. light and space). Shifting resource availability can shift competition from below- to aboveground (Hautier *et al.*, 2009), alter competitive outcome and biotic interactions within a community (Grassein *et al.*, 2014; Klanderud *et al.*, 2017) and ultimately shape species distributions (Wisz *et al.*, 2013). The resource availability and the constituents of an ecosystem determine amount (Hector *et al.*, 1999), stability (Isbell *et al.*, 2015) and multifunctionality (Hector and Bagchi, 2007; Manning *et al.*, 2018) of ecosystem functions and services provided.

Global change will affect ecosystems and their composition in the future (Sala et al., 2000; Parmesan and Yohe, 2003; Sunday, 2020) by changing the resource availability of an ecosystem and subsequently the ecosystem's ability to provide ecosystem services (Cardinale et al., 2012; Hooper et al., 2012). The different global change drivers (e.g. climate change, land-use change, nitrogen deposition, biotic interactions) will affect different types of ecoregions (e.g. terrestrial, marine, limnic) and ecosystems within them (e.g. tropical forests, temperate grasslands) to different degrees (Chapin et al., 2000; Steffen et al., 2011; Hooper et al., 2012). The magnitude of changes in biodiversity and provided ecosystem services are strongly linked to society's use of natural resource and are of global concern (Díaz et al., 2015; United Nations, 2015) for maintaining biodiversity (Pascual et al., 2017) or food security (Godfray et al., 2010; FAO et al., 2013; Springmann et al., 2018) as examples.

Climate change introduces novel weather regimes at given locations while migrating once prevalent climatic conditions to new geographic locations (Williams et al., 2007), both changing the extent of "thermal niches" and requiring species to track climate change (Loarie et al., 2009; Corlett and Westcott, 2013). Warmer temperatures, especially warmer winter and spring temperatures may advance start of the growing season whereas warmer autumns delay the end of the growing season; either would elongate the time available for plants to grow (Wipf et al., 2015; Asam et al., 2017). Further, warmer temperatures generally enable an overall faster metabolism leading to higher transpiration (Billings and Mooney, 1968; Larcher, 2003), increased decomposition (García-Palacios et al., 2013) and higher nutrient turnover via increased microbial activity (Wang et al., 2016). The faster decomposition can increase soil nutrient availability for plants alleviating belowground resource limitation, potentially inducing a shift from belowground to aboveground competition for light and space (Hautier et al., 2009; DeMalach et al., 2016). In contrast to a higher soil nutrient availability, warmer temperatures and the coupled higher evapotranspirative demand is likely to cause a faster soil-moisture depletion (Briffa et al., 2009). Such a temperature induced shift in soil resource availability may shift competitive outcomes within species of an established community and cause shifts in community composition and abundance of inherent species.

Mountainous or high altitude regions are temperature limited (similar to tundra or terrestrial high latitude habitats) and limited to a short growing season induced by cold temperatures (Körner, 2003). Higher temperatures and soil nutrient availability are likely to

favour fast-growing, resource acquisitive species which are able to leverage the newly available resources more quickly and outcompete slow-growing species with a resource conservative growth strategy (Stearns, 2000; Reich, 2014). In temperature limited non-woody ecosystems graminoids are likely to be favoured because of their growth form and often resource acquisitive strategy (Klanderud and Totland, 2005; Olsen et al., 2016; Rosbakh et al., 2017).

Altered temperature regimes and elongated growing seasons allow new species to migrate and establish where they were previously temperature limited. These new species might be stronger competitors under the novel climatic conditions than the resident species (Alexander and Edwards, 2010; Alexander et al., 2016). Cold-temperature limited grasslands with the respectively adapted species are undergoing changes in community composition induced by warming (Gottfried et al., 2012; Steinbauer et al., 2018). A thermophilization of the community takes place in high-altitude (alpine) communities especially (Jurasinski and Kreyling, 2007; Gottfried et al., 2012).

Changes in community composition to gradually increasing temperature happen slowly because of time lags introduced by dispersal, colonisation and establishment limitations of new species to arrive (Dullinger et al., 2012b; Essl et al., 2015; Alexander et al., 2017) as well as extinction lags of resident species (Dullinger et al., 2012a; Hylander and Ehrlén, 2013). Extinction lags are often caused by the highly adapted cryophilic species which are commonly slow-growing, perennial species (Körner, 2003).

### Climate change effects on biomass production and chemical composition

Climate change can directly and indirectly – via its effects on plant community composition – alter quantity and chemical composition of plant community biomass production. These two ecosystem functions are equivalent to forage quantity and quality, respectively, thus ultimately represent forage provision as an ecosystem service. Further, these two key ecosystem functions are strongly influenced by land-use and its interplay with climate change.

Warmer temperatures inducing an elongation of the growing season and an overall higher metabolic rate are likely to increase productivity of plant communities, as more time to

photosynthesize and, fix and allocate carbon is available (Billings and Mooney, 1968). Additionally, the faster decomposition of nutrients with warmer temperatures (García-Palacios et al., 2013; Wang et al., 2016) may alleviate belowground resource limitation thereby further increasing productivity of plant communities (Fay et al., 2015).

When warmer temperatures induce higher evapotranspirative demand of plant communities, it might lead to a faster soil moisture depletion (Briffa et al., 2009). Coupling this possible effect of temperature with increasing variability of precipitation can lead to communities being exposed to more frequent drought stress. Drought stress reduces community productivity (Grant et al., 2014) by causing closure of stomata and reduction of photosynthesis (Sack and Holbrook, 2006; McDowell et al., 2008). Hot summer temperatures coupled with deficient soil moisture availability reduce physiologic performance and can cause direct tissue damage (overheating) in mountain plant communities and lead to reduced productivity (De Boeck et al., 2016). Nonetheless, not only the magnitude or severity but also the timing of drought spells is of high importance for grassland productivity (Craine et al., 2012; Walter et al., 2012), as the impact of drought decreases as growing season prolongs likely caused by ecophysiological changes in the response to water stress and advanced phenology and maturity of plants (Pau et al., 2011; Craine et al., 2012).

Higher soil nutrient availability is affecting chemical composition respectively forage quality of plant communities (Sardans et al., 2012). Yet, the effects of warming on foliar element concentration depend on the plant species and the climate they grow in (Sardans et al., 2012). Plants as autotrophs are not as strictly bound to homeostasis as heterotrophs, a surplus of available nutrients can be taken up and stored (mainly in the vacuole) (Sternner and Elser, 2002). For example a surplus of available nitrogen can decrease plant tissue C:N ratio if growth and therefore carbon accumulation is limited by a different resource (Bracken et al., 2015). Stoichiometric flexibility allows maintenance of growth under less ideal conditions of nutrient availability (Sistla and Schimel, 2012), this flexibility is limited and changes in elemental ratios are and will become more vital in controlling ecosystem functioning (Peñuelas et al., 2013). The extent of stoichiometric flexibility of a species is coupled to its ability to leverage altered soil nutrient availability, ultimately changing communities' composition, stability, productivity and carbon cycling (Peñuelas et al., 2013).

In addition to the effects of climate change and warming, land-use has a major impact on biodiversity (Sala et al., 2000) and ecosystem services (Foley et al., 2005). Land-use intensity strongly affects the quantity (Beckmann et al., 2019) and quality of produced biomass (Rivas-Ubach et al., 2012; Sistla et al., 2015) via external nutrient input (fertilization) and the stimulation of re-growth (e.g. cutting or grazing). Broadly speaking, quantity and quality of production increases with increasing land-use intensity at the expense of biodiversity (Beckmann et al., 2019). In managed grasslands increasing land-use intensity further amplifies the negative effects of drought on the quantity of produced biomass, though the quality of managed grasslands' biomass is increasing under drought (Vogel et al., 2012; Grant et al., 2014; Ludewig et al., 2015; Li et al., 2018). As recently shown by Zistl-Schlingmann *et al.* (2019), substantial proportions of added nitrogen is quickly lost via gaseous emissions and leaching. The nitrogen taken up in plant tissue of mountain communities is often gained from mobilized soil organic matter stocks rather than from applied slurry fertilization (Zistl-Schlingmann et al., 2020). Thus, removal of biomass and the accumulated nutrients following mowing or grazing may cause soil nutrient stocks to become depleted over time (Zistl-Schlingmann et al., 2019). Also land-use intensification reduces biodiversity (Beckmann et al., 2019) leading to homogenization of landscapes (Gossner et al., 2016) and ultimately imperils ecosystem functions (Blüthgen et al., 2016).

### Linking plant species richness and community composition to provisioning of ecosystem services

Ecosystems provide services which are important for human well-being. These ecosystem services are provided by a community's resident species, with each species contributing to one or multiple ecosystem services to various degrees (Duffy, 2009; Isbell et al., 2011). To maintain multiple ecosystem services across space and time in a changing world, many species are needed (Isbell et al., 2011). This is because, first, species promoting one function across multiple years are not the same species promoting multiple functions within a year (Hector et al., 2010; Wilcox et al., 2017) and, second, species promoting one single ecosystem function differ between years under fluctuating environmental conditions. So, species being functionally redundant (in respect to a provided ecosystem function) under a specific set of environmental conditions in a single year can react asynchronously to changing environmental conditions thereby maintaining the emergent ecosystem service (Jentsch et al.,

2011). Ultimately, ecosystem productivity, stability of an ecosystem function (a constant provision of an ecosystem function under fluctuating environmental conditions) and ecosystem multifunctionality (the provision of multiple ecosystem functions and services) is affected by biodiversity, the amount of species within a system (see also Hector et al., 1999; Isbell et al., 2011; Loreau et al., 2002; Zavaleta et al., 2010).

The biodiversity-productivity relationship, which describes an increasing amount of produced biomass with increasing biodiversity, has a long history in ecology (Hector et al., 1999; Loreau et al., 2001) and was shown to hold true under natural fluctuating climatic conditions (Isbell et al., 2015) or experimental manipulated nutrient or water availability (Craven et al., 2016). The mechanisms driving the biodiversity-productivity relationship are, for example, complementarity effects based on different resource use efficiencies (Naeem et al., 1994; McKane et al., 2002) and co-limitations (Harpole et al., 2011) or positive interactions between species (Loreau and Hector, 2001). However, disentangling the cause (e.g. resource partitioning, abiotic facilitation and/or biotic feedback) from the consequence (e.g. increased productivity of diverse communities in contrast to monocultures) of complementarity effects remains a crucial task in forecasting the response of ecosystems to loss of species (Barry et al., 2019).

The stability of a community and its ecosystem functions is also related to biodiversity, known as the diversity-stability relationship (MacArthur, 1955; McCann, 2000) and was shown, for example, for temporal stability of ecosystems (Tilman et al., 2006). Though, different aspects of biodiversity are of importance for the diversity-stability relationship (Craven et al., 2018). This diversity-stability relationship is driven by species redundancy (Pillar et al., 2013) and asynchrony (Yachi and Loreau, 1999; Loreau et al., 2003). A weakening of asynchrony induced by warming reduces the temporal stability of biomass production in high-elevation mountain communities (Ma et al., 2017). Biodiversity can improve the resistance of an ecosystem to climatic extremes in general and to “mild” extremes in special (Isbell et al., 2015). However, it remains unclear if the biodiversity-stability relationship is of equal importance under “very” extreme climatic conditions (De Boeck et al., 2018).

In addition to increasing productivity and stability of a community and its provided ecosystem functions and services, biodiversity is also integral to ecosystem multifunctionality (Hector and Bagchi, 2007). With an increasing number of species, the multifunctionality

(including various ecosystem functions such as productivity, decomposition, soil nutrient status and various ecosystem services such as invasion control, ground water regulation and forage provision) of ecosystems also increases (Zavaleta et al., 2010). While vice versa a loss of species and a homogenization among communities reduced ecosystem multifunctionality (Hautier et al., 2018). Additionally, the loss of redundant species, which are able to buffer against fluctuations, can have large impacts on ecosystems in the future (Reich et al., 2012).

It was shown that loss of biodiversity induced by global change drivers directly and negatively affected ecosystem stability (Hautier et al., 2015). In particular, recovery after extreme events such as drought spells is positively affected by biodiversity (Kreyling et al., 2017), but the presence of invasive species within a plant community can nullify this positive effect (Vetter et al., 2020). Land-use changes also reduced ecosystem stability via reduced asynchrony across multiple trophic levels (Blüthgen et al., 2016). Further, land-use intensification reduces local species richness and causes a homogenization of species pools on larger spatial scales (Gossner et al., 2016) ultimately reducing ecosystem multifunctionality (Allan et al., 2015). To conclude, local species loss, which is more likely than global extinction, and spatial homogenization will affect provision, stability and multifunctionality of ecosystem services.

Thus, to study the effects of climate change, such as increasing temperature, precipitation variability, seasonality and, frequency and severity of drought events, and land-use intensity on the quantity and quality of biomass production of plant communities, as well as on changes in plant community composition is of high importance to maintain sustainable provision of ecosystem services of grasslands under future conditions.

### **3.3 Compatibility and reproducibility in science: methodological and conceptual challenges in science**

Global changes threaten ecosystems and their provided ecosystem services worldwide, creating challenges for societies around the world. Sustainable solutions based on scientific understanding are necessary to face these challenges. Modern infrastructure (e.g. internet or long distance travel opportunities) increase the interconnectivity of humankind, meaning globalization both threatens sustainability while providing new avenues for increased communication and collaboration to develop solutions. Science has become increasingly

specialized in the last century, fragmenting knowledge on levels of organization or biological scale (e.g. ecosystem science or molecular genetics) as well as ecosystems (e.g. tropical forests or temperate grasslands) and model organisms (e.g. *Arabidopsis* or *Drosophila*). *“As the twenty first century begins, biologists face the challenge of developing theories that can reconnect our detailed understanding of individual levels of biological organization and improve compatibility of data and hypotheses generated in the study of diverse biota and habitats”* (Wilson, 1998).

To address this challenge of connecting knowledge on different levels of organizations, habitats or organisms is a major effort in science. Meta-analysis (Gurevitch, 1993), coordinated-distributed experiments and observations (Fraser et al., 2013, 2014) or common protocols (Pérez-Harguindeguy et al., 2013; Halbritter et al., 2020), all offer opportunities to address this issue of global concern. Interdisciplinary collaboration to connect fields of expertise and degrees of specialization is important in achieving this goal.

At the end of the 20<sup>th</sup> century controlled manipulative experiments improved ecological theory, by testing hypotheses in a reductionist way. Though, upscaling these results often are limited by scope and scale of the experiments (Fraser 2013). To integrate and summarize large numbers of independent studies, meta-analyses have become a common tool for basic and applied science (Harrison, 2011). The strength of meta-analysis is to combine studies testing similar hypothesis, but with limited statistical power as sample size, geographic and temporal extent are limited by manpower (and funding) (Harrison, 2011; Fraser et al., 2013). These limitations often result in different conclusions between the single studies and impede a general conclusion of the tested hypothesis or theory. A meta-analysis provides the statistical framework to rigorously compare the single studies and helps to provide a “more unified” answer to the hypothesis (Gurevitch, 1993; Harrison, 2011). Meta-analysis has improved ecological theory by understanding generality between pattern and process (Hillebrand and Cardinale, 2010). Though, a growing body of literature highlights the difficulties of meta-analysis and their outcomes (Hillebrand and Cardinale, 2010; Whittaker, 2010). Weaknesses of meta-analysis can be of “philosophical” nature – for example the often missing expertise of the meta-analyst in each single studies system (Whittaker, 2010) – or “conceptual” nature – for example missing transparency and quality control of studies included (Hillebrand and Cardinale, 2010; Whittaker, 2010). The latter might often be caused by a wide variety of in ecology applied study design, measured parameters as well as the

methodology how they were measured (Harrison, 2011). Similar statistical approaches designed for meta-analysis of independent studies are used to analyse data of co-ordinated, distributed experiments and observations.

Using the global interconnectivity to extend the geographical and temporal scale and ensure high quality, transparency and compatibility of methods “coordinated distributed experiments” (CDE) (Fraser et al., 2013) are increasingly important tools for ecology. By standardizing experimental design and methodology, hypothesis driven experiments can be conducted across a vast geographic/ecologic gradient by multiple investigative teams at low cost and maintenance (*sensu* Fraser et al., 2013). Please note also “coordinated distributed observations” exist and profit by similar reasoning, such as the GLobal Observation Research Initiative in Alpine environments (GLORIA) network (Gottfried et al., 2012), HerbDivNet (Fraser et al., 2014) and DarkDivNet (Pärtel et al., 2019). The International Tundra Experiment (ITEX) was one of the first CDEs established and revealed first effects of global warming on ecosystems (Arft et al., 1999). Other prominent CDEs have been established (e.g. the International Drought Experiments (IDE) within DroughtNet and the Nutrient Network NutNet) demonstrating power in understanding the generality in patterns and processes of ecosystems around the world. For example NutNet revealed the effects of nutrient co-limitation on productivity (Fay et al., 2015), biodiversity (Harpole et al., 2016) and mechanisms controlling those effects on biodiversity (Borer et al., 2014). This existing networks not only offer great opportunities to answer upcoming (follow up) questions (Anderson et al., 2018) and develop novel techniques and data bases (Griffith and Anderson, 2019) but also can be used to “piggy back” on to gain further knowledge of initial underrepresented aspects of the ecosystem (Vicca et al., 2018). Additionally, with the effort spent to coordinate and make these multi-site studies comparable, the CDEs are urged to be maintained and ultimately established as Long-Term-Experimental-Research (LTER) “*capable of elucidating both ecological dynamics and ecological mechanisms*” (Knapp et al., 2012).

Though CDEs still represent a small fraction of experimental studies conducted around the world, hence improving data quality and compatibility by unified methodology of single-site studies is still very important. As already identified as a difficulty in conducting meta-analyses and post-hoc comparisons of ecosystems, the diversity of methods, sampling protocols, and data acquisition technologies should become more coordinated and unified

(Peters et al., 2014). For example plant traits reflecting species ecological strategy both are affected by and affect the environment (Lavorel and Garnier, 2002). Plant traits shape ecosystem processes and properties across various scales (Diaz et al., 2004; Wright et al., 2004; de Bello et al., 2010; Adler et al., 2013). Thus there was urgent demand for “*A handbook of protocols for standardised and easy measurement of plant functional traits worldwide*” (Cornelissen et al., 2003) which also was recently updated (Pérez-Harguindeguy et al., 2013). Unifying methodology allowed construction of trait data-bases (e.g. TRY (Kattge et al., 2011, 2019) and recycling data for regional (Schirpke et al., 2017) and global syntheses (Qi et al., 2019) or theoretical concepts (Reich, 2014). Common protocols will increase data re-usability and quality and allows for further generation and benefits from knowledge to ultimately enhance our biological and ecological understanding of pattern and process.

There are various classes of studies within ecology ranging from observations, experiments (natural and manipulative) to modelling (Knapp et al., 2012). The range of studies also represents a gradient from natural, highly complex conditions to artificial, complete controlled conditions and implicit different interpretation of results from pattern recognition to process understanding. In experiments the size of experimental units (Benton et al., 2007; Milbau et al., 2007) as well as number of experimental units (Schweiger et al., 2016; Kreyling et al., 2018) needs to be sufficient (or can be as minimal as needed) with regard to the hypothesis tested. Both, the high level of complexity and interactions in natural systems and the often non-linear response of ecosystems forces ecologists to apply multifactorial, multilevel experiments (Kreyling et al., 2018). The combination of multiple driving factors, irrespective of either being associated with the same “global change threat” (e.g. temperature and precipitation in climate change) or with differing threats (e.g. temperature and land-use intensity) are underrepresented in manipulative experimental studies (Song et al., 2019). By mixing natural experimental units and exposing them to natural complex changes in contrast to artificial controlled single factors, transplantations along latitudinal or altitudinal gradients offer a great opportunity (De Frenne et al., 2013; Nooten and Hughes, 2017) to address different global change drivers effects on ecosystems.

All of these conceptual advances – meta-analyses, coordinated distributed experiments, common protocols respectively methods and multilevel, multifactorial, and gradient experiments – either alone or in combination can be used as tool to “*reconcill[e] our*

*growing but fractured knowledge across multiple levels of organization, diverse types of organisms, and contrasting habitats [which] is a thrilling challenge” (Sterner and Elser, 2002). Finally, science might be able to timely support ecosystems to cope with global changes and nourish a world worth living for all.*

## 4. Outline and main findings of studies and summary of discussion and conclusions

In this section, I will first set the objectives and the conceptual framework of this thesis as well as the approach applied to generate new insights and contribute to the progress in science. Then, I will briefly summarize each of the six manuscripts presented as chapters of this thesis. Further, the main conclusion of the respective manuscripts will be highlighted and put into the thesis' conceptual framework and objectives. Lastly, the previously demonstrated scientific progress will be embedded in a broad scientific and societal context and potential future implications will briefly be sketched before in the next section new emerging research frontiers will be posed.

### 4.1 Objectives

The central goal of this dissertation is to improve our understanding of fundamental processes contributing to, altering and potentially limiting the provision of ecosystem services provided by grasslands. Until now, the response of grassland ecosystems especially to the combined effects and interplay between climate change and land-use management intensity have rarely been investigated, hence dissentient evidence exists.

Grasslands – as one of the major habitats on earth – are anthropogenically embossed while providing important ecologic and economic ecosystem services. As the world is undergoing substantial changes in the Anthropocene, it is crucial to understand how grasslands can maintain provided ecosystem services. Thus, it is of high importance to improve our understanding of the direction and magnitude of changes of grasslands' ecosystem functions and their emergent ecosystem services exposed to global changes. Ultimately hoping to potentially elucidate pathways to ensure stability and maintenance of provided ecosystem services in the future. These urgent challenges form the conceptual framework of this thesis.

The objectives of this thesis are

- i) to investigate the effects of climate change on provided ecosystem services of grassland plant communities, with special emphasis on mountain grasslands. The investigated

ecosystem functions are biodiversity (changes in species richness and community composition), productivity and chemical composition (with respect to forage quality and elemental stoichiometry) of grassland plant communities. Ultimately, aiming to improve knowledge on climate change impact on the provision of these ecosystem services for both scientists and stakeholders. For scientists, to improve understanding of the magnitude and direction of change on grasslands' ecosystem functions exposed to climate change and for stakeholders, to support potential management adaptation to maintain sustainable provision of ecosystem services under future conditions.

- ii) to contribute to the timely debate on reproducibility and compatibility of environmental science (i.e. climate change experiments). By exerting coordinated experiments, collaborating inter- and transdisciplinary and developing standardized methodology environmental science has the possibility to generate a more detailed process understanding and unravel generality in patterns and processes across large scale ecologic and climatic gradients.

To tackle these objectives and to advance scientific understanding of global change pressures and their interactions impacting species richness, community composition, productivity and chemical composition (with respect to forage quality and elemental stoichiometry) of grassland plant communities we used

- a) **a downslope translocation experiment** of intact, natural plant-soil communities in the European Alps spanning an elevational gradient of ~2000 m at six sites ranging from alpine (above climatic treeline, *sensu* Körner, 2003) via sub-alpine to montane. This translocation experiment is passively simulating climate change, especially climate warming, and was established as the project "Sustainable use of alpine and pre-alpine grassland soils in a changing climate" (SUSALPS I and II – Grant: Jentsch 2015 and 2018, UBT: FKZ 031B0516C). (The results are reflected in Manuscript 1, 2 and 3)
- b) **a plant mesocosm experiment** of controlled plant species richness and community composition exposed to 1000-year drought events in three European countries (Belgium, Germany, Bulgaria). This experiment was established as the BiodivERsA-project SIGNAL (Grant: Jentsch 2012, UBT: ID 01LC1201) and investigates the effect of invasive species on the biodiversity-productivity relationship under the impact of climatic extremes (Manuscript 4).

- c) **a standardized multi-species plant community phytometer** experiment involving 18 sites across a pan-European climatic gradient. This project was conducted at experimental research sites of various participants of “Climate Change Manipulation Experiments in Terrestrial Ecosystems – Networking and Outreach” (ClimMani – Grant Jentsch, UBT: ES1308). The experiment was initiated by the Department of Disturbance Ecology, University of Bayreuth, aiming to disentangle edaphic and climatic effects in grassland experiments (Manuscript 5).
- d) **an initiative by the ClimMani network** – an EU-cost action for networking and outreach – to support the creation of a comprehensive methods handbook to increase scientific compatibility and quality of data re-use & synthesis in climate change experiments (Manuscript 6).

Manuscripts 1 to 5 investigate the effects of climate change on species richness, community composition, productivity and chemical composition (with respect to forage quality and elemental stoichiometry) of grassland plant communities (Thesis objective i).

Manuscripts 2 and 4 to 6 use either standardized protocols in coordinated distributed experiments or develop respectively collect standardized, repeatable methods to increase compatibility and reproducibility in environmental science (Thesis objective ii).

## 4.2 Summary of manuscripts

Manuscript 1:

*Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes* – (2019; **Arctic, Antarctic and Alpine Research**)

**Bernd J. Berauer**, Peter A. Wilfahrt, Mohammed A.S. Arfin-Khan, Pia Eibes, Andreas von Heßberg, Johannes Ingrisich, Michael Schloter, Max A. Schuchardt, Anke Jentsch

Manuscript 1 investigates the initial response (i.e. after one year of exposure to novel conditions) of five mountain grassland communities to abrupt changes in climatic conditions. The five grassland communities originated from five different elevations and were translocated downslope in order to passively simulate climate change. Consequently, the grassland communities have been exposed to different intensities of climate change. Species

richness, an important component of biodiversity, was reduced in all communities by altered climatic conditions. Species loss was irrespective of the by translocation imposed intensity of climate change. The loss in species did not reflect any directional and deterministic shift caused by re-ordering of the competitive hierarchy of resident species towards thermophilic or fast adapting species, rather stochastic priority effects appeared to underlie compositional shifts. In contrast to local species loss, aboveground biomass increased under altered climatic conditions unless there was a critical reduction in precipitation coupled with warming, in which case we observed reduced aboveground production.

This study highlights the initial low resistance of mountain grassland communities to climate change and further emphasizes the importance of shifts in precipitation regimes in communities generally expected to be temperature limited. The low resistance of mountain communities to altered climatic conditions in general and of productivity in special to shifting precipitation regimes during the growing season, may have critical impacts on the stability of provided ecosystem services in the future.

#### Manuscript 2:

*Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change – (2020; **Agriculture, Ecosystems and Environment**)*

**Bernd J. Berauer**, Peter A. Wilfahrt, Björn Reu, Max A. Schuchardt, Noelia Garcia-Franco, Marcus Zistl-Schlingmann, Michael Dannenmann, Ralf Kiese, Anna Kühnel, Anke Jentsch

Manuscript 2 first establishes visible near-infrared spectroscopy (vis-NIRS) as a high throughput method to determine multivariate and complex forage quality and second proves its suitability to reveal the interacting effects of two major global change drivers – namely changes in climate and land-use intensity – on species rich, semi-natural mountain pasture communities. We successfully applied vis-NIRS to quantify forage quality (i.e. raw protein, raw ash and raw fat content – but not raw fibre) of two grassland communities originating from different elevations but within relative geographic vicinity exposed to climate change by downslope translocation as well as two commonly applied land-use intensities (intensive -vs- extensive) of the pre-alpine region. Intensive (five cuts and five slurry applications per year) land-use intensity increased protein and fat content of both translocated communities in

comparison to extensive (three cuts and two slurry applications per year) land-use intensity. Despite the increased forage quality of intensively managed communities, extensive communities also produced sufficient nutritional quality for dairy livestock. While effects of land-use intensity on forage quality are similar and unidirectional for both communities, the effects of climate change on forage quality depended on each community's geographic origin (thus also community composition).

This study first provides a monitoring tool to support adaptation of sustainable, climate smart agriculture under future conditions. This monitoring tool can easily be re-used and applied in different grassland ecosystems, offering a reproducible, high-throughput method to applied ecology. For instance, if forage quality (e.g. protein content) is sufficient for its purpose (e.g. dairy production) the amount of fertilization can be reduced, also reducing leaching and gaseous nitrogen loss after fertilization. Second, this study strengthens existing evidence on the importance of community specific effects of climate change on provided ecosystem services. The latter coupled with the local loss of species under warmed conditions (see Manuscript 1) further underpins the importance to potentially adapt land-use intensity under future climatic conditions to maintain sustainable provision of ecosystem services.

Manuscript 3:

*High land-use intensity diminishes stability of forage provision of mountain pastures under future climate variability* – (2021; **Agronomy**)

**Bernd J. Berauer**, Peter A. Wilfahrt, Max A. Schuchardt, Marcus Schlingmann, Anne Schucknecht, Anke Jentsch

In Manuscript 3, we tested the effect of climate change combined with two contrasting land-use regimes on the productivity and ecologic stoichiometry of mountain pastures. Aboveground biomass increased with translocation to warmer sites irrespective of management intensity. In the first year of translocation (2017), the positive effect of warming was amplified by high land-use intensity, but during an unusually dry growing season in 2018, aboveground biomass of intensively managed communities dropped drastically, while extensively managed communities were able to maintain their level of production. In general C:N ratio was higher under extensive than intensive management. Inversely to aboveground

biomass, C:N ratio of extensive managed communities increased under dry conditions. This changes in C:N ratio indicate flexibility of resource allocation, potentially suggesting higher investment into belowground root production or into more drought tolerant leaf tissue aboveground, which both might support stability of aboveground productivity.

This study adds extra evidence to the vulnerability of provided ecosystem services of mountain plant communities to in future more frequently occurring drought periods. Further, these findings express the necessity to flexibly adapt land-use management intensities to be able to maintain sufficient amounts and quality of produced aboveground biomass. The here stated results indicate an already low resistance (of aboveground biomass) and inherent low flexibility (of resource allocation) of intensively managed communities, which both might decrease even more by future intensification and warming. Ultimately, the effects of intensification chained with in future more often and severe drought periods might decouple biogeochemical cycles and disrupt ecosystem functioning.

Manuscript 4:

*Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought* – (2020; **Global Change Biology**)

Vanessa M.S. Vetter, Jürgen Kreyling, Jürgen Dengler, Iva Apostolova, Mohammed A.S. Arfin-Khan, **Bernd J. Berauer**, Sigi Berwaers, Hans J. De Boeck, Ivan Nijs, Max A. Schuchardt, Desislava Sopotlieva, Philipp von Gillhausen, Peter A. Wilfahrt, Maja Zimmermann, Anke Jentsch

In manuscript 4, the effect of two invasive species (*Lupinus polyphyllus* Lindl. and *Senecio inaequidens* DC.) originating from different ecozones on the resistance and resilience of productivity supported by biodiversity during drought was investigated. Native species richness increased productivity and increased recovery of communities after the extreme event, though did not increase communities' resistance during the extreme event. The resistance of communities was not changed if an invasive species was present, though the positive recovery-richness relationship present in native communities was nullified under invader presence. Yet, the performance of each of the two invasive species under extreme drought depended on their respective natural histories.

This manuscript highlights the disruptive effects invaders have on the stability of production in temperate grasslands in the face of future climatic extremes. Though, the invaders themselves might profit from drought depending on the ecozone they are originating, native species suffer and communities lose their insuring stabilizing effects.

Manuscript 5:

*Disentangling climate from soil nutrient effects on plant biomass productions using a multispecies phytometer* – (2021; **Ecosphere**)

Peter A. Wilfahrt, Nelson Abrantes, Mohammed A.S. Arfin-Khan, Michael Bahn, **Bernd J. Berauer**, Michael Bierbaume<sup>5</sup>, Ika Djuki<sup>6</sup>, Marleen van Dusseldor<sup>7</sup>, Pia Eibe<sup>1</sup>, Marc Estiarte, Andreas von Heßberg, Petr Holub, Johannes Ingrisch, Inger Kappel Schmidt, Lazar Kesic, Karel Klem, György Kröel-Dulay, Klaus S. Larsen, Krista Lõhmus, Pille Mänd, Ildikó Orbán, Sasa Orlovic, Josep Peñuelas, David Reinthaler, Dajana Radujković, Max A. Schuchardt, Andreas H. Schweiger, Julienne M.-I. Schweiger, Srdjan Stojnic, Albert Tietema, Otmar Urban, Sara Vicca, Anke J. Jentsch

Manuscript 5 disentangles edaphic and climatic effects, which often are confounding and difficult to separate in experiments, across a pan-European climatic gradient using a standardized multispecies phytometer. This phytometer is composed of three cosmopolitan but non-invasive perennial species representing a graminoid (*Dactylis glomerata* L.), a non-leguminous forb (*Plantago lanceolata* L.) and a leguminous forb (*Trifolium pratense* L.) with each six individuals regularly planted in a potted “phytometer” community. The phytometers grown in standardized substrate (vermiculite) revealed soil-independent climatic effects on communities’ biomass production. The edaphic effects were separated from the climatic effects by correcting the response of phytometers grown in site-specific local soil with the response of those grown in standardized substrate. This phytometer experiment revealed a surprisingly strong climate-independent effect of plant available phosphorous on productivity, while the effect of plant available nitrogen was relatively minor and dependent on climate. Further, species responded individually to climate-corrected environmental limitations, though aridity itself remained a negative constraint on above ground production.

The phytometer employed as a common metric can disentangle two important, often confounding factors in experiments and can be established as stand-alone site add-on in

climate change experiments in grassland communities. Additionally, the species specific, asynchronous responses to environmental limitations reveal the importance of species interactions within a community under future global change scenarios.

Manuscript 6:

*The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx) – (2020; **Methods in Ecology and Evolution**)*

Aud H. Halbritter, Hans J. De Boeck, Amy E. Eycott, Sabine Reinsch, David A. Robinson, Sara Vicca, **Bernd J. Berauer**, Casper T. Christiansen, Marc Estiarte, José M. Grünzweig, Ragnhild Gya, Karin Hansen, Anke Jentsch, Hanna Lee, Sune Linder, John Marshall, Josep Peñuelas, Inger Kappel Schmidt, Ellen Stuart-Haëntjens, Peter A. Wilfahrt, *the ClimMani Working Group*, Vigdis Vandvik

Manuscript 6 tackles the challenges of data re-use, synthesis and upscaling in climate change manipulation experiments resulting from a lack of common “best-practice” measurements of key responses and impacts of climate change across all compartments of the soil-plant-atmosphere continuum. To overcome these challenges and improve future generalization and process-understanding, the consortium of authors provided guidance on a minimum set of parameters related to climate change to measure, in-depth protocols for measuring (“best-practice” as well as minimum and special-case adaptations) impacts of climate change in five major categories (1. Site characteristics and data management | 2. Carbon and nutrient cycling | 3. Water cycling | 4. Species and interactions | 5. Stress physiology) occurring across the soil-plant-atmosphere continuum.

Due to the special format of this manuscript including its comprehensive methods collection in climate change experiments, the categories with personal contribution are 1., 2., and 5. as well as supporting literature research for the main manuscript.

This manuscript has the potential to improve reproducibility and compatibility of scientific research in general and especially related to climate change. It will further help young and/or new researchers in the field to improve first steps in climate change and scientific output and ultimately give rise to enhanced process- and macroecological understanding of climate change impacts.

### 4.3 Summarizing Discussion and Conclusion

This thesis aimed, first, to improve knowledge on provided ecosystem functions and services of grasslands impacted by climate change and land-use change for both scientists and stakeholders (Thesis objective i ), and second, to contribute to the timely debate of reproducibility and compatibility in environmental science by employing coordinated distributed experiments, common protocols and standardized methods to improve process understanding and allow for enhanced up-scaling to unravel large-scale ecological patterns (Thesis objective ii ). The in the previous manuscripts established findings conclude in challenges and opportunities in a broader scientific and societal context.

#### i) Cascading effects of climate change on grassland ecosystem services

The demonstrated decline in plant species richness of temperate mountain grassland communities following climate warming (Manuscript 1) is likely to have negative effects on the provisioning of ecosystem services. This loss of biodiversity is likely to diminish stability of productivity (Yachi and Loreau, 1999; Hautier et al., 2015; Craven et al., 2016) and opens up potential “windows-of-invasion” (*sensu* Vetter et al., 2019) for novel species (e.g. invasive species) to establish in communities. Invasive species were shown to interrupt the diversity-stability relationship (MacArthur, 1955) under climatic extreme conditions (Manuscript 4). Shifting precipitation regimes coupled with higher temperature causing more frequent and more severe drought periods lead to the local loss of species and novel community compositions. The altered (or reduced) plant species richness can reduce the stability of ecosystem functions and services provided by grassland communities.

The already weak resistance of aboveground biomass production under high (intensive) management-intensity (Manuscript 3) is likely to further weaken if biodiversity is reduced and community composition is altered (Blüthgen et al., 2016; Gossner et al., 2016; Hautier et al., 2018). Under changing climatic conditions, it will be crucial to monitor forage quality and adapt land-use practices to be able to maintain sufficient amount and quality of produced aboveground biomass (Manuscript 2 and Manuscript 3). With advancements in climate forecast (Reichler and Kim, 2008; Hourdin et al., 2017) and tools for the rapid estimation of forage quality (Manuscript 2) we hope to support stakeholders in monitoring

this key ecosystem service and thereby give opportunities for flexible adaptation of land-use regimes as shifting climatic conditions dictate.

The feedback of nitrogen loss via leaching (Fu et al., 2017) or gaseous loss from agricultural practices (Zistl-Schlingmann et al., 2019) to climate change and atmospheric nitrogen deposition (Galloway et al., 2008; Kanakidou et al., 2016), coupled with the negative impacts of land-use intensification on biodiversity (Beckmann et al., 2019) may in future reinforce the velocity of needed adaptations.

Therefore, future research should investigate the importance of biodiversity in general, and the role of individual species in particular, to the provisioning of ecosystem functions and services and help to develop sustainable management to support biodiversity and maintenance of provided ecosystem services. Further, research should investigate alternative practices to fertilization aiding to reduce the revealed phosphorus limitation across European grasslands (Manuscript 5), for example enhanced weathering.

#### ii) Progress for scientific compatibility and up-scaling in a globalizing world

With the use of standardized protocols employed in coordinated experiments across sites we have been able to show the imperilling effects of invasive species on the diversity-stability relationship (Manuscript 4) and unravel confounding factors, emphasizing an emerging phosphorus limitation across European grasslands (Manuscript 5). These findings add further insights to the long ongoing debate of the diversity-stability relationship (McCann, 2000; Ives and Carpenter, 2007) and add additional evidence to the increasing importance of phosphorus-limitation in grasslands (Craine and Jackson, 2010; Vitousek et al., 2010). As climate change received a high level of attention in society recently, the scientific progress of climate change research (ongoing since over 40 years) needs to be streamlined in a globalizing world. As multiple climate change experiments tackling similar or different research questions are executed all over the world a tool to standardize and compare results becomes increasingly necessary in order to identify generality in processes and responses (Beier et al., 2012; Vicca et al., 2012, 2018; Firbank et al., 2017; Korell et al., 2020). A comprehensive method handbook for measuring and monitoring parameters in climate change experiments (Manuscript 6) was established to improve comparability of data and allow for up-scaling and combination of single site studies. With this, we hope to generalize individual findings in climate change

research (at least to some extent) timely to be able to keep track with the fast changes imposed to society and environment in the Anthropocene.

The technical, physical and computational powers are increasing while the pure space occupied by novel devices is decreasing. Along with this ascent, we see the opportunity to apply visible near-infrared reflectance spectroscopy (vis-NIRS) technology (Manuscript 2) with small and affordable but optical and computational sufficient technical devices for stakeholders to monitor forage quality fast and affordable in the future and for scientists the opportunity to further test this technology on ecological important macromolecules.

Admittedly, a PhD-students' contribution to European wide collaborations tackling global concerns and advancing the debate of reproducibility and compatibility in science can only (most of the times) be a splinter. Nonetheless, applying common protocols in coordinated experiments we were able to reveal ecological patterns across large ranges and I am convinced that applying and progressing this effort will help environmental science to identify generality in processes and responses across wider scales bridging local scales.

## 5. Synopsis – Upcoming research frontiers to tackle

In this chapter, I will briefly sketch emerging research frontiers based on findings of the presented manuscripts.

### Linking manuscripts to emerging research frontiers

*Manuscript 1: Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes.*

The short-term (1 year of exposure) effects of climate change on aboveground biomass production and species composition revealed non-deterministic, priority effects on community composition and strong effects of precipitation regime on productivity.

The long-term effects of climate change on species composition of mountain grassland communities could reveal different processes. Changes in species composition occur with a time lag as dispersal, colonisation and establishment (Essl et al., 2015; Alexander et al., 2017) – as well as local extinction (Dullinger et al., 2012a) – are time dependent processes. So far scientific results on the impact of warming on shifts in plant community composition of mountain grasslands differ between long-term observational (Steinbauer et al., 2018) and short-term experimental (Debouk et al., 2015; Berauer et al., 2019) studies as well as projections (Gottfried et al., 2012; Corlett and Westcott, 2013). Future research should investigate deterministic changes, such as thermophilization (Gottfried et al., 2012) or increasing graminoid abundance (Klanderud et al., 2015), in community composition of high-elevation plant communities exposed to climate warming. This also includes the establishment of species (e.g. from surrounding vegetation at the recipient site) as novel competitors may have unexpected impact on communities response to climate change (Alexander et al., 2015). Studying long-term effects of climate change on mountain plant community composition, the timing of species establishment and local species loss will add further evidence on the mechanisms driving establishment and time dependency of colonisation in mountain plant communities. Further, these climate change induced shifts in plant community composition will improve the understanding of biodiversity effects on the temporal stability of productivity in mountain grasslands (Ma et al., 2017), thus advancing the research frontier of the fate of mountain plant communities under future climatic conditions.

*Manuscript 2: Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change*

Forage quality of mountain grassland communities was higher under intensive management, but still sufficient under extensive management. Chemometric (the statistical correlation of a samples spectral information and the known chemical component of interest) application to vis-NIRS was able to determine complex, biologic macromolecules of species rich mountain grassland communities.

In the most recent past the progress in engineering and information technology (IT) led to the development of pocket-size, plug-and-play, populace affordable hand-held spectrometers for rapid and applied use (see also Yan & Siesler, 2018 for more detailed information) as well as improved quality of high-end spectrometers for scientific use (see also Pasquini, 2018). The engineering and IT progress, coupled with the ability of chemometrics to quantify and qualify complex macromolecules, opens two research frontiers. One applied frontier to non-destructive, real time quantify forage quality as well as the second, scientific frontier, to elucidate stress induced secondary-metabolism reactions of plants.

A way forward for applied use of this findings would be to test the ability of hand-held spectrometers to determine complex organic and biologic macro-molecules in grasslands without exhaustive and expensive sample pre-preparations. Yet, the majority of wavelength selected in Manuscript 2 for modeling parameters of forage quality are either below 1000 or above 2000 nm and thus not within the covered range (~900 to ~1700 nm) of those newly developed hand-held spectrometer (Thong et al., 2017; Yan and Siesler, 2018; van Ruth and Liu, 2019). If those novel technical devices are capable to maintain and ensure high quality estimates of forage, this method can be applied by stakeholders around the world based on a growing database of cross-referenced molecules and support monitoring and adaptation of applied land-use regimes. In the near future instruments for stakeholders to live and on-line monitoring of forage quality will be available (as allready forecasted by Foley et al., 1998). This progress may sound very technical, nonetheless the possible advantage for society might be immense. To achieve this progress in a fast and efficient way, cooperation with economy (e.g. SCiO from Consumer Physics Inc., Israel) is promising.

More important than this application for stakeholders is the potential advance in science. Being able to measure complex biomolecules – such as induced-defense chemicals like terpenoids, glucosinolates or tannins (Keeling and Bohlmann, 2006; Carmona et al., 2011) or soluble non-structural carbohydrates to lower leaf water potential (Chaves, 1991) – at low cost and high quantity, would increase the understanding of plant species reactions to external stressors such as herbivores respectively drought. But to be able to do so, first the chemometric approach needs to be transferred and tested for other ecologic relevant biologic macromolecules. Combining together the availability of new high-end spectrometers for scientific use, the advances in statistical computation (Reichstein et al., 2019) and a controlled experiments manipulating a single external stressor (e.g. herbivory), a new way to quantify and qualify *in situ* chemical adaptation can be revealed and ultimately deepen process understanding of species reaction to external stressors.

***Manuscript 3: High land-use intensity diminishes stability of forage provision of mountain pastures under future climate variability***

Aboveground biomass production of extensive management remained constant under drought conditions, likely because these communities can flexibly change resource allocation to increase drought tolerance. Opposing, intensive management was not able to alter resource allocation and revealed a low resistance (indicated by a strong decrease in productivity) to drought conditions.

These findings open up two research frontiers, one to develop an “ecosystem” nutrient balance of mountain pasture grasslands and the second to investigate the importance of biodiversity and single species on the maintenance of ecosystem service provisioning.

To gather further insights into shifts of biogeochemical cycles of mountain communities imposed by climate change and land-use intensity, a promising way forward following up on the gained knowledge of aboveground ecological stoichiometry in Manuscript 3 would be building an ecosystem (i.e. plant-soil continuum) nutrient balance, pool and fluxes analysis.

Available resources [e.g. carbon see Canadell et al. (2007), nitrogen see Galloway et al. (2008) and phosphorous see Gilbert (2009)] are shifting at different extents (see Finzi et al., 2011

for an overview) causing critical effects on entire ecosystems and their respective biogeochemical cycles (Sardans et al., 2012; Peñuelas et al., 2013; Yuan and Chen, 2015). For example, phosphor (co-) limitation on productivity is increasingly recognized in grasslands (Bracken 2015) and was shown to be climate independent (Manuscript 5).

Investigating changes in soil available nutrient pools, primary producers' nutrient ratios and nutrient fluxes (gaseous and via leeching) in the plant-soil continuum to quantify both import and export of nutrients might give a more complete picture of possible occurring imbalances in biogeochemical cycles induced by climate change and land-use intensity. With concrete relation to the SUSALPS-consortiums expertise this would combine the determination of nutrient flux and turnover rates via respiration rates (e.g. Institute of Meteorology and Climate Research, Atmospheric Environmental Research; Karlsruhe Institute of Technology; see for example Zistl-Schlingmann *et al.* 2020), the determination of soil organic matter, soil organic carbon and dissolved nitrogen (e.g. Chair of Soil Science; TUM School of Life Sciences Weihenstephan; Technical University of Munich) and element content of plant aboveground biomass (e.g. Department of Disturbance Ecology, University of Bayreuth). Combining those three compartments of nutrient flux, pool and stock will allow to calculate ecosystem nutrient balance based on influx and efflux. A detailed understanding of ecosystem nutrient balance will help to further develop sustainable agricultural land-use and minimize potential occurring of nitrogen and carbon mining (Zistl-Schlingmann et al., 2019) from especially organic matter rich mountainous soils (Wiesmeier et al., 2012). The SUSALPS-consortium with its various collaborators spanning the entire range of the plant-soil-atmosphere continuum offers a great opportunity to develop "ecosystem" nutrient balances, which so far have rarely been addressed (Sterner and Elser, 2002).

To understand the underlying mechanisms of the different response of managed grasslands exposed to climate change, future research should investigate changes in plant community composition of managed grasslands under climate change and identify single species contribution to the provisioning of ecosystem functions across time.

To identify changes in community composition, shifts in dominance of species and plant functional types (i.e. graminoids, forbs and legumes) species specific cover of managed grasslands exposed to climate change should be estimated twice a year. The first survey at the early stage of growing season (mid of May) and a second at peak season (mid of July). Having

two inventories a year allows to track the early-flowering (Arfin Khan et al., 2018) species, which might not regrow after the first cut of the land-use regime, as well as cover the species composition building the dominant backbone under the specific land-use regimes. With this data shifts in managed plant communities exposed to climate change and the possible asynchronous reaction of species to interannual variability can be elucidated.

A long-term monitoring of biomass production, its chemical composition and plant community composition will help to identify key-species supporting temporal stability of production and its chemical composition.



## 6. List of manuscripts and declaration of own contribution

**Concept:** study idea, development of experimental design or development of structure for reviews and protocols

**Data acquisition:** organization, execution and conduction of data acquisition and processing together with the help of colleagues, students and interns

**Data analysis:** statistical analysis of data and their illustration in tables and figures

**Writing:** writing the manuscript, including literature research

**Editing:** proof-reading and grammar editing, including comments and inputs from co-authors and their integration in the manuscript and preparation for resubmissions after the manuscript was reviewed by the journals referees

Manuscript 1:

*Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes.*

**Bernd J. Berauer**, Peter A. Wilfahrt, Mohammed A.S. Arfin-Khan, Pia Eibes, Andreas von Heßberg, Johannes Ingrisch, Michael Schloter, Max A. Schuchardt, Anke Jentsch

*Arctic, Antarctic, and Alpine Research*, 2019, 51(1), 215-231. | **Corresponding author**

DOI: 10.1080/15230430.2019.1618116

Concept: 5%

Data acquisition: 70%

Data analysis: 80%

Writing: 65%

Editing: 60%

Manuscript 2:

*Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change*

**Bernd J. Berauer**, Peter A. Wilfahrt, Björn Reu, Max A. Schuchardt, Noelia Garcia-Franco, Marcus Zistl-Schlingmann, Michael Dannenmann, Ralf Kiese, Anna Kühnel, Anke Jentsch

*Agriculture, Ecosystems and Environment*, 2020, 296, 106929 | **Corresponding author**

DOI: 10.1016/j.agee.2020.106929

Concept: 10%

Data acquisition: 80%

Data analysis: 85%

Writing: 70%

Editing: 60%

Manuscript 3:

*High land-use intensity diminishes stability of forage provision of mountain pastures under future climate variability*

**Bernd J. Berauer**, Peter A. Wilfahrt, Max A. Schuchardt, Marcus Schlingmann, Anne Schucknecht, Anke Jentsch

*Agronomy*, 2021, 11(5), 910, 11050910 | **Corresponding author**

DOI: 10.3390/agronomy11050910

Concept: 10%

Data acquisition: 75%

Data analysis: 85%

Writing: 75%

Editing: 55%

Manuscript 4:

*Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought*

Vanessa M.S. Vetter, Juergen Kreyling, Jürgen Dengler, Iva Apostolova, Mohammed A.S. Arfin-Khan, **Bernd J. Berauer**, Sigi Berwaers, Hans J. De Boeck, Ivan Nijs, Max A. Schuchardt, Desislava Sopotlieva, Philipp von Gillhausen, Peter A. Wilfahrt, Maja Zimmermann, Anke Jentsch

*Global Change Biology*, 2020, 26(6), 3539 - 3551

DOI:10.1111/gcb.15025

Concept: 0%

Data acquisition: 10%

Data analysis: 5%

Writing: 5%

Editing: 15%

Manuscript 5:

*Disentangling climate from soil nutrient effects on plant biomass production using a multispecies phytometer*

Peter A. Wilfahrt, Andreas H. Schweiger, Nelson Abrantes, Mohammed A.S. Arfin-Khan, Michael Bahn, **Bernd J. Berauer**, Michael Bierbaumer, Ika Djukic, Marleen van Dusseldorp, Pia Eibes, Marc Estiarte, Andreas von Heßberg, Petr Holub, Johannes Ingrisch, Inger Kappel Schmidt, Lazar Kesic, Karel Klem, György Kröel-Dulay, Klaus S. Larsen, Krista Lõhmus, Pille Mänd, Ildikó Orbán, Sasa Orlovic, Josep Peñuelas, David Reinthaler, Dajana Radujković, Max A. Schuchardt, Julianne M.-I. Schweiger, Srdjan Stojnic, Albert Tietema, Otmar Urban, Sara Vicca, Anke J. Jentsch

*Ecosphere*, 2021, 12(8):e03719.10.1002/ecs2.3719

DOI: 10.1002/ecs2.3719

Concept: 10%

Data acquisition: 10%

Data analysis: 0%

Writing: 5%

Editing: 5%

Manuscript 6:

*The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx)*

Aud H. Halbritter, Hans J. De Boeck, Amy E. Eycott, Sabine Reinsch, David A. Robinson, Sara Vicca, **Bernd J. Berauer**, Casper T. Christiansen, Marc Estiarte, José M. Grünzweig, Ragnhild Gya, Karin Hansen, Anke Jentsch, Hanna Lee, Sune Linder, John Marshall, Josep Peñuelas, Inger Kappel Schmidt, Ellen Stuart-Haëntjens, Peter A. Wilfahrt, *the ClimMani Working Group*, Vigdis Vandvik

*Methods in Ecology and Evolution*, 2020, 11(1), 22-37.

DOI: 10.1111/2041-210X.13331

Concept: 2%

Data acquisition: 2% (in this case extended literature and protocol review)

Data analysis: NA

Writing: 5%

Editing: 10%

Please note the special structure of this manuscript as one article plus five major methodological sections (1. Site characteristics and data management | 2. Carbon and nutrient cycling | 3. Water cycling | 4. Species and interactions | 5. Stress physiology). Personal contribution: Co-Author in main-manuscript and section 2; Technical editing of sections 1, 2 and 5.

## 7. Appendix – Scientific Contribution and Outreach

### I. Participation at conferences

Community	Conference	Date	Location	Contribution	Title
<b>BonaRes</b>	Status-Seminar	November 2016	Leipzig	Poster	Vegetation responses to climate warming: Disentangling soil and climate effects using plant community translocations alongside a model plant community approach
		February 2019	Leipzig	Poster	Productivity and community dynamics of alpine grassland in a changing climate – a translocation experiment
		February 2020	Leipzig	R-Statistic Workshop-Presentation (3h)	Advanced data handling and visualization
				Poster	Productivity and stoichiometry changes of contrasting managed montane pastures exposed to climate change
<b>BonaRes</b>	Conference	February 2018	Berlin	Poster	More on productivity and community dynamics of alpine grassland in a changing climate - a translocation experiment
<b>Gesellschaft für Ökologie</b>	Jahres-Treffen	December 2017	Ghent	Poster	Productivity and community dynamics of alpine grassland in a changing climate - a translocation experiment
		September 2018	Wien	Presentation (15 min)	Differing patterns of productivity and biodiversity of montane and alpine grasslands under climate change
-	International Mountain Conference	September 2019	Innsbruck	Summer-School	Ecosystem services of montane habitats under climate extremes (Group Speaker)
Workshop-Presentation (5min)				How to adapt land-use regimes with ongoing climate change?	

				Poster	How to adapt land-use regimes with ongoing climate change?
<b>Arbeitskreis Biogeographie</b>	Jahres-Treffen	May 2019	Frankfurt	Presentation (15 min)	How to adapt land-use regimes of montane grasslands with ongoing climate change?
<b>BayCEER</b>	Kolloqium	2017	Bayreuth	Poster	Productivity and community dynamics of alpine grassland in a changing climate - a translocation experiment
		2018	Bayreuth	Poster	More on productivity and community dynamics of alpine grassland in a changing climate - a translocation experiment
		2019	Bayreuth	Poster	Effects of climate change and land-use management on forage quality and quantity
<b>ClimMani</b>	Conference	October 2017	Utrecht	Poster	Shifts in aboveground productivity of alpine and pre-alpine grasslands to passively simulated climate change-insights of a translocation experiment along an elevational gradient
<b>SUSALPS</b>	Conference	September 2018	Garmisch-Patenkirchen	Presentation (15 min)	Consistent decreases in biodiversity but conditional increases in aboveground biomass of montane and alpine grasslands observed under experimental climate warming

## II. Scientific “networking”

### **ClimMani:**

Active participation in the EU-Cost-Action “ES1308 - Climate Change Manipulation Experiments in Terrestrial Ecosystems - Networking and Outreach (ClimMani)” from autumn 2016 on. Two week stay at University of Bergen (Department of Biological Sciences, Professor Vigdis Vandvik) to assist in writing and editing Manuscript 4 financed by a ClimMani-Shortterm-funding.

### **BonaRes R-Group:**

One of the funders of the “BonaRes R-Group” in autumn 2019. Taking leadership in organising and holding one of four R-Statistic Workshops at the “BonaRes-Statusseminar 2020” at the “Helmholtz Zentrum für Umweltforschung” (UFZ) Leipzig. Further active participation and development of this group of young researchers leading to a participation in the science reproducibility project: “*Fraser, H., Parker, T. H., Nakagawa, S., Fidler, F., Gould, E., & Gould, E. (2020, March 20). Many EcoEvo Analysts. Retrieved from osf.io/mn5aj*”.

### III. Teaching

Lecture Type	Title	Contribution	Year	Semester
Practica	Standortkundliche Feldmethoden	Preparation and execution of two field days on plant determination and vegetation analysis	2017	Summer
			2018	Summer
			2019	Summer
Practica	Experimental Ecology	Introductory lecture (90 minutes) and supervision of groups conducting field experiments during the entire semester	2017	Summer
			2018	Summer
			2019	Summer
Seminar	Natural risks and hazards	Preparation and execution of three day weekend seminar including three topic-presentations (each 20 min)	2017   2018	Winter
			2018   2019	Winter
			2019	Winter
Seminar	Resilience, stability and inertia	Preparation and execution of three day weekend seminar including introductory lecture (60 min) to "How to create and present a scientific poster"	2017   2018	Winter
			2018   2019	Winter
			2019   2020	Winter
			2020	Winter
Seminar	Projektseminar: Landschaftsökologie	Preparation, development and supervision of Master-students project on either topic "Impact of climate change on vegetation dynamics and productivity of subalpine grassland in Bayreuth and the European Alps" or "The effect of climate change on trophic-biotic relationships – enemy release or novel competitor / weapon?"	2017	Summer
			2018	Summer
			2019	Summer
Lecture	Disturbance ecology	Preparation and execution of two lectures (each 90 min) on "Grassland dynamics - ecosystem services, biodiversity & asynchrony" and "Disturbances and risk management under global change"	2019   2020	Winter

#### IV. Additional Participation in Manuscripts:

*Intensive slurry management and climate change promote nitrogen mining from organic matter-rich montane grassland soils* – (**Plant and Soil**, 2020, 456, 81-98)

Marcus Zistl-Schlingmann, Ursina Tobler, **Bernd J. Berauer**, Noelia Garcia-Franco, Peter Wilfahrt, Martin Wiesmeier, Anke Jentsch, Benjamin Wolf, Ralf Kiese, Michael Dannenmann

*Biotic and abiotic controls on carbon storage in aggregates from grassland soils in the Northern Limestone Alps of Germany* – (**Biology and Fertility of soils**, 2021, 57, 203-218)

Noelia Garcia-Franco, Roswitha Walter, Martin Wiesmeier, Luis C. Colacho Hurtarte, **Bernd J. Berauer**, Vincent Bunes, Ralf Kiese, Michael Dannenmann, Ingrid Kögel-Knabner

*Drought effects on montane grasslands negate benefits of earlier flowering phenology due to experimental warming* – (**Ecosphere**, 2021, DOI: 10.1002/ecs2.3661)

Max A. Schuchardt, **Bernd J. Berauer**, Andreas von Heßberg, Peter A. Wilfahrt, Anke Jentsch

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## 9. Manuscripts

Please note, Supplementary Material is provided online from the respective manuscripts' publisher website.



View from experimental site into the Stubai Valley, Austria



View from "Hammelsberg" on anthropogenically diverse used landscape in Perl, Germany.

9.1 **Manuscript 1** | Berauer *et al.* (2019) – Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes.



Experimental set-up of SUSALPS in 2017 at 2440 m a.s.l. in Furka pass, Switzerland.



## Low resistance of montane and alpine grasslands to abrupt changes in temperature and precipitation regimes

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### ABSTRACT

High-elevation ecosystems will experience increasing periods of above-average warmth and altered precipitation changes because of climate change. This causes uncertainties for community properties such as productivity and biodiversity. Increasing temperature may increase productivity by increasing growing season length and metabolic rate or decrease productivity by causing drought stress. Competitive outcomes between species may change with altered climatic conditions, causing shifts in community composition. This study investigates the resistance of above-ground biomass and plant community composition of montane and alpine grassland ecosystems to abruptly altered temperature and precipitation conditions. Intact plant-soil communities were translocated downslope spanning an elevational gradient of 2,090 m in the European Alps. We hypothesize that increasing temperature leads to (1) increased aboveground biomass in the absence of precipitation deficits, (2) decreased species richness, and (3) shifts in plant community composition. After one year of exposure to their new environment, aboveground biomass changes appeared to be dependent on precipitation regimes, whereas species richness declined consistently with changed climatic conditions. No deterministic shift in community composition was found. Abrupt changes in climatic conditions can lead to rapid responses of community properties, indicating that these high-elevation communities may have low initial resistance to future heat waves and droughts.

### ARTICLE HISTORY

Received 10 January 2019  
Revised 5 April 2019  
Accepted 7 May 2019

### KEYWORDS

Alpine; climate manipulation experiment; community ecology; elevational gradient; grasslands; resistance

### Introduction

Temperature rise because of anthropogenic climate change is expected to be most extreme at high latitudes and elevations (Ceppi et al. 2012; Gobiet et al. 2014; Pepin et al. 2015). Temperature is a fundamental regulator of chemical and biological processes and is likely to both directly and indirectly affect plant community properties (Rustad et al. 2001). Properties such as biomass production and species diversity can be tightly linked (Fraser and Pither 2015; Hautier et al. 2015), and studying their joint responses to rapid environmental shifts can reveal additional properties of communities, such as resistance (Kreyling et al. 2017). Increased temperature within a single season can affect these properties in cold biomes by extending growing seasons for plants because of changes in snow regimes

(Myneni et al. 1997; Inouye and Wielgolaski 2003; Latenser and Schneebeli 2003; Klein et al. 2016; Asam et al. 2017). In addition, higher metabolic rates at higher temperatures (Billings and Mooney 1968; Lucht et al. 2002; Larcher 2003) may increase the plant productivity of cold-adapted ecosystems. Despite these potential gains in productivity, plants may simultaneously suffer from even short-term increases in temperature. At more extreme temperatures, this may occur directly via the damage of tissue or overheated photosystems (Larcher 2003; Buchner et al. 2015). Although, well before such extremes, temperature can indirectly reduce productivity via reduced soil-water availability resulting from increased evapotranspiration and altered precipitation regimes (De Boeck et al. 2016). These conflicting drivers may explain why

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 Supplemental data for this article can be accessed [here](#).

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experimental warming studies of grasslands revealed contradicting results on productivity effects. Negative effects of increased temperature on productivity have been found in both in situ warming experiments (De Boeck et al. 2008) and translocation experiments (Egli et al. 2004); positive productivity effects have also been found in both in situ warming experiments (de Valpine and Harte 2001; Wan et al. 2005) and translocation experiments (Sebastià 2007). Additional studies have found no effect of warming on productivity (Dukes et al. 2005) or results that varied temporally or with soil moisture conditions (Harte and Shaw 1995; Cantarel, Bloor, and Soussana 2013). But to our knowledge it remains unclear which underlying mechanisms prevail and which environmental conditions cause a change in the importance of both conflicting drivers. The stability and resistance of community diversity to changing conditions may conserve ecosystem functions, such as productivity (Hodgson, McDonald, and Hosken 2015; Donohue et al. 2016). Given this lack of consensus in warming experiments and the risk of increased warm and dry periods in cold-biome ecosystems, continued research on the resistance of communities to abrupt environmental changes is needed.

Resource acquisition and allocation strategies determine species coexistence, as plants compete for limiting resources (Harpole et al. 2016). Temperature changes may alter plant-resource availability through multiple pathways, such as increased decomposition rate (Gavazov 2010; García-Palacios et al. 2013) or the depletion of soil moisture (Schär et al. 1999; Jung et al. 2010). Increased microbial activity may increase nitrogen availability (Rustad et al. 2001; Wang et al. 2016), potentially alleviating belowground competition and shifting it toward aboveground competition for light (Hautier, Niklaus, and Hector 2009; Borer et al. 2014). Such gains or reductions in plant-resource availability may shift competitive outcomes among species of an established community, leading to species loss or community-structure reorganization. An extended growing season may also shift the competition pattern and subsequently the community composition because cold-adapted species might be less adapted to leverage the extended growing season into higher growth and fertility (Wipf 2010). This can result because of the sensitivity of increased exposure to frost events as the insulating effect of snow is lost (Wipf, Rixen, and Mulder 2006), or by advantaging early emerging species that preempt resource uptake (Mwangi et al. 2007).

Thus, in montane and alpine grasslands future warm periods are likely to promote fast adapting, fast growing, high-statured species such as graminoids, which are able to benefit efficiently from the changed resource

availability (Bret-Harte et al. 2004; Veen et al. 2015; Klanderud et al. 2017) and therefore outcompete others. Especially graminoids, with their taller stature, were shown to profit from increased temperature and nutrient availability and hence outcompete smaller-statured growth forms for light and space (Theurillat and Guisan 2001; Klanderud, Vandvik, and Goldberg 2015). In the absence of dispersal, this should lead to deterministic decreases in species richness by outcompeting species unable to adapt to the novel biotic and abiotic environment. These losses may be mitigated or even reversed, however, if novel plant species colonize into an existing community (Engler et al. 2009; Alexander, Diez, and Levine 2015; Rixen and Wipf 2017). Nonetheless, the life cycle and demographic properties of species will cause the processes underlying the colonization of new habitats to lag behind the speed of change (Alexander et al., 2017; Dullinger et al. 2012).

Here, we investigate community resistance by examining the initial, one-year responses of plant communities to simulated, abrupt changes in temperature and precipitation climatic conditions on montane and alpine grasslands using an experimental downslope translocation of intact plant-soil monoliths in the European Alps. We hypothesize that:

- (1) Aboveground biomass increases with increasing temperature in the absence of severe precipitation differences.
- (2) In the absence of colonization, increasing temperature leads to a loss of species because of shifts in interspecific competition reflected in shifting functional group dominance and light transmission.
- (3) Community composition will shift with increasing temperature and both diverge compared to the original composition (between translocation sites) and show decreased variability relative to its original composition because of deterministic competitive exclusion (within translocation sites).

## Materials and methods

### Study sites

This study was conducted along an elevation gradient ranging from 350 m to 2,440 m a.s.l. across Germany (DE), Austria (AU), and Switzerland (CH) in the European Alps. Six representative grassland sites ranging from colline to alpine ecosystems were selected for downslope translocation of intact plant-soil monoliths. These six sites were Bayreuth (DE, 350 m a.s.l.), Fendt (DE, 550 m a.s.l.), Graswang

(DE, 900 m a.s.l.), Esterberg (DE, 1,300 m a.s.l.), Stubai (AU, 1,850 m a.s.l.), and Furka (CH, 2,440 m a.s.l.). All of the selected grasslands are seminatural, with the exception of alpine Furka, where natural grasslands occur above the tree line. For a description of the environmental site conditions see [Table 1](#).

### Experimental setup

In the summer of 2016, a total of 126 intact plant-soil monoliths were taken from native grasslands by inserting in PVC tubes with a diameter of 30 cm (similar method to [Kreyling et al. 2017](#); [Wu et al. 2012](#)) using a modified jackhammer. This experimental unit size is sufficient to allow for studying the integrative responses to a multitude of community interactions in small statured grasslands ([Milbau et al. 2007](#)). The sampling depth was variable: At the four low- to mid-elevation sites (DE) monoliths were taken with a depth of 40 cm, but because of shallow soils and increasing stoniness, this depth was reduced to 25 cm for monoliths from Stubai (AU) and Furka (CH). The bottoms of the monolith units were left open to allow for water flow. The monoliths were taken with the turf 1–2 cm below the rim to minimize microclimatic effects while avoiding excess water runoff. The shallow soils of Furka (CH) prevented this, so the monoliths were instead filled from the bottom with local soil to achieve the desired distance between upper rim and turf. After excavation of the monoliths at their site of origin, they were translocated downslope to the recipient sites and dug into the extant vegetation with the rim of the monolith flush to its surrounding. Monoliths were installed in raised beds at the lowest (Bayreuth, DE) and highest (Furka, CH) location. This was done because of high groundwater tables at Bayreuth and to avoid excessive disturbance of the sensitive habitat at Furka. Monoliths of the four German sites, ranging from 1,350 m to 350 m a.s.l., were translocated to each site with a lower elevation than the monolith origin. Monoliths originating from Stubai (AU, 1,850 m a.s.l.) and Furka (CH, 2,440 m a.s.l.) were translocated only to Bayreuth to test the ecological limits of alpine and subalpine grasslands reacting to abrupt changes in environmental conditions. The high sensitivity of these grasslands to disturbance minimized the number of replicates that could be extracted, preventing translocation to intermediate elevations. At all study sites, additional sets of monoliths were translocated within the respective site as control monoliths (see [Table 2](#) for an overview of replicates from origin and recipient sites, as well as [Figure 1](#)). The installation of site-specific control monoliths allowed us to control

for unintended treatment effects resulting from the extraction and the PVC tubes themselves (e.g., isolation from surrounding soil, which might hamper or inhibit lateral subsurface flows, root damage, etc.).

### Measured environmental and ecological parameters

Within the first ten days after snowmelt in spring 2017, sensors for soil moisture (EcH<sub>2</sub>O 5-TM, Decagon Devices Inc., USA) were installed horizontal at 5 cm depth together with data loggers (EcH<sub>2</sub>O Em50, Decagon Devices Inc., USA) using one monolith from each origin at each recipient site. Data were recorded at 15 min intervals to allow fine scale resolution, but were aggregated to daily means for this study. Additionally, on-site weather station data were used to determine precipitation amounts and growing degree days (GDD) as a proxy for thermal time available for plant growth. The GDD was calculated as the sum of the area beneath a sinusoidal curve that was fit to the minimum and maximum daily temperature, with a lower bound of 5°C ([Baskerville and Emin 1969](#)). This provides a site-specific estimate for the thermal energy available for plants, although it should be noted that with increasing elevation, the thermal microclimate of plants becomes increasingly decoupled from air temperature ([Scherrer and Körner 2009](#)). Here, we present data for the entire calendar year until the date of harvest as well as for the site-specific growing-season length of 2017. Growing season was defined as the number of days between the first five consecutive days with mean air temperature above 5°C ([Zhang et al. 2011](#)) and the date of harvest; both dates are site specific.

During the 2017 growing season, aboveground biomass of each monolith was harvested 3 cm above ground level at the time of peak biomass at each site. Aboveground biomass was sorted into single vascular plant species, inventoried to provide species richness per monolith, and then dried at 60°C for 48 h and weighed. In 2016, all monoliths were harvested after translocation at peak season to provide a baseline of aboveground production in subsequent years. Because of harvests in the previous year, litter had not accumulated. Dead or senesced tissue from the 2017 growing season was included in the species-specific biomass. This represented one full year of exposure to the climate of the recipient sites.

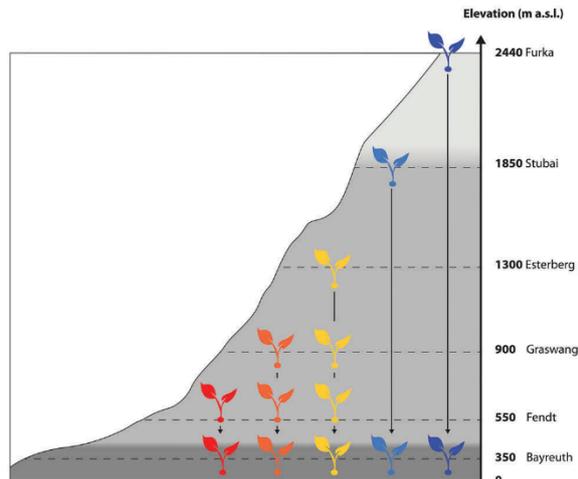
The percentage of green cover of each monolith was estimated weekly by a visual survey during the growing season in Bayreuth, always by the same observer (from March 16 to July 10, 2017). This was measured as a proxy of environmental stress, as senesced tissue

**Table 1.** Geographic, climatic, and plant sociologic characteristics of the study sites along the elevational gradient in the European Alps from low to high. All vegetation-period specific values are site specific. Data shown were calculated from on-site weather-station data. Long-term data for Esterberg are not available.

	Site (Country)					
	Bayreuth (DE)	Fendt (DE)	Graswang (DE)	Esterberg (DE)	Stubai (AU)	Furka (CH)
<b>Geographic</b>						
Elevational belt	Colline	Montane	Montane	Montane	Subalpine	Alpine
Coordinates	49°55'16"N 11°34'55"E	47°49'44"N 11°03'58"E	47°34'11"N 11°01'51"E	47°30'59"N 11°09'28"E	47°07'44"N 11°18'19"E	46°34'36"N 08°25'17"E
Elevation (m a.s.l.)	350	550	900	1,300	1,850	2,440
IMAT (°C)	8.2	8.6	6.6	NA	3	-0.5
MAP (mm)	724	959	1,285	NA	1,097	1,600
Growing degree days 2017	1,035	1,106	916	879	763	337
Total amount	982	1,046	860	779	664	285
Precipitation 2017 (mm)	275	513	643	556	550	658
Vegetation period	204	419	489	418	465	320
Total amount	8.1	7	6.3	5.3	5.9	0.5
Vegetation period	13.1	11.7	11.2	10.5	9.9	8.7
<b>Plant</b>	<i>Arrhenatheretum elatioris</i>	<i>Arrhenatheretum elatioris</i>	<i>Trisetum flavescens</i>	<i>Cynosuretum cristati</i>	<i>Trisetum flavescens</i>	<i>Caricetum curvulae</i>
Plant association	<i>Agrostis capillaris</i>	<i>Alopecurus pratensis</i>	<i>Dactylis glomerata</i>	<i>Anthoxanthum odoratum</i>	<i>Agrostis capillaris</i>	<i>Carex curvula</i>
Dominant species	<i>Anthoxanthum odoratum</i>	<i>Dactylis glomerata</i>	<i>Festuca rubra</i>	<i>Cynosurus cristatus</i>	<i>Trisetum flavescens</i>	<i>Helictotrichon versicolor</i>
	<i>Festuca rubra</i>	<i>Elymus repens</i>	<i>Festuca rubra</i>	<i>Elymus repens</i>	<i>Dactylis glomerata</i>	<i>Poa alpina</i>
	<i>Achillea millefolium</i>	<i>Lolium perenne</i>	<i>Trisetum flavescens</i>	<i>Festuca pratensis</i>	<i>Carex curvi</i>	<i>Nardus stricta</i>
	<i>Pilosella piloselloides</i>	<i>Poa angustifolia</i>	<i>Pimpinella major</i>	<i>Festuca rubra</i>	<i>Leontodon helveticus</i>	<i>Luzula lutea</i>
	<i>Plantago lanceolata</i>	<i>Poa pratensis</i>	<i>Plantago lanceolata</i>	<i>Lolium perenne</i>	<i>Leontodon hispidus</i>	<i>Campanula barbata</i>
	<i>Rumex acetosa</i>	<i>Taraxacum officinalis</i>	<i>Trifolium pratense</i>	<i>Trifolium pratense</i>	<i>Rumex acetosa</i>	<i>Homogyne alpina</i>

**Table 2.** Experimental setup. Number of monoliths translocated from origin (rows) to recipient sites (columns).

Recipient/Origin	Furka	Stubai	Esterberg	Graswang	Fendt	Bayreuth	Number Excavated
Furka	9	–	–	–	–	9	<b>18</b>
Stubai	9	9	–	–	–	9	<b>18</b>
Esterberg	9	9	9	–	–	9	<b>36</b>
Graswang	9	9	9	9	–	9	<b>27</b>
Fendt	9	9	9	9	9	–	<b>18</b>
Bayreuth	9	9	9	9	9	9	<b>9</b>
Number Translocated	<b>9</b>	<b>9</b>	<b>9</b>	<b>18</b>	<b>27</b>	<b>54</b>	<b>Σ 126</b>



**Figure 1.** Scheme of experimental setup. Each colored plant represents nine plant-soil monoliths, either translocated as control at the respective origin or to a specific recipient site. Colors of plants represent the investigated temperature gradient, from cold (blue) to warm (red). The grey scale of the mountain represents ecological zones spanned along this elevational gradient, ranging from colline (low elevation) to montane to alpine (high elevation).

may indicate either drought stress or water limitation (De Boeck et al. 2016).

We quantified light transmission to ground level by measuring the photon flux density of photosynthetically active radiation (PAR) immediately before harvest for all monoliths. We measured PAR above vegetation and at ground level to build a ratio of intercepted light by vegetation structure aboveground. This integrated measure of light transmission was used as a proxy for aboveground competition and shifting resource limitation (from below- to aboveground) in relation to hypothesis 2. We conducted these measurements using a light-ceptometer (AccuPAR LP-80, Decagon Devices Inc., USA) with the calibration set to 30 cm gauge length to capture PAR along the full diameter of the monolith.

### Statistical analyses

The treatment effects for all response parameters were calculated as relative changes compared to the control.

$$\text{Relative Change} = \left( \frac{\text{Sample} - \bar{X}\text{Control}}{\bar{X}\text{Control}} * 100 \right) \quad (1)$$

with *Sample* a single translocated monolith of a specific origin and  $\bar{X}\text{Control}$  the mean of all excavated and reinserted control monoliths of the same specific origin.

Data were checked for normality and homoscedasticity. All analyses were done using R Statistics version 3.3.1 (R Core Team) and the packages “nlme” and “lsmeans.” We tested the effects of translocation on aboveground biomass and species richness, using linear models with the origin of the monolith with either changes in elevation, GDD, or precipitation as predictors. Additionally, we tested for interactions between origin and each environmental variable. The effects of translocation on changes in relative proportion to community aboveground biomass of the plant functional groups graminoids, forbs, and legumes were tested in the same way to detect possible dominance shifts in communities’ plant functional group composition in relation to hypothesis 3. Then, to compare recipient site-specific differences, we used linear mixed-effect models to test whether recipient sites had differing effects on the relative changes in aboveground biomass and total species richness with site of origin as a random factor. We then used a post hoc TukeyHSD to examine each pairwise comparison. To test if the change in aboveground biomass or species richness at a single recipient site was significantly different from zero we used similar linear mixed-effect models without intercept.

To demonstrate the robustness of our monolith approach, we report several additional components of our monolith communities in the supplementary material. First, mean species richness and mean maximum relative abundance (percentage of total aboveground biomass produced by the most abundant species within a monolith) of each origin’s control and translocated monoliths demonstrates that our monoliths were of sufficient size for examining community dynamics. Second, we demonstrate that plant communities were initially indistinguishable following translocation and experimental setup in 2016. We examined

the origin-specific effects of translocations on aboveground biomass and richness to different recipient sites from initial data collected in 2016 using ANOVA, followed by a TukeyHSD post hoc test for origins with multiple recipient sites. This comparison was unavailable for the Esterberg site because of recent mowing immediately before monolith extraction.

We tested for mechanisms underlying changes in species richness and aboveground biomass by correlating the relative changes of (1) biomass on light transmission and species richness, (2) light transmission on species richness, and (3) graminoid dominance (relative abundance of graminoids) on species richness, using linear mixed-effect models with a unique origin-recipient site ID nested within the site of origin as random effects.

Because of the observed non-linearity, we used generalized additive models (GAM) to model the observed green cover versus day of the year. GAM was implemented through the “mgcv” package in R using cubic regression splines and cross-validation to select the optimal amount of smoothers for estimating how green cover was predicted by day of the year interacting with monolith origin. Thus, model estimates are composed of six smoothing functions and an intercept value.

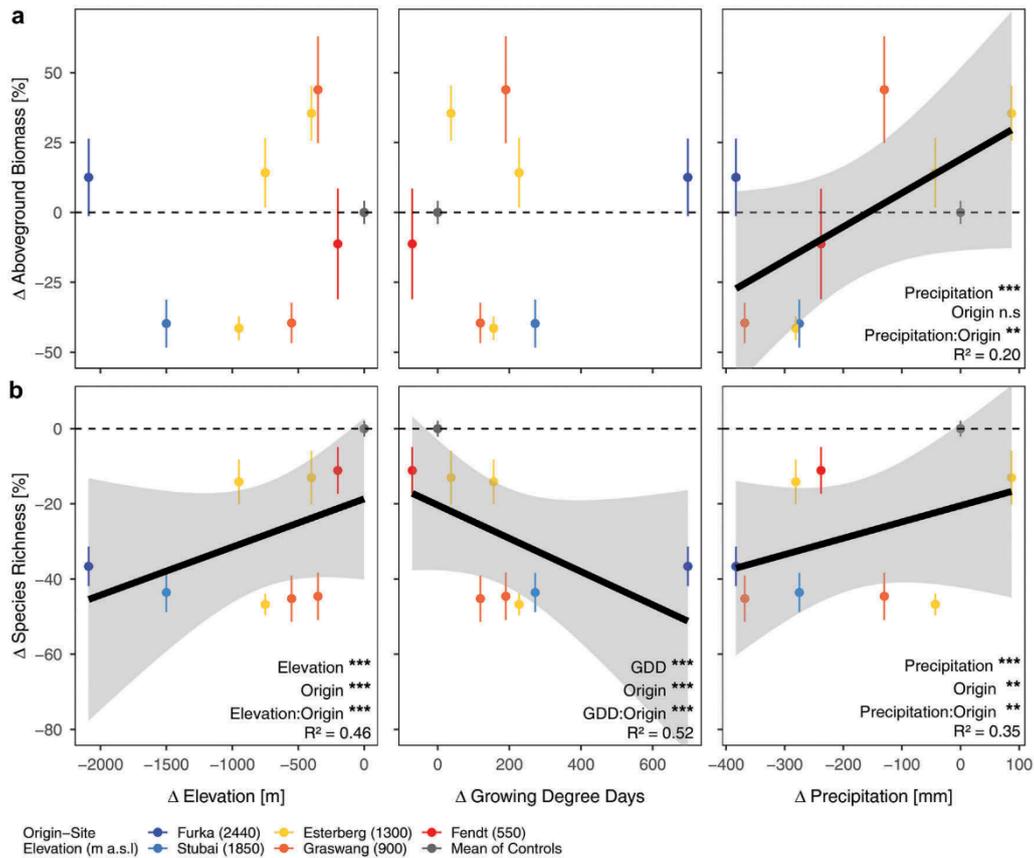
Differences in community composition were tested using the modified PERMANOVA approach proposed by Bacaro, Gioria, and Ricotta (2012). Bray-Curtis dissimilarity is an abundance-weighted beta diversity metric and was calculated for pairwise combinations of monoliths. For each combination of a translocation treatment ( $n = 9$ ) and its control ( $n = 9$ ) the following pairwise comparisons were made: Each monolith at the control site compared to all others at that site (labeled “within-control”), each monolith at the site of translocation compared to all others at that site (labeled “within-translocation”), and each monolith at the control site compared to each monolith at the translocation site (labeled “between”). To minimize the number of statistical comparisons made, we only tested for differences between the two “within” groups (e.g., does translocation to a given site cause monolith communities to become more or less alike one another than those at the site of origin?) and the “between” group to the “within-control” group (e.g., does translocation cause monolith communities to become more dissimilar than control monolith communities are to each other?). To avoid issues of inherent non-independence that arise as a result of each monolith being used for multiple comparisons, an  $F$  statistic was calculated for observed differences between each overall statistical comparison made, followed by 9999 Mantel randomizations of the observed values within the dissimilarity

matrix and recomputation of the  $F$  statistic. A  $p$  value was calculated based on the percentage of randomized  $F$  statistics that were larger than the observed  $F$  statistic. Because of the multiple comparisons made, we adjusted the 0.05 level of significance using Bonferroni corrections for the number of comparisons made within each origin. This allowed us to be cautious in our interpretation of significant differences, while avoiding being overly conservative in these adjustments.

## Results

### Aboveground biomass

Downslope translocation led to increased thermal time (GDD) for all translocated monoliths, except for the translocation from Fendt (550 m a.s.l.) to Bayreuth (350 m a.s.l.). However, the increased thermal time of Fendt relative to Bayreuth is entirely the result of the five additional days until harvest in Fendt, as the sites were otherwise equivalent. All monoliths that were translocated to the lowest site (Bayreuth, 350 m a.s.l.) experienced a substantial decrease in precipitation (Table 1). After a full year of exposure to these new environments, changes in aboveground biomass of the montane and alpine grassland communities did not correlate with elevational distance or thermal time (GDD) changes because of translocation (effect of elevation:  $p = .17$ ,  $R^2 = 0.05$ ; effect of GDD:  $p = .7$ ,  $R^2 = 0.04$ ). However, aboveground biomass positively responded to changes in precipitation (effect of precipitation:  $p < .001$ ,  $R^2 = 0.2$ ; Figure 2a). Recipient sites of translocated plant communities had different influences on the relative change of aboveground biomass ( $p < .001$ ;  $F = 18.51$ ). Aboveground biomass increased for all communities that were translocated to the intermediate recipient sites Graswang (900 m a.s.l.) and Fendt (550 m a.s.l.) on average by 35 percent ( $p < .01$ ) and 29 percent ( $p < .01$ ), respectively, even where precipitation was slightly reduced (Figure 2a). However, the overall aboveground biomass decreased for communities that were translocated to the warmest, driest elevation site Bayreuth (350 m a.s.l.) by an average of 24 percent ( $p < .05$ ; see Figure 3a). However, the aboveground biomass of communities originating from Fendt (decrease) and Furka (increase) showed no significant change after translocation to the warmest and driest site. Of note is the contrasting pattern of aboveground biomass between the two lowermost recipient sites, which both had reduced precipitation relative to higher sites but to different extents. This indicates a threshold of soil-water limitation. We found no significant effect of change in elevational distance, thermal



**Figure 2.** (a) Aboveground biomass and (b) Species richness change of plant communities in response to changes in elevation, growing degree days and precipitation resulting from downslope translocation. Significant influence of altered environmental conditions are shown as a black line with grey-shaded 95% confidence intervals. Significance of model factors indicated by asterisk (\*\*\*)  $p < .001$ ; \*\*  $p < .01$ ; \*  $p < .05$ ; n.s non-significant) and overall model  $R^2$  are displayed in the lower right corner of the respective panel. Mean and standard error are displayed in all graphs. For the control monoliths mean and standard error was calculated for all controls grouped. Color code of legend is valid for all panels.

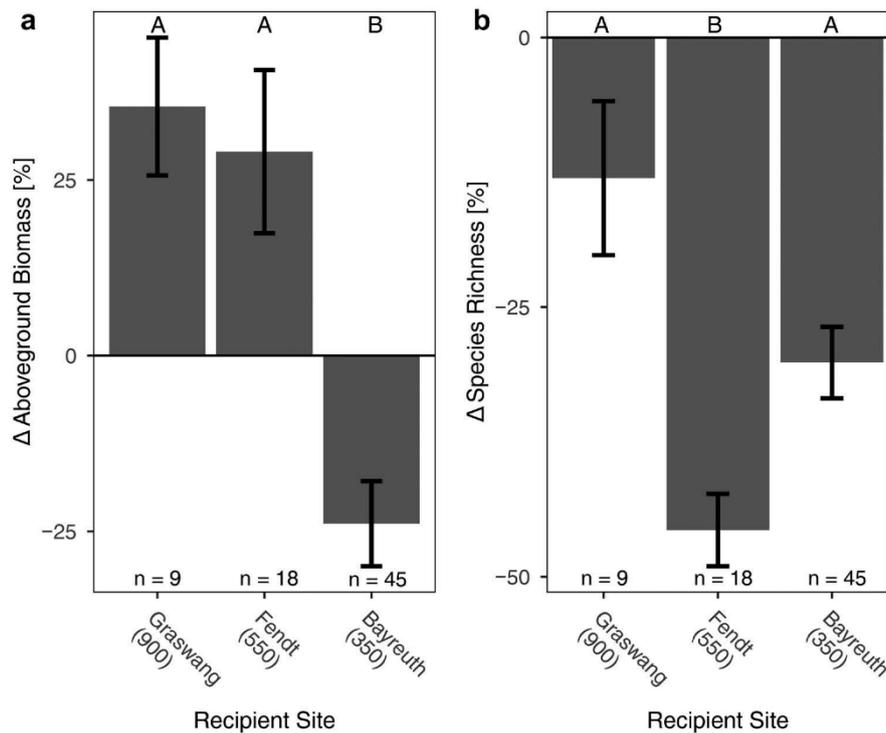
time (GDD), or precipitation on the relative proportion of the plant functional groups graminoids, forbs, or legumes to community aboveground biomass (see Supplementary Table 1).

### Species richness

The species richness of plant communities at all recipient sites consistently decreased with translocation. Thus, after the first year of exposure after downslope translocation, the species richness decline of montane and alpine grasslands was highly correlated with the change in elevation, thermal time, and precipitation (effect of elevation:  $p < .001$ ,  $R^2 = 0.46$ ; effect of GDD:  $p < .001$ ,  $R^2 = 0.52$ ; effect of precipitation:  $p < .001$ ,  $R^2 = 0.35$ ; Figure 2b). The recipient sites had a significant influence on the relative decrease of

species richness ( $F = 6.6$ ;  $p < .01$ ). Species richness decreased in monoliths translocated to Graswang (900 m a.s.l.), Fendt (550 m a.s.l.), and Bayreuth (350 m a.s.l.) by a mean relative change of 13 percent ( $p < .05$ ), 46 percent ( $p < .001$ ), and 30 percent ( $p < .001$ ), respectively (Figure 3b).

The mean species richness of the control monoliths ranges from 6.0 species to 20.4 species. While there is variation between origins, we see a significant loss of species in four of five origins at their respective transplanted sites (ranging from a mean species richness of 5.33 to 15.4; Supplementary Figure 1a). Further, the mean maximum relative abundance of control monoliths ranges from 29.1 percent to 48.5 percent, suggesting that single species (and thus individuals) are not dominating the monoliths. For translocated monoliths this generally increased (origin means ranging from 40.6 percent to



**Figure 3.** (a) Aboveground biomass and (b) species richness change in plant communities (monoliths) after one year of passive warming by translocation. Relative change for plant communities of all origins translocated downslope to the respective recipient site (m a.s.l. given) grouped together. Replicates at each recipient site given at the bottom of each panel. Mean and standard error are displayed in all graphs. Letters indicate significant differences between recipient sites as results of TukeyHSD post hoc tests conducted after ANOVA  $p < .001$ .

68.2 percent), showing a significant increase in two of the five origins (Supplementary Figure 1b).

### Green cover

The amount of green cover in plant communities after a full year of exposure to warming at the lowest site (Bayreuth, 350 m a.s.l.) decreased during mid-summer in periods coinciding with low soil moisture measured in the monoliths (Figure 4). After translocation to the lowest elevation site, the rates of increase and amount of green cover of all plant communities from higher elevations—irrespective of elevational distance translocated—differ significantly from the local control communities of the lowest site ( $p < .001$  for all origins,  $R^2_{\text{DOY} \times \text{Origin}} = 0.77$ ). Plant communities from the lowest elevation site (Bayreuth) showed high resistance to dry periods regarding green cover. In contrast, green cover of plant communities originating from intermediate elevation decreased after an initial, less pronounced dry period of ten days in late May with only one 0.87 mm precipitation event. Notably, plant communities from the highest elevation alpine site (Furka) were generally slower to green up, maintained

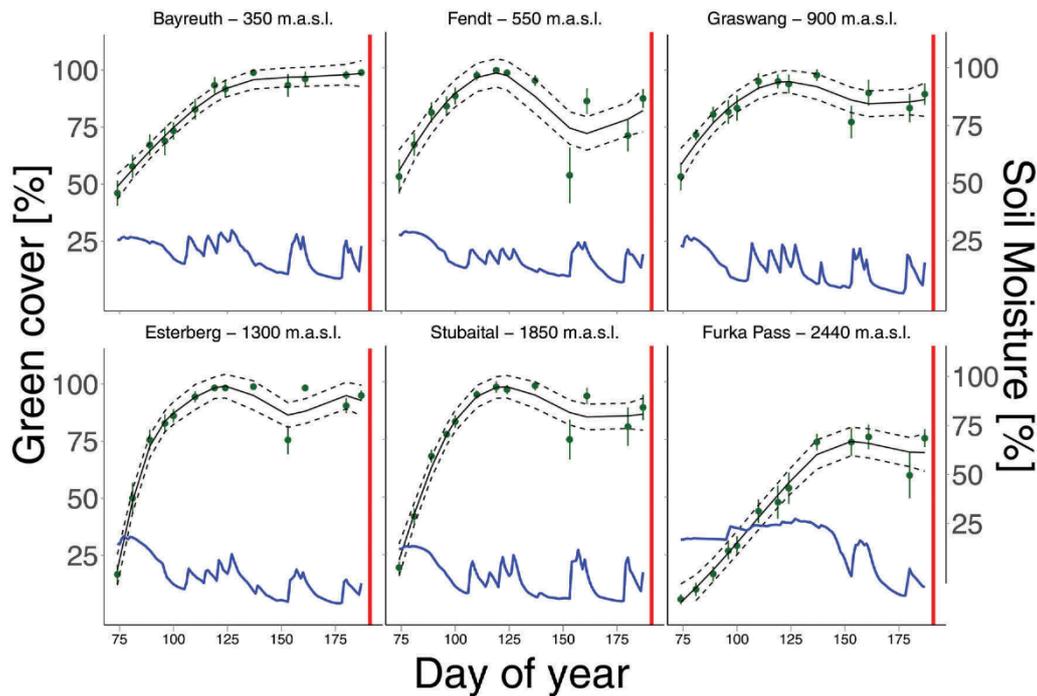
higher soil-moisture values during the initial dry period, and decreased in green cover only after a second dry period of 17 d without any precipitation in mid-June.

### Light transmission and graminoid proportion within plant communities

Light transmission at the ground level of plant communities decreased significantly as aboveground biomass increased ( $p < .001$ ,  $R^2 = 0.15$ ; see Figure 5a). Yet, species richness was uncorrelated to both aboveground biomass ( $p = .23$ ; see Figure 5b) and light transmission at ground level ( $p = .35$ ; see Figure 5c). A significant but weak correlation of decreasing species richness with increasing relative proportion of graminoids to community aboveground biomass was found ( $p < .01$ ,  $R^2 = 0.12$ ; see Figure 5d).

### Beta diversity

Bray-Curtis dissimilarity (abundance-weighted beta diversity) did not vary significantly between within-site of origin (control) and within-translocated



**Figure 4.** Green cover of translocated plant communities to the lowest elevation site (Bayreuth) showing different speed in greening up and different reaction to low soil-moisture availability. Green cover modeled as GAM shown as solid green line with 95 percent confidence intervals (dashed lines). Blue lines indicate soil moisture for the specific site of origin at the lowest elevation site (350 m a.s.l.). Red line shows harvest date at the recipient site (Bayreuth).

(warmed in respect of GDD) communities for any site. However, dissimilarity values between control and translocated communities did differ significantly for four of eight translocation combinations spanning a range between 200 m and 2,090 m of elevational distance. These significant differences in beta diversity were found for plant communities originating from the highest alpine site (Furka to Bayreuth, 2,090 m elevational distance,  $p < .001$ ) and from intermediate montane sites (Esterberg to Bayreuth, 950 m elevational distance,  $p < .01$ ; Graswang to Bayreuth, 550 m elevational distance, and Graswang to Fendt, 350 m elevational distance, both  $p < .05$ ), with a fifth translocation (Esterberg to Fendt, 750 m elevational distance) showing a marginal significance after Bonferroni adjustments ( $p = .08$ ). For a visual display of community dissimilarity results see Figure 6.

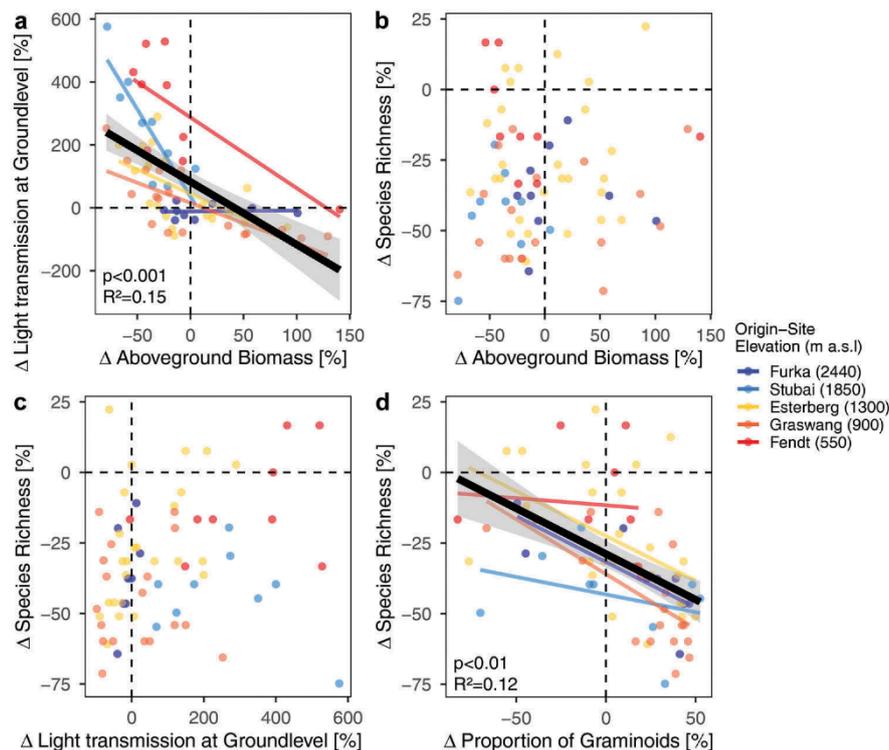
#### Initial state after experimental setup in 2016

In 2016, aboveground biomass increased in the monoliths originating from alpine Furka (2,440 m a.s.l.) and montane Graswang (900 m a.s.l.) after translocation to the lowest site, Bayreuth (350 m a.s.l.;  $p < .05$ ), while all other translocations were nonsignificant (Supplementary Table 2). This

suggests that the short-term residence in Bayreuth may have increased biomass shortly after translocation, but this influence was not evident in the 2017. Species richness in 2016 showed only one significant difference within origins, as Graswang (900 m a.s.l.) monoliths translocated to Fendt (550 m a.s.l.) had higher species richness than those in other sites ( $p < .05$ ; Supplementary Table 2). This result did not persist in 2017, and was in fact inverted. Ultimately, the process of downslope translocation did not appear to negatively affect the monoliths, which may have been indicated by initial loss of species or aboveground biomass.

#### Discussion

Abrupt warming following downslope translocation of plant-soil monoliths from montane and alpine grasslands revealed rapid changes in productivity and diversity, indicating low resistance. Consistent with our hypothesis, aboveground biomass increased with temperature in mid-elevation sites where the precipitation regime was similar, but decreased at the lowest elevation site where warming was coupled with a strong reduction in precipitation. Downslope translocation consistently led to species loss, again consistent with our hypothesis.



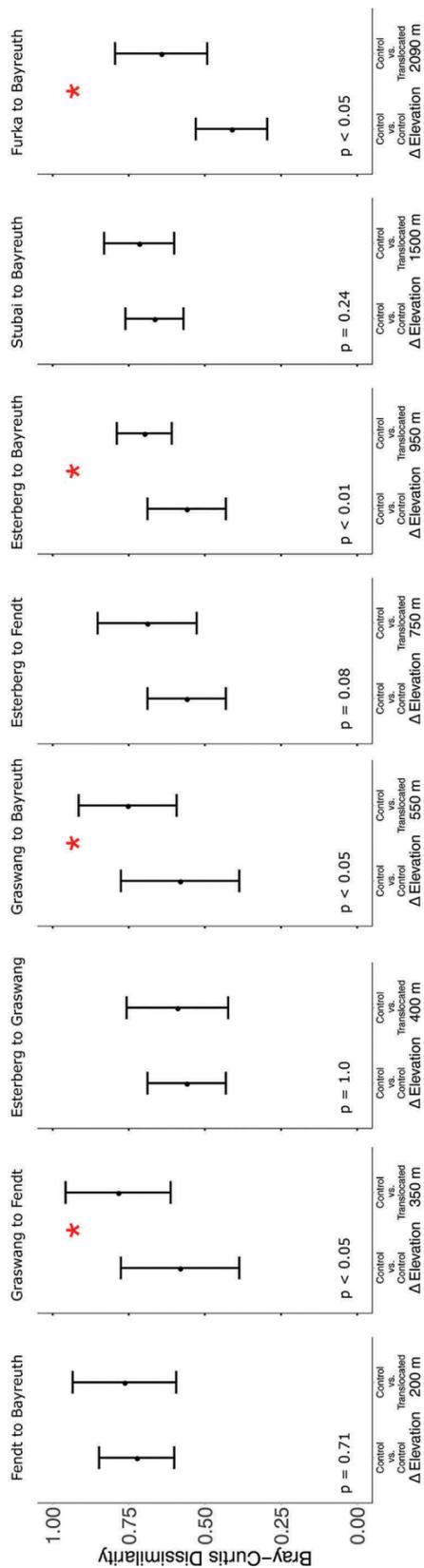
**Figure 5.** Changes in aboveground biomass and species richness showing relationship of relative change compared to the specific controls of (a) aboveground biomass versus light transmission, (b) aboveground biomass versus species richness, (c) light transmission versus species richness, and (d) proportion of graminoids versus species richness. Black lines with grey-shaded 95 percent confidence intervals are the overall model estimate;  $R^2$  and  $p$  values are given if significant. Colored lines represent site-of-origin model estimates.

However, contrary to our expectations the detected loss of species richness was independent of variations in aboveground biomass and light transmission. This outcome suggests that increased aboveground competition did not drive species loss. Richness decline was significantly stronger with increasing graminoid abundance, although it was still observed at weaker levels in monoliths that showed reduced graminoid abundance. Together with the observed beta-diversity changes between translocation sites, but not *within* translocation sites, we conclude that community composition shifts were neither highly deterministic nor converging toward similar communities.

#### **Initial aboveground biomass response to abrupt warming**

The observed increases in aboveground biomass of grassland communities at intermediate translocation range is consistent with an increase in the length of the growing season because of an earlier snow melt

(Inouye and Wielgolaski 2003; Latenser and Schneebeli 2003; Ernakovich et al. 2014; Gobiet et al. 2014) and faster metabolic processes (Lucht et al. 2002; Larcher 2003). The loss in aboveground biomass within plant communities that were translocated to the lowest site (350 m a.s.l.) may be the result of increasing water limitation (Schär et al. 1999, 2004; Kreyling et al. 2017), which is likely stressful for all the translocated plant communities that originated from regions of higher precipitation. The natural drought periods at the end of May (10 d) and in mid-June (17 d) at the lowest site (Bayreuth, 350 m a.s.l.), coupled with high summer temperatures, may have led to increased transpiration demand in the plants and a higher evaporation rate. This led to depleted soil moisture (Seneviratne et al. 2010; Quesada et al. 2012; Kreyling et al. 2016; Wolf et al. 2016) as shown by prolonged troughs in the soil-moisture trend lines in Bayreuth for monoliths of all origins. Low soil-moisture availability reduces stomatal conductivity, photosynthetic rate, and indirectly growth and carbon allocation (De Boeck et al. 2006, 2007; McDowell et al. 2008). Of note is the opposing



**Figure 6.** Dissimilarity in community composition among plant communities translocated along an elevational gradient from 2,440 m a.s.l. to 350 m a.s.l. Bray-Curtis dissimilarity values among indicated plant community (monolith) groupings for each monolith origin-translocated pairing. Panels are sorted according to elevational distance traveled by plant communities via translocation. Plotted values are means of all possible pairwise values in the indicated grouping with standard deviation error bars. PERMANOVA was used to test “within control dissimilarities” versus “between control/translocated” dissimilarities. Red asterisks indicate the significance between the translocated and control dissimilarities at  $p < .05$  after adjusting for the multiple comparisons made within each origin group, additional  $p$  values are given in the lower left corner of each panel.

direction of aboveground biomass changes between monoliths translocated to the two lowest elevation sites (350 and 550 m a.s.l.). These sites had similar amounts of thermal time, but only half the amount of precipitation fell at the lowest site (Table 1). Together, this suggests that drought stress induced by high temperature and low precipitation at the lowest site drove the observed decrease in aboveground biomass at the lowest site. The slight reduction in precipitation experienced by communities that were translocated to the intermediate recipient site did not result in reduced aboveground biomass. We speculate that a critical threshold of reduced precipitation underlies the observed contrasting pattern of aboveground biomass between the two lowest recipient sites. This is in line with previous studies stating that aboveground biomass is more sensitive to soil water content than temperature under low precipitation regime (Fei et al. 2015; Winkler, Chapin, and Kueppers 2016). High-elevation plant communities have been shown to be prone to direct tissue damage by overheating after being translocated to lower elevation if transpirational cooling of the plant tissue is impeded because of limited soil-moisture availability (De Boeck et al. 2016). All of these drought effects together with the reduction of precipitation explain the observed reduction in aboveground biomass of plant communities from mid-elevations (550–1850 m a.s.l.) at our lowest and warmest site in Bayreuth at 350 m a.s.l. It underpins the importance of jointly considering both temperature and precipitation effects in experiments seeking the ecological impacts of either variable.

The occurrence of drought stress is supported by the rapid reduction in community green cover within plant communities originating from intermediate elevation (900–1,850 m a.s.l.) compared to the local control originating from the lowest elevation (350 m a.s.l.) during natural drought events. Interestingly, alpine plant communities from the highest elevation (2,440 m a.s.l.) show a delayed decrease in green cover starting only during the second natural drought event (mid-June) compared to the ones from mid-elevations (550 m–1850 m a.s.l.) starting during the first natural drought event (late May). Those high-elevation plant communities green up slower, which may be caused by photoperiod regulation of plant growth (Ernakovich et al. 2014). The measured soil moisture of the alpine plant communities remained higher than the measured soil moisture in plant communities from intermediate elevations throughout the year at the lowest elevation site. This may indicate that the edaphic properties of this system buffered the community response, although caution is warranted given the lack of replication in soil-moisture measurements.

Hence, our results suggest that montane grasslands may be less resistant to periods of water scarcity than grasslands of high, alpine elevation. Alternatively, as long-lived and clonal species inhabit plant communities of high, alpine elevation, they could be particularly slow in response to changing environments (Dullinger et al. 2012; Gritsch, Dirnböck, and Dullinger 2016).

### **Initial diversity response to abrupt warming**

The consistent loss of plant species with downslope translocation is remarkable. In particular, mid- to high-elevation plant communities experienced significant species losses within one year at all recipient sites relative to the control mesocosms that were extracted and reinserted at their site of origin. However, as the loss of species richness was correlated with the degree of elevational distance, both temperature and precipitation change, our results suggest that the observed species loss is the result of complex interactions between multiple altered environmental drivers changed by translocation. Interestingly, species richness losses were highest at the second lowest site, where thermal time was similar to the lowest site and precipitation was similar to higher elevation sites. This could be an indication of water limitation restraining competitive exclusion at the lowest elevation site and merits further observation in future years. We note that because of the timing of translocations, monoliths are likely to experience limited colonization pressure from the matrix vegetation in this first year of sampling, meaning that future years could see a reversal in this species-loss pattern as the monoliths are colonized. Previous studies conducted at longer temporal scales did not detect a loss of plant species richness throughout time because of either natural global warming (Vittoz et al. 2009; Steinbauer et al. 2018) or experimental warming (Price and Waser 2000). Conversely, short-term experimental studies detect a loss of species richness after experimental warming (Debouk, de Bello, and Sebastià 2015; Sebastià, Kirwan, and Connolly 2008), which is aligned with the results presented here. This discrepancy between long- and short-term studies might be because of the time lag of colonization (Alexander et al., 2017; Dullinger et al. 2012). Additionally, observational studies can generally consider larger spatial scales than experimental studies, which inherently incorporates the role of habitat heterogeneity into maintaining diversity. Nonetheless, short-term studies reveal important aspects of community dynamics, such as the biotic conditions that promote resistance to abrupt changes (Kreyling et al. 2017). Our study shows a decrease in species richness that was stronger in communities with increased graminoid dominance. Evidence of alpine habitats suggests that shifts in plant-community composition can occur based on abundance

shifts toward more thermophile species that outcompete more cold-adapted species (Gottfried et al. 2012). This has also been attributed to competitive interaction among various plant functional groups (Theurillat and Guisan 2001) and species resource acquisition and allocation strategies (Aerts 1999; Diaz et al. 2004). With shifting environmental conditions, the relative strengths of community assembly mechanisms and species interaction have been shown to shift as well (Gellesch et al. 2013; He, Bertness, and Altieri 2013), including temperature-limited ecosystems such as arctic (Klanderud, Vandvik, and Goldberg 2015), montane, and alpine habitats (Callaway et al. 2002). While in our study competition was not measured per se, the observed loss of species in relation to beta-diversity patterns, light availability shifts, and functional group shifts informs species interaction effects.

Despite the consistent species loss, observed beta-diversity shifts within one year after translocation were less consistent. Most notably, plant communities originating from subalpine Stubai (1,850 m a.s.l.) showed a significant loss of species, but no significant shift in community dissimilarity when comparing translocated communities with their high-elevation controls. Because our dissimilarity metrics are weighted abundances, this suggests a general loss of rare or low-abundance species with a maintenance of the dominant species in plant communities. The opposite was observed for plant communities originating from mid-elevation Esterberg (1,350 m a.s.l.), with no species loss because of translocation to the lowest elevation site, but a strong increase in abundance-weighted dissimilarity compared to the control communities remaining at the site of origin. This pattern is consistent with dominance shifts, where previously subordinate species are able to take advantage of their new environment at the expense of previously dominant species that are reduced in abundance but still persist as part of the population. Overall, this points to a high amount of local site specificity in predicting grassland changes.

While our observed loss of species is consistent with increasing interspecific competition from colder to warmer mountain habitats (Kikvidze et al. 2005), we found no evidence suggesting that aboveground competition induced this species loss, as we did not find any significant correlation of light transmission (a proxy for aboveground competition intensity, sensu DeMalach, Zaady, and Kadmon 2016) and the loss of species. Increased temperature has been observed to increase decomposition rates and thus increased nitrogen availability (Gavazov 2010; García-Palacios et al. 2013; Wang et al. 2016). An improved soil-nutrient status often leads to increased light competition (Hautier, Niklaus, and Hector 2009; Borer et al. 2014; DeMalach, Zaady, and

Kadmon 2016). Although we did not measure any soil parameters directly, we speculate that the lack of evidence of increased aboveground competition indicates a nonsignificance in changes of soil-nutrient competition in our monoliths. Potentially, any increased nitrogen availability with warming is masked by other temperature-regulated mechanisms, such as soil microbial activity or increasing asynchrony between nitrogen availability and plant growth (Ernakovich et al. 2014), causing a high level of belowground competition.

The observation of stronger species richness losses in plant communities with increased graminoid dominance is consistent with other studies that found graminoids to be most responsive to warming in cold-adapted habitats (Dormann and Woodin 2002; Brooker 2006; Winkler, Chapin, and Kueppers 2016). Nonetheless, the relative contribution of plant functional groups to aboveground biomass did not show a correlation with the degree of change of either temperature or precipitation. Furthermore, as species richness losses were also observed in plant communities with strong shifts toward forbs, our results of community composition suggest that non-deterministic processes such as priority effects prevail during initial phases of severe environmental change. If the variance of beta diversity within translocated communities converges with warming (i.e., increased similarity among translocated monoliths), this would support the idea of deterministic processes (Chase and Myers 2011; Kreyling, Jentsch, and Beierkuhnlein 2011; Segre et al. 2014). Overall, this suggests that translocated plant communities are not filtered in the initial stages according to a system-wide competitive hierarchy of resident species. Rather, within-monolith priority effects may allow previously subdominant species to take advantage of the more favorable temperature and to outcompete the now subordinate species (Suding et al. 2005), indicating a role of community asynchrony (Ma et al. 2017). Our results capture only the first-year initial dynamics of the translocated plant communities. As the monoliths continue to be exposed to their new environments, colonization pressure from the matrix vegetation will likely mitigate or even reverse our observed changes in species richness and composition.

The shift in precipitation regime at our lowest site highlights the uncertainty of precipitation regimes in future climate scenarios and how they are coupled to the effects of temperature on productivity and diversity of grasslands (Backhaus et al. 2014; Grant et al. 2014a, 2014b). Our results underline the importance of understanding the interplay of temperature and precipitation (Easterling et al. 2000; Schär et al. 2004) in driving grassland community dynamics, especially for abrupt climatic changes. These findings highlight that water supplementation at

our lowest and driest site may yield additional insights onto the interactive effects of precipitation and temperature and will be an avenue of future research in this study. This study suggests that climate warming may increase aboveground biomass of montane grasslands as long as it is not coupled with a decrease in precipitation or sustained periods of drought. These montane grasslands are likely not adapted to drought stress during the growing season. The consistent loss of species in the first year indicates significant and rapid reordering of competitive outcomes in these communities, which may lead to unpredictable outcomes in the future at larger temporal and spatial scales. Shifts in composition can lead to challenges for conservation as well as for economy. Species loss or homogenization decreases community asynchrony (Gross et al. 2014; Hautier et al. 2018) and stability (Hautier et al. 2015), and the ability of those communities to buffer extreme events is also reduced, causing less secure ecosystem services in future climates (Wilcox et al. 2017). As disparities in responses of biomass production and richness to environmental changes in these systems remain, continued observational and experimental studies are warranted, and joint consideration of temperature and precipitation are critical.

### Acknowledgments

We thank Christian Körner, Erika Hiltbrunner, and Michael Bahn for giving valuable feedback during the process of writing, and Ralf Hafner for kindly visualizing the experimental setup.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Funding

This study was supported by the German Federal Ministry of Education and Research (BMBF) in the framework of the BonaRes project SUSALPS (Sustainable use of alpine and prealpine grassland soils in a changing climate) under Grant (project number: 031B0027C).

### Author contributions

BB, PW, MS, and AJ conceived the ideas; BB, MS, and AJ designed methodology; BB, PW, MAS, MASAK, PE, AH, and JI collected and processed data; BB, PW, and PE analyzed data; BB led the writing of the manuscript; PW, MASAK, PE, AH, JI, MS, MAS, and AJ assisted writing the manuscript. All authors critically contributed to the drafts and gave final approval for publication.

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9.2 **Manuscript 2** | Berauer *et al.* (2020) – Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change.



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Sampling procedure of vis-NIRS in the „dark spectrometry“-lab of the TUM, soil science



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## Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change



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## ARTICLE INFO

## Keywords:

Forage quality  
Grassland communities  
Climate change  
Sustainable agriculture  
Spectrometry  
Land-use intensity

## ABSTRACT

With a growing human population facing multiple global change drivers (i.e. climate change and land management change), the future of food security is of major importance. Sustainable agriculture is therefore key to ensure food supply and food security under future climatic conditions. Forage provision (composed of forage quantity and forage quality) is an important ecosystem service of grasslands for dairy production. However, monitoring forage quality in semi-natural species-rich grasslands is rarely done due to the inherent complexity in determining forage quality, high variability within natural systems and financial and workload restrictions. Here, we i) demonstrate the ability of visible-near-infrared spectroscopy (vis-NIRS) to predict forage quality of bulk samples of species-rich montane pastures and ii) show its potential to reveal effects of two key global change drivers, climate change and land management, on forage quality. Spectral information and chemometrics allowed us to predict three (ash, fat and protein) out of four analyzed forage quality parameters with high accuracy. Land management intensity strongly influenced species-rich grasslands' protein and fat content, whereas altered climatic conditions influenced ash and fat content. High management intensity increased protein content of high- and mid-elevation pastures by 22 % and 30 % and fat content by 19 % and 20 % respectively. Though forage quality was improved by intensive land management, extensive land management generally revealed sufficient forage quality for livestock. Vis-NIRS provides a rapid, cost-efficient and high-throughput technique to analyze forage quality, revealing effects of global change drivers on forage quality of grasslands. This approach will help to support stakeholders assure optimal nutrition feeding of livestock and achieve steps towards sustainable agriculture.

### 1. Introduction

Food demand is increasing with increasing human population (FAO, 2013; Godfray et al., 2010), however, land area suitable for agricultural production is limited (Azar, 2005; Stoll-Kleemann and O'Riordan, 2015; West et al., 2014) and intensification is unlikely to provide sustainable solutions (Allan et al., 2015; Foley et al., 2005; Gossner et al., 2016; Laliberté et al., 2010). Thus, to meet future human food demands, agriculture must sustainably increase production from less land through efficient use of natural resources and with lowest impact on the environment (Hobbs et al., 2008). Forage provision for cattle and dairy is

a major ecosystem service of agricultural grasslands. Forage provision determines carrying capacity and performance of livestock (Bailey et al., 1996; Schauer et al., 2005) and consists of two parts: quantity (yield or production) and quality (the nutritional value for livestock) (Beeri et al., 2007). Ongoing climatic changes as well as changes in land management intensity are likely to change both components of forage provision (Martin et al., 2014). While forage quantity is straightforward to assess, gaining measures of forage quality (i.e. crude protein, crude fat, crude fiber and crude ash) is challenging due to being multivariate and costly in time and money. Nonetheless, forage quality is equally important for maintaining a sufficient supply of energy and nutrients

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<https://doi.org/10.1016/j.agee.2020.106929>

Received 17 December 2019; Received in revised form 4 March 2020; Accepted 16 March 2020

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for livestock from plants. Failure to mitigate and adapt agriculture to changing environmental conditions can reduce economic value of grasslands and can even be a threat to food security (Richards et al., 2019). To maintain ecologic and economic values of grasslands under future changing conditions, we need to understand the effects of climate and land management on forage quality, which requires an effective and rapid tool to monitor forage quality.

Forage quality of grasslands increases with increasing protein and fat content, but decreases with increasing fiber content (Deak et al., 2007; Li et al., 2018; Xu et al., 2018). Forage quality is affected by species composition and abundance (Khalsa et al., 2012) and soil resource availability (Niu et al., 2016), which are both in turn affected by climatic conditions and land-use intensity. Land-use intensification ultimately reduces ecosystem stability (Blüthgen et al., 2016) by homogenization of landscapes (Gossner et al., 2016) and loss of biodiversity (Allan et al., 2015; Flynn et al., 2009; Newbold et al., 2015). Further, chemical, physical and biological soil properties, such as nutrient mining, compaction (Smith et al., 2016) and soil biodiversity (Tsioufouli et al., 2015) are negatively affected by land-use intensity. Climate change decreases soil organic carbon (Puissant et al., 2017), increases gross nitrogen turnover (Wang et al., 2016), and causes shifts in both aboveground plant communities (Gornish and Tylianakis, 2013; Klanderud and Totland, 2005) and belowground soil communities (Blankinship et al., 2011). Consequently, climate change and land management can interact both synergistic and antagonistic (Hasibeder et al., 2015; Ingrisch et al., 2017; Karlowsky et al., 2018).

In grassland systems, there is a need for increased research and monitoring of forage quality under global change scenarios. Rapid progress during the last decades in physics and engineering has opened a variety of optical measurements, notably near-infrared spectroscopy (NIRS) and closely related techniques such as vis-NIRS (visible near-infrared spectroscopy). The wavelengths covered by vis-NIRS is extended at the lower range down to 350 nm in comparison to the lower boundary of NIRS (800 nm), thus offers a broader spectral range to detect organic complex molecules. In comparison to the wet-chemical standard analysis (i.e. improved and adjusted Weender-analytic), which is time-consuming, expensive and demands relatively high sample masses, NIRS is a cheap and fast high-throughput technique. This opens the opportunity for quick and efficient analysis of large sample numbers (Foley et al., 1998; Osborne et al., 1993; Shenk and Westerhaus, 1993). NIRS is a qualitative and quantitative analysis technique. It is based on the absorption and reflectance of the analyzed sample. The chemical composites of a sample (e.g. element, chemical bond, chemical structure) get excited by NIR radiation each to a specific degree, in turn reflecting a unique proportion of radiation for wavelengths and its molecular overtones (Bokobza, 2002). In organic substances in particular chemical bonds (such as CH, CC, C=C, CN, OH, NH) help to identify macromolecules of high complexity (Ludwig and Khanna, 2001). However, NIRS always needs to be calibrated to a chemical analyzed subset of samples. The process of predicting the parameter of interest for unknown samples requires development of multivariate models with accompanying quality checks. A further key in the successful application of NIR spectrometry lies in proper processing of spectral data and model validation to extract the appropriate information from the spectra (Gautam et al., 2015; Liland et al., 2016; Morais et al., 2019; Ng et al., 2018). After model development, NIRS allows for relatively rapid identification and quantification of several parameters of interest from a plant samples' spectra. Thus, with NIRS we can extrapolate the parameters of interest for a large set of samples out of a considerably smaller subset of samples (Foley et al., 1998; Lawler et al., 2006).

NIRS has advanced in its application in basic and applied science (Ozaki, 2012). In vegetation ecology, NIRS has been implemented in elemental stoichiometry, non-structural carbohydrate or forage quality analysis of e.g. aquatic grasses (Lawler et al., 2006), terrestrial plant functional types (Anderson et al., 2018) and single species (Murguzur

et al., 2019; Quentin et al., 2016), dry matter yield of mixed sown communities (Biewer et al., 2009), different plant organs (Ramirez et al., 2015), remote sensing techniques to estimate forage provision (Beerli et al., 2007) or even classify or identify communities and species (Durgante et al., 2013; Richter et al., 2016; Ustin and Gamon, 2010). Hitherto, the application of NIRS on forage quality of bulk samples from species-rich grasslands is scarce (Fekadu et al., 2010; Parrini et al., 2018) and to our knowledge has not been used to reveal effects of multiple global change drivers on forage quality. To be able to meet the goals of sustainable agriculture under future environmental and demographic conditions, we need to be able to rapidly track forage quality and understand the interplay between major global change drivers and their effects on forage quality.

The goal of this study is twofold. First, we test the ability and accuracy of vis-NIRS to predict classic forage quality (i.e. crude ash, crude fat, crude fiber and crude protein as determined from wet-chemical analysis) of mixed bulk samples of central European species-rich, semi-natural, montane grasslands. Second, we demonstrate the application of vis-NIRS by testing the influence of two major global change drivers, climate change and land management intensity, on forage quality of such grasslands. Our ultimate aims are to inform and improve grassland management with a flexible and readily accessible tool to measure and monitor forage quality of agricultural lands in the face of changing conditions.

## 2. Material and methods

### 2.1. Sample collection

Samples were collected within the land-use experiment of the Project: "Sustainable use of alpine and pre-alpine grassland soils in a changing climate" (SUSALPS). In 2016, intact plant-soil mesocosms of two extensively managed, montane grasslands Esterberg and Graswang were translocated downslope along an elevational gradient in the TERENO Pre-Alpine Observatory in order to simulate climate change (increased temperature and reduced precipitation). The elevational gradient of translocation ranges from 1260 m a.s.l. (Esterberg) via 860 m a.s.l. (Graswang) to 600 m a.s.l. (Fendt). In 2017 and 2018, the years of study, this elevational gradient represented the two year mean annual temperatures and mean annual precipitations of 6.3 °C, 1113 mm at high elevation (Esterberg), 7 °C, 1433 mm at mid-elevation (Graswang) and 9 °C, 1036 mm at low elevation (Fendt). Thus, a warming of +1 K from high- to mid-elevation, +2 K from mid- to low-elevation and consequently +3 K from high- to low-elevation. We focus on altered temperature and precipitation as they are likely to exert the strongest effect on plant physiology. However, we acknowledge potentially other climatic changes to co-occur by downslope translocation, but believe that these factors likely only have a minor impact on plant communities in this study given the limited geographic extent of translocation. Communities from Esterberg and Graswang were translocated downslope to lower elevational levels as well as reburied at the site of origin as controls. Translocation and reinstallation procedure is described in Berauer et al. (2019). For an overview of experimental site descriptions see Supplementary Table 1, additional climatic conditions are displayed in Supplementary Fig. 1. Each of the communities was assigned to either an intensive or extensive land management intensity (n = 6 for each origin x recipient x land management combination) leading to 60 translocated communities in total. Intensive managed communities were cut (3 cm aboveground; simulating grazing or mowing) and fertilized (slurry application; with nitrogen input equivalent to  $42 \pm 10 \text{ kg N ha}^{-1}$  per fertilization event) 5 times a year, whereas extensive managed communities were cut 3 times and received slurry only after the first and last cut within each growing season. These applied management regimes were selected because they are common practices within this region and elevational range and thus represent realistic agricultural practices for future grasslands. Community bulk

samples of aboveground biomass were collected according to the local farmers' practice within the growing season of each year. From 2016 until 2018, a total of 515 bulk samples of montane grassland communities were collected and used here for analysis. For a detailed overview of dates of land-use management application see Supplementary Table 2.

## 2.2. Sample preparation

All collected samples were dried at 60 °C for 48 h and weighed. Samples were then first homogenized at 2 mm using a shredder (SK1, Retsch GmbH, Germany) and subsequently milled to powder using a ball-mill (MM301, Retsch GmbH, Germany). With this procedure, we obtained a homogenous and representative mixture of semi-natural, species-rich grassland samples. For each sample, a minimum of 3.5 ml volume of powder was milled and filled to an Eppendorf tube.

## 2.3. Spectral measurement

All spectra were measured in the darkroom laboratory using a vis-NIR analyzer (SVC HR 1024-i, Spectra Vista Corporation, USA) with a 4° fore optic lens. Spectral range was 350 nm – 2500 nm with a spectral resolution of 3.3 (at 700 nm), 9.5 (at 1500 nm) and 6.5 (at 2100 nm). To be able to produce representative and repeatable spectra, an external source of light (VNIR Light Source, Hyspex, Norway) was used and installed to avoid any shade from either the equipment set-up or the sample material. For the spectral scan, previously milled bulk sample material (each representing a mesocosm with 30 cm diameter) was filled into a plastic petri-dish (3 cm diameter) covering the bottom up to at least 0.5 cm and subsequently scanned 3 times at random positions. Each spectra was corrected for the offset between the three detectors, as described in Kühnel and Bogner (2017) and then smoothed by the singular spectrum analysis (SSA) with a window length of 50 using the package *Rssa* of the software R (Golyandina et al., 2013; Golyandina and Korobeynikov, 2014; Korobeynikov, 2009). Subsequently, the three spectra per sample were averaged. Three of the 515 samples had to be removed due to insufficient quality of the spectral data.

## 2.4. Wet chemical analysis

Wet chemical analyses of forage quality were conducted following standardized Weender-analytics (SGS Germany GmbH, Hamburg, Germany). A subset of 70 samples was chosen based on NIR spectral data for cross referencing using the Kennard-Stone algorithm with an Euclidean distance metric (Kennard and Stone, 1969), which selects representative samples to systematically cover the spectral variance in all samples. The selected samples are a representative mixture of all experimental combinations (origin, transplant, treatment and year). The algorithm had a total of 336 samples to choose from, as prior to calculations, samples gathered in the year of experimental set-up as well as samples with less than 15 g of biomass had to be removed. This relatively high amount of biomass provides a minimum measure quantity for the analyzed parameters of forage quality: crude ash, crude fat, crude fiber and crude protein.

## 2.5. Statistical analysis

### 2.5.1. Chemometric: vis-NIRS model for prediction of forage quality

Partial-Least-Square regression (PLS) was applied to predict forage quality (ash, fat, fiber, protein) of species-rich grasslands' tissue samples. We used the entire measured spectra ranging from 350 nm to 2500 nm. First derivative of spectral data was calculated prior to calibration-modeling, as it improved model performance in comparison to raw, vector-normalized or first derivative of vector-normalized spectra.

For model development, we split the available samples ( $n = 70$ ) into a calibration set ( $n = 50$ ) and an external validation set ( $n = 20$ ).

For this split, we conducted Kennard-Stone algorithm, again systematically selecting samples to represent the maximum spectral variance and to mimic the initial sample selection. A calibration-model was built using Partial-Least-Square regression (PLS) in combination with a variable selection procedure (CARS; competitive adapted reweighted sampling) to obtain the most parsimonious and robust models (Li et al., 2009). The Monte Carlo based approach CARS selects for an optimum number of wavelength with root mean squared error (RMSE) and an optimum number of latent vectors by leave-one-out cross-validation (Li et al., 2009). We used 50 CARS iterations, repeated 100 times to identify the best model with the lowest RMSE in cross-validation. For the simulation, a maximum of 10 latent vectors was set. Model performance was evaluated by the coefficient of determination ( $R^2$ ), root-mean-squared error (RMSE) and residual prediction deviation (RPD; ratio between standard deviation of the prediction to standard error).

### 2.5.2. Application: effect of climate change and land management intensity on forage quality

To test for effects of climate change and land management on forage quality, first parameters of forage quality suitable for prediction via vis-NIRS were identified; then all 512 gathered samples were predicted using the best model selected from CARS-PLS. Subsequently, we used linear mixed effect models to test the effects of treatments on individual fodder quality parameters, always including Plot-ID as a random factor to account for non-independence of samples by repeated measurements. First, we analyzed the data of 2017 and 2018 separately with land-use management, climate change as fixed factors and the interaction between both global change drivers. Secondly, where ANOVA revealed significant effects of model terms with multiple comparisons, we performed a Tukey HSD post-hoc test. For all models conformity of model assumptions (normal distribution and homoscedasticity of variances) were checked.

We calculated effect sizes to report on relative differences between either land-use management or climate change effects on parameters of forage quality. For effects of land management intensity, we used extensively managed communities (with low cutting and fertilization frequency as described in 'Sample collection') and for climate change effects we used the on-site of origin installed controls as reference.

All statistical analysis were performed in R Version 3.5.3 "Great Truth" (R Core Group, 2019) using the packages *pls* (Mevik et al., 2019), *nlme* (Pinheiro et al., 2019), *lsmeans* (Lenth, 2018).

## 3. Results

### 3.1. Predicting forage quality of species-rich grasslands (bulk samples)

Three out of four forage quality parameters were identified as suitable for measurements with vis-NIRS, namely ash, fat and protein, but not fiber. With CARS-PLS we identified robust and parsimonious models with high accuracy and predictive power in both internal (cross) as well as in external validation (see Fig. 1A and B). The selected wavelengths and their relative importance to model performance are shown in Fig. 1C.

Across all four modeled parameters, the best model was obtained for protein with the model evaluation of external validation ( $R^2_{\text{val}} = 0.83$ ;  $RPD_{\text{val}} = 2.4$ ;  $RMSE_{\text{val}} = 1.06$ ) and of calibration ( $R^2_{\text{cal}} = 0.93$ ;  $RPD_{\text{cal}} = 3.76$ ;  $RMSE_{\text{cal}} = 0.47$ ). The model consisted of 8 latent vectors built upon 93 selected wavelengths, which corresponds to 4.3 % of the 2150 wavelengths within the spectra. The second highest accuracy was achieved for communities' ash content with model evaluations of external validation ( $R^2_{\text{val}} = 0.71$ ;  $RPD_{\text{val}} = 1.87$ ;  $RMSE_{\text{val}} = 0.79$ ) and calibration ( $R^2_{\text{cal}} = 0.83$ ;  $RPD_{\text{cal}} = 2.42$ ;  $RMSE_{\text{cal}} = 0.47$ ). The 9 latent vectors building this model used 29 wavelengths, corresponding to only 1.3 % of the spectra. For fat content model evaluation of validation ( $R^2_{\text{val}} = 0.73$ ;  $RPD_{\text{val}} = 1.69$ ;  $RMSE_{\text{val}} = 0.37$ ) and calibration ( $R^2_{\text{cal}} = 0.86$ ;  $RPD_{\text{cal}} = 2.65$ ;  $RMSE_{\text{cal}} = 0.15$ ) is sufficient. To build this model

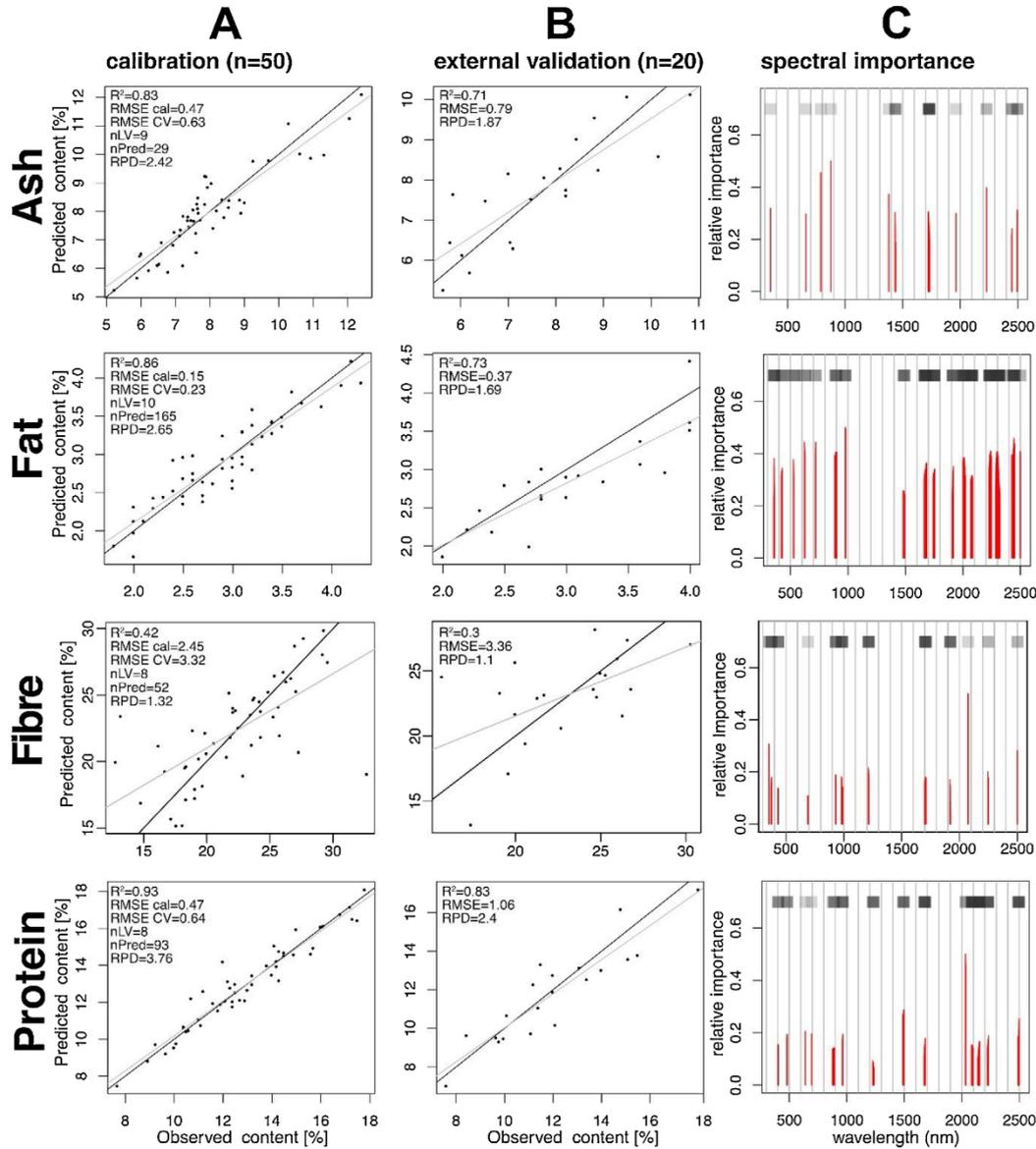


Fig. 1. Calibration (A, left panel), external validation (B, center panel) and importance of spectral regions (C, right panel) for parameters of forage quality of the selected best CARS-PLS model. Model descriptions and quality estimates are given in the top left corner of each respective panel. With  $R^2$ : coefficient of variation of the respective model; RMSE cal: root mean square error of calibration model; RMSE CV: root mean square error of internal cross-validation; nLV: number of latent vectors; nPred: number of predicting wavelengths; RPD: residual prediction deviation.

10 latent vectors of 165 wavelengths were built. These selected wavelengths represent 7.7 % of the entire spectra. For fiber we achieved insufficient model evaluations for prediction, both validation ( $R^2_{val} = 0.3$ ;  $RPD_{val} = 1.1$ ;  $RMSE_{val} = 3.36$ ) and calibration ( $R^2_{cal} = 0.42$ ;  $RPD_{cal} = 1.32$ ;  $RMSE_{cal} = 2.45$ ) indicate a high predictive error and inaccuracy.

For protein, ash and fat the RPD in combination with the respective  $R^2$  indicate “good” to “excellent” predictions, whereas the calibration for fiber is not usable (Malley et al., 2004; Saeys et al., 2005). Nonetheless, please note the deviation in model criteria between calibration and external validation for all three parameters, especially fat content.

For a detailed summary of model selection, estimates and quality criteria see Table 1.

The wet chemical determined percent concentration of dry matter of the forage quality parameters ranged between 5.24 and 12.42 (ash), 1.8 and 4.3 (fat), 12.8 and 32.5 (fiber) and 7.6 and 17.8 (protein) in the wet-chemical analyzed sub-set of samples. The range of the entire forecasted spectral dataset of the three parameters identified for robust prediction is 2.06 and 14.47 (ash), 0.72 and 5.31 (fat) and 5.95 and 28.70 (protein).

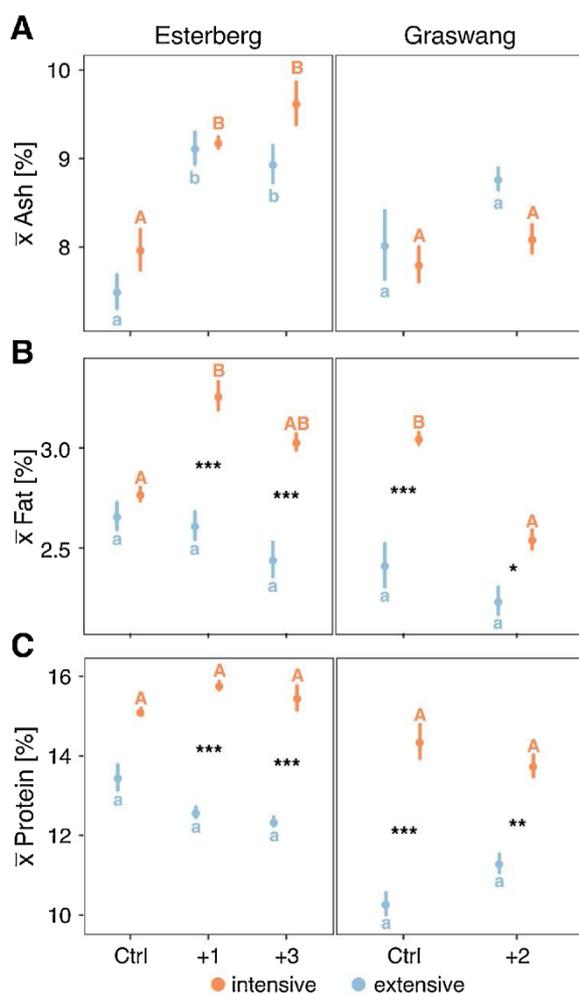
### 3.2. Effect of climate change and land management intensity on forage quality

Both climate change (increased temperature and altered precipitation) and land management intensities (different cutting and

**Table 1**

Description of CARS-PLS model performance and quality estimates of the selected best model. All models were built on first-derivate of spectra with allowed maximum latent vectors of 10. Dataset was split into calibration and external validation using the Kennard-Stone algorithm.

	Ash	Fat	Fibre	Protein
# latent Vectors	9	10	8	8
# Predicting Wavelength	29	165	52	93
<b>R<sup>2</sup></b>				
calibration	0.83	0.86	0.42	0.93
external validation	0.71	0.73	0.3	0.83
<b>RPD</b>				
calibration	2.42	2.65	1.32	3.76
external validation	1.87	1.69	1.1	2.4
<b>RMSE</b>				
calibration	0.47	0.15	2.45	0.47
internal validation	0.63	0.23	3.32	0.64
external validation	0.79	0.37	3.36	1.06



**Fig. 2.** Effects of land-use management and climate warming (x-axis) on forage quality in the year 2017 of both translocated communities. Shown are arithmetic mean and standard error of each parameter predicted with CARS-PLS. Asterisks indicate differences between land-use management within each climatic site (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ) and letters (colored according to land-use regime; additional upper and lower case for separation) indicate significant difference between climatic sites within each land-use regime.  $p$ -values of ANOVA and according TukeyHSD post-hoc test are displayed in Supplementary Table 6 and Supplementary Table 7.

fertilization frequency) influenced community forage quality using the full set of 512 samples. Here, we describe in detail the results of the year 2017 (Fig. 2). Similar analyses were also conducted for the data gathered in 2018. Results and summaries of analysis as well as effect size of both years can be found in the Supplementary Material (Supplementary Tables 3 – 7; Supplementary Fig. 2).

In general, intensive (five cuts and five slurry applications per growing season) land management increased both fat and protein content relative to extensively (three cuts and two slurry applications per growing season) managed communities originating from high-elevation Esterberg (19.4 % increase for fat and 22 % increase for protein) and mid-elevation Graswang (20.1 % increase for fat and 30.3 % increase for protein). Land management showed opposing trends on communities' ash content, with an increase of 5 % for intensive communities from high-elevation Esterberg and a decrease by  $-5.4$  % for intensive communities from mid-elevation Graswang compared to extensive communities. Climate change effects from warmer and drier conditions were highly variable across forage quality parameters and depended on the origin of communities (see Supplementary Table 3 for an overview of relative change by either land-use management or climate change).

### 3.2.1. Effect of management intensity on forage quality

Land management intensity affected fat ( $p_{\text{Graswang}} < 0.001$ ;  $p_{\text{Esterberg}} < 0.001$ ) and protein ( $p_{\text{Graswang}} < 0.001$ ;  $p_{\text{Esterberg}} < 0.001$ ) content of both translocated communities. Further the interaction between land-use intensity and climate change effected fat content ( $p_{\text{Esterberg}} < 0.05$ ) but only for communities from high-elevation Esterberg.

Intensive management in comparison to extensive management increased both fat (26.1 %;  $p < 0.001$ ) and protein (39.7 %;  $p < 0.001$ ) content of communities from mid-elevation at their site of control. Similarly to their site of control, fat (13.7 %;  $p < 0.05$ ) and protein (21.7 %;  $p < 0.01$ ) content of communities from mid-elevation Graswang increased by intensive management under altered (+2 K) environmental conditions (see Fig. 2B and C, the right panel).

Communities from high-elevation Esterberg did not show any significant difference introduced by land management intensity at their high-elevation control site. Yet, under altered environmental conditions after translocation to mid-elevation (+1 K warming) and low-elevation (+3 K) protein ( $p_{+1K} < 0.001$ ;  $p_{+3K} < 0.001$ ) and fat ( $p_{+1K} < 0.001$ ;  $p_{+3K} < 0.001$ ) content of communities originating from high-elevation Esterberg increased by intensive management. At +1 K warmed conditions protein content increased by 25.4 % and at +3 K by 25.2 %. Similar, fat content of high-elevation communities increased by 24.6 % at +1 K and 24 % at +3 K warmer conditions (see Fig. 2B and C, the left panel).

### 3.2.2. Effect of climate change on forage quality

Climate change affected ash content, but only in communities originating from high-elevation Esterberg ( $p_{\text{Esterberg}} < 0.001$ ). Whereas, fat content of communities originating from both sites was influenced by climate change ( $p_{\text{Esterberg}} < 0.01$ ;  $p_{\text{Graswang}} < 0.001$ ).

Ash content increased with warming irrespective of land management intensity at both +1 K ( $p_{\text{INT}} < 0.05$ ;  $p_{\text{EXT}} < 0.05$ ) and +3 K ( $p_{\text{INT}} < 0.01$ ;  $p_{\text{EXT}} < 0.05$ ) levels, but no difference between +1 K and +3 K warming was found for communities originating from high-elevation Esterberg.

Fat content of intensively managed communities originating from high-elevation Esterberg was 17.6 % higher at +1 K warming in comparison to the intensively managed controls ( $p < 0.001$ ). Contrasting to this, fat content of intensive managed communities of mid-elevation Graswang decreased by 16.5 % ( $p < 0.001$ ) under warmer conditions in comparison to the similar land management intensity at the control site.

#### 4. Discussion

Our results showed that visible-near-infrared spectroscopy (vis-NIRS) is a feasible tool to quantify forage quality in species rich communities undergoing structural shifts due to global change drivers. With CARS-PLS we identified robust, parsimonious and accurate models for ash, fat and protein but not for fiber content of communities' bulk samples. Applying these techniques to our experimental plant communities revealed strong and clearly differentiable effects of climate change – increasing temperature and decreasing precipitation – and land management regime – high versus low mowing and slurry application frequencies - on forage quality. Protein content was increased by higher mowing frequency and slurry application (Aavola and Kärner, 2008; Pavlů et al., 2011), but not affected by climate change, adding further evidence to inconsistent effects of warming on protein content (Dumont et al., 2015; Xu et al., 2018). Ash content varied with increases in temperature and decreases in precipitation, and fat content was interactively affected by both global change drivers (Grant et al., 2014; Li et al., 2018). vis-NIRS offers a high potential for monitoring and predicting changes in forage quality under changing environments (Anderson et al., 2018; Murguzur et al., 2019; Parrini et al., 2018).

##### 4.1. Predicting forage quality using vis-NIRS analysis

All three parameters with robust and parsimonious have a low RMSE of external cross validation. The RMSE for protein, ash and fat represents 4.7 %, 6.4 % and 8.1 % of the entire range spanned within all predicted samples. According to the criteria introduced by Saeys et al. (2005), our calibration range from “good” for fat and ash (on the brink) to “excellent” for protein. These good calibration model of protein is caused by the spectral relevance of nitrogen linked to the adsorption NH bond (Roberts et al., 2004), which is a major component of proteins, and the larger range of protein in our samples in comparison to ash or fat. This high accuracy predictions of forage quality are aligned with different studies of standardized sown communities such as alfalfa (De Boever et al., 1998), wheat (Cozzolino et al., 2006), ryegrass (Jafari et al., 2003; Smith et al., 2019), sown mixtures of varying complexity (Deak et al., 2007), as well as species-rich natural meadows (Sonia Andrés et al., 2005; Danieli et al., 2004; Fekadu et al., 2010; Parrini et al., 2018).

The wavelengths selected by CARS-PLS for all of the analyzed parameters of forage quality are within the region typical for organic molecules and the chemical bonds characterizing organic macro molecules of higher complexity (Kawamura et al., 2008; Ludwig and Khanna, 2001).

However, with respect to fiber content our approach did not lead to a model with high predictive power and accuracy. Studies of both sown, species-poor plant communities (Sanz-Sáez et al., 2012) and semi-natural, species-rich plant communities (Danieli et al., 2004; Parrini et al., 2018) successfully used NIRS to predict fibrous content, e.g. acid detergent fiber, neutral detergent fiber or crude fiber. Among the fibrous parameters, crude fiber was the one with the weakest model performance found by Danieli et al. (2004) and Parrini et al. (2018) in natural grasslands, but still remarkably higher than in our results. Despite the range of crude fiber in here is comparable to those reported in Danieli et al. (2004) and Parrini et al. (2018). We can only speculate, but this result might be based on the primary wet-chemical (Weender analytics) which allows only an approximate estimation of crude fiber, thus the follow-up prediction by vis-NIRS only propagates this uncertainty (S. S. Andrés et al., 2005; Sonia Andrés et al., 2005; Roberts et al., 2004). Spectral information is unlikely to cause the failure here, as wavelengths critical to determine fiber (characteristic chemical bonds CH and OH) are within our investigated spectral region (Kawamura et al., 2008).

##### 4.2. Effects of climate change and land management regime on forage quality

We detected effects of both global change drivers - climate change and land-use intensity – on forage quality. The effects of climate change on ash and fat content varied depending on land management and origin of community. Effects of warming on forage quality in mountain regions is sparse and so far did not reveal a consistent significant response to warming (Dumont et al., 2015). Yet, previous experimental studies on the Tibetan high plateau found an increase in fat and protein content and a decrease of parts of the fibrous fraction with warming but only under dry conditions (Li et al., 2018; Xu et al., 2018). This positive effect of warming on forage quality was mediated by changes in community composition induced by altered environmental conditions (Li et al., 2018; Xu et al., 2018) and was shown to also hold true on large geographical scales (Shi et al., 2013). The high variability in communities' response to climate change in our study may hint towards an indirect effect of climate change mediated by community composition, although we lack the data to test this. Cold adapted and montane communities are expected to shift towards more thermophilic species (Gottfried et al., 2012; Rumpf et al., 2018), higher graminoid abundance (Klanderud et al., 2015; Winkler et al., 2016) and are more susceptible to novel competitors (Alexander et al., 2015). Our translocated communities were only exposed to novel climatic conditions for two years. Yet, the early stages of changes in community composition by altered climatic conditions of the same grasslands without management showed non-deterministic changes in community composition change (Berauer et al., 2019). These effects on biodiversity are likely to be amplified by land management intensity, namely fertilization and cutting (Socher et al., 2013).

Land-use intensity increased forage quality by increasing fat and protein content of communities from both origins, thus improving forage quality in general. This effect was non-significant for the high elevation control of communities in Esterberg. We attribute this exception to the especially short growing season in 2017, which is also reflected in the land-use intensity in 2017 of this experimental site (see Supplementary Table 2). In 2018, protein content of high-elevation Esterberg control communities increased with land management intensity, further supporting our argument (see Supplementary Fig. 2)

Higher cutting frequency can increase leaf nitrogen and protein content (Pavlů et al., 2011; Walter et al., 2012; White et al., 2014) in comparison to lower cutting frequency. Conversely, reduction or cessation in cutting was shown to increase leaf dry matter content and reduce forage quality (Lavorel et al., 2011). Fertilization also increases forage quality via leaf nitrogen status in grasslands (Aavola and Kärner, 2008; Liu et al., 2010; Malhi et al., 2010). This matches our findings here of increased protein content (which incorporates the largest fraction of nitrogen in the organic molecules we tested). Whithead (2000) recommends a protein content ranging from 12 to 19 % to meet dairy cattles' requirements. Protein content of all intensively managed and most extensively managed communities are above this recommended threshold and only the protein content of extensive managed communities originating from mid-elevation Graswang are lower (Graswang<sub>ctrl</sub> = 10.3 %; Graswang<sub>+2K</sub> = 11.3 %). The amount of gaseous nitrogen oxides loss immediately after slurry application of similar grasslands (exposed to similar climate change and land-use intensity) was recently found to be higher than previously assumed rates of gaseous N losses (Zist-Schlingmann et al., 2019). This would likely reduce the amount of plant available nitrogen added by slurry application. With the possibility of “real-time” monitoring of forage quality, the timing and amount of fertilizer application could be adapted, which is key for sustainable grassland management (Wohlgemuth et al., 2019).

The lack of interaction between both manipulated global change drivers on forage quality indicate that both climate change and land management intensity are acting with independent mechanisms on forage quality, at least in the short-term.

Forage quality of species-rich communities is highly variable and can be influenced by species composition and abundance (Khalsa et al., 2012), nutrient availability (White et al., 2004) or stage of maturity (Waramit et al., 2011). Despite this natural variability, monitoring forage quality and adapting land-use regimes as needed remains an important task for sustainable agriculture under future conditions. Our study is the first that we know of to use NIRS to track forage quality in managed grasslands undergoing rapid change in response to global change drivers. Given the future threats these drivers pose to these grasslands' biodiversity and ecosystem function, such tools and monitoring schemes will be essential for maintaining sustainable agriculture under future conditions.

### Funding sources

This study was supported by the German Ministry for Education and Research (BMBF) funding the SUSALPS project ("Sustainable Use of Alpine and Pre-alpine Grassland Soils in a Changing Climate"); under Grant number: FKZ 031B0516C), which is part of the funding measure "Soil as a Sustainable Resource for the Bioeconomy-BonaRes".

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

We thank the project partners of SUSALPS. In special, the Karlsruher Institute of Technology (IMK-IFU) for strong support during the field campaign and land-use management applications and the Technische Universität München (Department of Soil Science) during processing from sample to spectra. We are grateful to the German Ministry for Education and Research (BMBF) for funding (SUSALPS; grant number: FKZ 031B0516C).

### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2020.106929>.

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9.3 **Manuscript 3** | Berauer *et al.* (2021) – High land-use intensity diminishes stability of forage provision of mountain pastures under future climate variability.



Driving cattle up to Brunnenkopfbalm in May 2020

Article

# High Land-Use Intensity Diminishes Stability of Forage Provision of Mountain Pastures under Future Climate Variability

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**Citation:** Berauer, B.J.; Wilfahrt, P.A.; Schuchardt, M.A.; Schlingmann, M.; Schucknecht, A.; Jentsch, A. High Land-Use Intensity Diminishes Stability of Forage Provision of Mountain Pastures under Future Climate Variability. *Agronomy* **2021**, *11*, 910. <https://doi.org/10.3390/agronomy11050910>

Academic Editor: Daniel Real

Received: 19 March 2021

Accepted: 4 May 2021

Published: 6 May 2021

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**Abstract:** Semi-natural, agriculturally used grasslands provide important ecologic and economic services, such as feed supply. In mountain regions, pastures are the dominant agricultural system and face more severe climate change impacts than lowlands. Climate change threatens ecosystem functions, such as aboveground net primary production [ANPP] and its nutrient content. It is necessary to understand the impacts of climate change and land-management on such ecosystems to develop management practices to sustainably maintain provision of ecosystem services under future climatic conditions. We studied the effect of climate change and different land-use intensities on plant-soil communities by the downslope translocation of plant-soil mesocosms along an elevation gradient in 2016, and the subsequent application of two management types (extensive vs. intensive). Communities' response to ANPP and leaf carbon (C), nitrogen (N), and phosphorus (P) content was quantified over the subsequent two years after translocation. ANPP increased with warming in 2017 under both management intensities, but this effect was amplified by intensive land-use management. In 2018, ANPP of intensively managed communities decreased, in comparison to 2017, from 35% to 42%, while extensively managed communities maintained their production levels. The changes in ANPP are coupled with an exceptionally dry year in 2018, with up to 100 more days of drought conditions. The C:N of extensively managed communities was higher than those of intensively managed ones, and further increased in 2018, potentially indicating shifts in resource allocation strategies that may explain production stability. Our results revealed a low resistance of intensively managed communities' ANPP under especially dry conditions. The ability to alter resource allocation likely enables a constant level of production under extensive management, but this ability is lost under intensive management. Thus, future drought events may leave intensive management as a non-sustainable farming practice, and ultimately threaten ecosystem services of montane pastures.

**Keywords:** climate change; grassland; land management; pasture; montane; nitrogen; primary production; stoichiometry

## 1. Introduction

On a global scale, grasslands as natural grasslands (35 mio km<sup>2</sup>) and as human transformed semi-natural grasslands (15 mio km<sup>2</sup> cropland and 31.5 mio km<sup>2</sup> pasture)

are among the largest terrestrial biomes [1,2]. Mountain grasslands are predominantly used as pasture for forage-based dairy and livestock production, forming the dominant habitat in the Alps and their foothills. However, these ecosystems are exposed to climate and land-use changes which will affect the provision of ecosystem services in the future. Mean annual temperature is predicted to rise over the 21st century [3], being most severe in high altitudes [4,5]. Besides the change in long-term average temperature, intra- and inter-annual temperature variability will increase [6], and has already increased in Europe [7]. Additionally, precipitation patterns may shift, and a reduction in precipitation during the summer months is projected for the Alps [5]. The coupled effects of climate change on temperature and precipitation are expected to increase the frequency and intensity of drought periods [8]. This likely impairs grasslands and their provided amount and chemical composition of biomass [9], causing persisting long-term effects [10]. To cope with limiting soil-moisture under drought conditions, plants can either increase their ability for acquiring limited resources by increasing root development [11], or be more efficient with their use of limited resource by constructing more water-use efficient and drought-tolerant leaf tissue [12,13]. Thus, it is necessary to understand mechanisms controlling managed communities' dynamic reaction to resource acquisition and allocation in response to fluctuating climatic conditions, as these mechanisms determine the stability of ecosystem functions. This will help to preserve provided ecologic and economic value under future climatic conditions.

Climate change and land-use intensity both have significant potential to change resource availability in managed grasslands. The strong seasonality and short growing season in cold-temperature limited mountain grasslands of the northern hemisphere strongly regulate productivity [14,15]. An earlier snowmelt caused by warmer temperature elongates the growing season length [16] and stimulates growth [17], though only if not water limited [18,19]. Further, warmer temperatures induced by climate change enhance the decomposition of soil organic matter [20], stimulate soil microbial activity [21], and will increase gross nitrogen turnover [22] and nitrification rates [23], ultimately increasing plant nitrogen availability while reducing phosphorus availability via immobilization [24]. In grasslands, where cold temperatures have caused the accumulation of large soil organic matter and total nitrogen stocks [25], the climate change-induced increase in gross nitrogen turnover may be pronounced. External nutrient input via fertilization (e.g., slurry application) directly increases soil nitrogen and phosphate ( $P_2O_5$ ) content. Acidification of soils can indirectly increase phosphorous solubility [26], yet low pH can also decrease phosphorous availability due to adsorption mechanisms (e.g., ligand exchange with aluminium or iron oxides) [27]. Classic knowledge states that the highest phosphorus solubility occurs at a pH ranging between 6.5 and 7 [28], which was recently confirmed by Penn and Camberato in 2019 [27]. Temperature induced increases in turnover rates and external nutrient input both alleviate belowground nutrient limitation and stimulate the growth of grasslands [29,30], and may be of high importance in nitrogen-limited grasslands at high altitudes or latitudes [30]. An increase in soil nutrient availability can also improve nutritive quality of produced biomass [31]. During the last few decades, land-use intensity in the Alps has changed, with grasslands at high altitude and with difficult terrain being abandoned due to high-cost management, while grassland management in valley bottoms has intensified [32].

Shifting nutrient availability under climate change and differing land-use intensities likely entails changes in uptake and allocation of nutrients, ultimately altering element contents and elemental stoichiometry of plant tissue [24,33]. For example, plants can alter their nutrient allocation and shift towards higher investment into above- rather than belowground biomass under a surplus of soil nutrient availability [34,35], which is reflected in stoichiometric flexibility in tissues [36,37]. The elemental stoichiometry of an organism is linked to its growth rate. A decreasing C:N or C:P ratio, equivalent to a lower amount of carbon per unit nitrogen, respectively, phosphorus, is coupled with an increasing growth rate [38]. Though the relationship of the N:P ratio to growth rate is not that simple, as, in

theory, it peaks at intermediate growth rate [38]; this can be confounded by excess uptake of a non-limiting nutrient under a single limitation of a different nutrient [36]. A recent meta-analysis by Yuan and Chen (2015b) [39] showed that fertilization with N, P, and their combination increases respective tissue element content. The ratios of C:N and C:P respond according to tissue element content across various plant functional types and habitats [31]. Additionally, leaf stoichiometry is strongly dependent on the phenology and maturity of plants [40], and is thereby affected by the frequency of biomass removal from land-use practices (e.g., mowing) [41]. Yet, the effect of warming-induced increases in nutrient availability on plant tissue element contents depends on plant type and the environmental conditions under which plants are growing [31]. Warming likely increases aboveground plant tissue C:N ratio of warm, moisture-limited temperate grasslands, as a result of increased structural carbon-rich compounds in leaves to reduce water loss [12], or by increased nutrient-use efficiency [42]. In contrast to this, effects of warming on cold-temperature limited systems with sufficient moisture remain ambiguous. Warming-induced changes of the C:N ratio were shown to either increase by increasing nutrient uptake [43], decrease via dilution of nitrogen by increased production [44], or reveal no effects [45]. In the long run, changes in nutrient availability and balances alter community composition [46], provided ecosystem services [47], multi-trophic interactions [48], and, ultimately, global coupled biogeochemical cycles [49].

Nutritive quality may decrease with warming [50], though these effects can be variable in high-elevation mountain grasslands [51], and often are mediated by community turnover [52,53]. Community composition and species richness are altered by climate change [54,55] and land-use [56], and community composition is key in determining forage quality and medicinal value [57]. For instance, increasing temperature and nutrient availability will likely favour fast-growing resource-acquisitive species [58,59]. In cold temperature adapted ecosystems, graminoids often increase under warmer climates [60], potentially reducing nutritive quality. Changes in nutrient availability, allocation, and plasticity within a community will affect the amount and stability of ecosystem services provided by managed grasslands, such as quantity and chemical composition of forage. Mountain grasslands are predominantly used for agriculture and are vulnerable to climate-change. Thus, it is of high importance to understand grasslands dynamic responses in elemental stoichiometry under future climatic conditions to maintain provided ecologic and economic value.

Here, we investigated the interacting effects of experimental, implemented climate change and contrasting land-use intensities (high/intensive vs. low/extensive) on forage provision (ANPP and elemental stoichiometry) of two montane grasslands within the German Alps. We hypothesize that (i) ANPP increases with both warming and high-intensity land-use, (ii) elemental stoichiometry changes due to increased nitrogen availability and uptake (decreasing C:N and increasing N:P ratio), with high-intensity management, and (iii) warming-induced changes of nutrient availability amplify the effects of land-use intensity on elemental stoichiometry.

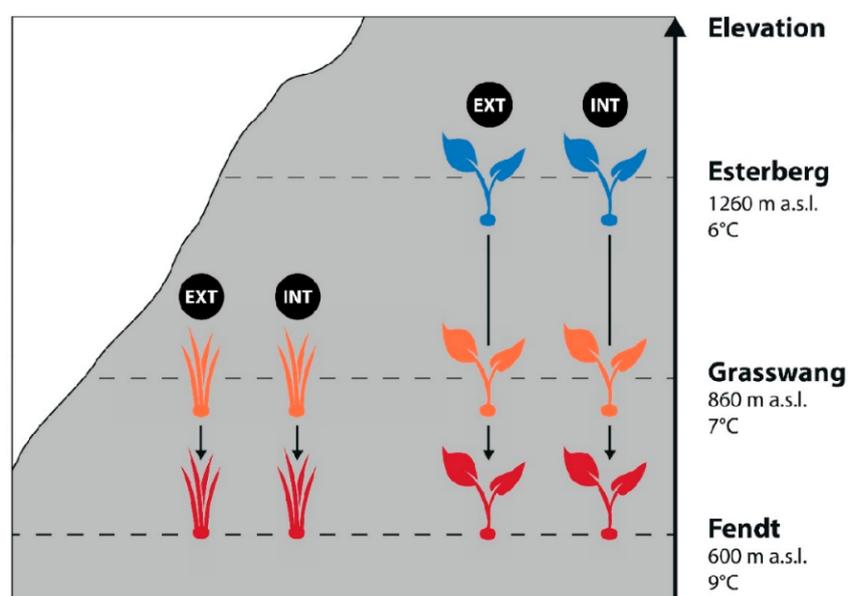
## 2. Materials and Methods

### 2.1. Experimental Setup

This study was conducted at three experimental sites of the project “Sustainable use of alpine and pre-alpine grassland soils in a changing climate” (SUSALPS; <https://www.susalps.de/en/>, accessed on 5 May 2021), along an elevational gradient within the German Alps. During the experimental campaigns in 2017 and 2018, this elevational gradient represented a temperature gradient of +3 K, spanning between the high-elevation site (Esterberg; 1260 m a.s.l.; mean annual temperature: 6 °C, mean annual precipitation: 1113 mm) via the mid-elevation site (Graswang; 860 m a.s.l.; 7 °C, 1433 mm), to the low-elevation site (Fendt; 600 m a.s.l.; 9 °C, 1036 mm). All of these sites are characteristic semi-natural grasslands of this region and are agriculturally used as cattle pastures. For

edaphic and plant sociologic information, see Supplementary Table S1 (or see Berauer et al. (2020) [51] for more detailed site descriptive information).

In 2016, intact natural plant-soil monoliths (communities) from the high-elevation and mid-elevation sites were translocated downslope to each lower elevational site to passively simulate climate change. Climatic control monoliths were reburied at the site of origin. Each translocated plant-soil community had a diameter of 30 cm and a depth of 40 cm. The procedure of translocation was as described in Berauer et al. (2019) [19]. A total replicate amount of 60 communities ( $3 \times 12$  from high-elevation and  $2 \times 12$  from mid-elevation) were translocated. Each set of 12 translocated communities was equally split into intensive and extensive land management ( $n = 6$  per origin  $\times$  recipient  $\times$  management combination; see Figure 1).



**Figure 1.** Scheme of experimental set-up. Each coloured plant symbol represents six plant-soil communities (replicates), translocated along an elevational and climatic gradient. The two different plant symbols represent communities from two different sites of origin, and colours represent climatic conditions, from cold (blue) to warm (red). The two contrasting—extensive (EXT) and intensive (INT)—land-use intensities are given on top of each respective row.

Intensively managed communities were cut five times per year and received a slurry application after each cut, whereas extensively managed communities were cut three times per year and received a slurry application only after the first and last cut per year, matching common agricultural practices of the region. For details on the chemical composition of the applied cattle slurry, see Supplementary Table S2. Cuts were conducted 3 cm aboveground using scissors. This cutting height was chosen to follow the scientific standards [61] that best represented aboveground biomass while avoiding damage to plant roots and allowing regrowth, even though this may not best represent typical agricultural practices. Fertilizer application consisted of 130 mL of slurry (corresponding to  $42 \pm 10 \text{ kg N ha}^{-1}$ ) spread homogeneously on the surface after each cutting. In total, intensively managed monoliths received  $\sim 250 \text{ kg N ha}^{-1} \text{ a}^{-1}$  and extensively managed monoliths received  $\sim 100 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , following typical amounts applied by farmers in this study region. The exact timing of land-use management application was linked to the local farmers' application as tightly as possible, and started in 2017.

## 2.2. Data Collection

After each cut, aboveground biomass bulk samples of each community (from now on “ANPP”—aboveground net primary production) were dried at 60 °C for 48 hours and weighed. ANPP was scaled to  $\text{g m}^{-2}$  (see Appendix A Formula (A1)).

Due to different time intervals between land management applications, we standardized biomass production by time (from now on called “productivity”). To do so, production was either divided by the number of days between two consecutive harvest dates or, in the case of the first cut of the year, the number of days between the start of the growing season and first cut (see Appendix A, Formula (A2)). We defined the start of the growing season as the first five consecutive days with mean air temperature above 5 °C [62]. This standardization allows for comparison with a similar standardized wetness index (see below).

To analyze leaf chemistry, we first ensured a homogenous and representative mixture and quality of dried community aboveground biomass bulk samples by shredding them to 2 mm [SK1, Retsch GmbH; Haan, Germany], and, afterwards, pulverizing them using a ball mill [MM301, Retsch GmbH; Haan, Germany]. Carbon (C in percentage of dry weight) and nitrogen content (N in percentage of dry weight) of mixed community bulk samples were analyzed using elemental analysis [Thermo Quest Flash EA 1112, Thermo Fisher Scientific; Waltham, USA], and phosphorous content (P in  $\text{g kg}^{-1}$  equivalent to permille) was analyzed using inductively coupled plasma optical emission spectrometry [Vista-Pro radial, Varian Inc.; Palo Alto, USA] after pressure digestion in 65%  $\text{HNO}_3$ , all following international standardized protocols (Bayreuth Center of Ecology and Environmental Research, central analytical chemistry laboratory; Bayreuth, Germany). We derived C:N, C:P, and N:P ratios (note, phosphorous was transformed to percent prior to calculation), as well as absolute amounts of elements (as produced biomass times relative portion of each respective element).

With the experimental set-up in 2016, sensors for soil temperature and moisture [EcH<sub>2</sub>O 5-TM, Decagon Devices Inc.; Pullman, USA] were installed horizontally at 5 and 15 cm depth, together with data loggers [EcH<sub>2</sub>O Em50, Decagon Devices Inc.; Pullman, USA]. Soil temperature and moisture were monitored at 30 min intervals at both depths in two separate monoliths, and in an additional third monolith at only 5 cm depth for each origin × recipient × treatment combination. Soil moisture was aggregated on a daily basis. Each site had operating weather stations and their precipitation and temperature data were used in this study.

We calculated the soil wetness index (SWI, e.g., Dirmeyer et al., 2000) [63]

$$\text{soil wetness index} = \frac{\theta - \theta_{WP}}{\theta_{FC} - \theta_{WP}} \text{ If } \theta > \theta_{FC} \rightarrow \theta = \theta_{FC} \quad (1)$$

with  $\theta$  as in situ measured soil moisture,  $\theta_{WP}$  as wilting point, and  $\theta_{FC}$  as field capacity. This wetness index represents plant available water at a given time. Field capacity and wilting point were calculated based on soil texture, including a relative amount of sand and clay, using the USDA’s Soil Water Characteristics program (following Saxton and Rawls, 2006 [64]), with soil texture data presented in Berauer et al. (2020) [51]. We excluded organic matter in the calculation, as we lacked data for 2017 and 2018.

The SWI was calculated for each occurring origin\*recipient\*treatment combination, aggregated across both soil depths, to quantify potential occurring drought condition. We use the SWI to calculate the number of days with no plant available water ( $\text{SWI} < 0$ ) during the growing season.

## 2.3. Statistical Analysis

We aggregated data per year (sum of ANPP, total N, total P, mean productivity, C, N, P content and C:N, C:P, and N:P) of each origin\*recipient\*treatment combination, and examined a Pearson’s correlation among them. Due to high correlations of many of these variables, we opted to analyze and report in detail ANPP, C:N, and N:P ratios,

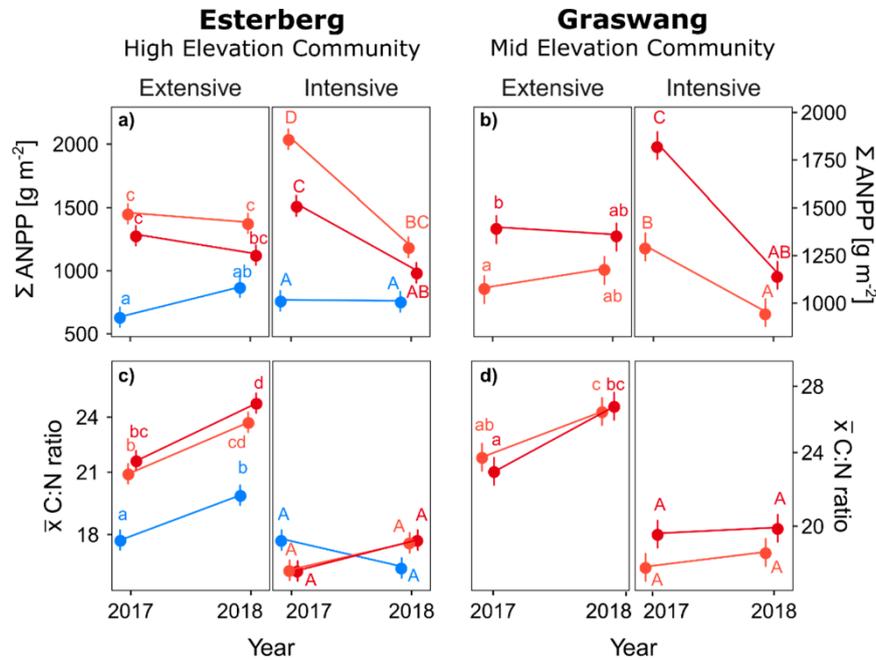
as the biological stoichiometry of plants is a powerful predictor of nutrient limitation and resource allocation strategies [65]. To test for effects of climate change and land-use intensity on the amount and elemental stoichiometry of ANPP over the course of the two studied years, we conducted generalized least square models with land-use intensity (LUI), recipient site as a proxy for climate change (CC), and year, all interacting with each other as predictors. To cope with the non-independence of repeated sampling of replicates (single experimental communities), we included an auto-correlation AR-1 model structure with a replicate nested in year. We conducted separate models for the two sites of origin, because of differing comparisons by experimental set-up and communities. When needed, we used a pairwise Tukey HSD as a post hoc test to test for significant differences if more than two comparisons were possible. Model assumptions of all models were confirmed visually (normal distribution: histogram, QQ plot and variance homoscedasticity: standardized residuals vs. fitted values; Zuur et al. (2009) [66]).

All statistical tests were performed using R-Version 3.6.1 [67], using the packages *nlme* [68] and *lsmeans* [69]. An exhaustive summary of mean and standard error of all measured parameters and model outputs is provided in Supplementary Tables S3–S5.

### 3. Results

Intensively managed communities had low resistance of ANPP in warmer climates when originating from both high-elevation ( $p_{LUI*CC*Year} = 0.0132$ ) and mid-elevation ( $p_{LUI*Year} < 0.001$ ,  $p_{CC*Year} = 0.0108$ ) sites. More specifically, ANPP in 2017 increased under warmer conditions for both intensively and extensively managed communities originating from high-elevation, but this effect was higher at mid-elevation (+1 °C: intensive +166%; extensive +128%) than the warmest low-elevation site (+3 °C: intensive +98%; extensive +101%, Figure 2a). In the second year of study (2018), extensive management maintained a similar level of ANPP, whereas ANPP of intensively managed communities decreased in comparison to 2017 at warmer sites (by −42% at +1 °C and −35% at +3 °C; Figure 2a). In 2018, the positive effect of warming in intensively managed communities only remained under +1 °C warming ( $p_{ctrl+1} < 0.05$ ), and was nullified at the warmest recipient site ( $p_{ctrl+3} = 0.402$ ). Communities originating from mid-elevation sites similarly showed increasing ANPP under warmer conditions for intensive (+41%) and extensive (+29%) land-use intensity, while, in 2018, no effect of warming was apparent under either land-use intensity. Again, ANPP of extensively managed communities remained constant in 2018, whereas intensively managed communities had less (−37%) ANPP than in 2017 under +2 °C warmer conditions (Figure 2b).

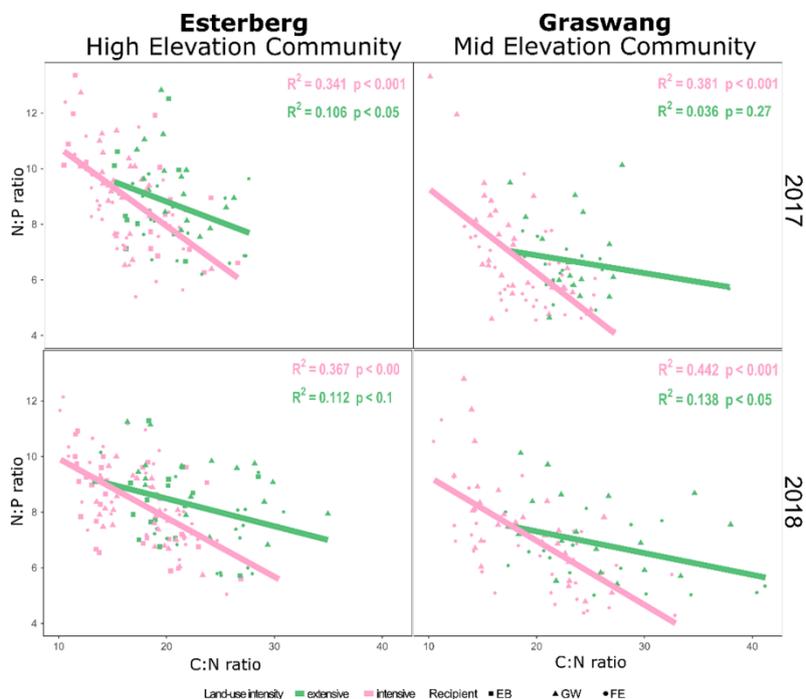
The C:N ratio of intensively managed communities remained constant across sites and years, while the C:N ratio of extensively managed communities originating from high-elevation sites increased with time ( $p_{LUI*Year} < 0.001$ ) and warming ( $p_{CC*Year} < 0.05$ ;  $p_{LUI*CC} < 0.001$ ). The increased C:N ratio of extensively managed communities (2017: +18% at +1 °C; +22% at +3 °C | 2018: +18% at +1 °C; +22% at +3 °C; Figure 2c) was primarily induced by a reduced nitrogen tissue content under warmed conditions in 2017 (−15% at both +1 °C and +3 °C) and 2018 (−13% at +1 °C and −15% at +3 °C, Supplementary Figure S1). Additionally, the C:N ratio increased in 2018 at climatic control site (+12%), +1 °C (+12%) and +3 °C (+13%), driven by a concordant but non-significant decrease in nitrogen and an increase in carbon content (see Supplementary Figure S2).



**Figure 2.** Yearly ANPP (a,b) of extensively managed (left column of a, b, c, d) communities of both high-elevation (a,c) and mid-elevation (b,d) origin remains stable over the two years of study, contrasted with the significant decline in ANPP of intensive managed (right column of a, b, c, d) communities in 2018. The C:N ratio (c,d) is higher under extensive management, and increases in 2018, in contrast to a constant low C:N ratio of intensively managed communities. Shown are mean and standard deviation. Letters indicate significant ( $p < 0.05$ ) differences within panel. Colours match recipient site colour code of Figure 1.

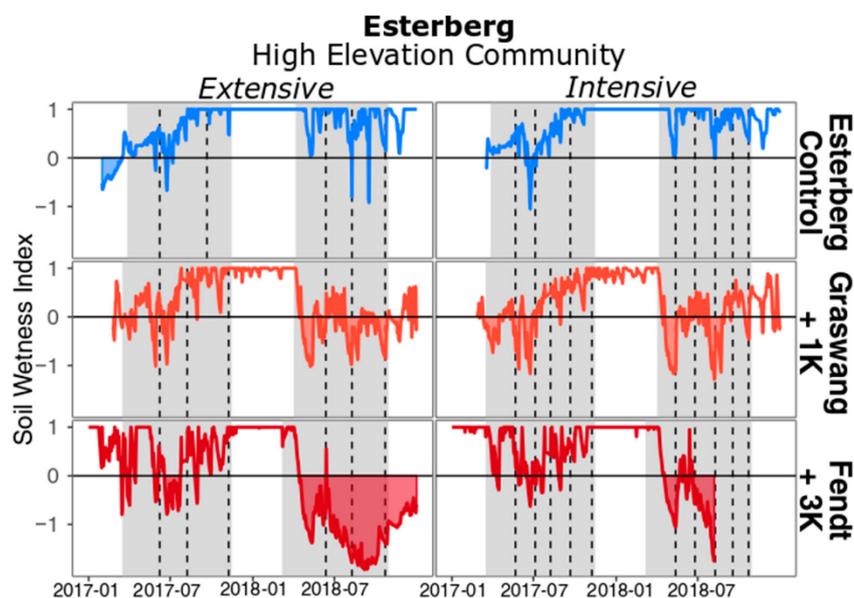
The C:N ratio of intensively managed communities originating from mid-elevation sites was invariant across time and climatic conditions. In contrast, the C:N ratio of extensively managed communities increased over time ( $p_{\text{LUP} \times \text{Year}} < 0.001$ ) at both the climate control (+10%) and +2°C site (+15%), but did not show differences between sites (Figure 2d).

C:N and N:P were negatively correlated, though this relationship was stronger in intensively managed communities (Figure 3). C:N and N:P ratios ranged from 16.3 to 26.6, and from 6.1 to 9.4, respectively. The range of N:P ratios is comparable for extensively (6.3 to 9.4) and intensively (6.1 to 9.2) managed communities. In contrast, the C:N ratio of intensively managed communities revealed a lower, narrower range (16.3 to 19.9) than the extensively (17.8 to 26.6) managed communities, demonstrating higher nutritive value of intensively managed communities and less stoichiometric plasticity.



**Figure 3.** Relationship between C:N and N:P ratios of high-elevation (left column) and mid-elevation (right column) communities for 2017 (top row) and 2018 (bottom row).

The strong differences in ANPP between both study years of intensively managed communities and for C:N ratio of extensively managed communities can be linked to the observed plant available water content during both study years (Figure 4, Supplementary Figure S3). The number of days without plant available water (number of days with SWI < 0) increased for all origin  $\times$  recipient  $\times$  management combinations in 2018, except in the high-elevation climatic control. Irrespective of site of origin and land-use intensity, the number of days without plant available water increased in 2018 in comparison to 2017 at mid-elevation sites by  $\sim$ 55 days, and at low-elevation sites by  $\sim$ 106 days. The increase in number of days without plant available water between both study years, irrespective of site of origin, was stronger under extensive than intensive land-use intensity at both mid-elevation (extensive:  $\sim$ 71 days; intensive:  $\sim$ 39 days) and low-elevation (extensive:  $\sim$ 122 days; intensive:  $\sim$ 90 days) sites (see Supplementary Table S6). The number of days without plant available might appear very high and unreasonable. Different methodological limitations are contributing to the pattern. First, both field capacity and wilting point were calculated based on measured soil texture, but not in situ determined. Second, soil moisture was tracked using TDR-sensors reporting soil volumetric water content. Prior to field installations, sensors were calibrated and ensured to be comparable against each other. Though, volumetric water content still slightly misrepresents gravimetric water content. Ultimately, these methodological difficulties might cause a slight overestimation of days without plant available water. Yet, as the sensors are comparable to each other, the induced systematic error is neglectable. Similarly, due to calculus, the fixed lower benchmark of wilting point will not result in changes of the here presented and discussed pattern.



**Figure 4.** Soil wetness index of communities originating from high-elevation site (Esterberg) under extensive (left column) and intensive (right column) land-use intensity, at their respective recipient site: control (top), +1 K (center), and +3 K (bottom). The growing season (grey shaded areas) of 2018 reveals more pronounced dry spells (area with wetness index < 0) than 2017, especially at mid- and low-elevation sites. Dashed lines indicate date of harvest.

#### 4. Discussion

Warmer environments led to increased productivity, as long as precipitation did not become limiting. The positive effect of warming on ANPP was amplified by intensive land-use management. Intensively managed communities were more productive and nutrient rich than extensively managed communities, regardless of warming. However, an especially dry year in 2018 revealed low resistance of ANPP under intensive land-use intensity, whereas extensive land-use intensity was able to maintain a constant level of ANPP, even under dry conditions, by shifting to a resource conservative strategy, indicated by higher C:N. Changes in elemental stoichiometry and content indicate a changed nutrient resource allocation under extensive land-use intensity, and may encourage investment in drought tolerance.

ANPP increases with warming in grasslands [21,70]. Specifically, cold-temperature limited grasslands were shown to profit from warming by increasing vegetation length and nutrient availability [71], as long as soil moisture conditions remained sufficient [18,19]. Higher nutrient input relieves nutrient limitations, leading to increased production [30]. On a global scale, land-use intensification increases ANPP at the cost of species loss [72]. Similarly, a supplied surplus of nitrogen by fertilization might cause the lower C:N ratio of intensive managed communities, as more nitrogen is available for plant growth and excess supply can be stored—so called luxury uptake [36]. Additionally, the generally lower C:N ratio of intensively managed communities in comparison to extensively managed communities might result from the two different cutting frequencies, yielding harvested tissue at different stages of maturity and phenology, both of which affect C:N ratio. Young tissues are known to be nutrient rich [40], adding evidence to the observed pattern.

The alternating resistance between both land-use intensities might be caused by different community compositions with different resource use strategies. Warming of cold-temperature limited grasslands causes loss of species [19] and changes in community composition [54,55], most likely favouring fast-growing resource acquisitive species [59],

i.e., graminoids [60]. The warming-induced loss of species is likely fuelled by increasing land-use intensity [73]. The asynchronous reaction of species—the loss of performance from one species suffering is filled by another species profiting from changed environmental conditions—to environmental changes and stressors affects communities' stability and the provision of ecosystem multifunctionality [74,75]. Ma et al. (2017) [76] found weakened species asynchrony induced by warming to be the main factor reducing the temporal stability of production. Our results add further evidence to suggest that ongoing climate change alters both mean [4,77] and variability of climates [8,78], which will have critical impacts on ecosystem functioning and stability in general [79,80], especially in mountain regions [14,32]. These effects can be direct or indirectly mediated via changed community composition and species dominance, though we lacked the data to test for composition effects here. Nutrient enrichment likely favours species with a high growth rate (acquisitive species) as the demand and supply of high N and P are met [36]. Though benefitting from high resource availability, acquisitive species are at a disadvantage under low resource availability [59]. Thus, we speculate the low resistance of intensively managed communities to be caused by the dominance of acquisitive species, whereas more species with a conservative resource strategy within the extensive communities are the potential cause of stability. This might also be supported by the constant C:N ratio of intensively managed communities, suggesting that species are less capable of changing resource allocation, or that resource conservative species were lost from these communities.

As the cutting regime remained constant for both land-use intensities in both years, the increase in C:N ratio of extensively managed communities is unlikely to be caused by changed maturity of harvested tissue. This poses the question of shifting resource allocation in extensively managed communities. The observed increase in C:N is driven by opposed changes in tissue carbon (increase) and nitrogen (decrease) content. The decreasing nitrogen tissue content might result from nitrogen immobilization and altered soil microbial communities in managed mountain grasslands under drought conditions [81]. However, because we did not detect a consistent nitrogen content pattern under intensive management, the consistent pattern in extensive management might hint towards shifting resource allocation belowground [11], though we lacked the data to test this. On the other hand, increasing leaf tissue carbon content, as increased structural investment to reduce water loss [12] or soluble non-structural carbohydrates to lower leaf water potential at stomatal closure [13], are possible mechanisms underlying the pattern observed in this study.

The observed N:P ratios for communities of both land-use intensities are below 10, suggesting N-limited growth conditions rather than P-limitation [46,82]. This potential N-limitation might be surprising, given the amount of slurry fertilizer added to the communities. Yet, it adds indirect evidence to a low slurry use efficiency of montane pasture communities, which ultimately may lead to C- and N-mining from soil organic matter [83]. Nonetheless, the N:P ratios are within the optimum range for agricultural managed grasslands in mountain regions of Switzerland proposed by Liebisch et al. (2013) [84], rating the optimum between 5.5 and 9. In a previous study, crude protein content of those montane pasture communities was shown to be sufficient for dairy production, irrespective of management intensity [51].

Human-induced changes in the availability of carbon, nitrogen, and phosphorous are reflected in changed elemental stoichiometry, and cause an inexorable imbalance in global nutrient cycles (especially a deficiency of phosphorous in comparison to carbon and nitrogen) already impacting managed ecosystems around the world [47]. Further intensification of agriculture—potentially exceeding sustainable limits of stock density or carbon export—can reduce soil organic carbon stocks [85] and, in a worst case scenario, can cause the decoupling of biogeochemical cycles [86]. In excess, the likely reduction of species richness by intensification [72] may further reduce ecosystem stability [87] and multifunctionality [88].

## 5. Conclusions

Our result revealed a low resistance of ANPP of intensively managed communities under dry conditions, whereas extensively managed communities were able to maintain production, likely by altering resource allocation. A communities' inherent ability to cope with interannual climate variability is crucial for maintaining stable ecosystem function. Thus, by restricting a community's plasticity in resource allocation strategies, intensive management may prove unsuitable for the sustainable provision of ecologic and economic services of montane pastures in future climates. We recommend shifting from intensive to extensive management in montane regions to preserve cultural heritage, in addition to the economic and ecologic value provided by these ecosystems.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11050910/s1>.

**Author Contributions:** B.J.B., A.J. conceived the ideas and designed the methodology. B.J.B., M.A.S., M.S., A.S. collected data. B.J.B. and P.A.W. analyzed the data. All authors contributed critically to interpretation of data, writing of draft and gave final approval for publication. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was conducted within the SUSALPS project (<https://www.susalps.de/en/>, accessed on 5 May 2021) funded by the German Federal Ministry of Education and Research, grant numbers 031B0027C and 031B0516C for UBT-DE, 031B0027A and 031B0516A for KIT.

**Data Availability Statement:** Data are available at the GitHub repository: ([https://github.com/BJBerauer/SUSALPS\\_ForageProvision](https://github.com/BJBerauer/SUSALPS_ForageProvision), accessed on 5 May 2021).

**Acknowledgments:** We thank the project partners of SUSALPS, especially the team from the Karlsruhe Institute of Technology (IMK-IFU) for their strong support during the field campaign and the land-use management applications. Sincere thanks go to the analytical chemistry keylab facility of the "Bayreuth Center for Ecological and Environmental Research" (BayCEER) for analyzing the elemental content and showing fundamental patience. This publication was funded by the German Research Foundation (DFG) and the University of Bayreuth in the funding program Open Access Publishing. BJB's personal gratitude goes to Ralf Hafner for helping to visualize the experimental setup.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A. Formulas

Upscaling of ANPP to [g m<sup>-2</sup>].

$$Production \left[ \frac{g}{m^2} \right] = \left[ Production \left[ \frac{g}{monolith} \right] * \left[ \frac{1}{(\pi * r^2)} \right] \right] \quad (A1)$$

With  $r = 0.15$  m,  $1/\pi * r^2$  equalling [1/surface area] of monolith equals the multiplier from [g/monolith] to [g m<sup>-2</sup>].

Calculation of productivity as production scaled by time

$$Productivity \left[ \frac{g}{m^2} * d^{-1} \right] = \frac{Production}{Days \text{ between cutting}} \quad (A2)$$

$$Days \text{ between cutting} (Midseason) = Cutting[DOY] - Previous \text{ Cutting}[DOY]$$

$$Days \text{ between cutting} (1st \text{ cut of the year}) = Cutting[DOY] - Start \text{ Growing Season}[DOY]$$

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9.4 **Manuscript 4** | Vetter *et al.* (2020) – Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought.



SIGNAL experimental site at the botanical garden of University of Bayreuth in autumn 2016



# Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought

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## Funding information

ERA-Net BiodivERsA, Grant/Award Number: 01LC1201; German Federal Ministry of Education and Research (BMBF); BonaRes, Grant/Award Number: 031B0027C; Belgian Federal Science Policy Office (BELSPO); Bulgarian Science Found

## Abstract

Higher biodiversity can stabilize the productivity and functioning of grassland communities when subjected to extreme climatic events. The positive biodiversity–stability relationship emerges via increased resistance and/or recovery to these events. However, invader presence might disrupt this diversity–stability relationship by altering biotic interactions. Investigating such disruptions is important given that invasion by non-native species and extreme climatic events are expected to increase in the future due to anthropogenic pressure. Here we present one of the first multisite invader × biodiversity × drought manipulation experiment to examine combined effects of biodiversity and invasion on drought resistance and recovery at three semi-natural grassland sites across Europe. The stability of biomass production to an extreme drought manipulation (100% rainfall reduction; BE: 88 days, BG: 85 days, DE: 76 days) was quantified in field mesocosms with a richness gradient of 1, 3, and 6 species and three invasion treatments (no invader, *Lupinus polyphyllus*, *Senecio inaequidens*). Our results suggest that biodiversity stabilized community productivity by increasing the ability of native species to recover from extreme drought events. However, invader presence turned the positive and stabilizing effects of diversity on native species recovery into a neutral relationship. This effect was independent of the two invader's

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own capacity to recover from an extreme drought event. In summary, we found that invader presence may disrupt how native community interactions lead to stability of ecosystems in response to extreme climatic events. Consequently, the interaction of three global change drivers, climate extremes, diversity decline, and invasive species, may exacerbate their effects on ecosystem functioning.

#### KEYWORDS

alien invasive species, biological invasion, climate extreme, disturbance, ecosystem functioning, grassland ecosystem, plant–environment interaction, recovery, resilience, resistance

## 1 | INTRODUCTION

Loss of biodiversity tends to affect ecosystem functioning and stability of grasslands negatively and is likely to affect human society (Cardinale et al., 2012; Hautier et al., 2015, 2018; Tilman, Isbell, & Cowles, 2014). A more diverse plant community leads to an overall more stable community functioning under a wider range of conditions when species react in asynchrony due to compensatory responses (sensu insurance hypothesis: de Mazancourt et al., 2013; Loreau & de Mazancourt, 2013; Tilman et al., 2014; Yachi & Loreau, 1999). Increased stability maintains community productivity while the availability of free resource declines (Gross et al., 2014; Tilman, Reich, & Isbell, 2012). Global change drivers lead to exogenous changes in resource availability and the introduction of non-native species, leading to uncertainty as to whether the diversity–stability relationship persists in the face of extreme climatic events (De Boeck et al., 2018) and invasion (Pinto & Ortega, 2016).

The frequency and magnitude of extreme climatic events, such as drought (Dai, 2013), are predicted to increase in Europe as a result of climate change (Hewitson et al., 2014). Altered drought regimes in semi-natural grasslands might lead to plant mortality, species composition shifts, degradation and desertification, and erosion (Craine et al., 2012; Reichstein et al., 2013; Wang et al., 2011). Strong droughts can cause lasting effects on community composition by selecting for drought tolerant species (e.g. Mediterranean species), resulting in dominance shifts and/or local extinction of drought intolerant species (Alba, NeSmith, Fahey, Angelini, & Flory, 2017; Reichstein et al., 2013; Török, Janišová, Kuzemko, Rusina, & Stevanovic, 2018). Such drought-induced changes in the plant community structure of grasslands might subsequently affect plant productivity as well as ecosystem functioning and the delivery of ecosystem services (Caldeira et al., 2015; Cantarel, Bloor, & Soussana, 2013; Reichstein et al., 2013).

In Europe, species invasions show a stable rate of increase (Butchart et al., 2010; Caldeira et al., 2015; Seebens et al., 2017) which might lead to large scale homogenization and reduced ecosystem (multi-) functioning (Dornelas et al., 2014; Hautier et al., 2018; Vellend et al., 2013). A globally unique feature of Europe is the extensive semi-natural grasslands, whose species-rich communities originate from millennia of low-intensive agricultural use (pastures, hay-meadows) on sites that would naturally support forests (Török

& Dengler, 2018). These semi-natural grasslands are of high importance for dairy and meat production as well as for biodiversity conservation, among other things (Dengler & Tischew, 2018; Török et al., 2018). Generally, European grasslands seem to be rather resistant against plant invasions, being one of the least invaded habitat types in Europe (Chytrý et al., 2008, 2009; Pyšek, Chytrý, & Jarošík, 2010). The exception is invasions by tall forbs, with the reasons not being fully understood (Dengler & Tischew, 2018). Invasives like *Lupinus polyphyllus* and *Senecio inaequidens* increasingly colonize semi-natural grasslands while the former tends to form dominance stands, changing the vegetation structure and species diversity drastically (Hejda, Pyšek, & Jarošík, 2009; Lachmuth, Durka, & Schurr, 2010; Scherber, Crawley, & Porembski, 2003; Thiele, Isermann, Otte, & Kollmann, 2010; Volz & Otte, 2001). Those changes will likely also affect biotic interactions, abiotic processes and consequently ecosystem stability of the invaded habitats (Sousa, Morais, Dias, & Antunes, 2011; Strayer, 2012).

There is a decades-long scientific discussion about the role of biodiversity, in terms of species richness, for ecosystem stability with a large scientific consensus that biodiversity, in terms of (plant) species richness, infers greater temporal stability to ecosystems in case of disturbance or extreme events (e.g. Isbell et al., 2015; Kreyling et al., 2017; Tilman, Reich, & Knops, 2006). However, counterexamples exist (e.g. Hillebrand et al., 2018; Pfisterer & Schmid, 2002) and the question of whether the diversity–stability relationship is linear, hump-, or U-shape is not definite yet (Pennekamp et al., 2018). Ecosystem stability against extreme events—which is often measured as the reciprocal of temporal variability in community biomass (Cardinale et al., 2012)—can be separated into resistance (Pimm, 1984) and recovery (Hodgson, McDonald, & Hosken, 2015). Here we consider resistance as the degree of change of an ecosystem property in response to an extreme climatic event; no change would indicate complete resistance. Recovery is defined as the degree to which an ecosystem property returns to control or predrought levels after the cessation of the extreme event (Bahn & Ingrisch, 2018; Hodgson et al., 2015; Kreyling et al., 2017). Resistance and recovery can be affected differently by biodiversity (De Boeck et al., 2018; Kreyling et al., 2017; Van Ruijven & Berendse, 2010), with the nature and duration of the extreme event potentially playing an important role. Resistance may be more important during ‘press’ events (long-lasting extremes with

brief periods of recovery, e.g. the Californian 2012–2016 drought), as acclimation responses (Zhou, Medlyn, & Prentice, 2016) and species reordering (Evans, Byrne, Lauenroth, & Burke, 2011) have more time to develop. These efficient longer term defences are less likely to manifest during short but intense pulse events (such as in the current study). On the other hand, alleviation of stress following a pulse event is usually more pronounced, promoting fast recovery. In the case of pulse droughts, nutrient flushes upon rewetting can further stimulate recovery (Dreesen, Boeck, Janssens, & Nijs, 2014). Several recent studies on pulse events have indeed found that plant species richness increased the recovery but not the resistance of grasslands (e.g. Kreyling et al., 2017; Van Ruijven & Berendse, 2010). However, counter examples highlighting the importance of biodiversity for the resistance of grasslands against extreme pulse drought events also exist (e.g. Tilman & Downing, 1994).

It is unclear if the diversity–stability–relationship is maintained in the presence of an invader (Pinto & Ortega, 2016). Climate extremes might impact all of the mechanisms conferring ecosystem stability (Cardinale et al., 2012; De Boeck et al., 2018), thus, enabling the establishment of non-native species in the first place (Hautier et al., 2018; Török et al., 2018; Wardle, Bardgett, Callaway, & Putten, 2011). Invasive species might be able to affect the diversity–stability relationship by altering the ability of communities to resist to and/or recover from an extreme event (Wilsey, Daneshgar, Hofmockel, & Polley, 2014). A highly competitive invader or an invader that tolerates abiotic stress more effectively than the native species might be able to outcompete natives before or during an extreme event respectively (Diez et al., 2012). Even with increased resistance of the invader, such indirect competitive effects could diminish the overall resistance of an ecosystem (e.g. to biomass fluctuations) if competitive pressure leads to native species loss (Bernard-Verdier & Hulme, 2019). The same accounts for recovery: if the invader recovers more quickly from harsh climatic conditions then it might impede the partitioning of resources after stress release as the invader instead captures the majority of available resources (De Boeck et al., 2018; Diez et al., 2012).

Here we quantified the effects on community productivity of two invasive species in Europe, the legume *L. polyphyllus* Lindl. and the non-legume forb *S. inaequidens* DC. We further studied their impacts on community resistance and recovery of biomass production to an extreme climatic event (ambient conditions, extreme drought manipulation) in field mesocosms differing in diversity (1, 3, 6 species) at three sites across Europe (Germany, Belgium, Bulgaria). We hypothesized (a) a positive diversity–recovery relationship in native communities exposed to drought, (b) that the presence of invasive species disrupts this relationship, and (c) that extreme drought events facilitate the studied invader species in these semi-natural grasslands.

The work presented here contributes to the global framework of the diversity–stability debate led by long-term, globally distributed grassland experiments such as Drought-Net and Nutrient Network. These investigate the diversity–stability effect across large spatial and temporal scales, taking into account different disturbances yet generally not including issues related to invasive species (Anderson et al., 2018; but see Flores-Moreno et al., 2016).

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental design

We tested the effects of invaders on the diversity–stability relationship of grassland communities in a coordinated–distributed mesocosm experiment in the field at three climatically (Table S1) and ecologically different sites across Europe: Belgium (BE), Bulgaria (BG), Germany (DE). Richness levels and species composition, including invader presence, were experimentally established in mesocosms. Then, we exposed them to an artificial severe drought event to study the joint effects of drought, invasion, and species richness on biomass production. The experiment was carried out with three fully crossed factors: (a) invader presence (three factor levels: native species only [no invader]; native species and the invader *L. polyphyllus*; native species and the invader *S. inaequidens*); (b) climatic extremes (two factor levels: severe drought, ambient control); (c) community richness (three factor levels: 1, 3 and 6 species).

The coordinated experiment was implemented using buried field mesocosms. At each site, 132 mesocosms were set up: 72 mesocosm with native communities and 60 mesocosms with invader presence (Figure S1). Each mesocosm had 18 individuals planted, split evenly among the number of species assigned to it. For mesocosms with only native species, 12 locally frequent, native species that naturally occur together on the local soil were selected for each site (Figure S1). From these, 12 different compositions were created which were considered as replicates for the species richness levels (3 sites  $\times$  3 species richness levels  $\times$  12 species compositions  $\times$  2 climate treatments = 216 native species mesocosms in total). Invader monocultures were replicated three times for each invader, while the other two richness levels each had six unique assemblages using the site-specific native species and one invader, yielding a total of 30 compositions  $\times$  2 climate treatments  $\times$  3 sites = 180 mesocosms with invader presence or monocultures of invaders (Figure S1). Each unique species composition was exposed to both a drought treatment and ambient weather conditions (control) at each of three sites. Native study species included three functional groups (forbs, graminoids, legumes) with four species representing each functional group per site (Table S2).

All plants were grown from seed under standardized conditions at each site and planted into field mesocosms in early spring 2014 (more than 3 months before the start of the drought manipulation). Seeds were collected from autochthonous populations close to the study sites (relying on expert knowledge; Table S2). Each mesocosm consisted of a PVC tube of 30 cm diameter and 50 cm height. The base of mesocosms was closed with root matting, permeable for water but impermeable for roots. Consequently, rooting depth was limited to 50 cm in order to standardize the climate treatment effects. This may have interfered with deep-rooting strategies (e.g. Nippert & Knapp, 2007) in turn limiting niche differentiation and the potential diversity effects (Dimitrakopoulos & Schmid, 2004). Mesocosms were buried in the soil to ensure realistic temperature

and drainage patterns, and filled with local soil substrate (homogenized, sieved to 2 cm). Mesocosms were planted with 18 pregrown plant individuals in a systematic arrangement, avoiding conspecific neighbours, and ensuring that edge/centre ratios were similar for each species. Each species had the same number of individuals per mesocosm within each species richness level. Thus, invader mesocosms of species richness level 1 had an invader fraction of 100%; mesocosms of species richness level 3 had an invader fraction of 33.3% (6 out of 18 individuals), and mesocosms with a species richness level of 6 had an invader fraction of 16.7% (3 out of 18 individuals). Upon planting, plants were cut to a height of 6 cm above ground level to standardize the initial conditions. No fertilization was applied. Mortality was checked regularly, and dead individuals were replaced during the first month after planting. Non-target species were weeded out at a monthly interval.

## 2.2 | Invader species

Two non-native species invasive to Europe, the legume forb *L. polyphyllus* Lindl. and the non-legume forb *S. inaequidens* DC., were selected for this study. Both species exhibit rapid population growth, an ability to alter their local environment, and are habitat generalists, making them interesting study subjects for invasion processes in European semi-natural grasslands (additional information can be found in the supporting information, p. 3; EPPO, 2006; Fremstad, 2010; Global Invasive Species Database [GISD], 2015; Lauterbach & Nehring, 2013; Scherber et al., 2003). *L. polyphyllus* (Fabaceae), the garden lupine, is native to the western parts of North America and Canada (Beuthin, 2012) and was introduced in Europe in the early 1900s (Fremstad, 2010). *S. inaequidens* (Asteraceae), the South African ragwort, is native to South Africa and Lesotho and was introduced in Europe during the late 19th century (Ernst, 1998; Lachmuth et al., 2010). To date, the occurrence and the impact of both invasive species in Europe is more localized (Dengler & Tischew, 2018; Lachmuth et al., 2010), but both invaders are expected to profit from climate change in terms of increasingly favourable conditions and a possible expansion in range (GISD, 2015; Heger & Böhmer, 2006; Lauterbach & Nehring, 2013).

We expect the Mediterranean type invader *S. inaequidens* to cope well with drought (GISD, 2015). Additionally, *S. inaequidens* is highly efficient in capturing free resources (Dassonville et al., 2008; GISD, 2015) and thus, might be able to compromise the resistance as well as the recovery of our native communities. *L. polyphyllus* is a deep-rooting legume which is able to store nutrients in its rhizomes in the event of disturbance and to resprout when conditions are more favourable (Fremstad, 2010; Volz & Otte, 2001). We expect *L. polyphyllus* to recover more quickly from the extreme event, and thus to disrupt the partitioning of resources after stress release. Consequently, we expect *L. polyphyllus* to hamper the recovery of our native species. However, due to its profound root system *L. polyphyllus* might also be able to outcompete native species during the extreme event.

## 2.3 | Climate treatment

We simulated a pulsed drought event using rainout shelters with 100% rainfall reduction for specific periods during the local growing season. The rainout shelters covered the buried mesocosms and additionally >1.5 m as lateral buffer zones. A randomized block design was applied at each site with either two or three blocks (with each block containing both a rainout shelter and a control). Mesocosms were completely randomized within each drought treatment-block combination. Drought length was standardized across sites with the aim to be extreme compared to past conditions (De Boeck et al., 2019; Schär et al., 2004) and on the basis that such events might become common in the future (Dai, 2013; Seneviratne et al., 2012; Hewitson et al., 2014). Drought length was calculated for each site as 1.5 times the number of consecutive days with <2.5 mm precipitation estimated from the statistical 1,000 year recurrence of such events based on historical data of local precipitation (series length: BE = 111 years, DE = 63 years, BG = 30 years), and constrained within the local growing season (months with mean temperature >5°C and precipitation sum [mm] >2 × mean temperature [°C]; Kreyling et al., 2017). Thus, the extremity of the manipulation is relative to each site, thereby increasing comparability. Ecologically, this is a more meaningful standardization of drought length than simply applying the same drought length to systems under different climatic conditions and, consequentially, different evolutionary adaptation of species and plant traits. The drought treatment started 2/5 of the way into the site-specific growing season (see Table S1 for starting dates). The durations of drought were 76 days in Germany, 85 days in Bulgaria, and 88 days in Belgium. In case of natural drought during the manipulation period, mesocosms growing under ambient weather conditions were irrigated (DE: 4 × 10 mm; BE and BG: never necessary). The drought manipulations were ended by irrigating the droughted mesocosms with 20 mm and the ambient mesocosms with 5 mm, to ensure a temporal synchrony between the postmanipulation rainfall events.

## 2.4 | Biomass production

Above-ground biomass (B) was harvested at three dates during the experiment: (B0) 2 weeks before the start of the drought treatment ('before drought'), to allow for a standardized quantification of biomass production during the drought period; (B1) directly at the end of drought ('end of drought'); and (B2) at peak biomass the following growing season ('peak following year', used for assessing recovery after drought). Biomass was always harvested at 3 cm above ground, and included all plant material rooted inside the mesocosms. We discarded all biomass of species rooted outside, but growing into, the mesocosm communities (Cancellieri, Mancini, Sperandii, & Filibeck, 2017). We did not sample root biomass. Species-specific biomass harvests were conducted directly after the drought (harvest B1) and at the peak of the following year (harvest B2; BG and DE only). Community biomass harvests were conducted at harvest B0. We

sampled the same mesocosms repeatedly because regular cutting 2–5 times per year is the management regime applied in these semi-natural grasslands across Europe (Blüthgen et al., 2012; Dengler & Tischew, 2018; Ellenberg, 1996).

## 2.5 | Response parameters

We used relative measures of resistance and recovery to examine different facets of ecological stability (Donohue et al., 2016; Kreyling et al., 2017; Pimm, 1984). Both metrics are dimensionless, and thus directly comparable between sites and communities with different levels of productivity. We calculated resistance to and recovery from drought for each unique species composition by comparing biomass production between drought treatment and ambient control as:

$$\text{Resistance} = \frac{(B1)_{\text{drought}}}{(B1)_{\text{control}}},$$

$$\text{Recovery} = \frac{(B2)_{\text{drought}}}{(B2)_{\text{control}}},$$

where B1 is the biomass of each community at the end of the drought period and B2 is the biomass of each community at the peak of biomass production in the following year. The resistance index equals 1 for complete resistance and 0 for no resistance (no biomass production during drought). The recovery index equals 1 for complete recovery and is <1 for incomplete recovery. Values >1 indicate overcompensation. Community compositions with <1.5 g dry weight per mesocosm under control conditions (1.5% of all cases) were disregarded because of their high relative uncertainty (e.g. incremental differences in cutting height can have strong relative effects) and their potential to disproportionately inflate errors (grand mean over all measurements is  $31.4 \pm 1.8$  [SE] g per mesocosm).

Survival of invader species as well as native species growing within invader mesocosms were recorded at the end of the drought treatment (B1 harvest). To further quantify the effect of invader presence on native species, we additionally analysed the biomass production of native species growing with and without invader presence. Methods and results of the parameter survival, and the impact of invader presence/absence on native species' biomass production can be found in the supplemental material (Tables S3 and S4).

## 2.6 | Statistical analysis

All analyses were conducted using the statistical software R 3.4.2 (R Core Team, 2017). We used linear mixed-effects models to test the productivity–richness relationship for native and invader mesocosms under ambient conditions (only for harvest B1). Here we tested the impact of the explanatory variables *species richness* and *invader presence*, as well as their interactions, on above-ground

biomass production (dependent variable). With a second linear mixed-effects model we tested if resistance and recovery to climate treatment depended on the explanatory variables *species richness* and *invader presence* as well as their interactions. A third linear mixed-effects model was used to evaluate the difference in the individual biomass of the two invader species (dependent variable). Here we used the fixed-effects *climate treatment*, *species richness*, and *species* (explanatory variables) as well as their interactions. Model 3 was run four times, once each for the B1 and B2 harvests. Results for the B2 harvest can be found in Figure S2. We additionally used model 3 to test the difference in the invader biomass per community biomass (relative invader biomass) of the two invader species (Figures S3 and S4) using the same fixed-effects as in the analyses of the individual invader biomass.

In case of significant interactions between the fixed-effects *invader presence* (models 1 and 2) or *species* (model 3) with the other explanatory variables (*climate treatment*, *species richness*), we ran additional linear mixed-effects models separately for each level of the categorical variables such as *invader presence* or *species* to determine if there were significant differences in the mean values within this group caused by climate treatment/species richness with Bonferroni correction for multiple testing.

We accounted for possible random effects due to the blocked structure and the multisite character of the experiment by nesting *blocks* within *sites* in all linear mixed-effects models. As we tested for general trends across three countries, we have considered country effects as random factors in our models, not as fixed factors. We only allowed the intercept to vary as a function of the block design, but did not include any other main factors into the random term. *Species richness* was introduced into the models as a linear numeric variable, but note that log-linear and factorial response produced qualitatively the same results.

Models were fit with the *lmer* function in the *lme4* package (version 1.1-12; Bates et al., 2014) and results were extracted with the *ANOVA* function in the *lmerTest* package (Kuznetsova, Brockhoff, & Christensen, 2016) in R. ANOVA tables for the respective models can be found in the supporting information document (part 2, pp. 13–22). We visually checked if the model assumptions of homoscedasticity and normal distribution of residuals were violated. Linear models are robust even towards rather severe violations of the model assumptions (Lo & Andrews, 2015; Wilson, 2007). Consequently, we only transformed our response variables in case of severe violations of the model assumptions. Transformation of the response variables produced qualitatively the same results as non-transformed responses. The response variables *resistance* and *individual biomass* were  $\log(x + 1.1)$ -transformed while the response variable *productivity* was  $(1 + x)^{0.4}$ -transformed (note that  $\log(x + 1.1)$ -transformation produced qualitatively the same results) to meet the model assumptions of homoscedasticity and normal distribution of residuals.

Additionally, we checked for effects of different sample sizes by permutated subsampling of the mesocosms containing only natives and did not see qualitatively different effects.

### 3 | RESULTS

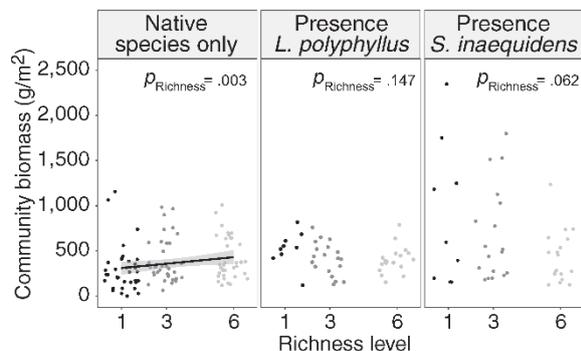
#### 3.1 | Richness–productivity relationship

Under ambient weather conditions, native species productivity increased with species richness (ANOVA subset natives:  $p_{\text{richness}} = .003$ ;  $\text{biomass}_{\text{natives richness level 1}} = 288 \text{ g/m}^2$ ,  $\text{biomass}_{\text{natives richness level 3}} = 395 \text{ g/m}^2$ ,  $\text{biomass}_{\text{natives richness level 6}} = 417 \text{ g/m}^2$ ; Figure 1, ANOVA-Table S1). But invader presence weakened this diversity effect (ANOVA  $p_{\text{richness} \times \text{invader presence}} < .001$ ; ANOVA-Table S1). Overall, invader communities were more productive than native communities (ANOVA-Table S1). In invaded communities, we observed a trend of increasing productivity at lower richness levels, and thus a higher invader fraction biomass (Figure 1; ANOVA-Table S1).

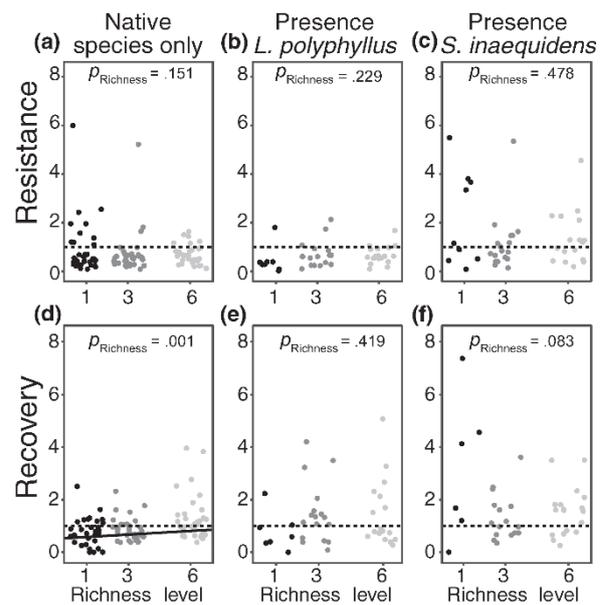
Native species growing with the invader *S. inaequidens* were 31.3% smaller than natives growing without invader presence (ANOVA  $p_{\text{invader presence}} < .001$ ; ANOVA-Table S2; Table S4). Mean individual biomass of native species growing with *L. polyphyllus* was 7.1% higher compared to native species growing alone (Table S4, ANOVA-Table S2).

#### 3.2 | Native community resistance and recovery from drought

Species richness did not have a significant effect on drought resistance of biomass production (ANOVA  $p_{\text{richness}} = .411$ ; Figure 2a; ANOVA-Table S3), while the ability of native plant communities to recover from a severe drought event did increase with



**FIGURE 1** Community biomass of the mesocosms growing under ambient weather conditions at the B1 harvest (after drought) shown as a function of richness (black = richness 1, grey = richness 3, light grey = richness 6) considered across all countries, separately for each invasion status. Shown is the community biomass of each mesocosm per invasion status ( $n_{\text{natives}} = 213$ ;  $n_{\text{Lupinus polyphyllus}} = 90$ ;  $n_{\text{Senecio inaequidens}} = 90$ ). Data points were jittered along the x-axis at each richness level to improve visibility of the data distribution. Black solid lines display mixed-effects model fits of the three submodels for every invader presence level (Bonferroni-corrected significance level:  $p < .017$ ; ANOVA-Table S1), and grey shades indicate their respective 95% confidence intervals



**FIGURE 2** Resistance (B1 ANPP harvest; a–c) and recovery (B2 ANPP harvest; d–f) to a severe drought event, shown as a function of richness (black = richness 1, grey = richness 3, light grey = richness 6), separately for each invasion status (resistance:  $n_{\text{natives}} = 107$ ;  $n_{\text{Lupinus polyphyllus}} = 45$ ;  $n_{\text{Senecio inaequidens}} = 45$ ; recovery:  $n_{\text{natives}} = 106$ ;  $n_{\text{Lupinus polyphyllus}} = 45$ ;  $n_{\text{Senecio inaequidens}} = 45$ ). All mesocosms with a ratio above 1 (the dashed line) showed higher biomass production in drought compared to ambient conditions, while all mesocosms with ratios below 1 showed lower biomass production in drought than under ambient conditions. Bonferroni-corrected significance level  $p < .017$  for the three submodels of invader presence in recovery to drought. Data points were jittered at each richness level to improve visibility of the data distribution

species richness level (ANOVA subset natives:  $p_{\text{richness}} = .001$ ;  $\text{recovery}_{\text{natives richness level 1}} = 0.78 \pm 0.10$ ,  $\text{recovery}_{\text{natives richness level 3}} = 0.86 \pm 0.07$ ,  $\text{recovery}_{\text{natives richness level 6}} = 1.29 \pm 0.15$ ; Figure 2d, ANOVA-Table S4; Table S5; significant higher level interaction of model 2 across all invader presence levels: see below).

#### 3.3 | Invader impact on resistance and recovery

Invasion status did not alter the richness–resistance relation compared to the (non-significant) relation observed in the native species mesocosms (ANOVA  $p_{\text{richness} \times \text{invader presence}} = .379$ ; Figure 2a–c; ANOVA-Table S3). However, invader presence changed the drought resistance of the affected plant communities (ANOVA  $p_{\text{invader presence}} = .011$ ; ANOVA-Table S3). Depending on the invader species, drought resistance—in terms of sheer biomass production—increased in *S. inaequidens* communities (+83.2%) and decreased in *L. polyphyllus* communities (–18.2%; Figure S7) compared to native species communities.

Invader presence altered the richness–recovery relationship in our experiment (ANOVA  $p_{\text{richness} \times \text{invader presence}} = .026$ ; Figure 2d–f;

ANOVA-Table S4). The presence of both invasive species nullified the positive effect of species richness on the recovery of plant communities (ANOVA subset *L. polyphyllus*  $p_{\text{richness}} = .419$ ; ANOVA subset *S. inaequidens*  $p_{\text{richness}} = .083$ ; ANOVA-Table S4; Figure 2e), possibly due to their high productivity in monocultures (*S. inaequidens*) and intermediate richness levels (*L. polyphyllus*). Both invader species showed increased recovery compared to native species across all species richness levels (ANOVA-Table S4).

### 3.4 | Invader performance

Performance per individual of the two invaders in response to the drought treatment differed marginally (ANOVA  $p_{\text{species} \times \text{climate treatment}} = .081$ ; ANOVA-Table S5a; Figure 3). Under drought, *L. polyphyllus* on average produced less biomass than under ambient conditions (-67.9%), while the drought treatment showed no significant impact upon *S. inaequidens* (on average +12.9% more biomass compared to control mesocosms, but this effect was not significant; ANOVA-Table S5a subset *S. inaequidens*; Figure 3). During the treatment phase of the first year (B1 harvest, resistance), native species showed marginally different effects on the invaders (ANOVA  $p_{\text{species} \times \text{richness}} = .058$ ; ANOVA-Table S5a; Figure 3): under ambient conditions, *L. polyphyllus*' biomass production showed a tendency to be negatively affected by interspecific competition, although this effect was superimposed by the strong negative effect of drought upon the individual biomass of *L. polyphyllus* (ANOVA subset *L. polyphyllus*:  $p_{\text{richness}} = .056$ ;  $p_{\text{climate treatment}} = .007$ ; ANOVA-Table S5a; Figure 3). We did not observe a significant effect of species richness on the drought resistance of *S. inaequidens*.

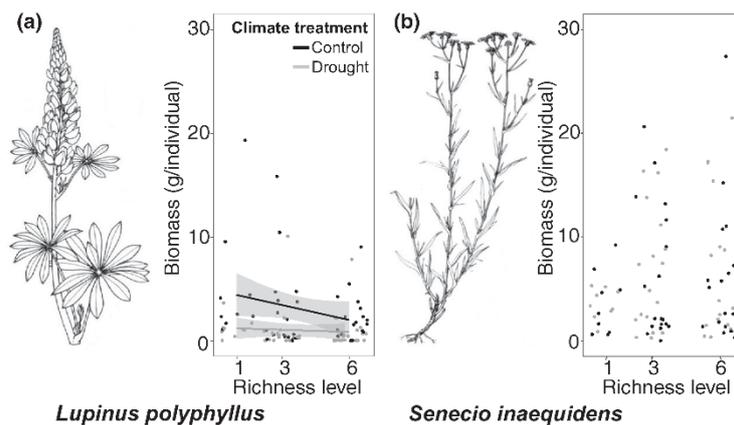
One year after the extreme event (harvest B2, recovery), performance of the invader species was affected by both, climate treatment and species richness (ANOVA  $p_{\text{species} \times \text{climate treatment} \times \text{richness}} = .027$ ;

ANOVA-Table S5b). Formerly drought-treated *S. inaequidens* individuals growing in interspecific competition with native species were able to regrow significantly more biomass than individuals growing in intraspecific competition (monocultures) or under ambient weather conditions (ANOVA subset *S. inaequidens*  $p_{\text{climate treatment} \times \text{richness}} = .0098$ ; Figure S2; ANOVA-Table S5b). While drought recovery of *L. polyphyllus* seemed not to be dependent on climate treatment or species richness (Figures S2 and S4; ANOVA-Table S5b).

Invader fraction per community biomass at the end of the drought treatment (B1 harvest), as expected, decreased with increasing species richness level in both invader species (ANOVA  $p_{\text{species} \times \text{richness}} = .055$ ; ANOVA-Table S6a). However, the steepness of the decline varied between the two invader species. *S. inaequidens*, contributed 47.2% to the total biomass even in the six species high-diversity mesocosms (ANOVA subset *S. inaequidens*  $p_{\text{richness}} < .001$ ; ANOVA-Table S6a; Figure S3), while the percentage share of *L. polyphyllus* was <20%, outside monocultures (ANOVA subset *L. polyphyllus*  $p_{\text{richness}} < .001$ ; ANOVA-Table S6a; Figure S3). One year after the extreme event (B2 harvest, recovery), the relative share of invader species to the total community biomass was still determined by species richness, but had decreased in total and was more similar between the invader species (ANOVA  $p_{\text{richness}} < .001$ ; ANOVA-Table S6b; Figure S4).

### 3.5 | Survival

Invader presence during drought (B1 harvest) changed the viability of plant communities depending on invader species and species richness level (ANOVA  $p_{\text{richness} \times \text{invader presence}} = .002$ ; Table S3a, ANOVA-Table S7); Survival of *S. inaequidens* communities showed a tendency to decrease with species richness level (ANOVA subset *S. inaequidens*  $p_{\text{richness}} = .042$ ; ANOVA-Table S7), while those of



**FIGURE 3** Individual invader biomass at the end of the drought treatment (B1 harvest) presented as a function of richness separately for every climate treatment. The mean biomass of an individual calculated for every mesocosm is shown, separately for (a) *Lupinus polyphyllus* ( $n_{\text{control}} = 45$ ;  $n_{\text{drought}} = 45$ ) and (b) *Senecio inaequidens* ( $n_{\text{control}} = 45$ ;  $n_{\text{drought}} = 45$ ). Data points were jittered at each richness level to improve visibility of the data distribution. Solid lines display mixed-effects model fits of the invader specific submodels (Bonferroni-corrected significance level  $p < .025$ ; ANOVA-Table S5a), and grey shades indicate their respective 95% confidence intervals

*L. polyphyllus* communities increased with decreasing invader fraction (ANOVA subset *L. polyphyllus*  $p_{\text{richness}} = .015$ ; ANOVA-Table S7). We observed a decrease in the survival differences of native plant communities versus invader plant communities with decreasing invader fraction (ANOVA  $p_{\text{richness} \times \text{invader presence}} = .002$ ; ANOVA-Table S7). Survival of *L. polyphyllus* individuals after drought was lower, while the survival of *S. inaequidens* individuals was higher (Table S3b) compared to native species (Table S3a). Presence of invader species decreased the viability of natives (natives within *L. polyphyllus*/*S. inaequidens*; Table S3b) compared to native plant species communities (Table S3a).

The drought treatment decreased the viability of native communities by 11.1%, of *L. polyphyllus* communities by 11.9% and those of *S. inaequidens* by 5.6% (ANOVA  $p_{\text{climate treatment}} = .072$ ; ANOVA-Table S7).

## 4 | DISCUSSION

### 4.1 | Presence of the two studied invader species disrupts the positive richness–recovery relationship of semi-natural grasslands in the face of drought

We found a positive diversity–stability relationship of our native species communities for recovery after drought across three European sites. Invader presence disrupted this positive relationship irrespective of the overall invader performance under drought conditions.

One year after the extreme drought event, community productivity had fully recovered and in some cases even overcompensated, particularly in the high-diversity mesocosms of native communities. Native species richness improved the drought recovery of biomass production in our multisite experiment and, thus, provided ecosystem stability in the face of extreme climatic events in uninvaded assemblages. These findings are consistent with previous studies showing positive relationships between diversity and recovery after extreme climatic events independent from the overall productivity of the communities (Allison, 2004; DeClerck, Barbour, & Sawyer, 2006; Kreyling et al., 2017; Van Ruijven & Berendse, 2010; Vogel, Scherer-Lorenzen, & Weigelt, 2012).

The presence of *L. polyphyllus* and *S. inaequidens* nullified the positive richness–recovery effect found in our native communities. The disruption of the positive richness–recovery relationship in the presence of invasive species is most likely due to their high productivity and the decreasing relative invader fraction with increasing richness. *S. inaequidens* communities showed a stronger resistance and recovery than *L. polyphyllus* communities and the native species communities. This resulted in the neutralization of the positive diversity–stability relationship of native communities wherever *S. inaequidens* contributed more to the community biomass. *S. inaequidens*' ability for enhanced nutrient uptake modifies ecosystem functions by depleting the topsoil nutrient pools and thus reduces the above-ground biomass of the invaded sites (Dassonville et al., 2008; GISD, 2015). Consequently, *S. inaequidens* might have been able to

capture free resources more efficiently than natives both during and after the drought event, likely leading to competitive advantages due to greater growth and development. The highly competitive invader *S. inaequidens* might be able to dominate native communities through increased stress tolerance (Daehler, 2003; Davis, Grime, & Thompson, 2000; Diez et al., 2012), more efficient uptake of limiting resources during the extreme drought (Funk & Vitousek, 2007; Huston, 2004; Vilà & Weiner, 2004), and by more rapid uptake of available resources after stress relief compared to slower growing native species. Thus, *S. inaequidens* seems to increase the competitive pressure on the native species during the drought event and likely disturbs the partitioning of resources after stress release.

*L. polyphyllus* communities also showed a higher and overcompensating recovery compared to native species, despite an average drought resistance in terms of above-ground biomass production. This high ability to recover from a severe drought event in *L. polyphyllus* suggests increased resource allocation to the roots. *L. polyphyllus* is resistant to above-ground biomass removal due to its rhizomes, which enable the invader to resprout multiple times as well as to spread vegetatively by polycormons up to 0.2 m/year (Volz, 2003; Volz & Otte, 2001). Consequently, *L. polyphyllus* might be able to dominate native communities due to its ability to recover quickly after an extreme event (Daehler, 2003; Davis et al., 2000; Diez et al., 2012), and is likely to benefit from the reduced competitive strength of drought-affected native species. However, *L. polyphyllus* does not seem to disturb the partitioning of resources after stress release as natives growing with the non-native legume produced more biomass in both control and drought conditions compared to native species without invader presence.

Many invasive species show such opportunistic traits (Burns & Winn, 2006; Daehler, 2003; Funk, 2008; Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006). Consequently, the presence of competitive and stress tolerant invaders might be able to overturn the positive diversity–stability relationship of native grassland communities (Wilsey et al., 2014). While stability, in terms of biomass production, of native species communities seems to have profited from species richness, that is native species growing in a mixture of potentially asynchronous species, stability of the invader mesocosms seems to be inferred largely by highly productive invasives and their respective share in the entire community (Wilsey et al., 2014). Consequently, the overall stability of the native versus the invader communities may be inferred by two different mechanisms but might ultimately lead to stable—in terms of sheer biomass production—grassland communities (Wilsey et al., 2014). However, both invasive species tested in this study are pasture weeds and their presence might lead to a reduction in fodder value and thus, to economic losses (since the quality of hay production is reduced due to the presence of those species) especially if they occupy large parts of the affected grasslands (Bossdorf, Lipowsky, & Prati, 2008; Hensgen & Wachendorf, 2016). Additionally, invader presence in semi-natural grasslands will certainly lead to significant change with respect to species richness, habitat provision, nutrient cycling, and water regulations (Klinger et al., 2019; Ramula & Sorvari, 2017; Thiele et al., 2010).

Resistance to the applied pulsed drought event was unrelated to species richness in our study. Current state of the art

knowledge reports contrasting effects of sudden pulse events (De Boeck et al., 2018; Dreesen et al., 2014; Kreyling et al., 2017; our study) versus prolonged chronic drought events in grassland communities (De Boeck et al., 2018; Zhou et al., 2016). Prolonged chronic drought events give time to trigger acclimation processes and therefore ecosystems have time to build up resistance in the case of press events (De Boeck et al., 2018; Zhou et al., 2016); while in pulse drought events stability is usually inferred via recovery due to a sudden increase of available resources at the end of the climatic event (De Boeck et al., 2018; Dreesen et al., 2014). However, interpretation of results is not always straight forward as the diversity–stability effect, in terms of insurance due to species asynchronous reactions to stress, might heavily depend upon the characteristics of the stressor (e.g. intensity), the affected community, and the response variable under consideration (Allison, 2004; Blake & Duffy, 2010).

The diversity effects observed in this experiment appear to be relatively small. This might be due to the chosen community composition used in the experiment, or due to the relatively short spatial and temporal scales tested. Previous literature has shown that different stability components do not necessarily covary positively along a diversity gradient, but are rather likely to show opposing effects (Pennekamp et al., 2018). That is, species richness may have had no effect on resistance in this study, but might have increased temporal stability in the long run. Extreme drought can induce stochastic effects in community development and therefore impair predictability even under homogeneous abiotic conditions (Kreyling, Jentsch, & Beierkuhnlein, 2011). For example, if immigration and extinction follow different temporal dynamics, a reduction in environmental quality (here, due to drought) could lead to a temporal increase of species richness (Hillebrand et al., 2018). Additionally, recent evidence suggests that non-monotonic effects, that is hump- or U-shaped effects of diversity, on overall ecosystem stability are likely (Pennekamp et al., 2018). Thus, depending on the ecosystem under consideration biodiversity may increase stability when biodiversity is low, and decrease stability in cases of high biodiversity or the other way round in cases of a U-shaped relationship (Pennekamp et al., 2018). Note that the level of abiotic stress tested in this study (drought-induced biomass reduction of 28%) is comparable to those of natural drought events such as the Central European summer heat waves in 2003 and 2018 (Ciais et al., 2005; Toreti et al., 2019) as well as preceding studies on diversity–stability relationships (Isbell et al., 2015; Kreyling et al., 2017; Pfisterer & Schmid, 2002; Van Ruijven & Berendse, 2010).

#### 4.2 | Extreme drought events lead to a facilitation of the two studied invader species in semi-natural grasslands

Our results revealed facilitation of invasive species in semi-natural grasslands due to extreme drought events, though the pathway to this effect varied between the two invaders. *S. inaequidens* showed increased resistance of biomass production during drought as well

as an increased ability to recover from such an extreme event. The increased biomass (relative to plants in ambient conditions) of *S. inaequidens* in the recovery to drought indicates a high potential of the species to acquire free resources, such as those released from soil C and N mineralization that follows re-wetting of the dry soil (sensu Birch effect; Birch, 1958; Borken & Matzner, 2009; Ingrisch et al., 2018) and the decreased survival of natives in the presence of *S. inaequidens*. Such an effect was not visible in the recovery of biomass production of *L. polyphyllus* (relative to plants in ambient conditions), nor was the survival of the non-native legume better compared to the native species in either climate treatment (even to the contrary). Windows of opportunity for establishment and spread of invasive species arise in the time lag between when invasive species are able to recover and when abiotic conditions become suitable again for native communities (Diez et al., 2012). According to our results, *L. polyphyllus* might not be able to use the ‘invasion window’ (Diez et al., 2012) arising during the drought event, but the slightly improved recovery of the legume invader might be sufficient enough to outcompete native species after the drought event. A recent study indicates that a slight performance advantage of *L. polyphyllus* under combined extreme drought and competition effects might be enough for the legume invader to outcompete native species during phases of increased abiotic stress and thus, to use the arising ‘invasion window’ (Vetter et al., 2019). But, it is likely that *S. inaequidens* will profit from a future increase of extreme climatic events (Hewitson et al., 2014) by using this key temporal aspect—the arising invasion window during drought—to expand its competitive advantage over native species and thus increasing its cover.

The invader impact upon native species performance—in terms of reduction in biomass production of the native species—was more pronounced in *S. inaequidens* relative to *L. polyphyllus*. The observed asymmetric competition is likely due to *S. inaequidens* being a better interspecific competitor and the combination of both stressors seemed to be beneficial for its biomass production. *L. polyphyllus* seemed to be a weaker interspecific competitor which suffered under the presence of the native species as well as the drought treatment, thus it struggled with both, the single effect of competition as well as the combination of competition and extreme climatic events. Native plants growing with *L. polyphyllus* in communities did not suffer from the presence of the non-native legume—in terms of reduced biomass production—as strongly as native plants growing within *S. inaequidens* communities. *S. inaequidens* seems to be better adapted to a possibly drier future climate (EPPO, 2006) than *L. polyphyllus*. *S. inaequidens* and *L. polyphyllus* have different plant–soil relationships, with the former being adept at depleting topsoil nutrient content (Dassonville et al., 2008; GISD, 2015) and the latter being a legume and potentially increasing the plant available nitrogen in the invaded habitats (Otte & Maul, 2005; Thiele et al., 2010; Volz, 2003). In other words, where *S. inaequidens* directly competes with natives, *L. polyphyllus* may facilitate native species due to (a) direct fertilization or (b) by using the rhizobia's nitrogen instead of the available soil nitrogen content, thus resulting in higher net soil N availability for the native species in both ambient and stressful environmental conditions.

In sum, we showed that the interaction of extreme climatic events and invasive species might not only disrupt the ability of native communities to recover from drought, but that extreme events might also facilitate non-native invaders, at least if they are well adapted to the future climate (Wilsey et al., 2014). Generalizing from our two target invader species, invaders of warm origin may profit from a drier future climate in Central Europe, while invaders from cold regions may struggle with the combined effects of drought and native species competition.

#### ACKNOWLEDGEMENTS

This work was funded by ERA-Net, BiodivERsA (SIGNAL; <http://www.bayceer.uni-bayreuth.de/signal/index.php?lang=de>; Funding ID 01LC1201), the German Federal Ministry of Education and Research (BMBF), BonaRes (SUSALPS; <https://www.susalps.de/en/>; Funding ID: 031B0027C), the Belgian Federal Science Policy Office (BELSPO), and the Bulgarian Science Found. We thank numerous students and technicians for field assistance, and two anonymous reviewers for their thoughtful recommendations, which helped to further improve our manuscript.

#### CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

#### AUTHOR CONTRIBUTION

A.J. and J.K. conceived the project and the experiment. Organization of the experimental sites and data collection was split per site: H.J.D.B., I.N., and S.B. contributed experimental data for site BE; D.S. and I.A. contributed experimental data for site BG; and A.J., B.J.B., J.D., J.K., M.A.S.A.-K., M.A.S., P.v.G., P.A.W., V.M.S.V., and M.Z. contributed experimental data for site DE. B.J.B., M.A.S. and V.M.S.V. designed the figures; and M.A.S. and B.J.B. produced the graphics. V.M.S.V. assembled and analyzed the data; and V.M.S.V. wrote the manuscript with substantial input from J.K. and P.A.W. All other co-authors contributed substantially in revising the manuscript.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.11766231.v1>. R scripts are available from the corresponding author upon reasonable request.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Vetter VMS, Kreyling J, Dengler J, et al. Invader presence disrupts the stabilizing effect of species richness in plant community recovery after drought. *Glob Change Biol.* 2020;26:3539–3551. <https://doi.org/10.1111/gcb.15025>

9.5 **Manuscript 5** | Wilfahrt *et al.* (2021) – Disentangling climate from soil nutrient effects on plant biomass production using a multispecies phytometer.



Phytometer experimental site in 2017 Doman-nek, Czech Republic

## Disentangling climate from soil nutrient effects on plant biomass production using a multispecies phytometer

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**Citation:** Wilfahrt, P. A., A. H. Schweiger, N. Abrantes, M. A. S. Arfin-Khan, M. Bahn, B. J. Berauer, M. Bierbaumer, I. Djukic, M. van Dusseldorp, P. Eibes, M. Estiarte, A. von Hessberg, P. Holub, J. Ingrisch, I. K. Schmidt, L. Kesic, K. Klem, G. Kröel-Dulay, K. S. Larsen, K. Löhmus, P. Mänd, I. Orbán, S. Orlovic, J. Peñuelas, D. Reintaler, D. Radujković, M. Schuchardt, J. M.-I. Schweiger, S. Stojnic, A. Tietema, O. Urban, S. Vicca, and A. Jentsch. 2021. Disentangling climate from soil nutrient effects on plant biomass production using a multispecies phytometer. *Ecosphere* 12(8):e03719. 10.1002/ecs2.3719

**Abstract.** Plant community biomass production is co-dependent on climatic and edaphic factors that are often covarying and non-independent. Disentangling how these factors act in isolation is challenging, especially along large climatic gradients that can mask soil effects. As anthropogenic pressure increasingly alters local climate and soil resource supply unevenly across landscapes, our ability to predict concurrent changes in plant community processes requires clearer understandings of independent and interactive effects of climate and soil. To address this, we developed a multispecies phytometer (i.e., standardized plant community) for separating key drivers underlying plant productivity across gradients. Phytometers were composed of three globally cosmopolitan herbaceous perennials, *Dactylis glomerata*, *Plantago lanceolata*, and *Trifolium pratense*. In 2017, we grew phytometer communities in 18 sites across a pan-European aridity gradient in local site soils and a standardized substrate and compared biomass production. Standard substrate phytometers succeeded in providing a standardized climate biomass response independent

of local soil effects. This allowed us to factor out climate effects in local soil phytometers, establishing that nitrogen availability did not predict biomass production, while phosphorus availability exerted a strong, positive effect independent of climate. Additionally, we identified a negative relationship between biomass production and potassium and magnesium availability. Species-specific biomass responses to the environment in the climate-corrected biomass were asynchronous, demonstrating the importance of species interactions in vegetation responses to global change. Biomass production was co-limited by climatic and soil drivers, with each species experiencing its own unique set of co-limitations. Our study demonstrates the potential of phytometers for disentangling effects of climate and soil on plant biomass production and suggests an increasing role of P limitation in the temperate regions of Europe.

**Key words:** aridity; climate gradient; nitrogen; nutrient availability; phosphorus; phytometer; plant productivity; resource limitation.

**Received** 23 March 2021; **accepted** 30 March 2021. Corresponding Editor: Debra P. C. Peters.

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## INTRODUCTION

Understanding vegetation responses to multiple global change factors is a central goal in ecology (Franklin et al. 2016). Key to addressing this challenge is disentangling the co-occurring and covarying environmental factors that drive responses such as primary production (Dormann et al. 2013). Vegetation biomass production is co-dependent on precipitation (Huxman et al. 2004), temperature (Larcher 2003), soil nutrients (Fay et al. 2015), inherent characteristics of the vegetation (Michaletz et al. 2014), among other things. An additional challenge emerges as these drivers often have non-linear effects (Knapp et al. 2017) and non-additive interactions (Wang et al. 2017). Isolating drivers of primary production can improve our understanding of vegetation dynamics in the face of global change. Yet, controlling for multiple influential factors while allowing for natural variation in others is a challenging task, particularly along environmental gradients at continental scales.

Soils control productivity through water regulation (Vicca et al. 2012), the availability of essential soil nutrients, such as nitrogen (LeBauer and Treseder 2008), phosphorus (Vitousek et al. 2010), additional macro- and micronutrients (Fay et al. 2015), and soil properties such as texture and pH (Van Sundert et al. 2018). Temperate regions have long been considered nitrogen-limited systems (LeBauer and Treseder 2008), but anthropogenic

nitrogen deposition and agricultural runoff may alleviate this limitation, potentially causing other nutrients to become limiting (Kaspari and Powers 2016). Increased soil N often shifts nutrient limitations to soil P (Vitousek et al. 2010), although K limitations are also common (Sardans and Peñuelas 2015). Furthermore, the ratio of soil N to P can strongly influence productivity (Penuelas et al. 2013) and community composition (Wassen et al. 2005) as pathways by which plants uptake soil nutrients, such as mycorrhizal associations, become more or less effective (Lambers et al. 2008). The interaction of soil properties in driving plant productivity makes isolating single soil resource effects challenging.

Climatic drivers, similarly to soil properties, are multi-faceted (Nemani et al. 2003) and interact with soil properties to drive plant productivity. Long-term climate–soil interactions, such as soil pH being linked to the climatic water balance (Slessarev et al. 2016), create uncertainty as to the relative importance of climate versus soil in driving productivity due to covariation between drivers. Moreover, more abrupt changes in climate increase the complexity. For instance, chronic fertilization can lead to decreased drought resistance in grassland ecosystems (Bharath et al. 2020), drought and fertilization can interactively change community composition (Van Sundert 2021) and decrease N and P availability to plants by altering water availability (He and Dijkstra 2014), and plant water availability

from precipitation is regulated by soil properties (Vicca et al. 2012). Thus, to even begin to unravel the complex soil dynamics underlying plant productivity, the backdrop of climatic variability must be considered.

The species composition of a community is an additional layer of complexity that can influence primary production through changes in diversity (Loreau and Hector 2001). Though, even at static species richness levels, species in local plant communities react asynchronously to changes in the environment to stabilize productivity (Wilcox et al. 2017). Additionally, species composition and productivity can change concurrently in response to environmental changes (Owensby et al. 1999), creating additional uncertainty whether productivity changes directly with the environment or is mediated by species composition (Hautier et al. 2020).

Recently, coordinated experimental protocols and collaborative approaches have been used successfully to disentangle the effects of these drivers across continental to global gradients (Halbritter et al. 2020). Coordinated studies have shown how primary production is controlled by multiple soil nutrients (Fay et al. 2015), species diversity (Fraser et al. 2015), increased temperature (Peñuelas et al. 2007, Reinsch et al. 2017), and mediation of drought impacts by biodiversity (Kröel-Dulay et al. 2015, Kreyling et al. 2017, Craven et al. 2018). Nonetheless, drivers remain partially obscured due to inseparable covariation between climate, soil properties, and species composition across study gradients and sites. Common metrics use protocols and materials to provide standardized quantifications of ecosystem properties, helping link cross-site studies (Halbritter et al. 2020). Yet, standardized metrics for plant community ecology remain elusive. As the diversity of communities and their constituent species play a clear role in mediating ecosystems responses to environmental changes (Hautier et al. 2015), disentangling soil and climate effects on primary production may be facilitated by standardizing plant communities.

In order to improve understanding of how soil and climate drivers independently and interactively drive plant biomass production, we developed a living reference system in the form of a multispecies phytometer that integrates community-level processes and is designed to

specifically separate climatic from edaphic effects. We expand on the traditional definition of a phytometer as a model plant community used within a single study (Clements and Goldsmith 1924) by introducing a standardized protocol and plant community grown in both local soil and a standardized substrate under different climate regimes. By testing this approach across a pan-European climate gradient, we address the following hypotheses: (1) Biomass production will decrease with aridity and increase with N and P availability, (2) biomass production in standard substrate will decrease with aridity but be unrelated to nutrient availability, (3) factoring out the biomass production from standard substrate in local soils (i.e., climate correction) will clarify soil relationships and potentially allow new relationships to biomass to emerge in local soils, and (4) species will have asynchronous responses to climate and soil drivers and the nature of these responses will be clearer using standard substrate and climate-corrected biomass.

## MATERIAL AND METHODS

### Site descriptions

Phytometers were installed at 18 experimental sites across 11 European countries (Table 1) in 2017. The habitats were predominantly grasslands. In the two shrubland and four forest habitats, the phytometers were installed in unshaded, open areas. The sites spanned a gradient in mean annual temperature from 2.9 to 15.5°C and in mean annual precipitation from 560 to 2005 mm. Local climate data were collected on-site or from weather stations at most 10 km away. All material necessary for germinating phytometer plants was distributed from the University of Bayreuth to ensure uniformity (see Appendix S1 for full list).

Phytometers were established in sets of 10 (five local soils, five standard substrates). Each phytometer consists of three species: *Dactylis glomerata* (grass), *Plantago lanceolata* (non-leguminous forb), and *Trifolium pratense* (leguminous forb). These species are cosmopolitan but non-aggressive perennial weeds. They naturally occur but have different sensitivities to environmental stressors, while also demonstrating sufficient survival rates across a range of climates, making them suitable candidates for global

Table 1. Summary of sites.

Site name	Country	Habitat	Latitude	Longitude	Elevation (m a.s.l.)	MAP (mm)	MAT (°C)	Start date of 50-d period	Precipitation (mm)	Aridity (mm)	Soil GDD (Standard)	Soil pH
Antwerp	Belgium	Semi-natural grassland	51° 9' 36" N	4° 24' 29" E	11	778	10.1	26 June 2017	104.1	110.2	712	8.1
Aveiro	Portugal	Mediterranean shrubland	40° 37' 48" N	8° 39' 0" W	4	916	13.9	3 July 2017	2	184.9	1059	6.9
Bayreuth	Germany	Semi-natural grassland	49° 55' 19" N	11° 35' 48" E	365	745	8.7	29 May 2017	144.9	115.3	830	4.1
Bílý Kříž	Czech Republic	Semi-natural grassland	49° 49' 48" N	18° 54' 30" E	890	1312	6.3	11 July 2017	105	70.8	663	3.6
Brandbjerg†	Denmark	Semi-natural grassland	55° 52' 48" N	11° 58' 12" E	15	742	9.7	29 June 2017	105	77.8	620	3.5
Domaríněk	Czech Republic	Semi-natural grassland	49° 52' 48" N	16° 23' 60" E	530	609	7.2	10 July 2017	107.2	104.6	715	6.2
Esterberg	Germany	Semi-natural grassland	47° 31' 12" N	11° 9' 36" E	1250	1450	2.9	29 May 2017	219.7	21.4	608	5.6
FAHM	Estonia	Forest	58° 13' 48" N	27° 18' 0" E	44	650	5.4	14 June 2017	76.4	49.0	576	4.5
Fendt	Germany	Semi-natural grassland	47° 49' 45" N	11° 3' 58" E	550	900	8.7	29 May 2017	241	2.8	765	5.3
GARRAF	Spain	Mediterranean shrubland	41° 18' 8" N	1° 49' 5" E	212	560	15.5	26 May 2017	27	296.8	1133	8.1
Graswang	Germany	Semi-natural grassland	47° 34' 12" N	11° 1' 48" E	850	1300	6	29 May 2017	282.9	43.1	716	6.8
Gumpenstein	Austria	Semi-natural grassland	47° 29' 44" N	14° 5' 53" E	700	1033	6.9	12 July 2017	424.1	185.4	828	6.4
Kacka Suma	Serbia	Forest	45° 17' 24" N	19° 53' 24" E	86	647	11.4	20 July 2017	65.6	204.1	1022	7.2
Kiskunság	Hungary	Grassland	46° 52' 16" N	19° 25' 16" E	108	594	11	4 June 2017	114.2	208.3	1025	8.1
Mols	Denmark	Semi-natural grassland	56° 22' 6" N	10° 54' 37" E	56	669	8.7	29 June 2017	113.2	69.6	644	4.1
Oldebroek	Netherlands	Semi-natural grassland	52° 24' 36" N	5° 55' 12" E	25	2005	10.1	3 July 2017	115.4	80.2	626	4.3
Waldstein†	Germany	Forest	50° 8' 24" N	11° 52' 12" E	775	1165.5	5.3	29 May 2017	182.1	3.7	714	7.3
Zöbelboden†	Austria	Forest	47° 50' 24" N	14° 26' 24" E	950	1645	7.8	16 June 2017	371.9	204.2	706	5.8

Notes: MAP, Mean annual precipitation; MAT, Mean annual temperature. Precipitation, aridity, and soil GDD are specific to the 50-d growth period of the phytometer trial. Aridity was the difference between site PET and precipitation during the 50-d trial, where PET was calculated using the Hargreaves-Samani equation. Growing Degree Days (GDD) is the area under the curve for temperature between 5 and 30°C. PRS adsorption values can be found in Appendix S2: Table S13.

† Site removed from analyses due to heavy herbivory.

distribution in such a phytometer (Joshi et al. 2001). By standardizing the plant community, we minimized variation from turnover in species, allowing a more focused inference of plant–environment relationships. Seeds for these species were provided from a single seed source located in Central Germany. Then, as early as March 2017, site partners began following a standardized protocol for plant germination, transplantation, phytometer installation, and data collection (Fig. 1a; Appendix S1).

#### Phytometer preparation

Seeds were germinated by the local site scientists in a standardized potting soil in a greenhouse or area with high natural light availability and regulated temperatures. Following an eight-week germination and growth period, plants were transplanted into the 30 cm diameter, 23 cm depth, phytometer pots, with

2–3 cm between the rim and substrate to ensure water penetration. Local soil pots consisted of soil from 5 to 30 cm below the surface at each site. The standard substrate was vermiculite (an inert, inorganic substance; 0–2 mm grain size), with 4 g of slow-release Osmocote fertilizer (see Appendix S1 for nutrient content) mixed evenly into the top 3 cm of substrate, a rate that likely removed nutrient limitations from these pots. Vermiculite was selected as a substrate to maximize the soil water holding capacity and isolate the water-related controls on plant performance derived from precipitation (i.e., the water supply to the soil) from the water controls derived strictly from the local soil properties (i.e., the soil capacity to store the water supplied by precipitation and to provide water to plants during rainless periods). Plants were planted systematically in exactly the same arrangement with an even density across the

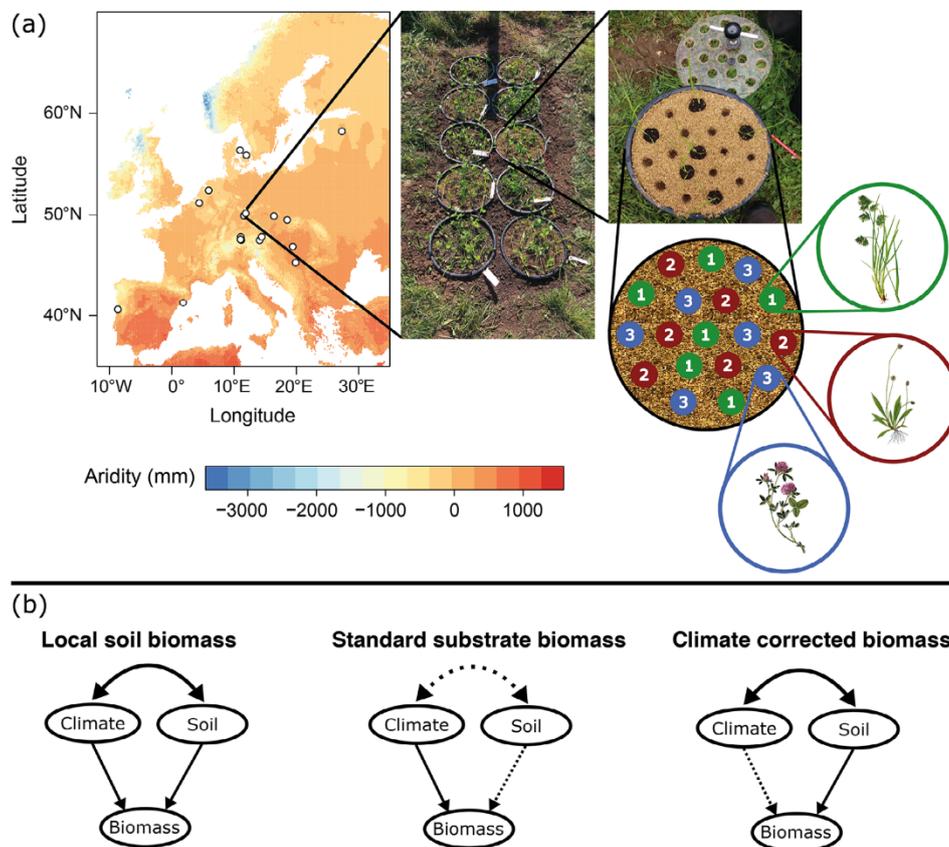


Fig. 1. (a) The phytometer workflow: from right to left, phytometers are planted in a standardized pattern with equal density of three globally distributed, perennial herbaceous species: *Dactylis glomerata*, *Plantago lanceolata*, and *Trifolium pratense*. Phytometer communities are planted in a standardized substrate and the local site soil. Study sites were distributed across an aridity (PET—precipitation; mm) and temperature gradient in Europe. The displayed gradient is calculated from mean annual PET and precipitation derived from WorldClim, while our study focused on 50-d values of this index (see Table 1). (b) The expected parsing of climate from soil effects, where local soils are influenced jointly by climate and soil. Standard substrate is influenced by only climate, which can be used to factor our climate influences from the local soil, clarifying the independent effects of soil. Dashed lines represent an expected absence of a relationship.

surface resulting in six individuals per species. A root-ingrowth core (3 cm diameter, 20 cm length, 2 mm mesh size) was installed in each pot in an area immediately surrounded by one of each species. To account for initial size differences due to varying greenhouse environments at the different sites, 25 individuals per species not planted in phytometer pots were clipped, dried at 60°C for 48 h, and weighed. Soil

temperature data loggers (HOBO TidbiT v2 Temp Logger) were installed at 5 cm depth at this time. Following this, plants were watered for ten days at a rate of 1 liter per pot per day to avoid transplant shock; this also meant that phytometer pots at the beginning of the natural growth period were at or near field capacity. Then, pots were transferred out to the field and buried up to the rim.

### *Phytometer harvest*

Phytometers were exposed to 50 d of growth in the local environment. This duration was chosen to accommodate possible sites with short growing seasons, but also limits inference to a juvenile stage of establishment where competition pressure may be lower than in more mature communities. Plants were inventoried for mortality (i.e., how many individuals were still present with green tissue), clipped 3 cm aboveground to avoid damaging roots, and all aboveground biomass was separated by species and each species was separated into green tissue and dead or senesced (i.e., brown) tissue to assess environmental stress. Root biomass in the root-ingrowth core was carefully washed free of substrate and collected. All biomass was dried at 60°C for 48 h and weighed. More details on any of these steps may be found in the full protocol (Appendix S1). Three sites (Brandenbjerg, DK, Waldstein, DE, and Zöbelboden, AU) showed clear visual signs of heavy vertebrate herbivory and were excluded from the analyses.

### *Environmental variables*

Plant Root Simulator (PRS) probes (Western Ag, Saskatoon, Canada) were installed in pairs in four local soil and four standard substrate pots for ten days prior to the harvest by placing them vertically in the top 10 cm of soil or substrate. These attract and adsorb soil ions, simulating plant root uptake, and indicate the availability of soil nutrients to plants in the final 10 d of the experiment when plant communities were most mature. Probes were analyzed in aggregate for each substrate type per site. This resulted in a single mean estimate for each ion per substrate type per site (i.e., variance was quantified only across sites). Soil pH was additionally measured in the local soil.

Climate data were matched to the 50-d growth period of the phytometer trial. We calculated two indices thought to be important to plant growth. First, we calculated an aridity index (PET—precipitation) (Thornthwaite 1948). PET was calculated using the Priestley-Taylor equation (Priestley and Taylor 1972), integrating daily minimum and maximum air temperature and relative humidity, daily clear sky radiation based on latitude and elevation, and solar radiation at ground level using the Hargreaves-Samani equation,

which estimates cloudiness based on the difference between maximum and minimum daily temperature (Hargreaves and Samani 1982). Aridity indices have the desirable quality of integrating temperature and precipitation into a single variable (Maliva and Missimer 2012), but may still imperfectly integrate all climate variables (Stocker et al. 2018) before even considering its non-independencies with soil factors. Thus, the appeal of our standardized substrate phytometer lies in being an integrative proxy for both measured and unmeasured climate variables (e.g., microclimate, cloud cover, wind, climate-mediated plant–soil feedbacks). Second, we calculated growing degree days (GDD) per soil type per site by fitting a sinusoidal function to the maximum and minimum soil temperature each day, calculating the area under the curve that was above 5°C and below 30°C where plants are expected to be most photosynthetically active, and then summing each day to produce an integrated value per site. Here, we relied on temperature values taken from soil data loggers buried at 5 cm depth, as this more closely represents the temperature the plants experience than typical weather station data collected at 2 m aboveground (Körner and Hiltbrunner 2018).

### *Statistical analyses*

*Empirical data.*—We explored how non-independence between climate and soil variables may influence patterns of biomass production. First, we used linear mixed-effects models to examine the relationship between biomass production at the community and species level to aridity, N availability, and P availability. Second, we used model selection to identify the most important predictors on biomass production. Third, we used interaction models to see how climate may have moderated soil effects on biomass production. In all three cases, we separately examined the response of biomass production in local soil phytometers, biomass production in standard substrate phytometers, and climate-corrected biomass. Climate-corrected biomass was calculated as local soil biomass production minus the mean of standard substrate biomass production at that site (Fig 1b).

In order to further understand causes of biomass changes in response to environmental drivers, we tested whether mortality, the proportion of brown tissue, or root:shoot biomass were

predicted by aridity, N availability, and P availability in local soil and standard substrate. For mortality, we used generalized linear mixed-effects models with a negative binomial distribution link function to account for the large number of zeros in the data using site as a random effect. For the proportion of brown tissue, a sign of environmental stress, we took the mean proportion of brown tissue within a site and soil type and used generalized linear models with a beta distribution link function. As beta distributions cannot use true zeros and ones, any observed zero was set at 0.01 and any observed one was set at 0.99. We used linear mixed-effects models for root:shoot biomass using site as a random effect. We expected that environmental stress (i.e., low resource availability) would increase mortality, the amount of brown tissue, and root:shoot production.

*Model selection.*—We investigated abiotic drivers of community and species biomass production in our local soil phytometers by first using linear mixed-effect models with site as a random effect to determine how biomass production in each phytometer pot was driven by our principal climate variable, aridity, and our principal soil variables, N and P availability, at the community and species levels. We also ran separate models with aridity and N:P availability as predictors to test whether N:P availability was similarly or more predictive. The soil variables were log10-transformed to achieve normality in the residuals. In order to examine the importance of additional variables, we used the “dredge” function in R package MuMIn (Barton 2020) to test which combination of soil properties, PRS ion adsorption of macronutrients (N, P, K, Mg, S, and Ca availability), climate predictors (aridity, soil GDD), and whether between site differences in initial size caused by differences in growth during the greenhouse phase affected final biomass. For this analysis, all variables were scaled to have a mean of zero and standard deviation of 1 to allow direct comparison of coefficients. Site was included as a random effect in all models. We selected all models that were within two of the model with the lowest AICc (Burnham and Anderson 2003) and used model averaging to produce a final fit. We calculated the relative importance of each predictor by refitting a model with all of the selected variables and calculating

the semi-partial  $R^2$  for each term using the r2glmm package (Jaeger 2017), following the Nakagawa and Schielzeth (2013) method of calculating a pseudo- $R^2$  of fixed effects in random-effects models. Ca and S availability were additionally log10-transformed to achieve normally distributed residuals.

We repeated the process in the standard substrate phytometers, albeit with several different predictor variables. Aridity and soil GDD were used to represent climate and macronutrient availability was used to determine whether soil resource supply differed. In the standard substrate, macronutrient availability effects could indicate different rates of nutrient release from the fertilizer in different climatic conditions. Soil pH was unmeasured in the standard substrate as it was assumed to be homogenous at the start of the experiment by design, with any subsequent changes being mediated by differences in climate. We additionally tested whether observed subsidence of the vermiculite substrate affected our results. As the subsidence was not measured at two sites, it could not be included in the full models. Therefore, we tested whether the residual variation of the best standard substrate model of the subset with shrinkage data was related to shrinkage using standard linear regression.

We isolated the effect of soil drivers from climatic drivers in the local soil phytometer by subtracting away the site mean of standard substrate phytometer biomass from each local soil phytometer biomass to obtain a “climate-corrected biomass” value. Using linear mixed-effect models, we followed the same linear mixed-effects model and model selection procedure using the same set of predictors as the uncorrected local soil model.

*Interaction models.*—We explored whether climate and soil had non-additive interactions in our three response variables (biomass in local phytometers, biomass in standard phytometers, and climate-corrected biomass values). We examined how aridity and soil GDD interacted with both N and P availability using linear mixed-effect models with all four two-way interactions between the climate and soil variables with site as a random effect. These variables were selected as drought conditions are known to interfere with plant uptake of N and P (He and Dijkstra 2014). We calculated the relative importance of each group (soil predictors, climate predictors,

and the four possible interactions) in predicting community biomass.

## RESULTS

### *Empirical data*

Linear regression of the principle biomass–environment relationships revealed that aboveground community biomass production in the local soil phytometers decreased with increasing aridity (pseudo- $R^2 = 0.81$ ,  $P < 0.001$ ; Fig. 2; Appendix S2: Table S1), increased with P availability (pseudo- $R^2 = 0.55$ ,  $P = 0.012$ ), and had no relationship with N availability ( $P = 0.84$ ). Species-specific biomass production of *T. pratense* had the same relationships as community biomass with aridity ( $P < 0.001$ , pseudo- $R^2 = 0.78$ ) and P availability ( $P < 0.01$ , pseudo- $R^2 = 0.52$ ). *Dactylis glomerata* and *P. lanceolata* showed qualitatively similar, though non-significant relationships, with aridity and P availability (Appendix S2: Table S1). No species had a relationship with N availability. Aboveground community biomass production in the standard substrate phytometers also decreased with the aridity gradient (Fig. 3; Appendix S2: Table S2; pseudo- $R^2 = 0.59$ ,  $P < 0.01$ ). Species-specific biomass production in standard substrate was negatively related to the aridity gradient for *T. pratense* ( $P < 0.01$ ); *D. glomerata* ( $P = 0.067$ ) and *P. lanceolata* ( $P = 0.66$ ) showed no significant relationship with aridity (Fig. 3; Appendix S2: Table S2). No relationships between biomass production and N or P availability were found (Appendix S2: Table S2), confirming that any uneven soil supply rates of N and P from the fertilizer were not driving biomass production.

Using the standard substrate phytometers to factor out the independent influence of climate, we found that the community climate-corrected biomass was positively correlated with P availability of the local soil (Fig. 3; Appendix S2: Table S3;  $P = 0.016$ , pseudo- $R^2 = 0.63$ ), but showed no significant relationship with N availability (Fig. 3;  $P = 0.47$ ). Species-specific climate-corrected biomass in the local soil phytometers had no significant relationships with N or P availability, though P availability had relatively high predictive power for all three as indicated by the semi-partial pseudo- $R^2$  (Appendix S2: Table S3; all pseudo- $R^2 > 0.3$ ).

Aridity was not significantly correlated with N or P availability in local soil or standard substrate. N:P availability was not a significant predictor of local soil (Appendix S2: Table S1), standard substrate (Appendix S2: Table S2), or climate-corrected (Appendix S2: Table S3) biomass production, though it often had similar predictive power to P availability.

Individual mortality increased with aridity in local soil ( $P < 0.01$ ; Appendix S2: Table S4) and in standard substrate ( $P < 0.001$ ). Percentage of brown tissue increased with aridity in local soil ( $P < 0.01$ ; Appendix S2: Table S5) and in standard substrate ( $P < 0.001$ ). Neither mortality nor percentage brown tissue showed a relationship with P or N availability in local or standard substrate (Appendix S2: Tables S4, S5). Root:shoot biomass showed no relationship to aridity, P availability, or N availability in either local soil or standard substrate (Appendix S2: Table S6).

### *Model selection*

In the local soil biomass production, top models for community biomass included 9 of 10 predictors and model averaging included aridity and soil GDD (50-d thermal radiance), P availability, Mg availability, and soil pH as significant predictors (pseudo- $R^2 = 0.92$ , Appendix S2: Table S7). Best-fit models for the species all had unique sets of predictors in the local soil phytometers, though each species had at least one climatic and one soil variable that were both significant and high in predictive power (Appendix S2: Table S7).

The top models in standard substrate biomass production of communities contained 7 of 9 predictors, but only aridity and soil GDD were significant (pseudo- $R^2 = 0.68$ , Appendix S2: Table S8). This demonstrated that potential variability in nutrient release from the applied fertilizer may have had some impact in driving biomass production but was relatively small compared with the direct effect of climatic drivers. In the standard substrate phytometers, species-specific top models all contained unique sets of predictors (Appendix S2: Table S8). Notably, no predictor was significant after model averaging for *P. lanceolata*, although the 8 predictors chosen in top models were able to account for 54.7% of its variation in biomass (i.e., semi-partial pseudo- $R^2$ ).

For climate-corrected community biomass in the local soil phytometers, the top model

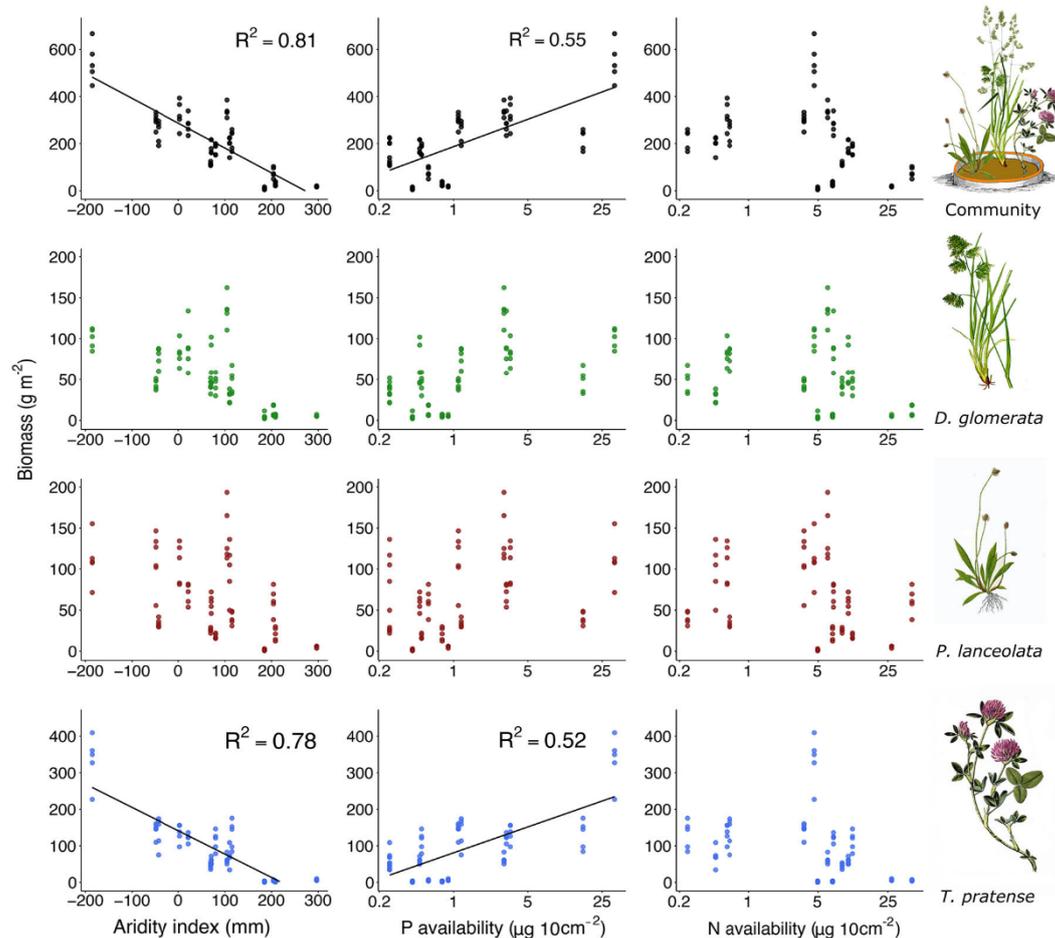


Fig. 2. Fifty-day aboveground biomass production of community and species in local soil across an aridity index (50-d PET—precipitation) and soil N and P availability. Soil predictor variables are log-transformed and black lines are the best-fit line from a mixed-effects linear model fit to the transformed data when  $P < 0.05$ . Significant relationships have the semi-partial pseudo- $R^2$  displayed for significant relationships.

contained all variables, though only P, K, and Mg availability were significant (pseudo- $R^2 = 0.76$ ; Appendix S2: Table S9). Notably, the climate-corrected biomass model revealed potassium as a significant predictor, which was not selected in the local soil model; potassium was also significant for *D. glomerata* and *P. lanceolata*. Top models for species-specific climate-corrected biomass all contained unique sets of predictors and significant predictors; P availability was the only consistent significant predictor for all species and *D.*

*glomerata* and *T. pratense* contained significant climatic variables despite the climate correction (Appendix S2: Table S9).

#### Interaction models

Linear models with two-way interactions between the two climate variables and P and N availability revealed no significant interactions in local soil (Appendix S2: Table S10), standard substrate (Appendix S2: Table S11), or climate-corrected biomass responses (Appendix S2:

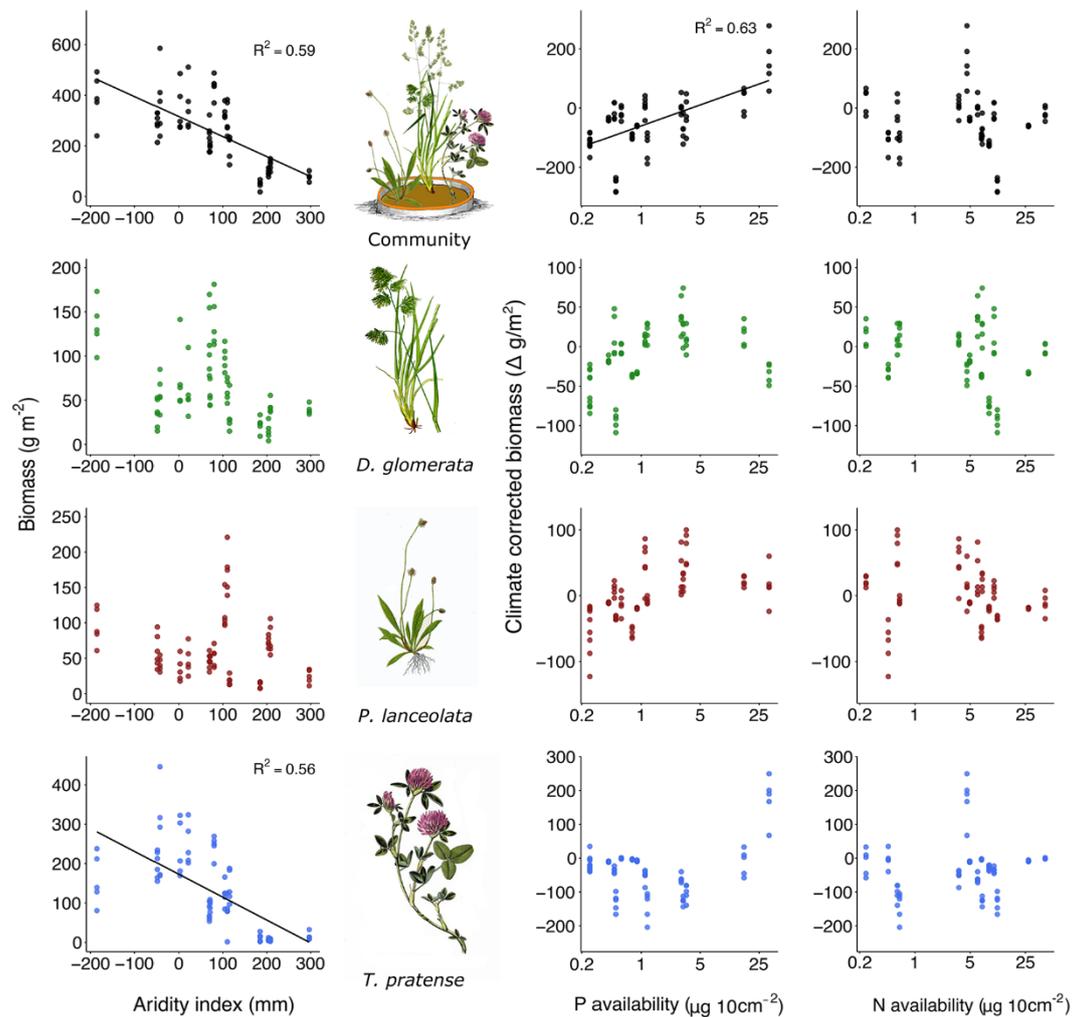


Fig. 3. Biomass response to aridity in the standard substrate and climate-corrected biomass response to P and N availability in the local soil phytometers at the community and species levels. Climate-corrected values are the 50-d biomass production in the local soil minus the mean biomass produced in the standard substrate during the same period. Negative climate-corrected values indicate sites where the standard substrate was more productive than the local soil and vice versa. Soil predictor variables are log-transformed and black lines are the best-fit lines from mixed-effect linear models fit to the transformed data when  $P < 0.05$ . Significant relationships have the adjusted  $R^2$  displayed.

Table S12). Including interactions in models also masked the previously detected significance of main effects (i.e., aridity and P availability; Figs. 2, 3). Post hoc model simplification also did not reveal any significant interactions.

## DISCUSSION

Our phytometer trial showed biomass production strongly decreased with aridity and increased with P availability, but surprisingly

had no relationship to N availability. Phytometers grown in local soil revealed strong effects of aridity, soil temperature, and several soil parameters on biomass production, but failed to clearly separate the effects. Phytometer biomass in standard substrate was explained predominantly by climate variables. Climate-corrected biomass in local soil reacted strongly to soil parameters, with reduced climatic influence. The emergence of a climate-corrected biomass relationship with potassium that was not evident in the local soil analysis demonstrates that studies crossing large climate gradients may unintentionally mask relationships with soil nutrients (Bruehlheide et al. 2018). This experimental separation of climatic and soil influences advances our ability to understand mechanistic controls on primary production, such as multiple-resource limitation (Fay et al. 2015) or non-linearity in climate responses (Knapp et al. 2017).

Aridity was a strong determinant of community biomass production across our European gradient in both local soil and standard substrate phytometers. High aridity led to increased mortality and senesced tissue, factors which would reduce aboveground biomass production. The weaker relationship of biomass with aridity in standard substrate may be partly explained by larger within site variance compared with local soil phytometers, which could result from the observed subsidence of the vermiculite substrate. Additionally, standard substrate phytometers had an overall lower range of biomass values across sites, which may artificially constrain  $R^2$  values. We recommend a modified standard substrate using quartz sand in combination with vermiculite in our revised protocol to mitigate these effects in future trials (Appendix S2: Fig S2). There is additionally the possibility that plant-soil feedbacks led to changes in pH or microbial communities in the standard substrate (van der Putten et al. 2013), though given the isolation from the surrounding soil any such changes should themselves be largely mediated by climate, and we did not detect any effect of climate on soil nutrient availability in the standard substrate. The upshot is that standardized substrate phytometers could be an important tool even in the absence of paired local soil phytometer, by detailing non-linearities of biomass production in response to climatic influences when

employed regularly along natural climatic gradients (Schweiger et al. 2016, Knapp et al. 2017, Kreyling et al. 2018). Additionally, this makes the use of standard substrate phytometers appropriate for clarifying soil influences as we do in this study.

Biomass production was responsive to multiple soil nutrients and properties, suggesting co-limiting factors (Fay et al. 2015). In the local soil phytometers, the role of multiple soil nutrients was clear. Correcting biomass values using the standardized substrate phytometers strongly shifted the predictive power of environmental variables from climate to soil nutrients and exposed potassium as a driver of biomass. Temperate grassland productivity is generally limited by N (LeBauer and Treseder 2008), but we surprisingly observed neither a direct nor an indirect relationship between biomass production and N availability in local soil or climate-corrected soil. High atmospheric deposition of N and fertilization of sites has the potential to shift soil nutrient limitations toward non-nitrogen sources, such as P (Vitousek et al. 2010) and K (Sardans and Peñuelas 2015). P limitation was apparent in both the local soil biomass production and climate-corrected biomass, as biomass production was highest in the presence of large P pools. K relationships became evident only after correcting for climate effects. However, increased K availability was actually associated with decreased biomass, potentially indicating that larger K pools were a result of decreased uptake resulting from decreased biomass. Plant nutrient uptake, and therefore nutrient demand, is not linear across plant ontogeny and resource gradients (Coleman et al. 1993), suggesting shifts to different resource limitations such as N would be possible as our phytometer communities mature.

Communities of species are more likely to experience multiple-resource co-limitation (Harpole et al. 2011). Here, species-specific biomass production was responsive to different soil nutrients and properties, suggesting that species were themselves uniquely co-limited by sets of resources (Harpole et al. 2016). *Trifolium pratense* reacted more strongly to climate, while the deeper rooting *Plantago lanceolata* showed more responsiveness to soil properties after climate correction, suggesting a more fundamental split between climate and soil as the dominant source

of variation between the species. Community-level data displayed clearer responses to drivers, supporting the use of multiple species that may asynchronously respond to environmental conditions to stabilize biomass production (Loreau and de Mazancourt 2013). The discrepancy between community and species-specific biomass responses is likely due in part to confounding biotic interactions, which are impossible to separate from abiotic factors in this current approach. For instance, in the climate-corrected biomass, there is a notable uptick in biomass production of *T. pratense* at the highest P available site, while *D. glomerata* and *P. lanceolata* appear to decrease or level off at this site. P may limit the rate of nitrogen fixation, which is demanding in terms of ATP, leading to high P soils benefiting N-fixers like *T. pratense* (Dynarski and Houlton 2018), which could in turn competitively suppress other species at this site, though we lack the data to test this. The initial starting biomass (i.e., from the greenhouse growth phase), appeared as a significant contributor in several species models, particularly for *D. glomerata*, implying this species may have benefitted from an unintended advantage of being bigger relative to its competitors at some sites. However, while this clouds the species-level responses, communities are by definition networks of interacting species, and the underlying trade-offs between species are integrated into the emergent processes that interest ecologists.

In conclusion, our phytometer approach for parsing climatic and edaphic contributions to biomass production revealed that aridity and P availability were the strongest determinants of biomass production. While our focus was on climate–soil interdependencies, we believe the phytometer approach offers a flexible protocol for investigating additional drivers. For instance, soil microbes undoubtedly drive plant biomass production, and this could be tested by titrating local soil microbes into standardized soil pots. Similarly, plant competition and environmental filters could be explored more thoroughly through the addition of monocultures and single individual pots. Our study adds to a growing body of evidence suggesting increasing P limitation in temperate zones and climate-dependent N effects on biomass production. As ecosystems continue to face multiple global change

pressures, unraveling independent and dependent effects of these drivers will only increase in importance.

## ACKNOWLEDGMENTS

This project was developed as part of the ES1308 ClimMani COST action. It was supported by the German Federal Ministry of Education and Research (BMBF) within the framework of the BonaRes project SUSALPS (Sustainable use of alpine and pre-alpine grassland soils in a changing climate; project number: 031B0027C). JP and ME acknowledge European Research Council Synergy grant ERC-SyG-2013-610028 IMBALANCE-P, the Spanish Government grant CGL2016-79835-P, and the Catalan Government grant SGR 2017-1005. PH, KK, and OU were supported by the National Programme for Sustainability (grant no. LO1415). MB, JL, and DR acknowledge the support from the Austrian Science Fund (FWF) project no P28572 and background logistic support from AREC Raumberg-Gumpenstein (Erich PÖTSCH). PM and KL were supported by the Estonian Ministry of Education and Research (Institutional Research Funding IUT34-9). GKD was funded by the Hungarian Scientific Research Fund (K112576, K129068). PAW and AJ conceived the study and designed the methodology; PAW and AHS analyzed the empirical data; PAW wrote the initial draft; all authors contributed data by maintaining one or more phytometer sites, helped improve the standardized phytometer protocol, and contributed substantially to revising the manuscript.

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## DATA AVAILABILITY STATEMENT

Data and code used for analyses are available from Figshare: <https://doi.org/10.6084/m9.figshare.14675640.v2>

## SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3719/full>



9.6 **Manuscript 6** | Halbritter *et al.* (2020) – The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx).



Members of the ClimMani Working-Group participating at the kick-off meeting for the “Methods-Handbook” in Finse, Norway.

# The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx)

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ClimMani COST Action (2014-2018), Grant/Award Number: ES1308

**Handling Editor:** Robert Freckleton

## Abstract

1. Climate change is a world-wide threat to biodiversity and ecosystem structure, functioning and services. To understand the underlying drivers and mechanisms, and to predict the consequences for nature and people, we urgently need better understanding of the direction and magnitude of climate change impacts across the soil–plant–atmosphere continuum. An increasing number of climate change studies are creating new opportunities for meaningful and high-quality generalizations and improved process understanding. However, significant challenges exist related to data availability and/or compatibility across studies, compromising opportunities for data re-use, synthesis and upscaling. Many of these challenges relate to a lack of an established 'best practice' for measuring key impacts and responses. This restrains our current understanding of complex processes and mechanisms in terrestrial ecosystems related to climate change.
2. To overcome these challenges, we collected best-practice methods emerging from major ecological research networks and experiments, as synthesized by 115

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experts from across a wide range of scientific disciplines. Our handbook contains guidance on the selection of response variables for different purposes, protocols for standardized measurements of 66 such response variables and advice on data management. Specifically, we recommend a minimum subset of variables that should be collected in all climate change studies to allow data re-use and synthesis, and give guidance on additional variables critical for different types of synthesis and upscaling. The goal of this community effort is to facilitate awareness of the importance and broader application of standardized methods to promote data re-use, availability, compatibility and transparency. We envision improved research practices that will increase returns on investments in individual research projects, facilitate second-order research outputs and create opportunities for collaboration across scientific communities. Ultimately, this should significantly improve the quality and impact of the science, which is required to fulfil society's needs in a changing world.

#### KEYWORDS

best practice, coordinated experiments, data management and documentation, ecosystem, experimental macroecology, methodology, open science, vegetation

## 1 | INTRODUCTION

Climate change is an increasing threat to biodiversity and ecosystem functions and services world-wide (IPBES, 2019; Pacifici et al., 2015; Schuur et al., 2015). Numerous studies, including experiments, long-term monitoring, resampling of historic data and various kinds of space-for-time approaches, have been and are being conducted. Such studies address climate and other global change-related questions for various players and processes in ecosystems across the soil–plant–atmosphere continuum. Plants are key players in our ecosystems: they make up the majority of biomass globally, are the main source of photosynthetically fixed carbon and serve as habitat, food sources and hosts for other organismal groups, while also competing with them for resources (Bar-On, Phillips, & Milo, 2018; Fanin et al., 2019). Plants interact with other plants, microbes, invertebrates and other organisms in multiple ways and on different trophic levels (e.g. decomposers, mutualists, pathogens, herbivores, predators). Together, they play a major role in controlling important terrestrial ecosystem processes such as carbon and nutrient cycling (Clemmensen et al., 2013). Improved understanding of the magnitude of climate change impacts, the underlying drivers and mechanisms and the ecological consequences at the individual, population, community and ecosystem scales across the soil–plant–atmosphere continuum, with a focus on first-order plant–animal interactions in detritivore and herbivore food web is urgently needed.

In climate change research, the most common empirical approaches include manipulative experiments *sensu stricto* (Elmendorf et al., 2012; Emmett et al., 2004; Guittar, Goldberg, Klanderud, Telford, & Vandvik, 2016; Wu, Dijkstra, Koch, Penuelas, & Hungate, 2011) and natural experiments that explicitly contrast or compare

different climates through space (i.e. climate gradient studies; Halbritter, Alexander, Edwards, & Billeter, 2013; Olsen, Töpper, Skarpaas, Vandvik, & Klanderud, 2016) and time (i.e. monitoring or resampling; Fitter & Fitter, 2002; Steinbauer et al., 2018). Climate change experiments *sensu stricto* are particularly useful in establishing cause-and-effect relationships, disentangling the effects of and interactions between different drivers and understanding underlying processes (De Boeck et al., 2015; Fraser et al., 2013). Natural experiments are useful to detect processes on large spatial and temporal scales, related to evolutionary processes and equilibria, and they also avoid experimental limitations and side effects. Hereafter, we refer to these collectively as 'climate change studies' *sensu lato* (if not otherwise specified).

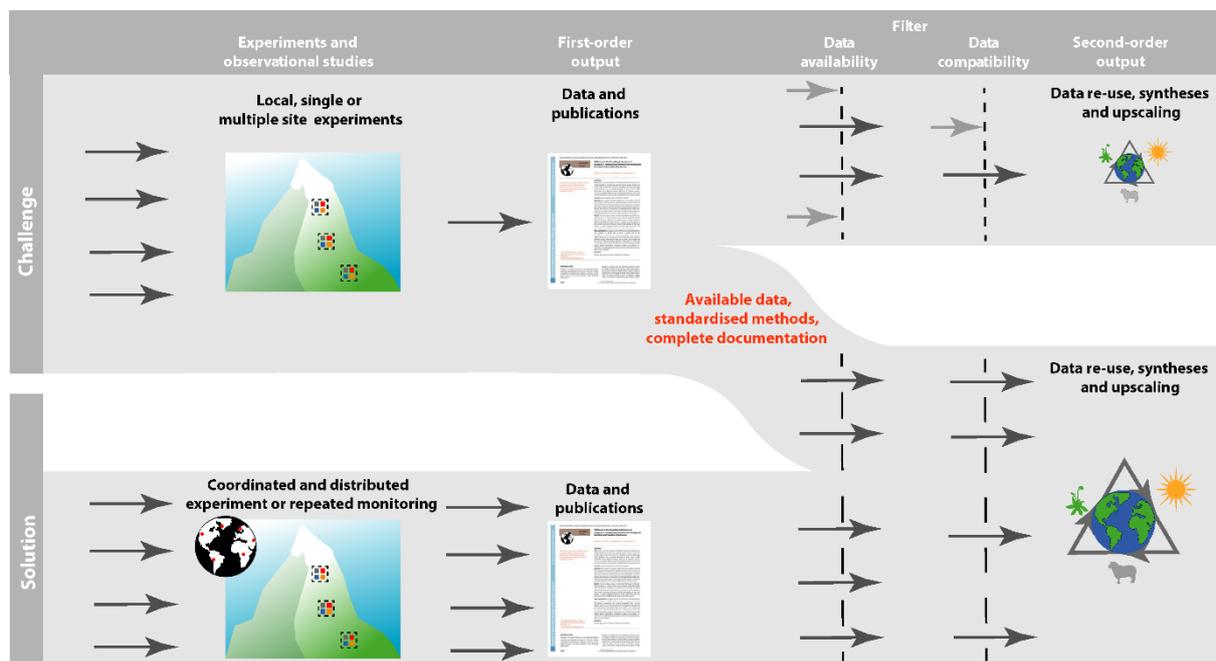
As the number of such climate change studies is quickly increasing, new opportunities for meta-analyses and syntheses are emerging to explore broad-scale patterns and context dependencies in effects, responses and processes, as well as for model development and evaluation. However, there are major challenges for meaningful and high-quality syntheses, including geographic and climatic biases in the available studies (Beier et al., 2012; Vicca et al., 2014), a lack of coordinated measurements and standardized protocols (Denny et al., 2014; Firbank et al., 2017; Vicca, Gilgen, et al., 2012) and a lack of basic and well-structured study information (i.e. covariates, metadata) needed for comparisons (Gerstner et al., 2017; Haddaway & Verhoeven, 2015). Similarly, model–experiment interactions are hampered by methodological inconsistencies across experiments, and by the lack of specific ancillary data to characterize sites and experimental treatments (Medlyn et al., 2015).

The majority of climate change studies are local or regional, and single- to multiple-site studies that are designed to address

specific research questions. As a consequence, these studies often have unique experimental and sampling designs (e.g. Countryside survey, Emmett et al., 2010; ExpeEr, Bertora et al., 2013; INCREASE, Schmidt et al., 2014). Individual research projects and networks invest considerable resources in collecting data for a number of environmental and biotic variables and in developing protocols for field measurements. This leads to a diversity of similar but not quite identical protocols, and hence to a diversity of ways to measure and quantify the same underlying effects and responses. While some of this variability may be due to good scientific reasons, protocol selection is often based on traditions and habits. This methodological diversity can hinder backward compatibility, and thus, syntheses across studies, for example due to incompatible quantification of treatment effects, lack of necessary covariates or response variables, and/or incompatible scale or units of measurements (Estiarte et al., 2016; Lu et al., 2013; Vicca, Gilgen, et al., 2012). These issues largely stem from using a diversity of protocols, and we refer to this as the '*data compatibility filter*' (Figure 1). Another issue that may hinder syntheses and meta-analyses is when key information is not available from the original studies. Data, covariates, metadata and detailed methodological information that are critical for the synthesis step may not be necessary for first-order publications and

are hence not reported, structured well or stored in an accessible location and format. We refer to this issue as the '*data availability filter*' (Figure 1), which is increasingly recognized in the scientific community and is, in part, addressed in recent guidelines on data management and open science practices (e.g. British Ecological Society, 2017; Nosek et al., 2015; and see protocol 1.5). Together, the data compatibility filter and the data availability filter significantly compromise second-order research outputs such as data re-use, synthesis and upscaling (Figure 1).

One way to address these challenges is through '*coordinated and distributed experiments*' (Fraser et al., 2013; Luo et al., 2011; Figure 1), exemplified by the DroughtNet (Knapp et al., 2017), ITEX (Arft et al., 1999), NutNet (Borer et al., 2014), INCREASE (Schmidt et al., 2014) and Tea Bag Index network (Keuskamp, Dingemans, Lehtinen, Sarneel, & Hefting, 2013). Similarly, broad-scale, long-term or repeated observational monitoring can be used, such as resampling studies (Steinbauer et al., 2018; Verheyen et al., 2016) or national phenological networks (Defila & Clot, 2001; Denny et al., 2014). An alternative to such formalized networks is to facilitate coordination and standardization of methods and sampling protocols across studies more generally (Figure 1). The handbook for plant trait measurements is an excellent example of the latter approach; a collection of standardized, documented, state-of-the-art protocols for trait-based plant ecological research, providing



**FIGURE 1** Challenges and solutions to the integration of information from first-order outputs of individual experiments or observational studies (i.e. the original data and publications) to second-order research outputs resulting from, for example meta-analyses, reviews and modelling (i.e. data re-use, synthesis and upscaling). The figure illustrates the major challenges to achieve such second-order outputs, summarized as two filters (dashed lines) relating to data availability and data compatibility across studies. Two general approaches to solve these challenges exist; either using formally coordinated and distributed experiments or using standardized methods, sampling protocols and reporting across individual and independent studies. The aim of this paper is to contribute to the latter approach by offering guidance on selection of response variables, protocols for standardized measurements of these variables and advice on data reporting and management in climate change studies

general methodological advice on how to select and measure traits, independent of the particularities of the individual experiments or study (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). This handbook has become a key resource for trait-based research as it has been hugely important in stimulating the use of standardized methods while at the same time providing an easily available resource for new researchers in the field. Its potential is evident from its high impact world-wide (the two editions of the handbook have been cited over 1,700 times in Web of Science to date, September 2019). Other similar efforts exist like the web resource PrometheusWiki, a handbook for standardized physiological protocols (Sack et al., 2010), or the International Biological Program Handbooks (e.g. Milner & Elfyn Hughes, 1968), as well as national or community standards such as the UK Countryside survey (Emmett et al., 2010) or the UK Environmental Change Network (Sier & Monteith, 2016).

The aim of this paper was to provide a handbook (ClimEx) of standardized field and laboratory methods across the soil–plant–atmosphere continuum applicable to a broad range of terrestrial ecosystem climate change studies (including both experiments and plot-based temporal and spatial gradient studies) world-wide. Because of their dominance in terrestrial ecosystems (Bar-On et al., 2018), we focus mainly on plant communities and the other organisms with which

they interact. We discuss a variety of organisms (i.e. microbes, fungi, invertebrates and first-order plant–animal interactions) where the connection to vegetation and ecosystem functioning is apparent. Through a community effort by experts across scientific disciplines (Box 1), we provide a comprehensive collection of state-of-the-art methods and sampling protocols on key variables of relevance for terrestrial climate change research (Table 1).

The protocols are grouped into five chapters: site characteristics and data management, carbon and nutrient cycling, water cycling, species and interactions, and stress physiology. In each protocol, we describe which response variables should be measured broadly across studies to facilitate data re-use, synthesis and upscaling, using a *Gold standard* (if possible) and, if applicable, we offer minimal requirement *Bronze standards*. We discuss *Special cases, emerging issues and challenges* that address adaptations needed for specific systems or situations and new methods under development. Finally, we give guidance on additional variables that should be measured more widely to improve inter-site and inter-study comparisons and model–experiment interactions.

Our intention is that the ClimEx handbook will be widely used and will stimulate standardized data collection and collaboration between projects within and across sub-disciplines. We acknowledge that different projects may have different specific needs and resources available, and therefore propose a ‘minimal set’ of variables and practices that, if more widely applied, would significantly increase each individual study’s potential contribution to second-order outputs. Many of the methods described in this handbook are also applicable more generally in ecosystem ecology and for other global-change drivers (e.g. nitrogen deposition, invasion, pollution, land-use change). Where relevant, this is indicated in the protocols.

### BOX 1 ClimMani and the protocol writing team

The EU Cost Action ClimMani (2014–2018) focussed on building and strengthening the experimental climate change research community. Key goals of this Action were to provide guidelines for best experimental practices, and to advance experiment–data–model interactions. ClimMani therefore set out to develop and promote common protocols as a community effort to guide future measurements and experiments, and to improve future inter-site comparisons, meta-analyses and model–experiment interactions.

In March 2017, 26 ClimMani members met for a workshop and initiated the collection of measurement protocols in climate change studies. The structure and outline for the project was developed, and leaders for the five chapters appointed. Researchers were identified that could lead protocols based on their scientific expertise. In total, the protocol writing team consisted of 85 authors, who wrote the protocols based on their expert knowledge and existing protocols in the literature. In a second round, 50 experts reviewed the protocols, after which the authors finalized the protocols by the end of 2017. A second workshop was organized in January 2018 to finalize and harmonize all protocols and agree on data presentation. In total, 115 scientists from 21 countries on four continents were involved.

## 2 | MATERIALS AND METHODS – A USER’S GUIDE

### 2.1 | How were the protocols produced and assessed?

We collected a large number of protocols from major terrestrial climate change experiments; ITEX, DroughtNet and NutNet to name a few, and complemented these with literature on methodology, method descriptions from publications and protocols from our own projects (see the appendix for full reference lists). From these sources, we extracted a list of response variables that are relevant and commonly used in terrestrial climate change research (Table 1). A core group outlined the writing process, the relevant response variables, the protocol structure and the final editing, while expert teams on each topic wrote the protocols (Box 1).

We focussed on variables that are relevant for understanding climate change impacts on the biodiversity and functioning of the soil–plant–atmosphere continuum, and/or which are particularly useful for data re-use, synthesis and upscaling. We do not comprehensively

cover plant functional traits as these are already extensively covered in the *New handbook for standardised measurement of plant functional traits worldwide* by Pérez-Harguindeguy et al. (2013), but provide a motivation and overview of relevant traits (see Chapter S4), and otherwise refer to this source.

## 2.2 | Content and structure of the protocols

For each protocol, we provide a short overview of the equipment and running costs, installation and maintenance effort, knowledge-needs and the type of measurements conducted (Table 1). Note that this overview is to a certain extent country-, habitat- and project-dependent (i.e. costs differ between countries, knowledge-needs for species identification might differ between a rainforest and a temperate bog). We give a short summary of the ecological background and its relevance to terrestrial climate change studies. We succinctly describe *What and how to measure*, providing sufficient detail to ensure reproducibility, and provide a reference list with more extensive literature on each method. We describe a *Gold standard*, that is the optimal measurement irrespective of economic, technical and practical constraints, and (if applicable) a *Bronze standard*, that is the minimal requirement for data of adequate quality, which can be advisable in specific situations. In the section on *Special cases, emerging issues and challenges*, we explain how the method can be adapted in specific cases and provide guidance on relevant challenges and opportunities. We further provide an extensive list of key references on the underlying theories, assumptions and applications of each method in the section on *Theory, significance and large datasets*, as well as under *More on methods and existing protocols*. These references may be especially useful for students, early-career scientists or research teams getting started in setting up new studies, and more generally for readers interested in exploring the wider literature related to a specific ecological response variable. Each chapter is available as a separate supplement for easier handling, but we encourage the climate change research community to be aware of aspects of other chapters outside their own scientific expertise.

## 3 | RESULTS – OVERVIEW OF THE HANDBOOK CONTENT

We produced a collection of 66 protocols, describing response variables ranging from elements and organisms to the ecosystem scale, organized into five supporting information chapters (Table 1). All protocols can be found in the online Supporting Information to this paper. In addition, the protocols are also available online on the ClimEx handbook webpage ([climexhandbook.w.uib.no](http://climexhandbook.w.uib.no)). To ensure that the handbook will also be a useful resource for the community in the future, comments and suggestions for updating the protocols can be made via the webpage. These comments and suggestions will be assessed by the authors of this paper and every update will be

tracked. The protocols should be cited as appendices to this paper, see individual protocols for details. In the following, we present a summary of these chapters, guidance on their use and examples of their relevance to climate change research.

### 3.1 | Chapter 1: Site characteristics and data management

**Systematic reporting of background information of the studied system**, data analysis and results is necessary to ensure that studies are reproducible and to enable data syntheses and analyses across studies, meta-analyses, modelling and upscaling (Gerstner et al., 2017; Haddaway & Verhoeven, 2015). Such information includes a basic site description, such as geographic location and abiotic and biotic properties, pre-experimental baseline conditions (e.g. climate, soil properties), basic methodological descriptions (e.g. sample size, timing, duration), a proper characterization of treatments and results (e.g. units, effect sizes), and well structured and documented data management. Surprisingly, this necessary basic information is often incomplete or missing in scientific publications (Hillebrand & Gurevitch, 2013).

The **site characteristics and data management chapter** (Supporting Information S1) therefore describes which key site, study system, and study design variables and information should be collected, and how this information is best reported. We first give practical guidance on how to design and set up a climate change study that may serve multiple uses beyond the needs of the particular project. Then, we describe basic site description parameters (e.g. coordinates, elevation, land-use history, vegetation) and physical (e.g. soil horizon, pH), chemical (e.g. nutrient availability) and meteorological variables. Although some of this information may not directly relate to the particular research question or hypothesis of the original project, reporting all relevant information is essential as it puts studies in a larger context and is key to making data and results useful beyond the particular research for which they were designed.

Arguably, improved collection and reporting of background information about the sites and study systems are probably the largest benefit of this handbook in terms of opportunities for second-order outputs (Figure 1). At the same time, these data are non-focus variables in most studies and therefore typically have low priority. To stimulate systematic and standardized collection and reporting of key background variables, we therefore provide an overview of the most critical variables, both overall and specifically for different kinds of data re-use, synthesis and upscaling (Table 2). Here, we also indicate the spatial scale (plot/treatment/site) at which the different variables should preferably be collected for different opportunities of data re-use. The first '*minimum requirement*' column in Table 2 lists variables that *all* climate change studies – regardless of research question and focus – should measure and report. Background data of particular relevance for specific kinds of second-order outputs (i.e. meta-analyses, community modelling, ecosystem modelling, spatial and temporal upscaling) are indicated in the other columns (also see discussion below).

**TABLE 1** List of all protocols in the five chapters: 1) Site characteristics and data management, 2) Carbon and nutrient cycling, 3) Water cycling, 4) Species and interactions and 5) Stress physiology. For each protocol, ranges of equipment costs and running costs (specified in euros, € = <100€, €€ = 100–1,000€, €€€ = 1,000€–10,000€, €€€€ = >10,000€), installation effort (installing or applying on-off measurements), maintenance effort (maintaining instrumentation or repeated measurements), knowledge-need specified in three categories low (L), medium (M) and high (H), and measurement mode as manual measurement (MM) and/or data logger (DL) are specified

		Equipment costs	Running costs per exp. unit	Installation Effort	Maintenance Effort	Knowledge-need	Measurement mode
1. Site characteristics and data management	1.1	Optimizing the study design	-	-	-	L	-
	1.2	Geographic location and basic site description	-	-	-	L	-
	1.3	Soil type and physical characteristics	-	-	L	L-M	MM, DL
	1.4	Soil chemistry and nutrient availability	-	-	L	M	MM
	1.5	Meteorological measurements	-	-	M	M	MM, DL
	1.6	Open science practice, reproducible workflow, and data management	-	-	-	L	-
2. Carbon and nutrient cycling	2.1.1	Above-ground plant biomass	€-€€	L-M	L	L-M	MM
	2.1.2	Below-ground plant biomass	€-€€€	M	L-M	M	MM
	2.1.3	Leaf-scale photosynthesis	€€€	€€	M	M	MM
	2.1.4	Plant respiration	€€€	€	M-H	H	MM, DL
	2.1.5	Root exudation (in situ)	€-€€€	€-€€€	H	L-H	MM
	2.1.6	Foliar stoichiometry and nutrient resorption	€	€-€€	L-M	-	MM
	2.2.1	Soil microbial biomass - C, N, and P	-	€	L	M	MM
	2.2.2	Root and soil colonization by mycorrhizal fungi	€	€	M-H	-	MM
	2.2.3	Soil CO <sub>2</sub> (and other trace gas) fluxes	€€-€€€	€-€€	M-H	M	MM, DL
	2.2.4	Soil carbon and nutrient stocks	€	€	M	-	MM
	2.2.5	Nutrient mineralization	None-€	€-€€	L-M	M	MM
	2.2.6	Foliar litter decomposition	€	€	L	L	MM
	2.2.7	Root decomposition	€	€	M-H	L	MM (DL)
	2.2.8	SOM decomposition	€€	€€-€€€	M	M	MM
	2.2.9	Soil leaching	€€	€€	M	L	MM
	2.2.10	Soil water erosion	€€	€	M	M	MM
	2.2.11	Biological nitrogen fixation	-	€€-€€€	M	M	MM
	2.3.1	Ecosystem CO <sub>2</sub> and trace gas fluxes	€€-€€€	€-€€	M-H	L-H	MM, DL
2.3.2	Total below-ground carbon flux	€€€	€€€	H	M	MM, DL	
2.3.3	Upscaling from the plot scale to the ecosystem and beyond	€-€€€	NA	M	M	MM, DL	
3. Water cycling	3.1	Soil moisture	€€	€	M	M	DL
	3.2	Soil hydraulic conductivity	€€	€	L	L	MM
	3.3	Soil water retention	€€€	€	M	M	MM
	3.4	Soil water potential	€€€	-	M	L	DL
	3.5	Soil temperature (thermal regime)	€€	€	M	M	DL
	3.6	Soil wettability or water repellency	€	-	-	-	MM
	3.7	Sap flux	€-€€	€-€€	M	M-H	DL
	3.8	Ecosystem water stress	€€	€	M	M	DL

(Continues)

TABLE 1 (Continued)

		Equipment costs	Running costs per exp. unit	Installation Effort	Maintenance Effort	Knowledge-need	Measurement mode	
4. Species and interactions	4.1	Sexual plant reproduction	€	€	L	L	M	MM
	4.2	Seed viability, germinability and dormancy	€	€	L	L	L-H	MM
	4.3	Plant demography	€€	€	L	L	M	MM
	4.4	Bud dormancy depth	€	€	L	M-H	L	MM
	4.5	Above-ground plant phenology	€-€€	€	L-M	H	M	MM, DL
	4.6	The soil seed bank (buried seed pool)	€	€	M	H	H	MM
	4.7	Propagule rain	€	€	L-M	L-M	H	MM
	4.8	Plant community composition	€	-	L-M	-	H	MM
	4.9	Soil microbial community composition	€	€€-€€€	M	-	M-H	MM
	4.10	Soil micro- and mesofauna community composition	€	€	L-H	L-M	M-H	MM
	4.11	Macroinvertebrate community composition	€	€	L-M	M-H	H	MM
	4.12	Pollinator composition	€€€	€	L	L	H	MM
	4.13	Pollinator visitation	€€€	€	L	L	M	MM
	4.14	Plant pathogen and invertebrate herbivory	€	-	L	L	M	MM
	4.15	Vertebrate herbivory	€-€€	€	L	L	M	MM
	4.16	Functional traits	-	-	-	-	-	-
5. Stress physiology	5.1	Chlorophyll fluorescence	€€-€€€	€	L	M	M-H	MM
	5.2	Chlorophyll and carotenoid content	€-€€	€	M	L	M	MM
	5.3	Non-structural carbohydrates	€€€	€	M	M	M	MM
	5.4	Lethal dose (LD50) to quantify stress tolerance exemplified by frost tolerance	€€€	€	M	L	M	MM
	5.5	Leaf temperature	€€-€€€	€-€€€	M	M	M	MM, DL
	5.6	Leaf thermal traits	€€-€€€	€-€€€	M-H	-	M	MM
	5.7	Stomatal conductance	€€-€€€	€	L	L	M	MM, DL
	5.8	Psychrometry for water potential measurements	€€	€	M	M	M	DL
	5.9	Pressure-volume curves - TLP, $\epsilon$ , $\psi_0$	€€	€	M	L	M	MM
	5.10	Maximum leaf hydraulic conductance	€€€	€	L	L	M	DL
5.11	Metabolomic profiling in plants using mass-spectrometry	€€€	€€	H	M	H	MM	
5.12	Reflectance assessment of plant physiological status	€€-€€€	€	L	L	M	MM, DL	
5.13	Stable isotopes of water for inferring plant function	€€€	€	M	M	H	MM, DL	
5.14	BVOCs emissions from plants and soils	€€€	€€€	H	H	H	MM	
5.15	Water-use efficiency	€€	€	L	L-M	L-M	MM, DL	
5.16	Leaf hydraulic vulnerability to dehydration	€€€	€	L	L	M	DL	

**TABLE 2** Guidance on the selection of variables to optimize different types of data re-use, synthesis and upscaling. The minimum requirement column lists variables that should be measured and reported in all climate change studies. The meta-analysis, community models, ecosystem models, and temporal and spatial upscaling columns list variables required for these specific kinds of data re-use. The critically important variables are in grey cells, whereas advised variables are in white cells. The letters indicate at which level the variables should be measured: S = site, T = treatment and P = individual plot. The last column gives the relevant specific protocols in the Supporting Information

Variable	Minimum requirement	Meta-analyses	Community models	Ecosystem models	Temporal and spatial upscaling	Protocol reference
Site description						
History: Soil and land management/ Disturbance/ Nutrient input/ Contamination	S	S				1.1.1
Location: Coordinates/ Name/ Country	S	S	S	S	S	1.1.2
Elevation/ Slope/ Aspect	S/P	S/P	S	S	S	1.1.3
Climate data <sup>a</sup> : Mean annual temperature/ Precipitation/ Seasonality/ Length of growing season	S	S	S	S	S	1.1.4
Dominant vegetation/ Life strategy (density, volume, cover, or biomass)/ Plant functional type	S	S/T/P	S/T/P	S/T/P	S	1.1.5
Abiotic and biotic properties of the ecosystem						
Soil type	S	S	S	S	S	1.2.1
Soil horizons (layers)	S		S	S		1.2.1
Plant rooting depth	S	S	S	S	S/T/P	1.2.2
Stone content	S	S		S		1.2.3
Bulk density	S	S		S		1.2.4
Soil texture/ Particle size analysis	S	S		S		1.2.5
Soil pH	S	S/T/P	S	S/T/P	S/T/P	1.3.1
Soil organic matter (SOM) <sup>b</sup>	S	S/T/P	S	S/T/P	S/T/P	1.3.2
Soil C, N and P <sup>c</sup>	S	S/T/P	S	S/T/P	S/T/P	1.3.2
Cations (exchangeable base cations and cation exchange capacity)	S	S		S		1.3.2
Plant community composition and abundance	S	T/P	T/P	S		4.8
Leaf area index	S	S	S	T/P		4.15
Plant traits, e.g. leaf mass per area (LMA)	S	T/P	T/P	T/P	T/P	4.15
Air temperature <sup>d</sup>	S	S	S	S	S	1.4.2
Soil temperature <sup>d</sup>	S/T	S/T/P	S	S/T/P	S/T/P	1.4.3, 3.5
Fraction of absorbed photosynthetically active radiation (FAPAR)/ Photosynthetically active radiation (PAR) <sup>e</sup>	S	S	S	S	S	1.4.4
Relative humidity	S	S				1.4.5
Precipitation (± Snow depth and duration) <sup>d,f</sup>	S	S			S	1.4.6
Soil moisture <sup>g</sup>	S/T	S/T/P	S/T/P	S/T/P	S/T/P	1.4.7
Rain through-fall <sup>g</sup>	T	S		P		1.4.8

<sup>a</sup>From nearby meteorological station; >30 years of data are needed for meaningful means.

<sup>b</sup>Gold standard is via CN analyser; Silver standard Walkley-Black method; Bronze standard is loss-on-ignition (LOI).

<sup>c</sup>Organic C and N are essential; P is not always measured and is variable, so only continuous measurements are useful; for calcareous soils, inorganic C is important; gold standard is organic and inorganic C, N and P; bronze standard is LOI measurement converted to soil C by dividing by 2.

<sup>d</sup>Included in standard automated meteorological station.

<sup>e</sup>Usually easily measured as part of automated weather station; necessary if plant growth responses are studied.

<sup>f</sup>Snow cover duration in alpine systems, where snow is important.

<sup>g</sup>Important in forests and in rainfall experiments.

<sup>h</sup>Continuous measurements.

Alongside the planning of the study design and data collection, the 'data cycle' in a research project – creating, processing, documenting, sharing, storing and re-using data – should also be planned, follow standardized practice and be well structured and documented (British Ecological Society, 2018; Poisot, Bruneau, Gonzalez, Gravel, & Peres-Neto, 2019). Good data management practice is a key element of 'reproducible' research practice, a term describing a holistic approach to reproducible science (Stark, 2018). We argue that good data management practice is a key step in overcoming the challenges related to the 'data availability filter' (Figure 1) and thus in creating opportunities for data re-use, synthesis and upscaling. Funding bodies and publishers are increasingly recognizing this, and now often require data management plans and open science practice (British Ecological Society, 2018). We therefore include guidance on open science practice, reproducible workflow and data management in this chapter.

### 3.2 | Chapter 2: Carbon and nutrient cycling

The **cycles of carbon and nutrients** involve many ecological processes that are all sensitive to climate change (Bai et al., 2013; Yue et al., 2017), including photosynthesis, above- and below-ground plant growth, autotrophic and heterotrophic respiration, organic matter decomposition and nutrient mineralization. Even minor changes in any of these processes, or in the balance between them, can have implications for biodiversity and ecosystems, which again can impact strongly on ecosystem goods and services such as provisioning of food and fibre, water regulation and carbon sequestration (Trumbore, Brando, & Hartmann, 2015).

In the **carbon and nutrient cycling chapter** (Supporting Information S2), we focus on the main components of the terrestrial carbon cycle, including above- and below-ground processes, pools and fluxes. Nutrients are also included, but limited to pools and processes that are linked to carbon cycling and ecosystem feedbacks to climate. We stratify measurements into three thematic protocols (plants, soil and ecosystems) that are particularly relevant when considering carbon and nutrient cycling processes within terrestrial ecosystems.

### 3.3 | Chapter 3: Water cycling

**Ecosystems play key roles in water and energy cycles**, with feedbacks to climate. The understanding of the water and energy exchange between the soil, plants and the atmosphere is still a major research challenge in climate change research because of difficulties in some of the measurements, which are needed to complete the water and energy balances. Water that enters the ecosystem via precipitation will be separated into evaporation, infiltration, transpiration by plants, drainage to groundwater and (temporary) storage in the soil. All these water fluxes need to be determined to fully understand the water and energy exchange between the ecosystem and the atmosphere.

In the **water cycling chapter** (Supporting Information S3), we start with soil hydraulic measurements, which include soil moisture,

hydraulic conductivity, water retention and water potential. The methods included are key to determining parameters that link to, or are used in, modelling the soil–plant–atmosphere continuum. We also provide guidelines to quantify the ecosystem water stress aiming to facilitate comparison and syntheses across studies. We further include measurements used to track the progress of water through the plant and back to the atmosphere.

### 3.4 | Chapter 4: Species and interactions

As discussed above, plants and the interactions in which they participate play key roles in terrestrial ecosystems. Impacts of climate change on ecosystems and their functioning are therefore to a large extent, mediated through **the plant–soil food web**, both through changes in the abundance and diversity of organisms, and through changes in their interactions (competition, mutualism, grazing, predation, decomposition, etc.).

In the **species and interactions chapter** (Supporting Information S4), we provide guidance on quantifying the consequences of climate change for key organisms, biodiversity components and ecological processes across the plant–soil food web. At the **level of populations**, we cover vital rates such as reproduction, recruitment, growth, mortality and phenology, as well as the overall impacts on the dynamics and growth rates of populations. At the **community level**, we provide guidance on the assessment of impacts on plant-, invertebrate- and microbial-species composition, abundance and diversity. For plants, we consider both above- and below-ground diversity, as well as community-level dynamics in the form of propagule rain. With regard to **species interactions**, we cover pollination, vertebrate and invertebrate herbivory, plant predation and pathogens, and decomposition. We also provide a short motivation for, and link to, the plant traits protocol (Pérez-Harguindeguy et al., 2013). This chapter does not cover organism responses at the individual level, which are dealt with in chapter 5 on stress physiology (see below).

### 3.5 | Chapter 5: Stress physiology

In uncovering how climate change will affect plants and ecosystems, ecophysiology provides the means to mechanistically predict when tolerance limits are exceeded, and therefore when changes in the functioning of individual plants, species and entire ecosystems should occur. However, generalizations drawn from ecophysiological measurements have specific methodological challenges related to factors such as microclimate versus tissue temperature (De Boeck, Velde, Groote, & Nijs, 2016; Michaletz et al., 2016), acclimation (Neuner & Buchner, 2012) and scaling up from leaves to whole organisms or even ecosystems.

In the **stress physiology chapter** (Supporting Information S5), we describe a series of physiological or related measurements that are useful in climate change research. We focus mostly on their use as

indicators of stress, attained through determination of compounds (e.g. chlorophyll and carotenoid content, non-structural carbohydrates), plant functional traits (e.g. reflectance, leaf hydraulic conductivity, leaf thermal properties, stable isotopes of carbon and water) and measurements that directly characterize or assess stress and tolerance.

## 4 | DISCUSSION – ADDING VALUE THROUGH BETTER COORDINATION OF CLIMATE CHANGE ECOLOGY

### 4.1 | The case for standardized data collection and reporting

Variation in the reporting of basic site and experimental characteristics or in measurement methodologies substantially reduces the potential data (re-)use for analyses and comparisons across studies. Comprehensive comparisons across studies are therefore often hampered by (a) unrecorded factors increasing the unexplained variance in the data, (b) incompatible reporting leading to loss of information in the data (e.g. necessitate a simplification from quantitative to factor or qualitative variables) and (c) failure to report key (co)variates reducing the sample size (e.g. if studies have to be excluded from a synthesis) (Gerstner et al., 2017; Haddaway & Verhoeven, 2015). Using standardized protocols and reporting – to the extent that these are appropriate for the individual study – will therefore substantially increase the potential downstream use of data and added value of individual studies (affecting both filters; Figure 1).

Another challenge relates to specific parameters that are important for syntheses and meta-analyses, but which are often not measured or reported in stand-alone studies (affecting the data availability filter; Figure 1). For example, nutrient availability and nutrient dynamics may not be important in all individual studies but are increasingly being recognized as key drivers of across-site variation in ecosystem properties, dynamics and responses to climate and global changes (De Graaff, Van Groeningen, Six, Hungate, & Van Kessel, 2006; Janssens et al., 2010; Ren et al., 2017; Stevens et al., 2015; Terrer, Vicca, Hungate, Phillips, & Prentice, 2016; Vicca, Luyssaert, et al., 2012). Carbon-cycle models have traditionally overlooked the role of nutrient availability, but the overwhelming evidence of its dominant role is now encouraging the modelling community to address the roles of nitrogen and, more recently, phosphorus (Goll et al., 2012; Peñuelas et al., 2013; Wang, Law, & Pak, 2010). While the scientific community is increasingly acknowledging the importance of nitrogen, phosphorus and other nutrients for understanding and projecting the carbon cycle, there is still a significant lack of informative and comparable datasets at regional and global scales (Vicca et al., 2018). In the *Site characteristics and data management* chapter, we therefore provide a section on what variables to measure to enable disentangling the role of nutrients in carbon synthesis studies (summarized in Table 2).

### 4.2 | The case for using standardized and relevant units and scales

Several of the key variables and covariates for inter-study comparisons (Table 2) include data and parameters that may be measured in different units or scales, depending on the research tradition in the specific fields. Such variables are, or can easily be made, comparable across studies, as long as the necessary information needed for conversions are also reported along with the original variables (these may relate to area/volume, climatic data, vegetation characterization, soil properties, nutrients; Table 2; affecting the compatibility filter, Figure 1). For example, changes in soil organic carbon in response to a manipulation are typically reported either per unit area or as a weight percentage. These metrics are both valid, but they are not comparable unless the data necessary for conversion (bulk density and sampling depth) are provided. The necessary information for recalculation or conversion across reporting traditions should therefore be recorded and reported.

A second challenge relates to the need to quantify the treatments as experienced by the biota. For example, precipitation manipulation experiments typically report the amount of water added or removed, but the manipulation as experienced by the biota may deviate substantially from what is reflected in the absolute or percentage change in precipitation. Specifically, soil water availability is influenced by many factors, including soil water-holding capacity, run-off, hydrological legacy, rooting depth and drainage (Vicca, Luyssaert, et al., 2012). Hence, assessing water availability in a standardized way will substantially improve our understanding of the sensitivity of ecosystems to the manipulations and facilitate cross-experimental comparisons (see protocol 3.8 on Ecosystem water stress).

### 4.3 | The case for upscaling through models

Various types of mechanistic models can be used to generalize and extrapolate the data collected from in situ climate change studies in space and time. The potential of model-data interaction and its potential high impact, however, is often forgotten during experimental planning. Here, we want to illustrate the importance of early project planning for future data use (Table 2). For example, soil pH as an easy and low-cost measure may have been traditionally measured at the site level (lowest resolution), but in order to be a useful variable in meta-analyses, ecosystem models and temporal and spatial upscaling, soil pH data are more valuable if measured at a higher resolution (e.g. treatment or plot level). Thus, investing time in considering the aspired impact of the data-to-be-collected already in the project planning phase can direct budget investments and will be beneficial for the wider experimental and modelling community.

Purely statistical models attempt to describe cause-and-effect based on independent measurements of key processes. On the other hand, process-based models are built on a theoretical

understanding of relevant ecological processes and provide understanding about specific responses to various environmental conditions. Here, we suggest three strategies for stimulating information flow between models and experiments and/or observational studies. First, data-model comparisons can be used as a tool to directly test hypotheses, where observations are compared directly against model output. Second, experimental and/or observational data can be used as for model calibration tool, where parameters and predictor variables measured at a site or within a treatment are used to calibrate the model. In this case, the uncalibrated response variables can be compared to the model output; flexible parameterization can be considered a hypothesis (i.e. a form of sensitivity analysis) and can be used to inform the final model selection. Finally, the model parameters can be used in process-based models to 'tune' or 'benchmark' the model, which means that the model is simply fitted to the model-derived data. In this latter case, the model provides no hypothesis test, but simply synthesizes the data within the logical structure provided by the model (Luo et al., 2012). Each of these strategies has its place, but the difference in objectives and data requirements needs to be recognized.

To give an example, sensitivity analyses often identify similar variables as important for similar models. The BIOME-BGC model, which describes biogeochemical processes, is especially sensitive to leaf area index, cumulative soil water-holding capacity, leaf mass to area ratio, temperature and some measure of nutrient status (White, Thornton, Running, & Nemani, 2000). Similarly, Yang et al. (2016) described a sensitivity test of a model predicting the distribution of plant functional types. They found that leaf area index, leaf nitrogen per mass and leaf mass per area provided a particularly powerful combination of predictions. When exercised with changes in temperature and precipitation, the model predicted, for example, that boreal forest, boreal steppe and tundra would lose significant area. By measuring and reporting the variables needed to meet the data requirements of different types of models (Table 2), the information flow between empirical studies and modelling will be increased.

#### 4.4 | The case for considering new mechanisms and drivers under future climates

As the climate warms and rainfall patterns intensify in many regions across the globe, mechanisms and driving factors that are currently of minor importance could become more dominant in the future. Thus, new variables might need to be considered for measurement protocols to adequately track modifications in ecosystems under a changed climate. In particular, drought events are expected to occur more often, to last longer or to be more severe in many regions (Prudhomme et al., 2014; Touma, Ashfaq, Nayak, Kao, & Diffenbaugh, 2015). Conversely, increased rainfall intensities and flash flooding during the growing season are also predicted (Kendon et al., 2014), leading to a short-term rise

in the availability of water. Drier conditions could have consequences for the drivers and mechanisms operating in biogeochemical cycles. For example, rain might periodically be absent, thus driving the ecosystem to pass a threshold and start relying on non-rainfall water inputs (occult precipitation, such as dew and fog) for plant, litter and soil processes (Gliksman et al., 2017; Hill, Dawson, Shelef, & Rachmilevitch, 2015; McHugh, Morrissey, Reed, Hungate, & Schwartz, 2015). Moreover, abiotic processes, such as photochemical and thermal processes, start to kick in after passing heat and drought thresholds (Austin & Vivanco, 2006; McCalley & Sparks, 2009). Methodologies need to be adapted to capture such changes in drivers and mechanisms. For example, litterbags to study decomposition should transmit UV radiation and should only minimally affect the energy balance of litter to prevent interfering with dew formation. In addition to the need of measurement devices to consider these mechanisms and allow their quantification, the relevant drivers have to be measured with affordable equipment, such as UV irradiance by UV sensors and duration of dew by leaf wetness sensors. In general, this illustrates how novel future climates and conditions will necessitate the consideration of new drivers and mechanisms.

## 5 | CONCLUSIONS

Climate change experiments and observational studies are increasingly being conducted across a broad range of habitats and regions. Experiments are generally designed to disentangle complex and interactive drivers, causal relationships, response surfaces and underlying mechanisms, whereas observational studies document large-scale temporal and spatial patterns. The growing interest in data re-use, synthesis and upscaling within and across experiments and observations highlights challenges and pitfalls regarding the downstream use of knowledge from these studies, especially regarding *data availability* and *data compatibility* across studies (Denny et al., 2014; Firbank et al., 2017; Vicca, Gilgen, et al., 2012) and the lack of basic study information or covariates necessary for comparison (Gerstner et al., 2017; Haddaway & Verhoeven, 2015; Halbritter et al., 2018).

To overcome these challenges, we provide a handbook with guidance on the selection of response variables, protocols for standardized measurements of these variables and advice on data reporting and management. The ClimEx handbook summarizes best-practice methodologies emerging from major ecological research networks and studies, as synthesized by 115 experts from across relevant research fields. Our ambition is that this community effort will facilitate awareness by the next generation (and the older generation) climate- and global-change scientists of standardized methods to promote data availability, compatibility and transparency. We envision improved research practices that will not only result in better returns on the time and money invested in individual research projects, but will especially facilitate second-order research outputs across projects and scientific disciplines. More general, this should lead to a better connection of

scientific communities and disciplines. The ultimate goal is to optimize the quality and impact of our science to fulfil society's needs in a changing world.

#### ACKNOWLEDGEMENTS

We thank the ClimMani core group for the opportunity to realize this handbook, Linn Vassvik, Christine Pötsch, William Erazo Garcia and Siri V. Haugum for assisting in the collection of existing protocols, and Cathy Jenks for reading all the protocols thrice, checking the language, flow, logic and all references. The authors were funded by different national and international research agencies while contributing to this work, but in the interest of the word count we are not mentioning these individually. This study was funded by the ClimMani COST Action ES1308 (2014-2018).

#### AUTHORS' CONTRIBUTIONS

H.J.D.B. and V.V. conceived the idea and initiated the project. A.H.H. and V.V. led the project, which included developing the original idea, elaborating on the structure, content and workflow of the paper, organizing meetings to discuss ideas, methodology, data collection, organizing the writing and reviewing of the protocols, and leading the writing of the manuscript. H.J.D.B., A.E.E., A.J., S.R., D.A.R., S.V. and P.W. led the collection and writing of the five protocol chapters and were involved in conceiving the ideas, designing methodology and contributing critically to the drafts of the manuscript. B.B., C.T.C., M.E., J.M.G., R.G., K.H., I.K.S., H.L., S.L., J.M. and J.P. contributed to developing ideas, selecting and describing methodologies, and writing drafts of the protocols and manuscript. The ClimMani working group contributed to writing and reviewing individual protocols (see each individual protocol for author and reviewer contributions). All authors gave final approval for publication.

#### DATA AVAILABILITY STATEMENT

This manuscript does not use data.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Halbritter AH, De Boeck HJ, Eycott AE, et al. The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). *Methods Ecol Evol*. 2020;11: 22–37. <https://doi.org/10.1111/2041-210X.13331>

## APPENDIX

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## 10. Acknowledgements

Ich möchte allen Danken die mich unterstützt und begleitet haben während allen Arbeitsschritten dieser Dissertation, und auch davor und darüber hinaus.

Mein größter Dank gilt Anke Jentsch und dem Lehrstuhl für Störungsökologie (Universität Bayreuth), die diese Dissertation ermöglicht hat. Vielen herzlichen Dank für die Gespräche auf beruflich wissenschaftlicher und auch auf sozial menschlicher Ebene, sowie die Möglichkeit mich Intellektuell weiter zu bilden und entwickeln.

Vielen Dank an: Peter Wilfahrt für die alltägliche Begleitung; Max Schuchardt für das praktische Händchen; Andreas v. Heßberg für die Artenkenntnis. Christine Pisl und Eva Strätz für den praktischen Support; Reinhold Stahlmann für den technischen Support; allen Sekretärinnen für den administrativen Support; Nobel, Pia, Hannah, Tom, Lukas, Negin und Marius für den fleißigen und flexiblen Support; den BioGeos und Carl Beierkuhnlein.

Ein besonderer Dank noch an das SUSALPS-Konsortium, dass sehr viel möglich gemacht hat und in dem ich mich immer Willkommen gefühlt habe.

Vielen Dank an alle die „dem GEO“ zu einem so tollen und offenen Arbeitsklima verhelfen.

Selbstverständlich gilt mein Dank auch meinen drei permanenten Mitbewohnern – Anja, Jul und Skylla – wir hatten viel Spaß, sind auch zusammen- und über uns hinausgewachsen. Es ist schön verschiedene Charaktere und Gesprächsthemen zu haben, aber immer einen Nenner zu finden – spätestens am nächsten Tag.

Meine Familie ist, war und wird immer für mich da sein. Ich bin unendlich dankbar euch zu haben Mama, Papa und Schwesterherz.

Nicht zu vergessen meine mehr als fabelhafte Begleitung in meinem Leben, wenn es schwer ist und auch wenn wir zusammen lachen. Wissenschaft ist nicht alles, Kunst bietet ein sehr facettenreiches Weltbild – oft aus anderen Blickwinkeln und es hilft auch mal Weisheit von einem anderen Löffel zu fressen. Danke für dich und die Zeit mit dir und deinen Kleinen.