Understanding plant-soil interactions of native and non-native plants under climatic extremes

Inaugural dissertation

of the Faculty of Science,

University of Bern

presented by

Shareen, Sanders

Supervisor of the doctoral thesis:

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General introduction

The study of soil has evolved significantly over the centuries, transitioning from a focus on its physical properties through soil classification and mapping, to a more distinctive understanding of its ecological functions and contributions to ecosystem services (Coleman and Crossley, 2017). All terrestrial life ultimately depends on soil and water as the base of networks that support everything from microscopic organisms to the largest land animals. However, soil is not merely a passive medium for plant growth; it is a dynamic environment that plays a crucial role in regulating community structure, buffering climate, maintaining or enhancing water and air quality, promoting overall ecosystem health and biodiversity. Microbial communities in particular are essential for nutrient cycling, organic matter decomposition, and the overall resilience of soil ecosystems (Van Der Heijden, Bardgett and Van Straalen, 2008; Aislabie, Deslippe and Dymond, 2013). Soil is now understood as a living entity, home to a diverse array of microorganisms, fungi, and fauna that contribute to its health and functionality (Doran and Zeiss, 2000). In recent years, the impact of climate extremes, such as intense droughts and heavy rainfall, has emerged as a critical factor in altering soil ecology (Meisner *et al.*, 2018). These extreme weather events can disrupt microbial communities, influence nutrient cycling, and affect the resilience of soil ecosystems, thereby reshaping the interactions between soil biota and plant communities (Bardgett and Caruso, 2020). The following section explores the key factors that shape soil ecology, focusing on how plant-soil interactions influence the

success of both native and invasive species. It also examines how these dynamics are affected by climate extremes, and whether plant-soil interactions can help explain invasion success in a changing environment.

Plant-soil interactions

Growing research interest in soil processes has made it increasingly evident that interactions between plants and the biotic and abiotic properties of soil play a crucial role in shaping plant communities, influencing above-ground ecosystems, and driving ecosystem responses to environmental change (Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2016). Simultaneously, the consequences of plant growth in the soil also influence the soil's nutritional, chemical, and microbial composition through its nutrient uptake, the release of root exudates, and interactions with soil microorganisms (Van der Putten *et al.*, 2013). Such changes in soil properties caused by plants, which subsequently affect the performance of secondary plants, are known as 'plant–soil feedbacks' (PSF); whereby, feedback loops encompass how soil influences plant growth, and plants, in turn, shape the biotic and abiotic compositions of the soil (Bever, 1994; Van der Putten *et al.*, 2013). Positive feedback effects occur through mechanisms like nutrient enrichment and beneficial microbial interactions, often due to a lack of species-specific pathogens or an accumulation of mutualists such as mycorrhizal fungi. In contrast, negative feedback can lead to declines in plant performance, driven by an abundance of soil pathogens, nutrient depletion, inhibitory allelochemicals, and a reduction in mutualists (Van der Putten *et al.*, 2013; Crawford *et al.*, 2019). Plants sharing similar functional traits, or conspecifics, tend to intensify negative plant-soil feedbacks by depleting shared soil nutrients and mediating a build-up of species-specific pathogens. Such negative feedbacks are often instrumental in enhancing biodiversity, as they facilitate the proliferation of heterospecific plants by hampering the dominance of conspecific plants (Kulmatiski *et al.*, 2008; Thakur *et al.*, 2021). Several studies have also speculated how these PSF's may influence the invasibility of the native community (Levine *et al.*, 2006; Inderjit and Cahill, 2015). However, invasive plants often encounter positive or neutral plant-soil feedbacks in novel environments due to the

release from species-specific antagonistic interactions that would normally restrict their spread in their native range. This gives them a competitive advantage over native plants, which are more affected by negative plant-soil interactions (Reinhart and Callaway, 2006; Maron *et al.*, 2014).

Invasion by non-native plants'

The spread of invasive plants species is a major component of ongoing anthropogenic global change threating the diversity of grassland communities (Carboni *et al.*, 2021). Invasive species are distinguishable to introduced species by their negative impacts on the novel environment through reduction in biodiversity, ecosystem services or the health of the ecosystem (Keller *et al.*, 2011). In order to efficiently reduce the spread of invasive plants, more research is required for the early detection of vulnerable communities where management strategies are most effective (Catford *et al.*, 2012). However, to predict which communities are vulnerable to invasion it is vital to establish which biotic and abiotic characteristics impact invasion success within a community. By furthering our understanding of the factors that determine the success or failure of non-native plants, we also gain valuable insight into the processes of community assembly and coexistence theory (Melbourne *et al.*, 2007). This knowledge can be implemented to facilitate the successful expansion of vulnerable plants (such as range-expanding species) into new habitats, helping to mitigate the effects of climate change by ensuring they can thrive in more suitable environments (Corlett and Westcott, 2013).

Only a small fraction of the non-native plant species that are intentionally or unintentionally introduced into novel environments are able to form self-sustaining populations (around 24%; Jeschke and Pysek 2018), while even fewer of these established plants are able to proliferate to the extent that they cause detrimental damage to the native ecosystem and are classed as invasive (around 18%; Jeschke and Pysek 2018). This has prompted a number of ecologists to delve deeper into the characteristics that set apart the most successful plant invaders. For example, many successful invasive plant species possess specific traits, such as fast growth and

propagule production, that contribute to their successful growth and establishment (Van Kleunen, Weber and Fischer, 2010). Invasive plants also typically lack a shared co-evolutionary history with native organisms, which means that invasive species entering native ecosystems may carry "novel weapon" that give them an advantage in agonistic interactions involving competition, grazing, and parasitism (Callaway and Ridenour, 2004). As these invasive plants arrive in new habitats, they also often undergo an "enemy release" phenomenon, where they leave behind their species-specific pathogens and herbivores (Wolfe, 2002; Mitchell and Power, 2003). As a result, invasive plants may face reduced pressures from biotic interactions, enabling them to allocate more resources toward growth and competitive capabilities (Blossey and Nötzold, 1995). Moreover, invasive plant species often exhibit greater phenotypic plasticity than native species, especially under conditions of high nutrient availability (Davidson, Jennions and Nicotra, 2011). This enhanced plasticity enables them to adapt to the abiotic and biotic factors of their new environment, and express traits that allow for effective nutrient acquisition, stress tolerance and competitive ability (Ren and Zhang, 2009). Indeed, previous studies have suggested that invasive plant species typically possess an enhanced ability to utilize increases in resources compared to native species, enabling them to exploit fluctuations, and enhancing their competitiveness in new environments (Davis, Grime and Thompson, 2000; Pearson *et al.*, 2018). In particularly this also enables invasive plants to thrive in disturbed environments where resource availability is temporarily increased (Hobbs and Huenneke, 1992; Diez *et al.*, 2012; Orbán *et al.*, 2021).

Connection between plant-soil interactions and invasion success

Several hypotheses posit altered biotic interactions, particularly between plants and their associated soil biota, as key determinants of plant invasive success (Inderjit and van der Putten, 2010). One important plant-soil interaction which has received a lot of attention is the symbiotic relations between plants and arbuscular mycorrhizal (AM) fungi. These fungi establish a symbiotic relationship with host plants by infecting and spreading within the root cortical cells, resulting in the exchange of nutrients, such as phosphorus (P) and nitrogen (N),

and water for carbon and lipid compounds (Wang *et al.*, 2017). Invasive plants have been shown to exploit such beneficial soil microbes in their non-native habitats more effectively than in their native ranges (enhanced mutualism hypothesis; Reinhart and Callaway 2006). Research indicates that invasive species associate with a richer community of AM fungi within novel habitats and selectively promote fungal taxa that offer more mutualistic benefits, thereby promoting their own growth and fecundity (Zhang *et al.*, 2010; Sheng *et al.*, 2022). Furthermore, while exotic plants accumulate local pathogens in their rhizospheres, they tend to suffer less from these pathogens compared to native species, thus gaining a competitive edge (Inderjit and van der Putten, 2010). However, variation in the native plant community can likewise shift the biotic and abiotic conditions of the soil with impact on the PSF effect on non-native plant species with impacts on the invasion success (Burns and Brandt, 2014). For example, plant traits along the slow-fast leaf economic spectrum can drive the balance between positive and negative PSF effects by influencing nutrient acquisition of the native community and the soil microbial community compositions, (de Vries *et al.*, 2012; Baxendale *et al.*, 2014). While there are several hypotheses that explain variation in the invasibility of different native ecosystems; few studies have specificity looked at the extent to which these mechanisms are driven by plant-soil interaction. However, these plant-soil interactions are especially relevant, particularly as many ecologists attribute the success of invasive plants due to their ability exploit increases in soil nutrient availability and their tendency to associate with a wide-range of beneficial microbes (Sun and He, 2010; Pearson *et al.*, 2018; Sheng *et al.*, 2022; Sun *et al.*, 2024). As such, any mechanisms that shift the nutritional and microbial composition of the soil may influence the invasibility of the community through plant-soil interactions.

The biotic resistance hypothesis focuses on how native biodiversity can prevent or limit the establishment of non-native species. Native ecosystems with high functional diversity may reduce the success of invaders by having more complex interactions, including herbivory, predation, disease, and competition (Levine, Adler and Yelenik, 2004). According to the hypothesis, diverse plant communities occupy more ecological niches, making it harder for non-native species to establish and increasing the likelihood of encountering strong competitors and

natural enemies (Levine, Adler and Yelenik, 2004). Although soil biota's role in biotic resistance has recently garnered attention (Zhang *et al.*, 2020a; Yuan *et al.*, 2024), its significance was previously underestimated, even though it has been shown to impact invasive species' performance (Reinhart and Callaway, 2006; Dawson and Schrama, 2016).

The invasion meltdown hypothesis proposes that the introduction of one invasive plant species can alter an ecosystem in ways that facilitate the success of other non-native species to establish (Simberloff and Von Holle, 1999). For example, a nitrogen-fixing invasive species might enrich soil nutrients, benefiting other non-native plants (Simberloff and Von Holle, 1999). Invasive plants can also indirectly change native community structures by reducing native enemies and promoting mutualists (Zhang *et al.*, 2020b; Chen and van Kleunen, 2022). Additionally, alien species from different origins often have fewer shared enemies compared to native species, potentially increasing their invasion success (Zhang *et al.*, 2020b). This lack of similarity can increase the invasion of a non-native plant species as there is less overlap in shared enemies than with members of the native community (Kempel *et al.*, 2018; Crawford *et al.*, 2019).

These hypotheses provide insights into the biotic and abiotic characteristics of the native ecosystem that allow for predictions of the invasion success (or failure) of non-native plant species; as such they are crucial for understanding the vulnerability of ecosystems to invasions (Guo *et al.*, 2015). However, to fully grasp the dynamics of these theories in a rapidly changing world, it is essential to integrate perspectives on climatic extremes. Environmental stresses such as severe droughts can disrupt ecosystem functioning and biotic interactions, as such shifting PSF’s and the invasibility of the native community (Diez *et al.*, 2012; van der Putten *et al.*, 2016).

Invasion success due to extreme climatic events: can plant-soil interactions help us understand the links?

The most recent IPCC report (2023) suggests that mean global annual temperatures will continue to increase in the near term (2021–2040) due to increased cumulative CO2 emissions in nearly all considered scenarios. The report suggests that global average warming is likely to surpass a global mean increase of 1.5°C in the 21st century. It emphasizes the need for significant reductions in greenhouse gas emissions in the next decade to align with pathways limiting warming to 2°C. However, it points out that current pledges (NDCs) until 2030, without increased ambition, will lead to higher emissions, resulting in a median global warming of 2.8°C by 2100 (IPCC 2023). Modelled simulations show that these different warming scenarios have distinct impacts on the occurrence of extreme climatic events (Lange et al., 2020).
Extreme climatic events are defined as statistically rare reductions in soil moisture content; below the fifth - tenth percentile of the historically norm for the given ecosystem (Zscheischler *et al.*, 2013; Dalezios, Dunkel and Eslamian, 2017). Extreme climatic events represent the tails of the distribution of climatic occurrences, falling below the fifth to tenth or the ninetieth or ninety-fifth percentile of the historical norm for a given ecosystem (Dalezios, Dunkel, and Eslamian 2017; Zscheischler et al. 2013). Ecosystems facing such extreme climatic events demonstrate more extreme responses, especially where critical ecological thresholds are surpassed, influencing how the ecosystem recovers from the perturbation (Smith, 2011; Zhang et al., 2019). For example, while organisms and populations can acclimate to average shifts in soil moisture content, extreme precipitation events present a greater challenge as they are typically short in duration and surpass the capacity of organisms to acclimate (Gutschick and Bassirirad 2010; Smith, 2011).

The impact of climate extremes on the facilitation of invasive species has drawn much attention, with research focusing on the creation of "invasional windows" (Diez et al., 2012). This concept suggests that climate extremes may create favourable conditions for invasive species, thereby influencing their successful establishment. Extreme climatic events decrease

the capacity of native plants to utilize resources, as well as, limiting their growth and/or reproduction. In many cases this causes abrupt and widespread mortality of resident species (Ciais *et al.*, 2005; Niu *et al.*, 2014; Xu *et al.*, 2019; Yuan *et al.*, 2021; Thakur, Risch and van der Putten, 2022). By destabilising the native community, extreme climatic events increase the availability of resources (e.g. nutrients, space) and decrease the strength of biotic interactions to introduced species (Jiménez *et al.*, 2011; Schrama and Bardgett, 2016). As native species struggle to recover from the stress imposed by these events, invasive species may capitalize on the resulting ecological opportunities, leading to shifts in species composition and potentially long-term changes in ecosystem function (Jiménez *et al.*, 2011).

As such, the facilitation of invasion through extreme climatic stress may depend on the whether these extreme climatic events elicit extreme detrimental ecological responses in the native community (Smith, 2011; Zhang *et al.*, 2019). However, the extremeness of ecological responses to climatic extremes also depends on other biotic and abiotic characteristics of the native community, such as, the biodiversity, nutrient availability and plant-soil interactions (Isbell *et al.*, 2015; van der Putten *et al.*, 2016; Valliere *et al.*, 2017). Furthermore, successful invasion occurs across multiple stages, each representing different hurdles that have to be overcome by a non-native species in the new environment (Richardson *et al.*, 2000; Blackburn *et al.*, 2011). While extreme climatic events may facilitate the initial establishment of a non-native plant species, at other stages such perturbations likewise negatively impact the performance of the non-native plant species with neutral or even detrimental effects on invasion success.

Extreme drought and flooding significantly affect plant productivity and soil microbial communities by disrupting water availability. Drought conditions reduce plant nutrient uptake due to impaired plant functioning, decreased nutrient cycling, and lower microbial activity (He and Dijkstra, 2014). Similarly, flooding can hinder a plant's ability to absorb water and nutrients by reducing root hydraulic conductivity (Elzenga and van Veen, 2010). These water stress conditions lead to shifts in soil microbial composition, driven by changes in root exudates and

plant-microbial interactions. For instance, during drought, plants allocate less carbon belowground, leading to declines in beneficial microbes that promote plant growth and offer protection from pathogens (Williams and de Vries, 2020; de Vries *et al.*, 2023). Prolonged saturation lead to anaerobic conditions that likewise favour pathogenic organisms over beneficial microbes (Martínez-Arias *et al.*, 2022). When extreme drought is followed by flooding, the rewetting process often triggers a surge in microbial activity, temporarily increasing nutrient cycling and availability (Borken and Matzner, 2009; Barnard, Osborne and Firestone, 2013; Leitner *et al.*, 2017; Song *et al.*, 2017; Xu *et al.*, 2020; Brangarí, Manzoni and Rousk, 2021). Additionally, these extreme events can have lasting impacts plant-soil interactions, affecting subsequent plant growth and competition (van der Putten *et al.*, 2016). Through reduced plant functioning and plant-microbial interactions, these extreme abiotic stressors result in soil microbial communities which are overall less specific to the plant species inhabiting the soil (Fry *et al.*, 2018). As such, known mechanisms whereby certain plant characteristic cause more negative or positive PSF become less clear. For example, in the case of invasional meltdown, extreme drought or flood may hinder ability of an invasive plant to accumulate beneficial microbe from which another invasive plant species can benefit (Simberloff and Von Holle, 1999). Furthermore, an extreme climatic events may also decrease the diversity of soil microbes (Yang *et al.*, 2021) thereby reducing biotic resistance and facilitating the growth of an invasive plant through the reduction in potentially agonistic biotic interactions (Elton, 1958; Levine, Adler and Yelenik, 2004). As such, these climate extreme driven PSF's may play a significant role in the facilitation and resistance to invasion, where changes in soil nutritional availability and microbial communities contribute to the success of invasive species (Meisner *et al.*, 2013).

Scope and objectives of the thesis

In this PhD thesis, I aim to investigate how extreme climatic events impact plant-soil interactions and, consequently how this influences the performance of native and non-native plant species. The relevance of this research is particularly important given the current

trajectory of global climate change, which predicts an increase in the frequency and intensity of extreme weather events. Understanding how extreme climatic conditions impacts plant-soil interactions and the invasion process can help us predict which ecosystems are most vulnerable to invasions and inform management strategies to mitigate these effects. Moreover, by studying plant-soil interactions under these extreme conditions, we can also develop insights into the successful expansion of vulnerable plants into new habitats, helping to mitigate the effects of climate change by ensuring they can thrive in more suitable environments. As such, this work also contributes to a broader understanding of plant-soil interactions in the context of global environmental change and provide a framework for both managing invasive species and assisting native plants in adapting to climate-induced stress.

For this purpose, I have experimentally measured plant biomass and traits responses to climatic extreme events to investigate: 1) how extreme drought, extreme flooding and sequential extreme drought and flooding events impact the PSF effect of invasional meltdown (Chapter 1), 2) how intraspecific competition impacts the extreme drought recovery of a range-expanding and native congener in the presence of mycorrhizal fungi (Chapter 2; Sanders et al. 2024), and 3) how extreme drought and in the presence of mycorrhizal fungi impact the PSF effect on plant communities differing in plant traits and diversity (Chapter 3). I then formulated a framework wherein I speculate how extreme drought mediated invasibility also depends on the ecological response of the native communities as well as the ecological barriers at different invasive stages (Chapter 4). Finally, I summarize the key insights gained from the research presented in this thesis. From linking these chapters together, I am able to present a comprehensive understanding of particular soil attributes which impact plant performance both during and after an extreme drought event through plant-soil interactions. I further explain how the impact of these plant-soil interactions on plant performance differ in the context of the plant community as well as how the plant community responses to drought extremes (Figure 1).

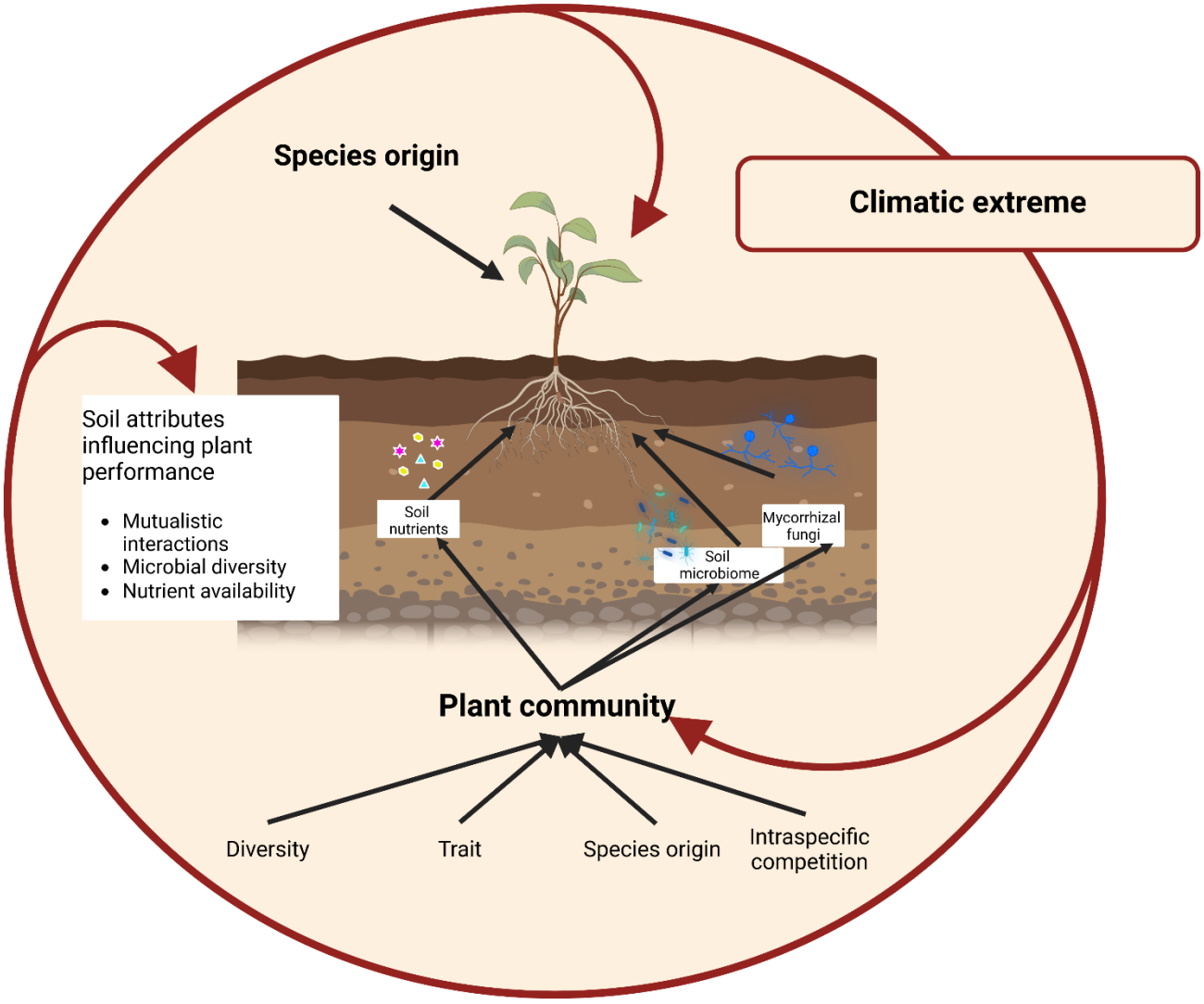

Figure 1. A conceptual framework illustrating how extreme drought and plant community composition alters the plant-soil interactions of a focal plant. Soil attributes, including mutualistic interactions, microbial diversity, and nutrient availability, impact plant growth through plant-soil interactions. These interactions are further modulated by the plant community's diversity, traits, species origin, and intraspecific competition. The figure also highlights how extreme drought plays crucial roles in altering these soil attributes and the extent to which the plant community influences these soil attributes with further impacts on the performance of the focal plant. Created on bioRender.ch

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Chapter 1

Drought soil legacies enhance the performance of invasive plants more than native plants in conspecific soils

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Abstract

Invasive plant species can alter ecosystem dynamics, potentially leading to invasional meltdown, where the impact of the invasive species facilitates the success of a secondary invasive species. The plant-soil feedback (PSF) effect of an invasive plant could play a crucial role in invasional meltdown, as soil biotic and abiotic legacies are major drivers in mediating plant performances. However, extreme climatic events, such as drought and flooding, can further modify PSF processes by disrupting water availability, altering soil nutrient cycling, and reshaping microbial communities. These changes may amplify or mitigate invasional meltdown by influencing the plant-soil interactions of invasive plants. This study explores whether belowground processes, shaped by plant legacies of varying origins and life history strategies, drive invasional meltdown. Moreover, we test how such plant- and soil-mediated effects on invasional meltdown change under different kinds of extreme climatic conditions. In the PSF experiment, we conditioned soils with one of four invasive or four native plant species under drought, flood, and sequential drought and flood events. The performance of a secondary native or invasive plant species was then assessed in each soil during the feedback phase. Contrary to expectations, invasive plants did not exhibit superior performance in soils conditioned by other invasive plants. Instead, invasive species performed better than native species in their own soils (conspecific soils) when these soil had a legacy of drought, suggesting that post-drought-induced nutritional and microbial pulses could boost positive PSF in invasive plants. As drought events become more frequent, we suspect that invasive plants may become increasingly dominant, forming denser populations and enhancing their spread potentially due to weakened conspecific negative PSF. Management strategies must account for how environmental changes, like intensified drought, may disproportionately favour invasive species.

Keywords: Plant soil feedback, climatic extremes, soil drought legacy, plant life history, phylogenetic distance, plant root traits

Introduction

The introduction of invasive plant species has been recognized as a significant driver of shifts in ecological processes, often leading to profound impacts on ecosystem functioning (Pyšek *et al.*, 2012). As such, invasive plant species have become a global concern due to their detrimental effects on native ecosystems (Rai and Singh, 2020; IPBES, 2023). Numerous studies have highlighted how the introduction of an invasive plant species can drastically impact the biodiversity (Powell, Chase and Knight, 2011; Castro-Díez *et al.*, 2016; Livingstone, Isaac and Cadotte, 2020), nutrient cycling (Ehrenfeld, 2003), as well as, numerous above and belowground trophic interactions in a native community (Levine *et al.*, 2003). These habitat modifications by invasive plants can change the invasibility of the ecosystems, in particular, they can facilitate the invasion success of secondary non-native plant species (other non-native plants that could exploit the habitat modified by an already invasive plant); this is known as invasional meltdown (Simberloff and Von Holle, 1999). For instance, an invasive species may directly facilitate the success of a secondary invasive plant species by altering the nutrient content of soils; for example, nitrogen-fixing invasive plants can increase the nitrogen available in the soil which profits other invasive plant species (Maron and Connors, 1996). Indirectly, invasive plants can also alter the community composition of native plants, invertebrates, and microbes through biotic interactions, potentially benefiting a secondary invasive plant (Kuebbing, 2020; Zhang *et al.*, 2020). For instance, an invasive plant can suppress the abundance of dominant competitive native plant species, thereby indirectly facilitating secondary invasions (Kuebbing, 2020; Cavieres, 2021). In a study by Sun et al. (2024), the high density of the invasive plant *Conyza canadensis* caused both a reduction in the performance of native plant species and the facilitation of invasive plant species (Sun *et al.*, 2024). Furthermore, Zhang et al. (2020) found that fungal communities differed more between invasive plant species than between native species or between native and invasive species, when studying plant soil legacies on a secondary invader. This lack of similarity resulted in

greater performance and competitive ability in invasive plants when grown on soils conditioned by other invasive species (Zhang *et al.*, 2020). Together, this growing body of research has highlighted how belowground processes play a major role in determining the invasibility of native habitats (Reinhart and Callaway, 2006; Inderjit and Cahill, 2015; Dawson and Schrama, 2016; Zhang *et al.*, 2020).

Belowground biotic and abiotic effects are often studied through plant-soil feedback experiments (Van der Putten *et al.*, 2013). Plant–soil feedback (PSF) refers to the phenomenon where plants influence the performance of subsequently growing plants (also known as feedback phase plants) by altering the biotic and abiotic characteristics of the soil they grow in (Bever, 1994; Van der Putten *et al.*, 2013). PSFs are shaped by the nutritional and microbial composition of the soil, which is influenced by the growth and interactions of resident plants, known as conditioning phase plants (Kulmatiski *et al.*, 2008; Van der Putten *et al.*, 2013). The composition of soil biota plays a crucial role in this feedback, with positive feedback occurring due to a lack of species-specific pathogens and an accumulation of mutualists, and negative feedback resulting from an abundance of pathogens and a reduction in mutualists (Van der Putten *et al.*, 2013; Crawford *et al.*, 2019). Plants with similar functional traits, or conspecific plants, often create more pronounced negative PSFs by competing for the same soil nutrients and accumulating species-specific pathogens. These negative PSFs are commonly associated with the maintenance of plant diversity through relaxing the interspecific competition among heterospecific plants (Kulmatiski *et al.*, 2008; Thakur *et al.*, 2021). Invasive plants entering a new habitat often encounter positive or neutral PSF due to the release from antagonistic interactions that would normally restrict their spread in their native range (Reinhart and Callaway, 2006; Maron *et al.*, 2014).

The variation in PSF further depends on the life history strategy of the plants. Annual plants have been suggested to experience and exert more negative PSFs than perennials, perhaps due to their higher investment in rapid growth, which comes at the cost of their ability to defend

against soil pathogens (Kulmatiski *et al.*, 2008). A recent study showed that annual plants experienced more negative PSF responses compared to perennials, however, these PSF responses were consistent when growing either in soils conditioned by annual or perennial plants (Wilschut *et al.*, 2023). As such, these negative PSF on annual plants appeared to be more dependent on their own susceptibility to microbial pathogens than the microbial community composition (Wilschut *et al.*, 2023). Such differential PSF effects and responses between annuals and perennials may likewise shape how invasive plants respond to PSF and benefit from or contribute to invasional meltdown, depending on the life-history traits.

The impact of PSF on invasional meltdown may also differ in the context of climate extremes which can amplify or diminish the feedback effects by altering soil conditions, such as moisture, thereby affecting soil biota and nutrient cycling. Such climatic effects on the PSF make the outcomes of invasional meltdown even more unpredictable (van der Putten *et al.*, 2016; de Vries *et al.*, 2023). For instance, increasing frequency and magnitude of extreme drought and flooding can directly influence plant productivity and the composition of soil microbial communities by disrupting water availability (Niu *et al.*, 2014; Bardgett and Caruso, 2020; Thakur, Risch and van der Putten, 2022). Drought reduces plant nutrient uptake by lowering plant functioning, slowing nutrient movement in the soil, and decreasing microbial activity and nutrient cycling (He and Dijkstra, 2014). Similarly, flooding can hinder a plant's ability to absorb water and nutrients by reducing how well roots conduct water (Elzenga and van Veen, 2010). The decline in plant productivity due to water-stress can further influence the soil microbial community composition, through changes in root exudates and shifts in plant-microbial interactions. For example, under drought, plants reduce their belowground carbon allocation, causing a decline in beneficial microbes that support plant growth and provide pathogen protection (Williams and de Vries, 2020; de Vries *et al.*, 2023). Conversely, during periods of extreme rainfall, the increased availability of water can initially enhance microbial activity; however, prolonged saturation may lead to anaerobic conditions that likewise favour pathogenic organisms over beneficial microbes (Martínez-Arias *et al.*, 2022). However, when

flooding sequentially follows a period of extreme drought, the process of rewetting can play a critical role in shaping microbial community dynamics.

Rewetting typically stimulates a pulse of microbial activity, leading to a temporary increase in nutrient cycling and availability (Borken and Matzner, 2009; Barnard, Osborne and Firestone, 2013; Leitner *et al.*, 2017; Song *et al.*, 2017; Xu *et al.*, 2020; Brangarí, Manzoni and Rousk, 2021). Indeed, the legacy of prior drought conditions can still persist after rewetting , influencing the composition of microbial communities even after moisture levels are restored (Meisner *et al.*, 2013). For instance, the history of extreme drought significantly altered the microbial community's response to subsequent rewetting, resulting in a notable reduction in microbial richness and a shift in community composition (Meisner *et al.*, 2018). This shift suggests that the drought legacy not only impacts the immediate microbial response but also shapes the long-term community composition, potentially influencing soil health and plant interactions. Moreover, these extreme climatic events can have lasting effects on plant-soil feedback (PSF), altering the subsequent growth and competitive ability of plants (van der Putten *et al.*, 2016). As such, this PSF may play a significant role in mediating invasional meltdown, where changes in soil nutritional availability and microbial communities contribute to the success of invasive species (Meisner *et al.*, 2013). Understanding these dynamics is crucial for predicting how ecosystems will respond to the increasing frequency of extreme weather events, as altered microbial communities can have lasting implications for nutrient dynamics, plant growth, and overall ecosystem resilience (Van Der Heijden, Bardgett and Van Straalen, 2008). Furthermore, such drought mediated shifts in the nutritional and microbial composition of the soil may disproportionately favour invasive over native plant species, as invasive plants are both more adept at exploiting increases in nutrient availability and less adversely affected by microbial interactions in their non-native range (Davis, Grime and Thompson, 2000; Reinhart and Callaway, 2006)

In this study, we conducted a PSF experiment to investigate whether the impact of invasional meltdown differed in the context of climate extremes (single and sequential events). We also investigated whether species relatedness and plant life history strategies (annuals and perennials) played a role in mediating the impacts of invasional meltdown. We first predict that invasive plants will grow better in soils conditioned by other invasive plants species compared to soils conditioned by native species, due to the accumulation of beneficial soil microbes or a reduction in antagonistic interactions. We further predict that climatic extremes, such as drought and flooding, will suppress the effect of invasional meltdown due to the disruptions in plant functioning and plant-microbial interactions. However, rewetting after the extreme drought will trigger a temporary pulse in microbial activity, which may enhance the growth of invasive species more than native species.

Methods

To evaluate the impact of extreme climatic and plant life-history strategies on soil-mediated invasional meltdown, we conducted a plant-soil feedback experiment consisting of two phases. In the conditioning phase, biotic soil legacies were created from separate monocultures of native and invasive plants species which conditioned the soil, and by the occurrence of an extreme climatic event. In the subsequent feedback phase, we tested this soil legacy by assessing its impact on the performance of a singular conspecific plant species, heterospecific native or invasive plant species (Supplementary figure S.1).

Study species

Plants – Four invasive and four native *Asteraceae* species were chosen for this study. The invasive species (non-native in Europe) consisted of two perennial (*Solidago canadensis* and *Senecio inaequidens*) and two annual (*Erigeron annuus* and *Matricaria discoidea)* plant species. All the invasive plant species chosen have been observed within Switzerland and, apart from *Matricaria discoidea* which is invasive within Europe (Pyšek et al. 2009), all the invasive species are on the “black list” of harmful neophytes in Switzerland (Bundesamt 2022). Each of the four

chosen native species were the congeners of the invasive plants: two perennial (*Solidago virgaurea* and *Senecio jacobeae*) and two annual (*Erigeron acris* and *Matricaria chamomilla)* native plant species.

Germination

Seeds for *Solidago canadensis*, *Senecio inaequidens*, *Erigeron annuus* and *Senecio jacobeae* were collected by hand between July to November 2021 in various locations surrounding the city of Bern, Switzerland (Supplementary Figure S.2). Seeds for *Matricaria discoidea, Matricaria chamomilla, Solidago virgaurea* and *Erigeron acris* were purchased from seed companies (*Matricaria discoidea, Matricaria* chamomilla: Weberseeds, netherlands, *Solidago virgaurea*: Saemereien, Switzerland and *Erigeron acris*: UFA, Switzerland). The same seeds were used for both the conditioning and feedback phases of the experiment. All seeds were stored at 4°C before germination. For surface sterilisation, seeds were bleached for 15 minutes in a 30% bleach solution (commercial bleach with sodium hypochlorite) and rinsed with deionised water. Seeds were placed on germinating soil containing peat, peat substitute, compost, sand and organic fertiliser (Landi, Switzerland), using black containers (18 cm x 14 cm x 5 cm). The germinating soil was prepared by sieving out large particles using a 5 mm mesh and sterilising the soil twice in an autoclave (Systec VX-150, Systec GmbH & Co., Germany) at 121°C for 20 minutes. The two cycles were separated by at least 48 hours to target more resistant fungal species that opportunistically spread in the soil. Seeds were moistened using deionised water, and moisture was retained by partially placing a lid on the container.

Experimental soils

The soil used in our experiment was a mixture of 40% quartz sand, 25% compost, 25% silty soil and 10% vermiculite (pH = 5.9; organic matter content = 0.63 kg kg-1). The soil was hand-mixed after bigger particles – such as stones, clay and wood – were removed from the potting soil with a coarse-meshed sieve of 5 mm mesh. The mixed soil was gamma sterilised in June 2022 (20–60 kGy; STERIS, Däniken). The sterilised soil was then distributed into plant pots (width: 10.5 cm, depth: 10.5 cm, height: 22 cm, 1.8 Ltr), with a total of 1.8 kg of dry soil weight in each

pot. Water was steadily added to each pot until an initial water volumetric content of 25-30% was achieved (adding ~450ml of water).

Natural soils containing the roots and surrounding rhizosphere were collected from three separate locations for each plant species within the city of Bern, Switzerland, in June 2022 (see Supplementary Material for map S.3). These soils were collected to obtain plant population specific rhizosphere microbiomes for the conditioning phase. For each natural field soil sample, 150 g of soil was mixed with 300 ml of deionised water and hand-shaken for about 10 minutes. The mixture was then passed through a 125 µm mesh filter to allow a wide range of microbial species to pass through (Koide & Li, 1989). The resulting microbial wash was collected in containers and stored at 4°C until the start of the experiment. After all the pots were filled with sterilised soil, 150 ml of the microbial wash inoculum was added to the soils of each respective plant species. Each of the microbial wash inoculums (three replicates per species) was applied separately to different pots within each plant and climatic treatment group in a fully factorial manner.

Conditioning phase

From each plant species four individual seedlings ranging between 5 – 7 cm in height were transplanted into pots containing soil and inoculum in July 2022. Pots were then randomly allocated along five tables within a greenhouse (u. Zollgasse 77, 3072 Ostermundigen) of the Institute of Plant Sciences at the University of Bern. Plants were grown under a 14-hour daily light cycle, with daytime temperatures maintained between 18 and 26 °C and nighttime temperatures between 16 and 24 °C. Heating was provided if temperatures fell below these ranges, while a cooling system was activated if temperatures rose above 26 °C. Due to insufficient insulation, temperature, light intensity, and humidity still varied drastically within the greenhouse, but these conditions were continuously monitored throughout the experiment to account for fluctuations (Supplementary Fig. S.4). Soil water content was estimated by weighing the pots and keeping track of losses in soil weight (Supplementary Fig. S.5). We let the plants condition the soil for a total of ten weeks (Supplementary Fig. S.6). After four weeks the

plants were exposed to either extreme drought, extreme flooding or sequential drought + flood event, where and extreme drought was followed by extreme flooding. Control treatment plants were continuously well-watered throughout the experiment to a water content of 20-25%. For the extreme drought treatment, no water was added for four weeks, which resulted in a water content estimation close to 0 % for several weeks. For the extreme flood treatment pots were placed into containers filled with stagnant water (18 cm x 14 cm x 5 cm) and regularly watered from above. Soil water content within this treatment was estimated to be close to ~40%, which exceeds the 95th percentile of soil moisture variability observed in Switzerland (Supplementary Fig. S.7). In sequential drought + flood treatments plants similarly received no water for two weeks, after which they were placed in stagnant water and watered regularly for another two weeks. In this way, both our single and sequential events took place for four weeks during the conditioning phase. After eight weeks from the start of the experiment, the plants were watered in a way for two weeks to create similar water content levels among the soils of all pots (Supplementary Fig. S.5). Each treatment combination was replicated ten times, resulting in a total of 384 pots (8 plant communities x 4 climatic treatments x 10 replicates) and 1536 plant individuals.

Harvest and response variables of the conditioning phase

During the harvest, the aboveground shoot tissue of each plant was removed just above the soil level. Soils (containing roots) were removed from the pots and loosened within separate plastic bags; once thoroughly loosened, roots were removed from the loosened soil and meticulously washed in order to remove attached substrates. Equipment used while handling soils from different pots, including gloves, were thoroughly sterilised using a 70% ethanol solution. Fine root samples of about 1 g (fresh weight) were taken from each pot for root trait measurements. The fresh weight of the remaining roots was measured and then dried in an oven for 3 days at 40°C along with the plant shoot to measure the dry biomass of each plant. Due to the intertwining of roots, it was not possible to measure the dry biomass of each individual plant, as such, the total root biomass per pot was measured. The fresh leaf and root samples were weighed and scanned using an Epson Perfection V850 Pro Scanner, and were analysed using ImageJ and RhizoVision Explorer v2.0.3 (Schindelin *et al.*, 2012; Seethepalli *et al.*, 2021) to

collect data on root diameter and specific root length (SLR). Specific root length was estimated as the ratio of root length to its dry mass.

Feedback phase

Conditioned homogenised soils containing root fragments from each pot in the conditioning phase were transferred to new 0.49 litre pots using sterilised equipment (width: 9 cm, depth: 9 cm height: 9.5 cm). Soils from each conditioning treatments were used to fill 40 feedback pots with five replicates for each of the eight plant species used in the conditioning phase (32 conditioning treatments x 8 feedback plant species x 5 replicates = 1280 pots). Within each conditioning treatment, the soil from eight replicates were used to each fill the pots of four different plant species in the feedback phase, while four replicates from each conditioning treatment were used to fill the pots of 2 different plant species in the feedback phase. Each pot in the feedback phase was filled with around ~400 g of conditioning phase soils (Supplementary Fig. S.1). For each plant feedback species, one seedling with a height of around 1 cm, was transplanted within each pot and then randomly allocated among 7 tables within the greenhouse. Temperature, light and humidity settings matched those used in the conditioning phase and were constantly monitored to account for differences across the four tables in the two climate chambers (Supplementary Fig. S.4). Plants were left to grow for four weeks during this feedback phase and were regularly watered. During the feedback phase, few plants got unintentionally infected with thirps (Thysanoptera) and whiteflies (Aleyrodidae) in the greenhouse facility; these plants were treated by lightly wiping the leaves with an insecticide (Kendo Gold, Maag Garden, Switzerland), after which we began to water all plants from below so to limit soil contamination. Given that these infections had nominal effects on plant growth and were also random, we included them in our data analysis. The feedback phase was run for four weeks to capture the early growth patterns in eight plant species across the conditioning phase treatments. All plants were harvested and biomass values (shoot and root) were measured in the same way as in the conditioning phase.

Data analysis

All data was analysed in R statistical software v4.2.2 (Team, 2013). Phylogenetic trees of eight plant species and distances were calculated using the "ape" and "v.Phylo.maker" packages (Jin and Qian, 2019; Paradis and Schliep, 2019). All other figures were created using the ggplot2 package (Wickham, 2009). Mixed-effects linear models were run on all response variables measured in the conditioning and feedback phase using the "lme4" package (Bates *et al.*, 2015). The random effects in the models accounted for the three distinct plant groups within each species, each inoculated with one of three replicates of the natural field soil inoculum. To explore how PSF differed depending on the origin of the feedback plant and the origin of the conditioning plants in the context of climate extremes, models were run with the fixed effects of feedback plant origin, conditioning phase soil group (conspecific soil, native heterospecific soil or invasive heterospecific soil) and the climatic treatment (control, drought, flood or drought + flood) (e.g. variable ~ Feedback plant origin * soil conditioning group * climatic treatment + (1|Soil inoculum group); Table 1).

A separate model was run to explore how PSF differed depending on the phylogenetic distance between the feedback and conditioning plants (by replacing "soil conditioning group" with "phylogenetic distance" in the model). Another model was also run to explore how the life history strategy of the feedback and conditioning plant impacted the PSF effect under the climatic treatments (e.g. variable ~ Feedback life history * feedback plant origin * conditioning life history * climatic treatment + (1|Soil inoculum group); Table 1). For plant variables measured during the conditioning phase, another mixed-effects model was run to explore how plant responses to the climatic treatments differed in conditioning phase depending on the plants origin, plants life history strategy (e.g. variable ~ Plant origin * plant life history strategy * climatic treatment + (1|Soil inoculum group); Table 1). The treatment effects in these mixed models were evaluated with a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for the estimation of degrees of freedom, using the lmerTest package (85).

Table 1. Summary of the mixed-effects linear models used to analyse plant responses based on the plant's origin, soil conditioning group, phylogenetic distance, life history strategies, and climatic treatments. Each model was run with specific fixed effects and a random effect accounting for the replicates in soil inoculum.

Model	PSF phase	Model input	Purpose
1	*Feedback*	variable ~ feedback plant origin * soil conditioning group * climatic treatment + (1|Soil inoculum group)	Explored how PSF varied based on feedback plant origin, conditioning soil type (conspecific, native heterospecific, invasive heterospecific), and climatic treatments.
2	*Feedback*	variable ~ feedback plant origin * feedback life history * conditioning life history * climatic treatment + (1|Soil inoculum group)	Investigated how life history strategies of feedback and conditioning plants influenced PSF under different climates.
3	*Feedback*	variable ~ feedback plant origin * phylogenetic distance * climatic treatment + (1|Soil inoculum group)	Examined PSF effects in relation to the phylogenetic distance between feedback and conditioning plants under different climates.
4	*Conditioning*	variable ~ plant origin * plant life history strategy * climatic treatment + (1|Soil inoculum group)	Assessed how plant responses to climatic treatments differed by origin and life history during the conditioning phase.

Model assumptions (e.g., homogeneity of variance and normality of residuals) were inspected visually for each linear model. To meet the model assumptions, some response variables were square-root or log-transformed (indicated in Table 2 & Supplementary Table 1). Conditional R^2 values were taken as the proportion of total variance explained through both fixed and random effects of the linear models and their statistical significance was obtained from the r2glmm package (Jaeger *et al.*, 2017). For linear model 1 (Table 1), effect sizes of plant responses to the

water treatments compared to the control were calculate using the effsize package (Torchiano, 2020). For this effect-size analysis all response variables that were previously transformed to meet model assumptions were back transformed. Further t-tests were performed to compare these water treatment effect sizes between invasive and native feedback phase plants within their respective soil conditioning groups. Additionally, pairwise comparisons were conducted using the emmeans package (Russell *et al.*, 2024), with adjustments made for multiple comparisons using the Sidak method. Each group comprised multiple plant species, contributing additional variation that our models could not account for, as they were not designed to differentiate between species within groups. Graphs illustrating the responses of individual species are provided in the supplementary information (see Supplementary Information for individual species responses in both the conditioning and feedback phase Fig S.8, S.9 & S.10). To explore the strength of relationships between two variables for understanding of potential mechanisms driving feedback responses, Pearson correlation coefficient and significance levels were calculated and displayed on the plots using the "stat_cor" function in the ggplot2 package (Wickham, 2009).

Results

Feedback phase – Species origin

Across all plants in the feedback phase, the impact of the climatic treatments in the conditioning phase lead to large differences in plant biomass accumulation (linear model 1: shoot biomass: $\text{F-value}_{df} = 130.89_{1,1237}$, p-value <0.001, root biomass: $\text{F-value}_{df} = 115.54_{1,1254}$, p-value <0.001; Table. 2; Supplementary Fig. S.11). In particularly, feedback phase plants grew substantially better and invested more in shoot growth in soils with a legacy of drought. However, for the shoot biomass this drought mediated increase in plant biomass was greater in invasive feedback phase plants compared to native plants (linear model 1; $\text{F-value}_{df} = 5.45_{1,1237}$, p-value <0.01; Table. 2; Supplementary Fig. S.11). An analysis comparing the effect sizes of each climatic treatment (from linear model 1; Table 1) showed that this drought-induced biomass increase was significant only in conspecific soils, where invasive feedback phase plants

accumulated significantly more biomass (shoot and total) than native plants (Fig. 1). Shoot and root biomass also differed in the context of the climatic treatment and the conditioning soil (linear model 1: shoot biomass: F-value$_{df}$ = $3.32_{1,1237}$, p-value <0.01, root biomass: F-value$_{df}$ = $4.01_{1,1254}$, p-value <0.001; Table. 2). In particular, shoot biomass in control treatments was greater in the soil from native heterospecific conditioning plants compared to invasive heterospecific conditioning plants (Supplementary Fig. S.12).

Table 2. Results from linear mixed-effect models (linear model 1 (see Table 1)) testing the effects feedback species origin (SO), conditioning phase soil group (e.g. conspecific soil, native heterospecific soil or invasive heterospecific soil; CS) and the climatic treatment (CT) for the dependent variables measured in the feedback phase. Bold values are statistically significant (p-value < 0.05). Conditional R^2 represents the combined effects of fixed and random effects used in our models. The random intercept of soil inoculum type had a variance of 0 across all dependent variables. df stands for degrees of freedom.

Response Variable	Species origin (SO)		Conditioning soil (CS)		Water treatment (WT)		SO x CS		SO x WT		CS x WT		SO x CS x WT		Model
	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	R-squared (conditional)
Total biomass	$0.47_{1,1256}$	0.49	$1.47_{2,1256}$	0.23	$127.80_{3,1256}$	**<0.001 *****	$0.53_{2,1256}$	0.74	$4.27_{3,1256}$	**<0.01 ****	$3.76_{6,1256}$	**<0.01 ****	$1.47_{6,1256}$	0.18	0.33
Shoot biomass	$2.67_{1,1236}$	0.10	$2.58_{2,1237}$	0.07	$130.89_{3,1237}$	**<0.001 *****	$0.37_{2,1236}$	0.69	$5.45_{3,1237}$	**<0.01 ****	$3.32_{6,1237}$	**<0.01 ****	$1.75_{6,1237}$	0.11	0.34
Root biomass	$0.82_{1,1255}$	0.37	$0.38_{2,1255}$	0.68	$115.54_{3,1254}$	**<0.001 *****	$0.61_{2,1254}$	0.54	$1.72_{3,1255}$	0.16	$4.01_{6,1254}$	**<0.001 *****	$0.75_{6,1255}$	0.61	0.31
Root:Shoot	$7.38_{1,1232}$	**<0.01 ****	$0.44_{2,1232}$	0.65	$14.17_{3,1231}$	**<0.001 *****	$1.91_{2,1231}$	0.15	$0.74_{3,1232}$	0.53	$0.67_{6,1231}$	0.67	$0.42_{6,1232}$	0.86	0.06

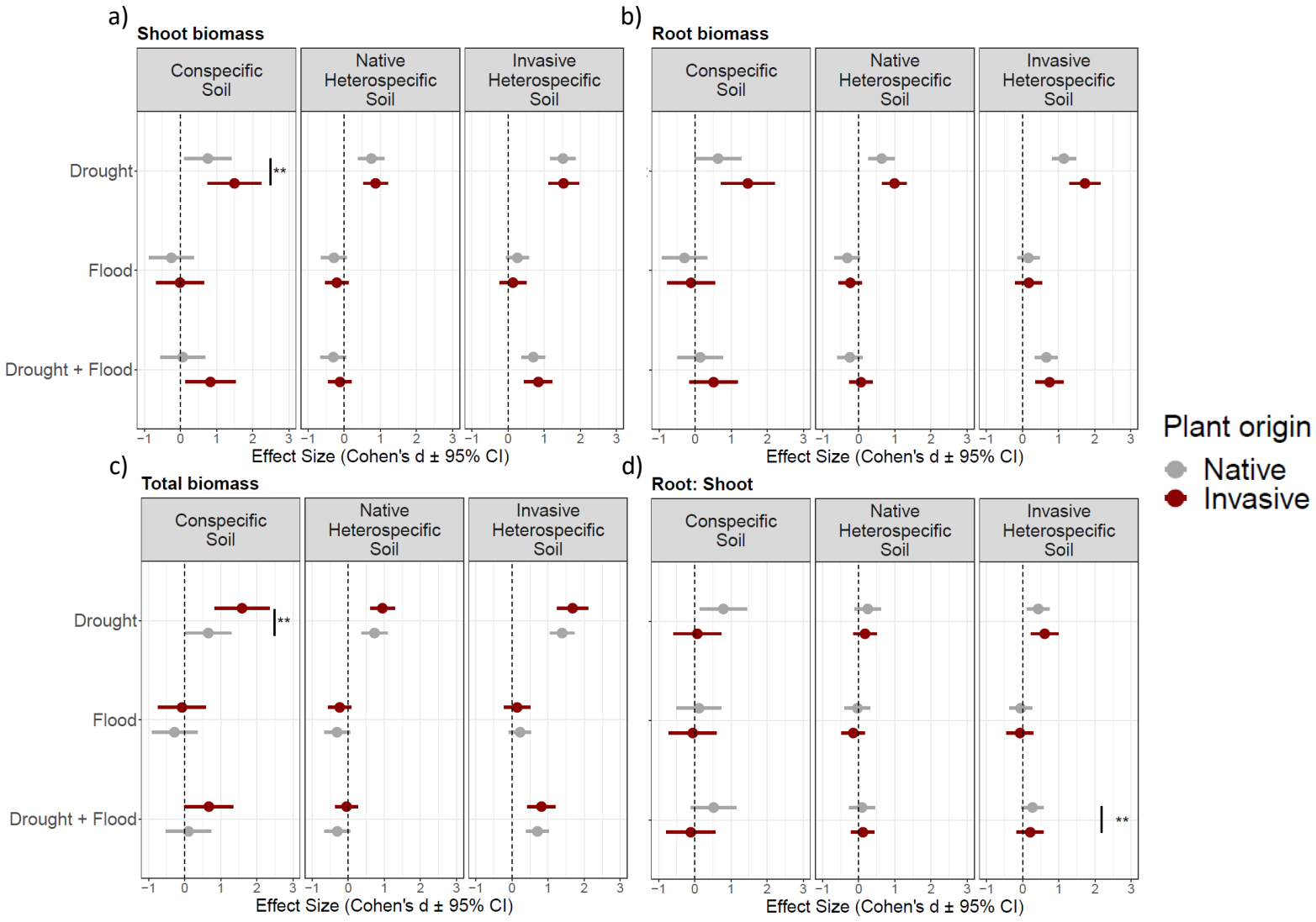

Figure 1. Forest plot showing the effect sizes (obtained from linear model 1 (see Table 1)) of water treatments on a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot ratio for feedback phase native and invasive plants. The effect size (Cohen's d ± 95% confidence intervals) was calculated for shoot biomass in invasive (red) and native (grey) feedback phase plants across the three water treatments: Drought, Flood, and Drought + Flood, compared to the Control. Treatments with significant differences between native and invasive species are denoted by asterisks based on p-values from t-tests (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The vertical dashed line at 0 indicates no effect. Results are faceted by the plant conditioning group.

The effects of climatic treatments during the conditioning phase led to a significant variation in root biomass allocation across all feedback phase plants (linear model 1: $F\text{-value}_{df} = 14.17_{1,1231}$,

p-value <0.001). Specifically, feedback phase plants grown in soils with a legacy of drought or drought + flood invested substantially more in root biomass. Native plants, however, consistently allocated a higher proportion of their biomass to roots compared to invasive feedback phase plants, regardless of the climatic treatment (linear model 1: F-value$_{df}$ = $7.38_{1,1232}$, p-value <0.01; Supplementary Fig. S.11).

Feedback phase – Life history strategy

Results showed that biomass accumulation of feedback phase plants was influenced both by the distinct life history strategies of the feedback phase plants (linear model 2: shoot biomass: F-value$_{df}$ = $31.48_{1,1212}$, p-value <0.001, root biomass: F-value$_{df}$ = $85.99_{1,1232}$, p-value <0.001, total biomass: F-value$_{df}$ = $56.68_{1,1232}$, p-value <0.001; Supplementary Fig. S.13). In general, annual feedback phase plants accumulated less biomass (both shoot and root) than perennial feedback phase plants, however this trend was further influenced by the origin of the feedback phase plant species (linear model 2: shoot biomass: F-value$_{df}$ = $15.17_{1,1212}$, p-value <0.001, root biomass: F-value$_{df}$ = $40.38_{1,1232}$, p-value <0.001, total biomass: F-value$_{df}$ = $28.76_{1,1232}$, p-value <0.001; Supplementary Fig. S.13). Specifically, native perennial plants accumulated the most biomass, significantly more than both native and invasive annuals and invasive perennials, likewise native annual plants accumulated the least biomass, significantly less than both native and invasive perennials and invasive annuals. However, within invasive plants there was no significant difference between annual and perennial plants (Supplementary Fig. S.13). Across all feedback phase plants biomass accumulation was also impacted by the life history of the conditioning plant, with all plants producing less biomass in soil conditioned by annual plant species compared to perennial plant species (linear model 2: shoot biomass: F-value$_{df}$ = $5.10_{1,1212}$, p-value <0.01, root biomass: F-value$_{df}$ = $4.28_{1,1232}$, p-value <0.05, total biomass: F-value$_{df}$ = $4.98_{1,1232}$, p-value <0.01; Supplementary Fig. S.13).

Root biomass allocation in the feedback phase plants was influenced by the distinct life history strategies of annuals and perennials (linear model 2: F-value$_{df}$ = $89.76_{1,1207}$, p-value <0.001; Supplementary Fig. S.13). In general, annuals allocated less biomass to roots compared to

perennials. This trend was more pronounced in native plants (linear model 2: F-value$_{df}$ = $24.01_{1,1207}$, p-value <0.001), however, invasive plants also showed a significant decrease in root allocation in annual plants compared to perennial plants. Notably, this pattern between native annual and perennial plant species shifted depending on climatic treatment (linear model 2: F-value$_{df}$ = $3.19_{1,1207}$, p-value <0.05; Supplementary Fig. S.13). Specifically, in soils with a legacy of drought, the difference in root allocation between native annual and perennial plants disappeared.

Feedback phase – Phylogenetic distance

Phylogenetic distance between the feedback and conditioning plant species impacted the biomass accumulated across all feedback phase plants (linear model 3: shoot biomass: F-value$_{df}$ = $25.35_{1,1244}$, p-value <0.001, root biomass: F-value$_{df}$ = $19.47_{1,1264}$, p-value <0.001, total biomass: F-value$_{df}$ = $22.29_{1,1264}$, p-value <0.001; Fig. 2). Particularly feedback phase plants accumulated more biomass in soils conditioned by plants species with a greater phylogenetic distance to the feedback species (Fig. 2). This impact of phylogenetic on the plant shoot biomass accumulation of feedback phase plants differed in the context of the climatic treatment during the conditioning phase (linear model 3: shoot biomass: F-value$_{df}$ = $5.01_{1,1245}$, p-value <0.01, total biomass: F-value$_{df}$ = $3.36_{1,1264}$, p-value <0.05; Fig. 2). In this case, this increase in shoot with increasing phylogenetic distance was most prominent in soils with a legacy of drought. For root biomass, the influence of phylogeny on biomass accumulation varied based on the origin of the feedback phase plants, with a stronger correlation observed in native plants compared to invasive plants (linear model 3: F-value$_{df}$ = $4.73_{1,1264}$, p-value <0.05; Fig. 2).

Differences in biomass allocation between the roots and shoots of the feedback phase plants did not significantly differ with phylogenetic distance (Fig. 2).

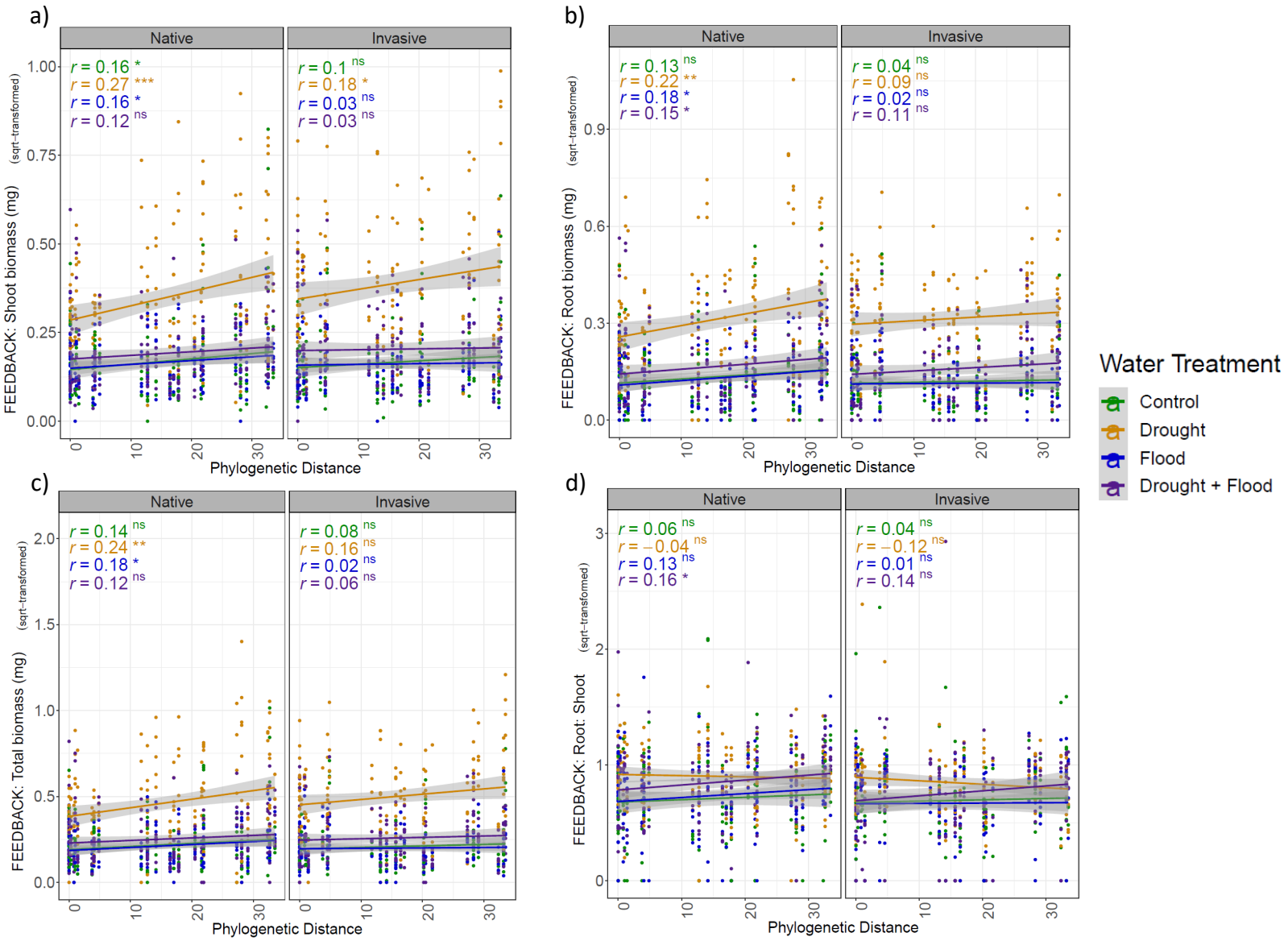

Figure 2. Correlation between the phylogenetic distance between feedback and conditioning plant species and the a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot ratio of feedback phase plants. The e Pearson correlation coefficient (r) and the corresponding significance level are shown on the graphs. The asterisks (*) indicate statistical significance, where $p < 0.05$ is represented by *, $p < 0.01$ by **, and $p < 0.001$ by ***, while "ns" denotes non-significant correlations ($p \geq 0.05$).

Conditioning phase – plant root traits

Root traits varied significantly depending on plant experimental treatment groups. Perennial plants had thicker roots, characterized by a greater root diameter, and shorter roots with a

smaller specific root length (SRL) compared to annual plants (linear model 4: root diameter: F-value$_{df}$ = $123.37_{1,324}$, p-value <0.001, SRL: F-value$_{df}$ = $29.40_{1,321}$, p-value <0.001; Supplementary Fig. S.15). Among native species, the differences in SRL between annuals and perennials were more pronounced than those observed within invasive species (linear model 4: F-value$_{df}$ = $10.15_{1,368}$, p-value <0.01). Invasive plants generally exhibited slightly larger root diameters than native plants (linear model 4: F-value$_{df}$ = $4.63_{1,324}$, p-value <0.05). Additionally, plants subjected to drought conditions displayed an increase in SRL (linear model 4: F-value$_{df}$ = $8.53_{1,321}$, p-value <0.001; Supplementary Fig. S.15).

Correlations between response variables

Further analysis was conducted to elucidate how root traits during the conditioning phase influenced drought-driven growth differences between native and invasive feedback phase plants in conspecific soils. The analysis showed a positive correlation between root diameter and biomass accumulation in invasive species grown in conspecific soils with a drought legacy. In contrast, this relationship was less clear for native plants or those grown in heterospecific soils. Specific root length also negatively correlated with biomass accumulation in invasive species in conspecific soils with drought legacies, but this trend was absent in native plants and those grown in heterospecific soils (Fig. 3). Across all feedback phase plants, the biomass accumulated in the feedback phase negatively correlated with the biomass accumulated by the plant in the conditioning phase (e.g. for the total biomass in the feedback and conditioning phase: r = -0.24, p-value <0.001; Supplementary Fig. S.16). Likewise, the biomass accumulated across all feedback phase plants positively correlated with the SRL of plants in the conditioning phase (e.g. for the total biomass in the feedback phase: r = 0.11, p-value <0.001; Supplementary Fig. S.16).

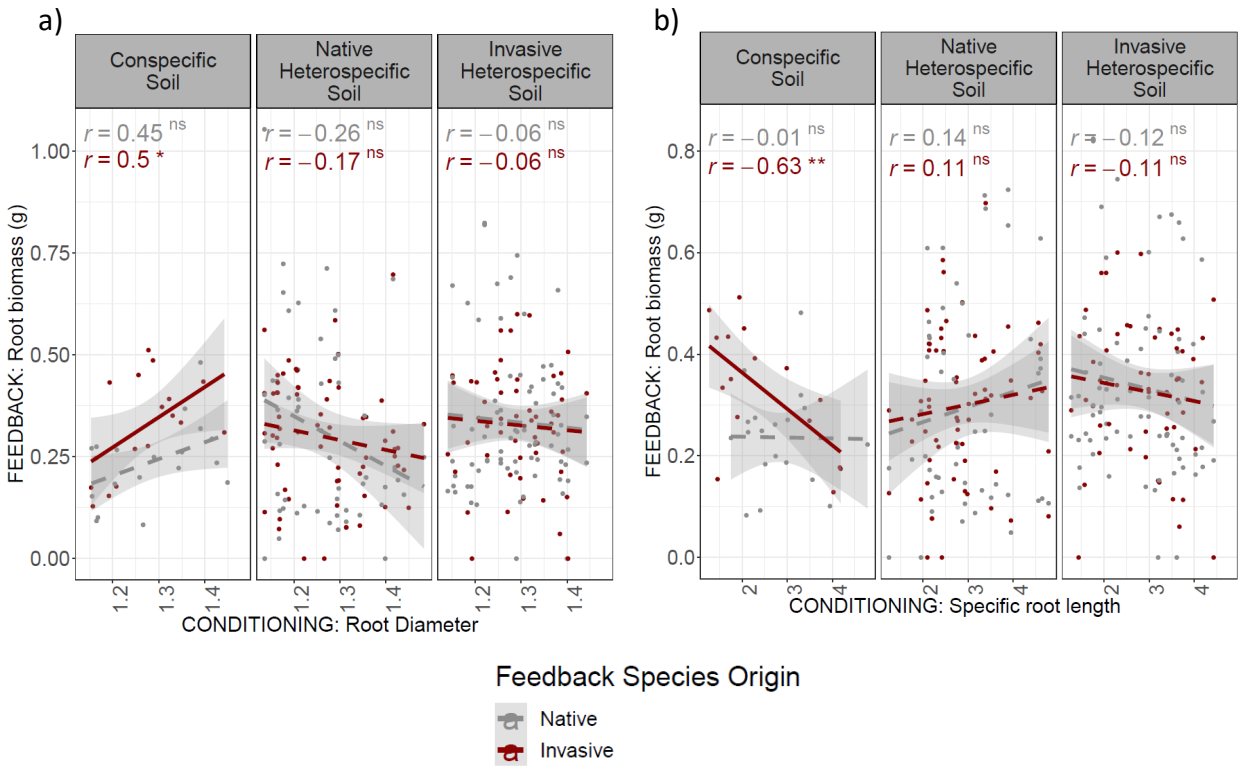

Figure 3. Correlation between root trait response variables of plants subjected to drought during the conditioning phase (a: root diameter and b: specific root length (SRL)) and belowground biomass accumulated in the feedback phase. The correlation statistics are displayed within each panel, showing the Pearson correlation coefficient (r) and the corresponding significance level. The asterisks (*) indicate statistical significance, where $p < 0.05$ is represented by *, $p < 0.01$ by **, and $p < 0.001$ by ***, while "ns" denotes non-significant correlations ($p \geq 0.05$).

Discussion

The concept of invasional meltdown, which suggests that the presence of invasive species can facilitate the establishment and success of other invasive species (Simberloff and Von Holle, 1999) was not explained through soil legacies in our study. Contrary to expectations, many invasive plant species exhibited poorer performance in soils conditioned by heterospecific invasive plants compared to those conditioned by heterospecific native plants (e.g., Supplementary figure S.12). Moreover, there was no significant difference in the performance of native versus invasive feedback phase plants when grown in invasive heterospecific soils (Fig.

1). However, our study did suggest that native and invasive plants disproportionately response to the soil legacies shaped by the climatic treatments, particularly drought, during the conditioning phase (Fig 1 & 2). More importantly, we show that soil legacies of extreme drought facilitate the performance of non-native invasive plants in their own conspecific soils more than native plant species.

The legacy of drought conditions in the soil had a marked effect on feedback phase plant performance, enhancing plant growth and leading to more positive plant-soil feedback (PSF). Rewetting soils after a drought tends to trigger a microbial pulse in the soil, thereby temporarily increasing nutrient cycling and availability through osmotic diffusion (Borken and Matzner, 2009; Barnard, Osborne and Firestone, 2013; Leitner *et al.*, 2017; Song *et al.*, 2017; Xu *et al.*, 2020; Brangarí, Manzoni and Rousk, 2021). However, this surge in microbial activity and performance may also lead to a rise in abundance of certain microbe, such as species-specific pathogenic microbes, which may dampen the positive effects of improved nutrient availability. This was evident specifically for native plants in conspecific soils with a drought legacy, where they experienced a reduced positive PSF (Fig. 1). Furthermore, native plants during the feedback phase performed significantly worse in soils conditioned by closely related species when these soils had a legacy of drought (Fig. 2). In contrast, invasive plants continued to perform well in soils with a drought legacy, regardless of the conditioning plant's identity (Fig. 1 & Fig. 2). Previous studies have suggested that invasive plant species are able to utilise beneficial soil microbes more effectively within their non-native ranges (Callaway *et al.*, 2004; Reinhart and Callaway, 2006; Sun and He, 2010; Sheng *et al.*, 2022) . We specifically show that invasive plant species grow comparably well in all soils with a drought legacy and are less hampered by the negative PSF associated with limiting similarity compared to native plants. This may either result from less species-specific pathogen or a greater accumulation of mutualistic microbes that can interact with the conspecific invasive plants compared to conspecific native plants (Mitchell and Power, 2003; Lekberg *et al.*, 2013; Maron *et al.*, 2014).

Further analysis of the root traits of the conditioning plant subjected to drought helped elucidate the potential role of beneficial soil microbes in mediating this reduction of negative conspecific PSF in invasive feedback phase plants. Specifically, in conspecific soils, the root diameter of conditioning plants positively correlated with root biomass accumulation in invasive plants during the feedback phase, while specific root length of conditioning phase plants showed a negative correlation with root biomass in these same invasive species from the feedback phase. Such correlations imply that the performance of the invasive plants in conspecific soils may be driven by the biotic responses of their conspecific conditioning plant under extreme drought. However, this relationship was less clear for native plants or plants grown in heterospecific soils. These root traits provide valuable insight into the extent of collaboration between the conditioning plants and mutualistic soil microbes, such as mycorrhizal fungi (Bergmann *et al.*, 2020; Rutten and Allan, 2023). The presence of thicker and shorter roots suggests a greater degree of 'outsourcing' in resource acquisition, indicating that these plants may rely more heavily on their fungal partners for nutrient uptake. This aligns with the concept of a collaboration gradient, where plants can choose between 'do-it-yourself' strategies, characterized by thin, efficient roots, and 'outsourcing' strategies that involve investing in mycorrhizal relationships (Bergmann *et al.*, 2020; Rutten and Allan, 2023). Notably, the invasive species performed best in soils conditioned by plants exhibiting root traits indicative of greater outsourcing, suggesting that these soils may harbour a higher abundance of beneficial soil microbes which potentially led to more positive PSF. These findings suggest that drought legacies may enhance the competitive advantage of invasive species, which could widen their invasional window in new environments (Sanders et al. 2025).

Among several limitations of our study, one key limitation is the focus on a specific subset of invasive and native species, which may constrain the broader applicability of the findings. While the results highlight important mechanisms related to drought legacies and PSF, these dynamics could vary significantly with different plant species, soil types, or climate conditions. Additionally, our experiment was conducted in controlled conditions that may not fully capture

the complexity of natural ecosystems, where multiple biotic and abiotic interactions occur simultaneously. Future studies should aim to incorporate a wider range of species, soil conditions, and climatic scenarios to better understand the broader implications of drought-mediated feedbacks on invasive species success.

We conclude that soil mediated drought legacies may enhance the competitive advantage of invasive species as they are less inhibited by their own specific PSF, potentially increasing their success in new environments. As climate change intensifies and drought events become more frequent, invasive plants may become even more dominant, forming denser populations. This could lead to greater invasional success in the establishment and spread of invasive plant species under future climatic conditions, potentially reshaping ecosystems and biodiversity. Management strategies addressing invasive species should consider the potential that changing environmental conditions, such as more frequent droughts, may disproportionately enhance the performance of invasive plants compared to native species.

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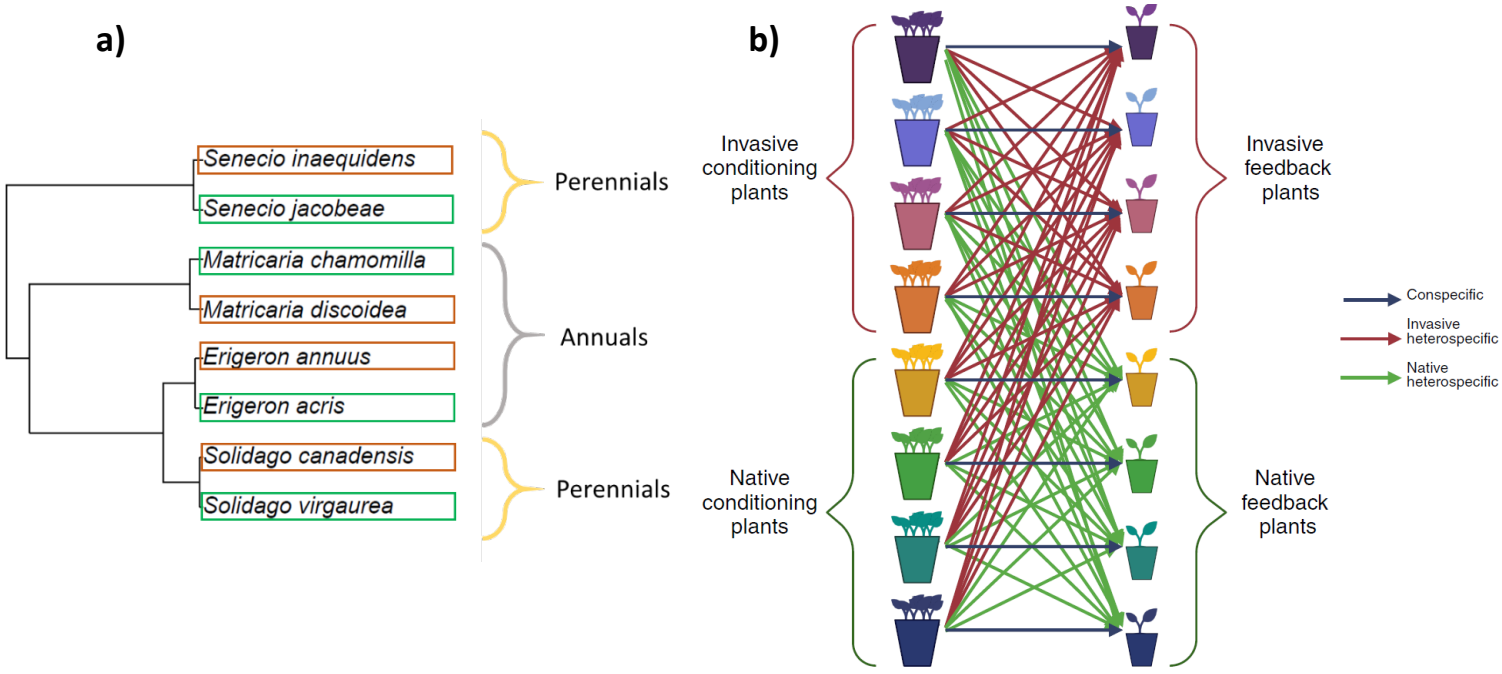
Supplementary material

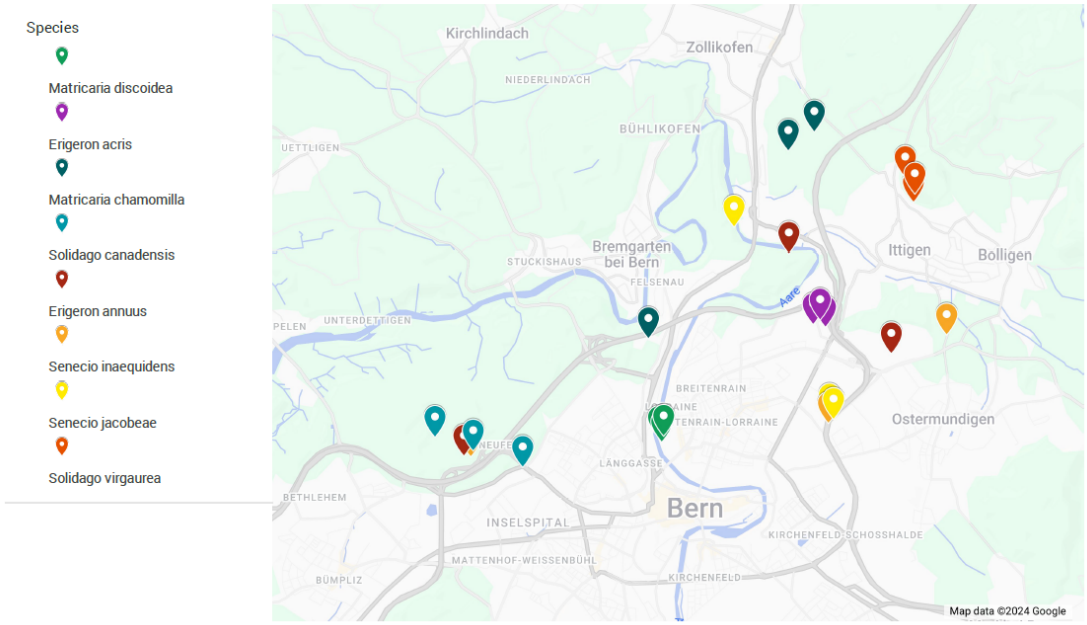


Supplementary figure 1. Schematic of the experimental design of the study showing a) the phylogeny of the different plant species used in the study and how they were divided by species origin (invasive= red and native species = green) and life history strategy. Schematic showing b) how the design of the conditioning and feedback phase allowed for investigation of plants grown in conspecific, native heterospecific and invasive heterospecific soils (created using Biorender).

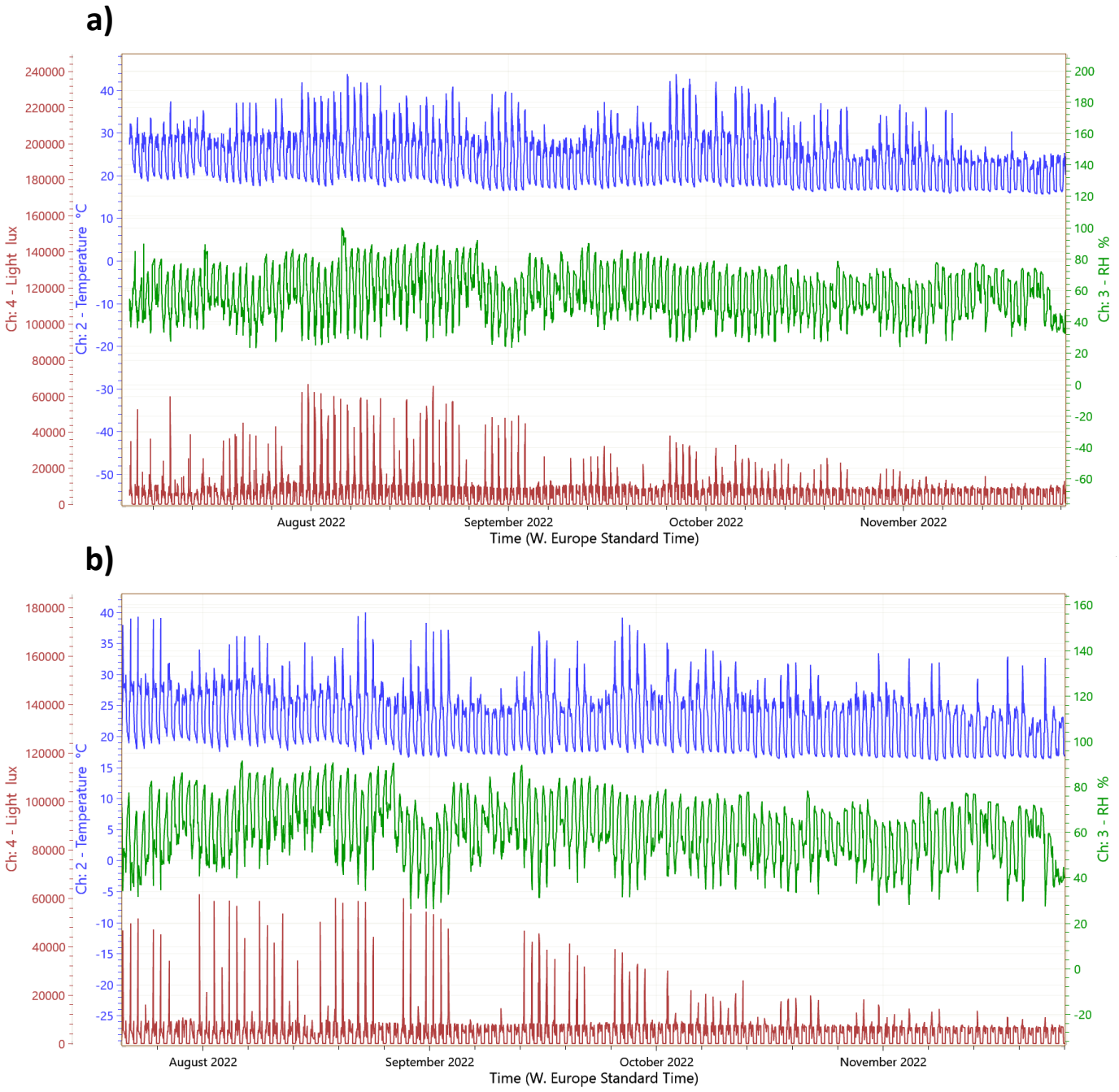
Seed collection locations

Supplementary figure 2. Map of locations where seeds where were collected for the experiment between July to November 2021.

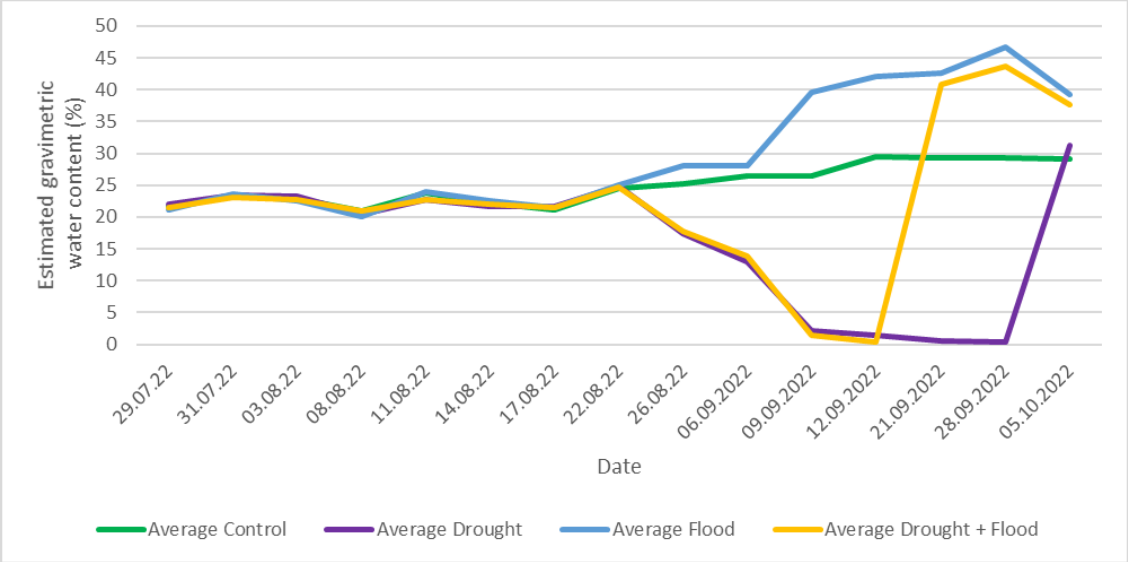
Species specific soils



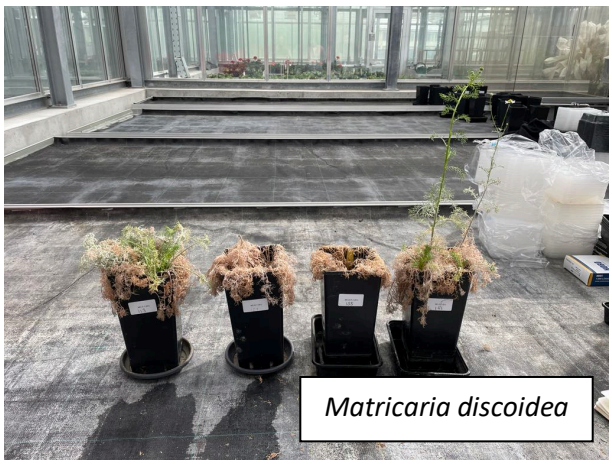
Supplementary figure 3. Map of locations where species specific soils where were collected (in June 2022) for the microbial wash used in the soil inoculum.

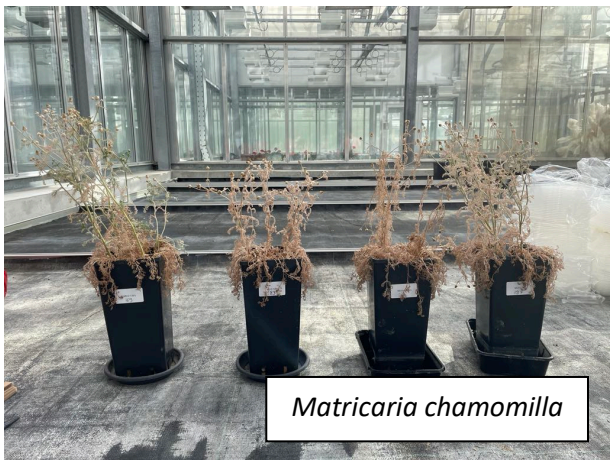


Supplementary figure 4. Climatic conditions measured inside the greenhouse throughout the experiment (both conditioning and feedback phase) each logger was randomly moved each week to different tables and postions (u. Zollgasse 77, 3072 Ostermundigen). Temperature (blue), relative humidity (green) and light (red) recorded from logger one (a) and two (b).

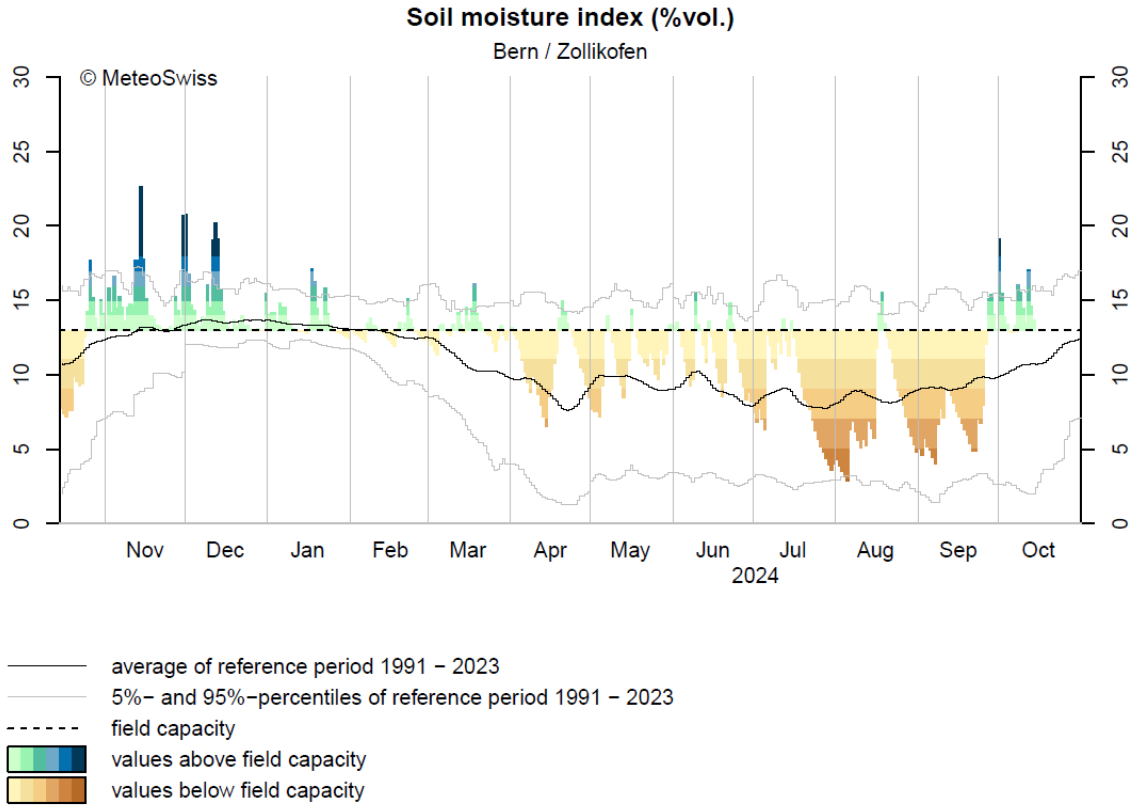


Supplementary figure 5. Estimated gravimetric water content (%) of soils from each climatic treatment throughout the conditioning phase of the experiment.





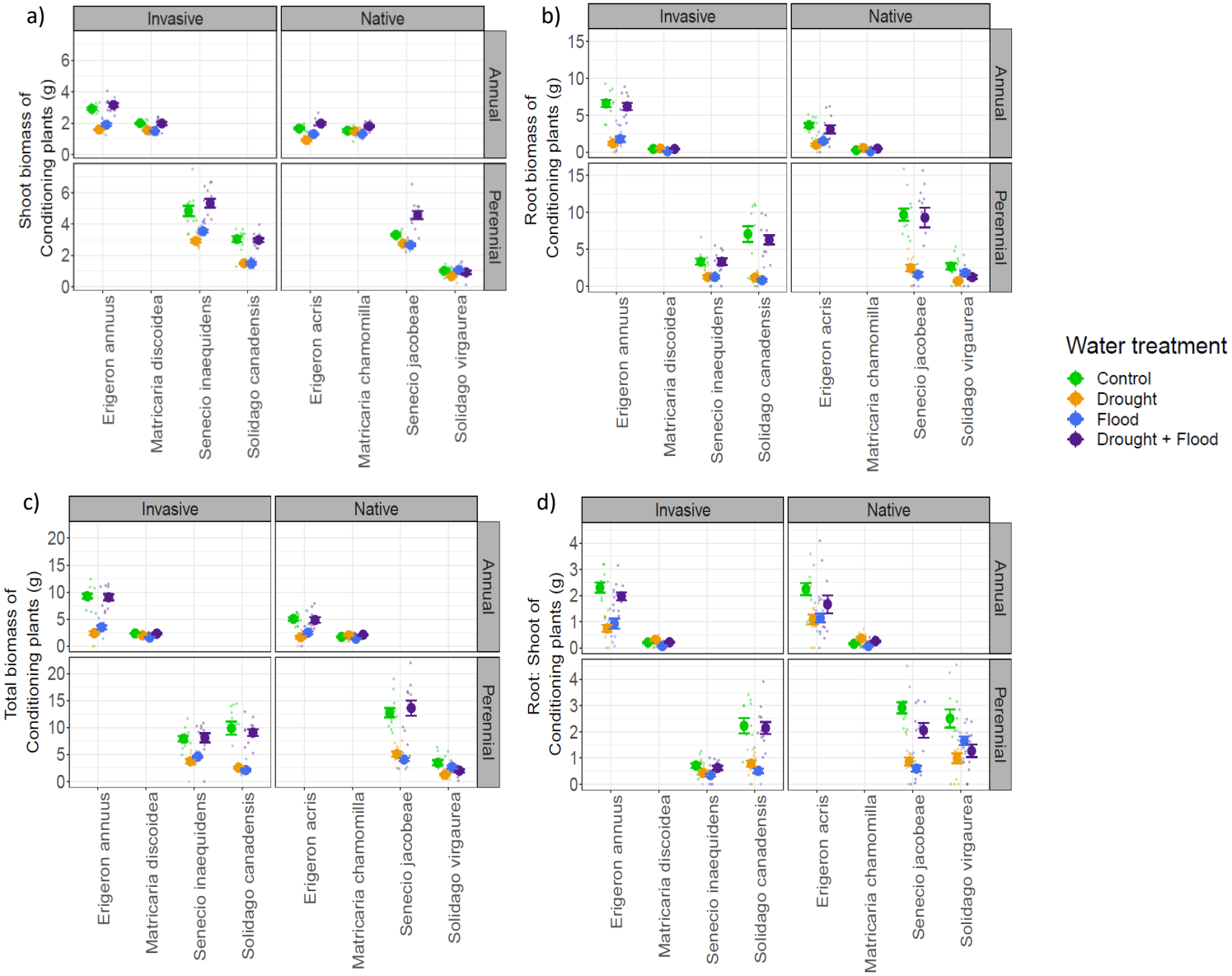
Supplementary figure 6. Photos of each conditioning plant species before plants were harvested. Climatic treatments from left to right are: control, drought, drought + flood and flood.



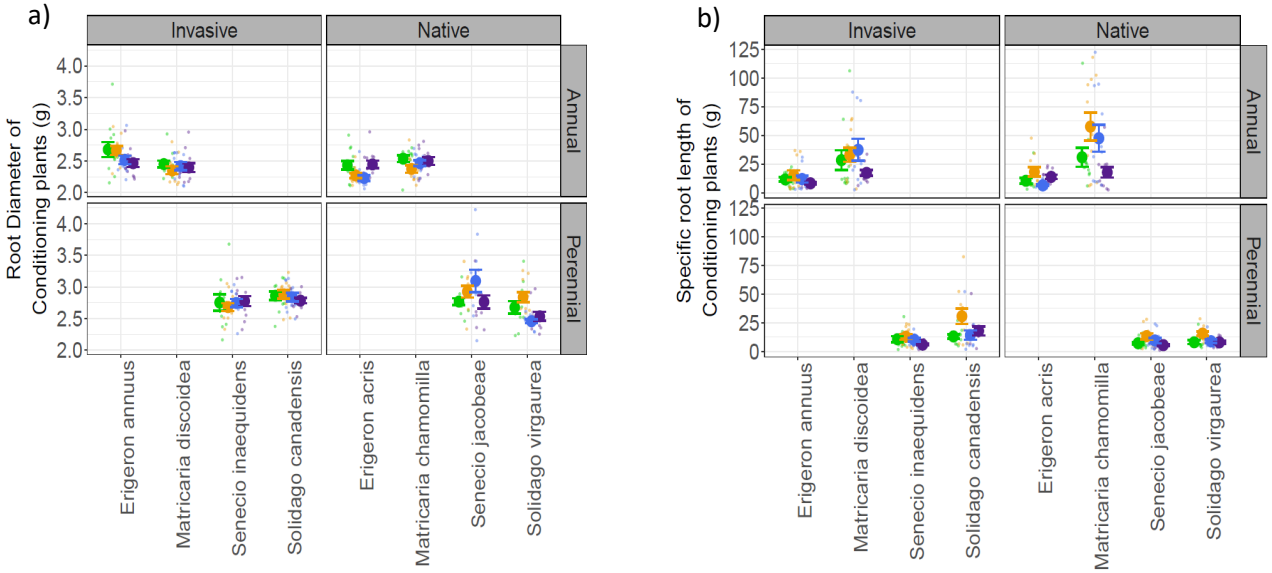
Supplementary figure 7. Soil moisture index over time at Bern/Zollikofen from November 2023 to October 2024. The solid black line represents the average soil moisture from 1991 to 2023, while shaded areas indicate values above (blue-green) and below (brown-yellow) field capacity. The dashed line marks field capacity, and the grey band shows the 5th to 95th percentile range of historical values. Credit: MeteoSwiss.

Supplementary table 1. Results from linear mixed-effect models testing the effects species origin (SO), plant life history strategy (LH) and the climatic treatment (CT) for the dependent variables measured in the conditioning phase. Bold values are statistically significant (p-value < 0.05). Conditional R2 represents the combined effects of fixed and random effects used in our models. df stands for degrees of freedom.

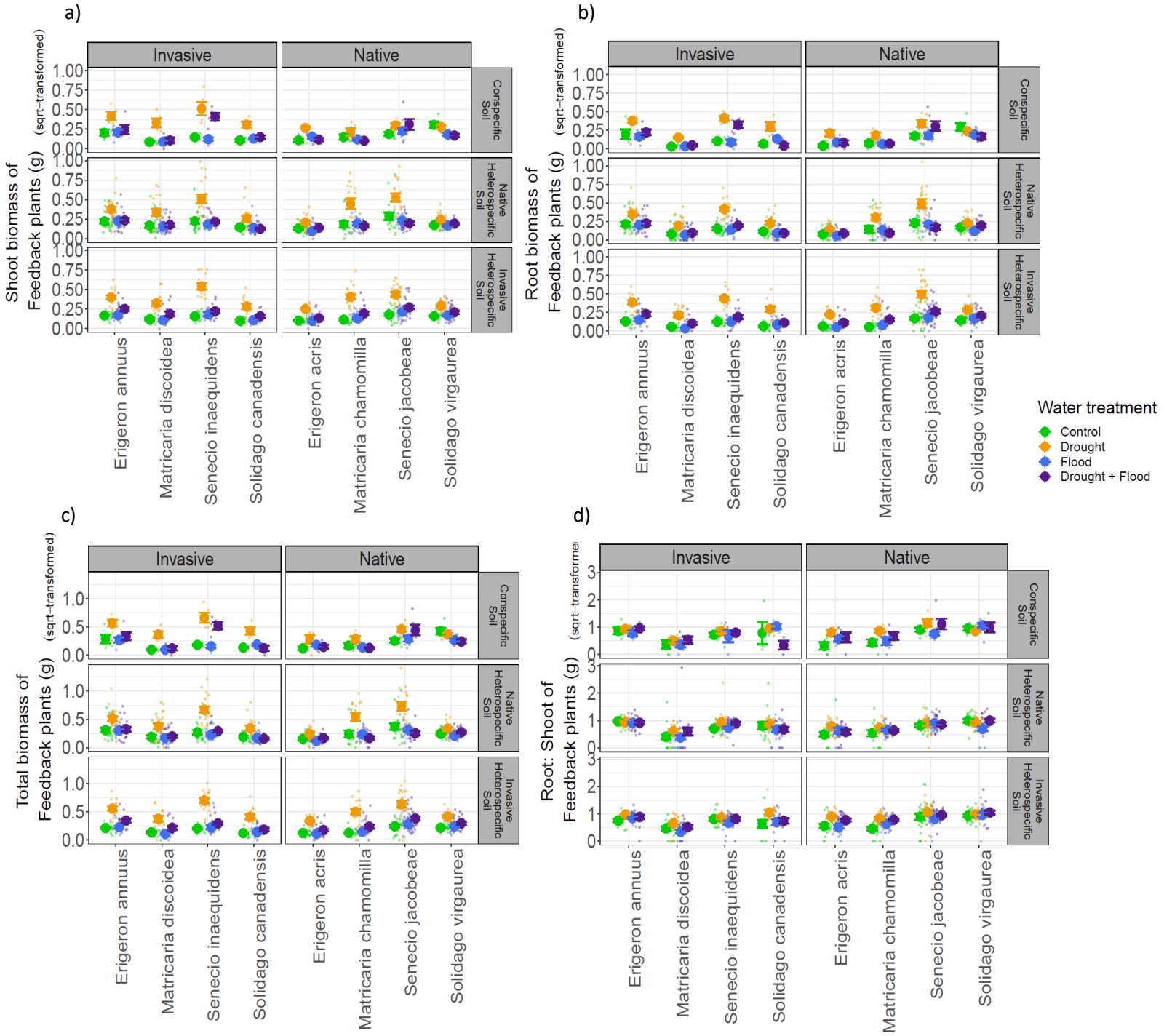
Response Variable	Species origin (SO)		Life history (LH)		Water treatment (WT)		SO x LH		SO x WT		LH x WT		SO x LH x WT		Random intercept (soil innoculum)	Model
	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	Variance	R-squared (conditional)
Total biomass (log-transformed)	$16.60_{1,368}$	<0.001 ***	$59.82_{1,368}$	<0.001 ***	$47.38_{3,368}$	<0.001 ***	$0.68_{1,368}$	0.41	$1.68_{3,368}$	0.17	$1.54_{3,368}$	0.20	$0.33_{3,368}$	0.80	0.00	0.38
Shoot biomass (log-transformed)	$90.53_{1,368}$	<0.001 ***	$54.36_{1,368}$	<0.001 ***	$30.77_{3,368}$	<0.001 ***	$5.87_{1,368}$	<0.05 *	$3.14_{3,368}$	<0.05 *	$0.33_{3,368}$	0.80	$0.76_{3,368}$	0.52	0.00	0.40
Root biomass (log-transformed)	$0.82_{1,368}$	0.37	$54.16_{1,368}$	<0.001 ***	$41.33_{3,368}$	<0.001 ***	$3.44_{1,368}$	0.06	$1.78_{3,368}$	0.15	$3.34_{3,368}$	<0.05 *	$0.51_{3,368}$	0.68	0.00	0.34
Root:Shoot (log-transformed)	$12.22_{1,368}$	<0.001 ***	$29.07_{1,368}$	<0.001 ***	$23.76_{3,368}$	<0.001 ***	$10.15_{1,368}$	<0.01 **	$1.28_{3,368}$	0.28	$2.49_{3,368}$	0.06	$1.29_{3,368}$	0.28	0.00	0.26
Root diameter (log-transformed)	$4.63_{1,324}$	<0.05 *	$123.37_{1,324}$	<0.001 ***	$0.91_{3,325}$	0.44	$0.52_{1,324}$	0.47	$0.14_{3,324}$	0.94	$2.16_{3,324}$	0.09	$2.37_{3,324}$	0.07	0.00	0.30
Specific root length (log-transformed)	$0.94_{1,321}$	0.33	$29.40_{1,321}$	<0.001 ***	$8.53_{3,321}$	<0.001 ***	$5.50_{1,321}$	<0.05 *	$0.44_{3,321}$	0.72	$0.17_{3,321}$	0.92	$0.21_{3,321}$	0.89	0.01	0.18



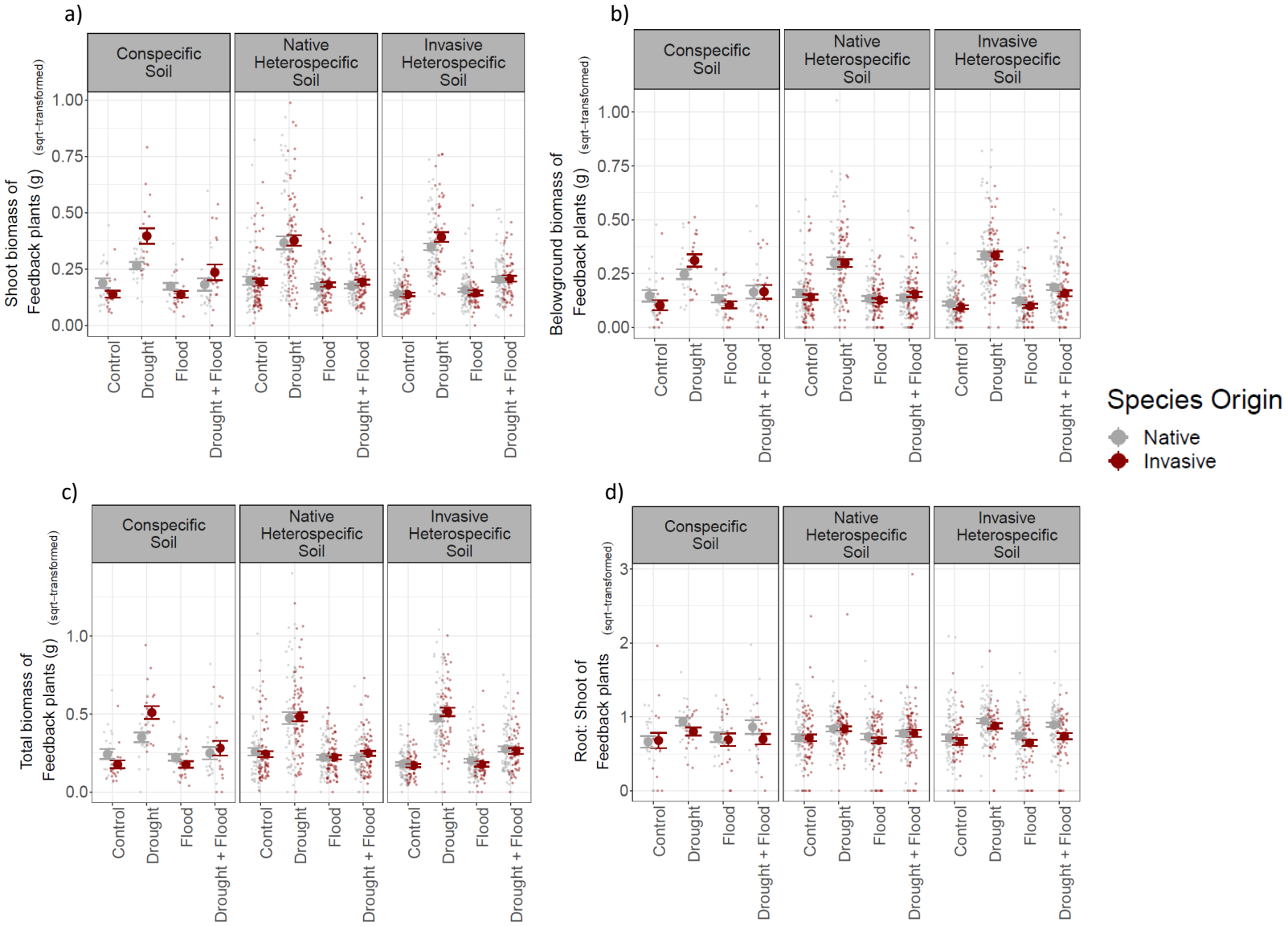
Supplementary figure 8. Species-specific a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of conditioning plant in response to the separate climatic treatments. Species origin (native or invasive) and life history strategy (annual or perennial) are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



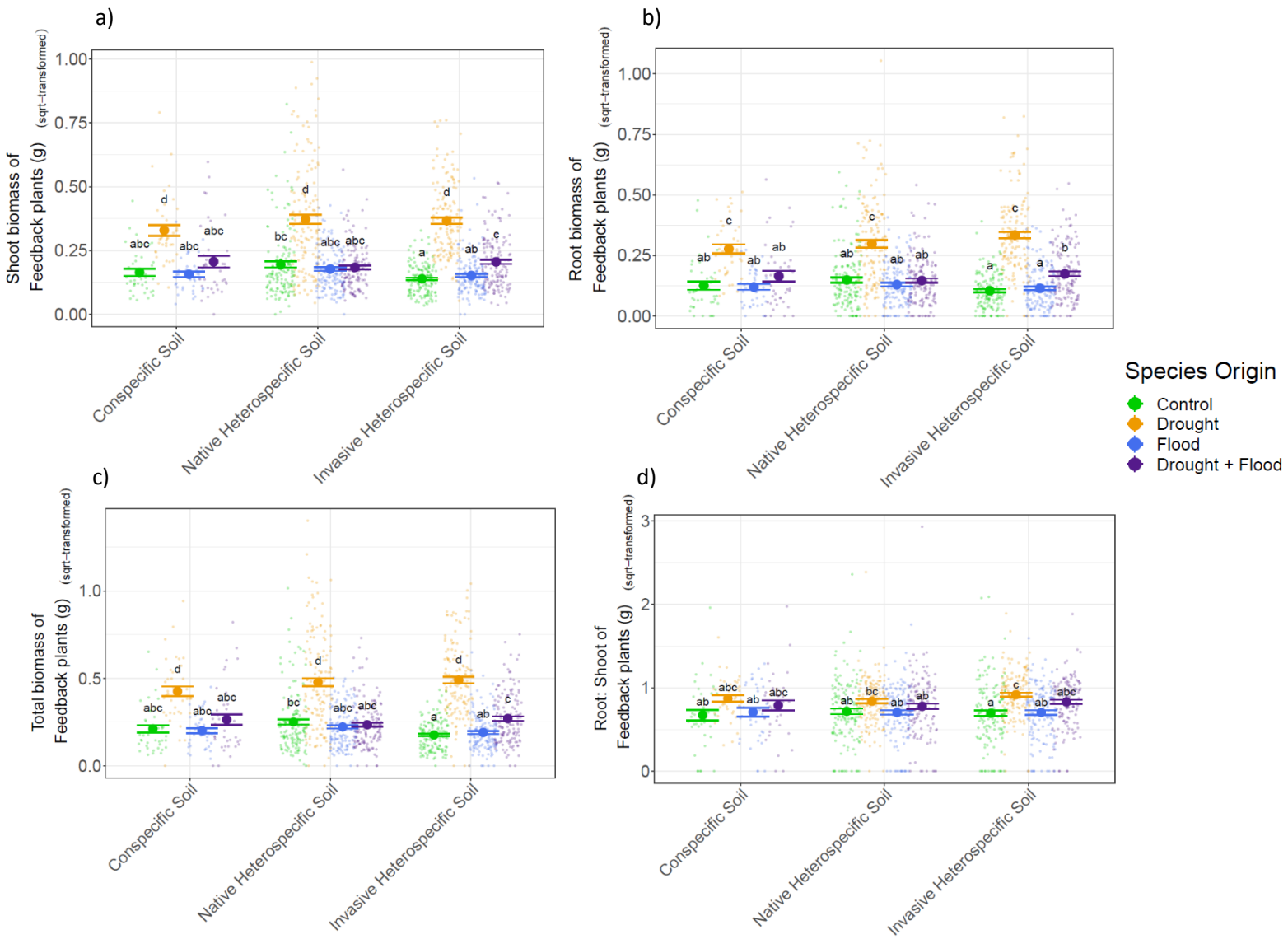
Supplementary figure 9. Species-specific a) root diameter and b) specific root length of conditioning plants in response to the separate climatic treatments. Species origin (native or invasive) and life history strategy (annual or perennial) are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



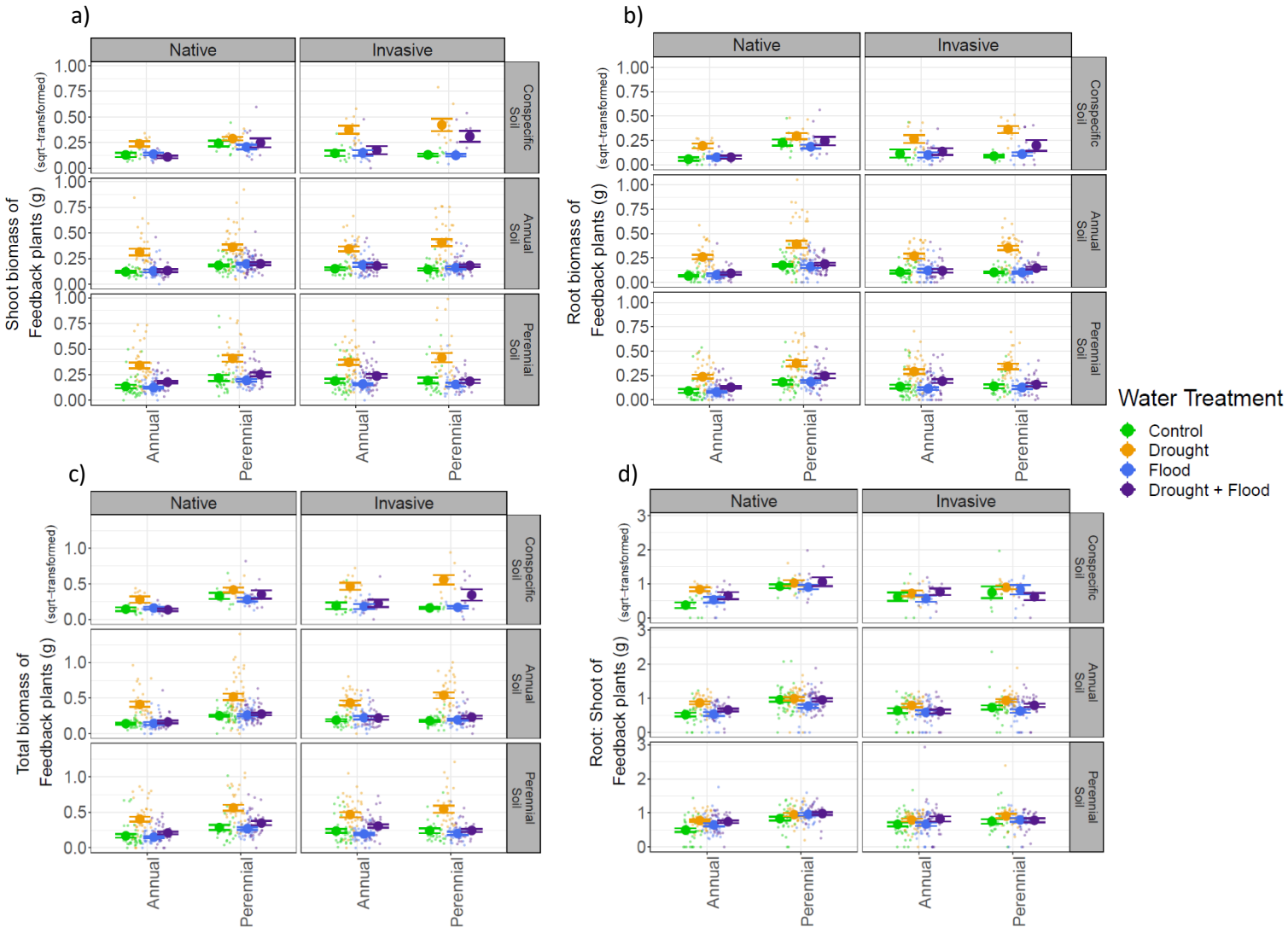
Supplementary figure 10. Species-specific a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of feedback plant response to the soil legacy of separate climatic treatments in the conditioning phase. Species origin (native or invasive) of the feedback plants (top) and the type of conditioning soil (conspecific, native heterospecific and invasive heterospecific) on which the feedback plants are grown (right) are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary figure 11. Averaged a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of feedback plants response to the soil legacy of separate climatic treatments (x-axis) in the conditioning phase. The origin of the feedback plants is shown using the colours grey (native) or red (invasive). The type of conditioning soil (conspecific, native heterospecific and invasive heterospecific) on which the feedback plants are grown are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary figure 12. Averaged a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of feedback plants response to the type of conditioning soil (conspecific, native heterospecific and invasive heterospecific) on which the feedback plants are grown in (x-axis). The plant biomass responses to the soil legacy of separate climatic treatments in the conditioning phase are shown using colours. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE). Different lowercase letters indicate significant differences among means for each variable in post-hoc tests ($p < 0.05$).



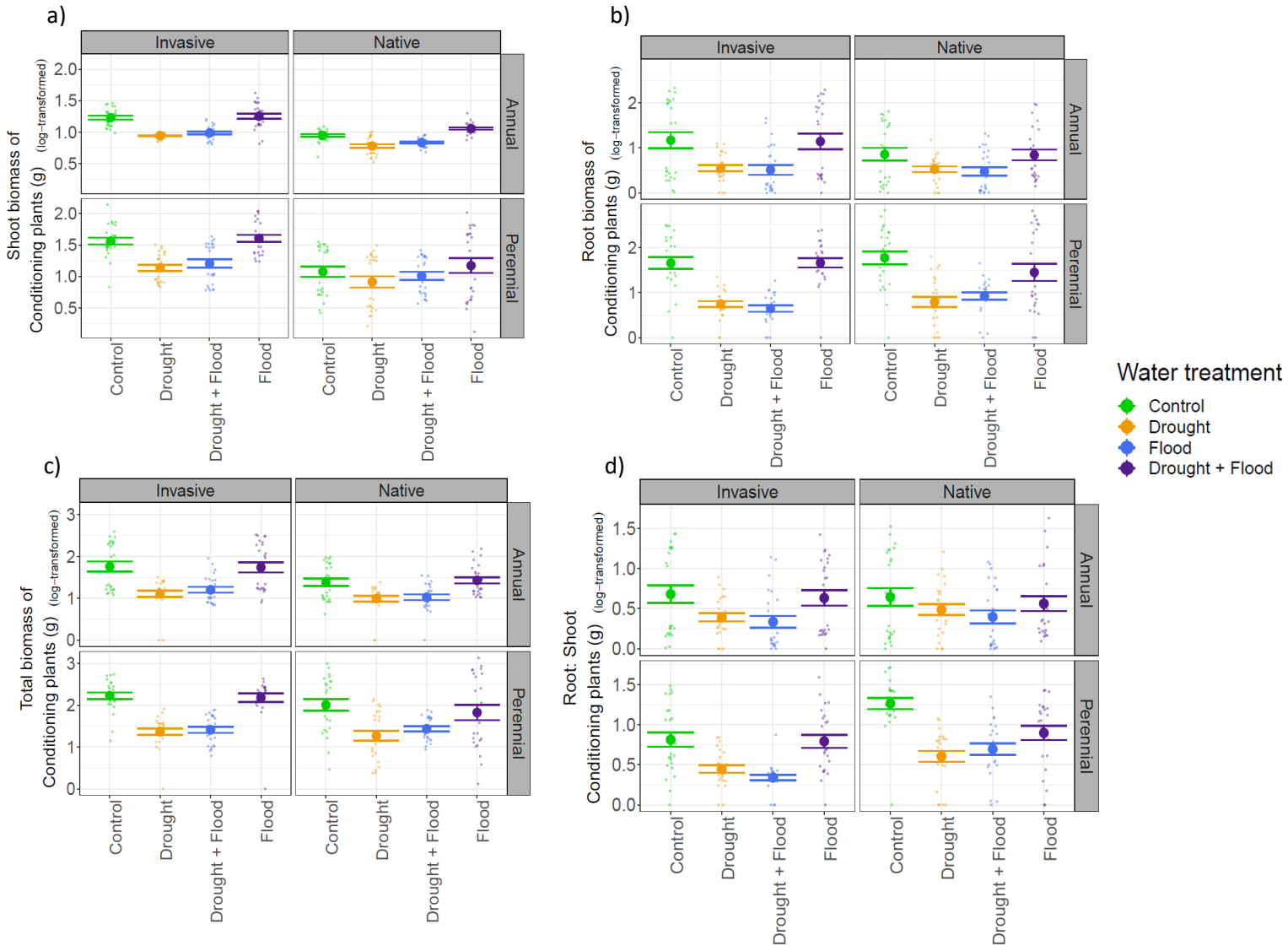
Supplementary figure 13. Averaged a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of feedback plants depending on their life-history strategy (x-axis). Species origin (native or invasive) of the feedback plants (top) and the type of conditioning soil (conspecific, native heterospecific and invasive heterospecific) on which the feedback plants are grown (right) are shown within separate facets. The plant biomass responses to the soil legacy of separate climatic treatments in the conditioning phase are shown using colours. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).

Conditioning phase – plant biomass

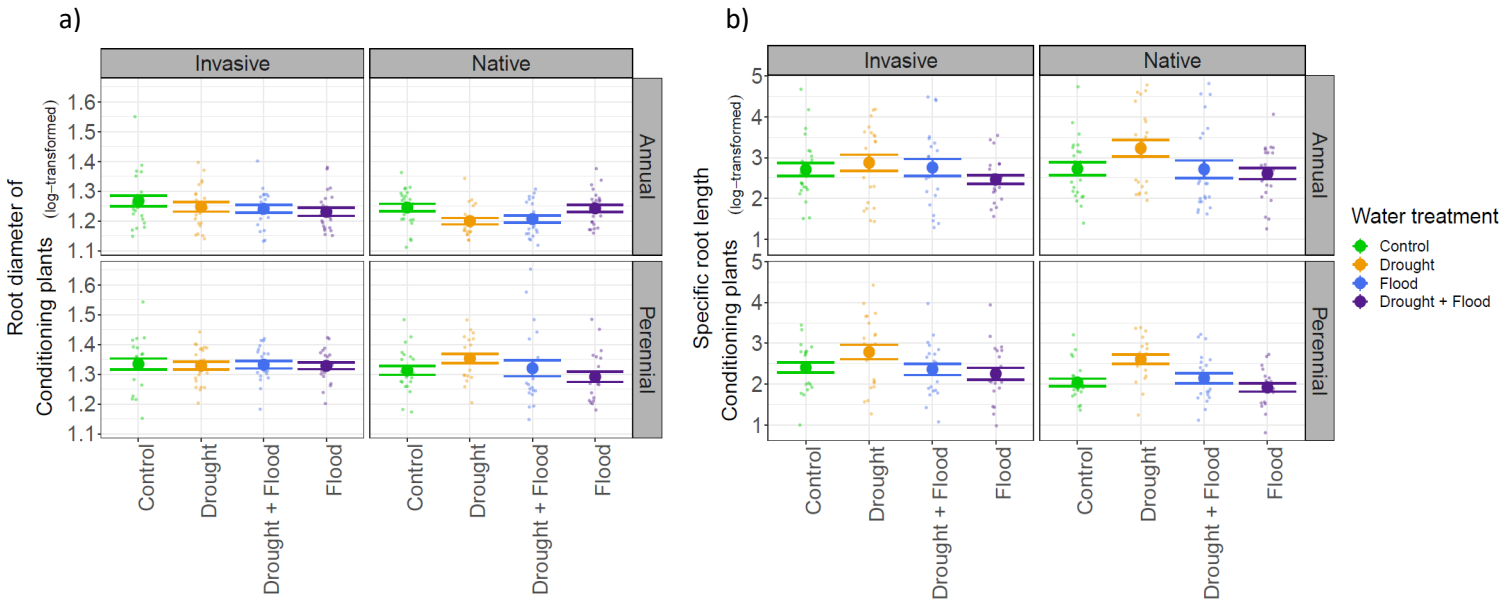
The total biomass accumulated by all eight conditioning plant species differed depending on the single effect of plant origin, plant life history strategy and the climatic treatment (linear model 4: Plant origin: $F\text{-value}_{df} = 16.60_{1,368}$, p-value <0.001, Plant life history: $F\text{-value}_{df} = 59.82_{1,368}$, p-value <0.001, climatic treatment: $F\text{-value}_{df} = 47.38_{1,368}$, p-value <0.001; Supplementary Fig. S.14). In particularly invasive conditioning plants accumulated substantial more biomass than native plants, while perennial plants accumulated more biomass than annual plants (Supplementary Fig. S.14). For the climatic treatment, plant subjected to drought or flood treatments accumulated substantially less biomass than plants that underwent sequential drought and flood treatments or were continuously watered (i.e., control treatments) throughout the experiment. Although invasive conditioning plants tended to accumulate higher total biomass than native plants, for the shoot biomass this difference was greater within perennial plants and plants which were subjected to the flood treatment or controls (linear model 4: Plant life history: $F\text{-value}_{df} = 5.87_{1,368}$, p-value <0.05, climatic treatment: $F\text{-value}_{df} = 3.14_{1,368}$, p-value <0.05; Supplementary Fig. S.14). Similarly, for the belowground biomass where perennial plants generally accumulated more biomass, this difference was greater for plants subjected the flood treatment or well-watered throughout (linear model 4: $F\text{-value}_{df} = 3.34_{1,368}$, p-value <0.05; Supplementary Fig. S.14).

Biomass allocation patterns (root: shoot ratio) differed depending on the single effects of plant origin, life history strategy, and climatic treatment (linear model 4: Plant origin: $F\text{-value}_{df} = 12.22_{1,368}$, p-value <0.001, Plant life history: $F\text{-value}_{df} = 29.07_{1,368}$, p-value <0.001, climatic treatment: $F\text{-value}_{df} = 23.76_{1,368}$, p-value <0.001; Supplementary Fig. S.14). In particular, invasive plants invested significantly less in root biomass compared to native plants, while annual plants allocated less to roots compared to perennials. Plants exposed to drought or sequential drought and flood treatments invested more in root biomass than those subjected to continuous flooding or well-watered conditions. Among native plants, perennials allocated more to root biomass than native annuals, whereas invasive plants consistently invested less in

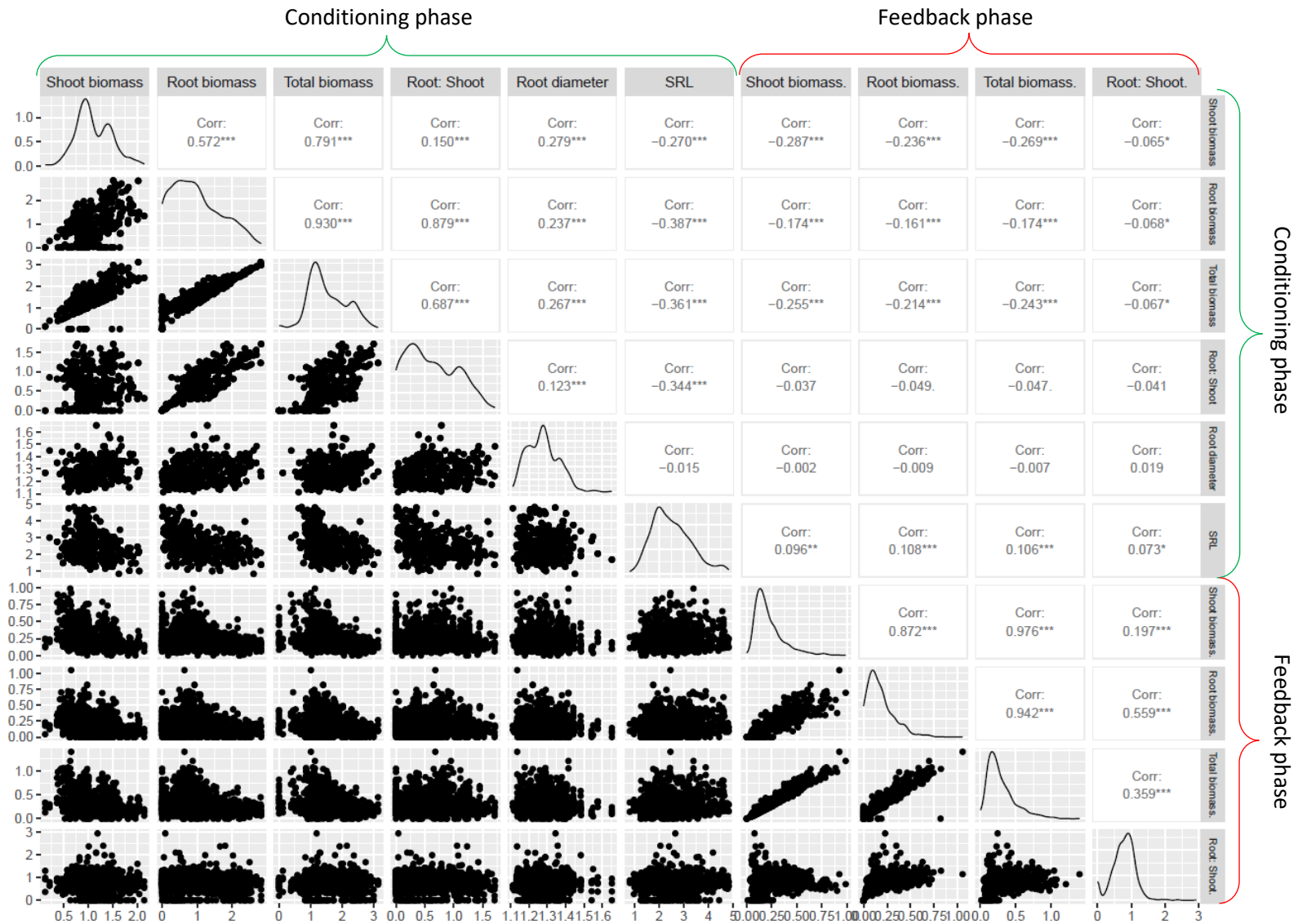
roots, with only a slight increase in root biomass investment within invasive perennial plants (linear model 4: F-value$_{df}$ = 10.15$_{1,368}$, p-value <0.01; Supplementary Fig. S.14).



Supplementary figure 13. Averaged a) shoot biomass, b) root biomass, c) total biomass and d) root: shoot of conditioning plant in response to the separate climatic treatments. Species origin (native or invasive) and life history strategy (annual or perennial) are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary figure 14. Averaged a) root diameter and b) specific root length of conditioning plants in response to the separate climatic treatments. Species origin (native or invasive) and life history strategy (annual or perennial) are shown within separate facets. Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary figure 15. Pairwise correlations between the plant response variables measured in the conditioning and feedback phase, with Pearson correlation coefficients shown in the upper panels. Facet headers along the x-axis and y-axis indicate the variables being compared. The variables were transformed prior to analysis to meet assumptions of normality.

Chapter 2

Intraspecific competition hinders drought recovery in a resident but not in its range-expanding congener plant independent of mycorrhizal symbiosis

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Photo credit: Priyanka Ambavane

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Abstract

Background and Aims

Understanding biotic interactions within plant populations and with their symbiotic partners is crucial for elucidating plant responses to drought. While many studies have highlighted the importance of intraspecific plant or mutualistic fungal interactions in predicting drought responses, we know little about the combined effects of these two interactions on the recovery of plants after drought.

Methods

We conducted an experiment to study the recovery after an extreme drought event of a native European plant species (*Centaurea jacea*) and its range-expanding congener (*Centaurea stoebe*), across a gradient of plant density and in association with an AM fungal species (*Rhizophagus irregularis*).

Results

Our results showed strong intraspecific competition in *C. jacea*, which constrained their post-drought recovery. We further found that AM fungi constrained root biomass recovery of *C. jacea* after drought under high intraspecific competition. The post-drought recovery in *C. stoebe* was high potentially due to its greater plasticity in the root diameter under drought conditions.

Conclusion

Strong intraspecific competition can constrain recovery in plants like *C. jacea* with lesser root trait plasticity after drought, independent of mycorrhizal symbiosis.

Keywords: Density-dependent effects, arbuscular mycorrhizal fungi, range-expanding plants, plant recovery, root traits, extreme abiotic stress

Introduction

Extreme drought events are becoming common and widespread across the biosphere as a result of anthropogenic climate change (IPCC 2023; Liu et al. 2018). The effects of such drought events on plant communities can be dramatic (Luo et al. 2019; Ploughe et al. 2018; Stampfli & Zeiter 2004). For instance, drought can act as a strong environmental filter to eliminate plant species that lack traits for drought tolerance from the plant community (Engelbrecht et al. 2007; Moeslund et al. 2013; Tilman & Haddi 1992). This allows plants with certain traits to persist during extreme drought and subsequently thrive due to reduced competition and surges in nutrient availability upon rewetting (Cleland et al. 2013; Leitner et al. 2017). With extreme drought events becoming more widespread and pronounced with climate change (IPCC 2023; Lange et al. 2020), understanding the mechanisms that underlie plant species recovery after extreme drought is critical to predict and manage ecosystem responses.

The persistence and recovery of plants during and after drought can further depend on their interaction with neighbouring plants (Cadotte & Tucker 2017; Kraft et al. 2014). Numerous recent studies have shown how intraspecific interactions alter plant responses to drought, resulting in either an amplification of negative drought responses (Foxx & Fort 2019; Guo et al. 2020) or facilitation through improved drought tolerance (Wang & Callaway 2021; Zhang et al. 2017). Neighbouring conspecific plants can strongly impede each other's persistence and recovery during and after drought through competition for space, nutrients and light (Foxx & Fort 2019; Guo et al. 2020). On average, such intraspecific interactions can be several folds stronger than interspecific competition in co-occurring plants, as these plants have greater niche overlap, which limits plant performance (Adler et al. 2018). Moreover, at higher plant densities, intraspecific plant competition could lead to reductions in average shoot and root biomass due to limited space and nutrient availability (Postma et al. 2021). This reduction of plant growth, especially root growth, can exacerbate the effects of a disturbance event, such as drought, by impairing water uptake, thereby inducing density-dependent mortality of plants (Casper & Jackson 1997). Examining intraspecific plant interactions is essential for understanding the responses of plant populations to drought. Specifically, as these interactions determine resource availability, competition, facilitation, and ultimately influence the recovery potential of plants within ecosystems.

Intraspecific plant competition can lower soil nutrient availability with subsequent effects on plant-soil biota interactions, such as symbiotic interactions between plant and mycorrhizal fungi (Ayres et al. 2016; Koide 1991). Arbuscular mycorrhizal (AM) fungi have been extensively studied to understand how their positive symbiotic relationships can mitigate the negative effects of drought on the host plant (Augé 2001; Jongen et al. 2022; Worchel et al. 2013). Fungal extraradical mycelia cover a surface area 10- to 1000-times larger than that of root hairs, making mycorrhizal fungi highly efficient in taking up water and nutrients (Goltaph et al. 2008; Marjanović & Nehls 2008). By infecting and spreading within the root cortical cells of host plants, AM fungi form a symbiotic relationship with plants where nutrients such as phosphorus (P) and nitrogen (N) and water are exchanged for photosynthesized carbon and lipid (Wang et al. 2017). Plants have been found to acquire up to 80% of their essential N or P through this symbiosis (van der Heijden 2008) and several meta-analyses have consistently shown that AM fungi can ameliorate the drought stress on plant performance (Delavaux et al. 2017; Hawkins & Crawford 2018; Jayne & Quigley 2014; Kivlin et al. 2013). However, studies investigating the effects of AM fungi on intraspecific competition often show a diminished beneficial effect of AM fungal colonisation compared to communities with interspecific competition (Tedersoo et al. 2020; Guo et al. 2022). This shift in response to mycorrhizal colonisation under intraspecific competition is likely because mycorrhizal fungi intensify competition between plants which overlap in niche and nutrient requirements (Tedersoo et al. 2020; Guo et al. 2022). In contrast, under interspecific competition, mycorrhizal fungi can promote the performance of weaker competitors and dampen competitive interactions (Hart et al. 2003; Wagg et al. 2011). Yet, we know little about how intraspecific plant competition and plant-mycorrhizal symbiosis can interactively affect plants' responses during and after drought events (Birhane et al. 2014; Hawkins & Crawford 2018; Zhang et al. 2011).

Drought can further amplify negative intraspecific competition within plant populations, which could weaken the benefits provided by mycorrhizal fungi to host plants (Hawkins & Crawford 2018). Alternatively, AM fungi can also relax drought-induced amplification of intraspecific plant competition. For instance, the same AM fungi that negatively affected biomass production of plants in ambient water conditions by intensifying intraspecific competition also reduced

intraspecific competition in drier soil conditions, subsequently benefitting plants (Duan et al. 2021; Zhang et al. 2011). The benefits of AM fungi to plant populations during and after a drought may vary depending on mycorrhizal responses to changes in water availability and the intensity of intraspecific plant competition (Birhane et al. 2014; Duan et al. 2021; Hawkins & Crawford 2018; Meisner et al. 2013; Zhang et al. 2011).

Here, we conducted a growth chamber experiment to study the post-drought recovery of two congeneric *Centaurea* plants; a common European resident plant (*Centaurea jacea)* and its congener range-expanding plant (*Centaurea stoebe*), which is expanding its geographic range from southern Europe to northern Europe in recent years (Wilschut et al. 2019). As a result of ongoing climate change, many species are expanding their native range to track their favourable climatic conditions (Anderson 2015; Walther et al. 2002). However, the ability of plants to expand their range is often constrained by the novel biotic and abiotic conditions of the new habitat (Morriën et al. 2010; Spence & Tingley 2020). Range-expanding plants like *C. stoebe* arriving from more arid environments may profit over native plants in drought conditions (Yang et al. 2022), although this may further depend on how intraspecific plant competition limits the *C. stoebe* growth in the presence of AM fungi.

We therefore experimentally manipulated the presence of an AM fungi species (*Rhizophagus irregularis*) and created a density gradient (to create a gradient of intraspecific plant competition) within *C. jacea* and *C. stoebe*. Through this, we aim to investigate the interactive effects of AM fungal colonisation and intraspecific plant competition on their species-specific drought recovery. We hypothesize greater post-drought recovery with decreasing intraspecific plant competition. We also hypothesize greater drought recovery of plants in the presence of AM fungi, though this AM fungi mediated recovery will be dampened at high plant densities (high intraspecific competition).

Material and methods

Study species

Plants – *Centaurea jacea* and *Centaurea stoebe* are herbaceous plants and belong to the family of Asteraceae. *Centaurea jacea* is a perennial flowering plant that is native and widespread throughout Europe. *Centaurea stoebe* is a biennial or short-lived perennial flowering plant that is also native to Europe but is expanding its northern European range due to climate warming (Broennimann et al. 2014; Lauber et al. 2018). Given that previous studies have shown both common and distinct responses of these two plant species to climate change manipulations despite being closely related (Koorem et al. 2021; Quist et al. 2020; Wilschut et al. 2019), we chose these plants to advance the current understanding of plant recovery after drought by exploring their intraspecific interactions and mutualistic interactions with mycorrhizal fungi.
Arbuscular mycorrhizal fungi – We inoculated our study soils with *Rhizophagus irregularis*, previously known as *Glomus intraradices* (Stockinger et al. 2009), a well-known model AM fungi species from the family Glomeraceae (Krüger et al. 2012; Tisserant et al. 2013). *Rhizophagus irregularis* can colonise the roots of numerous plant species, such as our study *Centaurea* species (Bunn et al. 2014; Thakur et al. 2019). As such, it is described as a generalist coloniser of plants with a widespread distribution (Basiru et al. 2021; Savary et al. 2018).

Experimental design

Seeds of both plant species (*C. jacea* and *C. stoebe*) were obtained from a seed company (UFA Samen, Switzerland) and were stored at 4°C before germination. For surface sterilisation, the seeds were bleached for 15 minutes in a 30% bleach solution (commercial bleach with sodium hypochlorite) and rinsed with deionised water subsequently. The germination was initiated on a moist filter paper (using deionised water) in Petri dishes kept in the dark for one week at room temperature (average of ~20 - 22 °C). Subsequently, seedlings were transferred carefully into a multi-pot tray containing sterilised soils (CAPITO line, Landi, Switzerland). We sterilized soils in an autoclave (Systec VX-150, Systec GmbH & Co., Germany) twice at 121°C for 20 minutes, and the two cycles separated by at least 48 hours to target more resistant fungal species that opportunistically spread in the soil. The seedlings in the multi-pot trays grew for one week in the climate chambers at 20°C/16°C at 16 hours day (i.e., with light) and 8 hours night (i.e., dark)

conditions. One-week-old seedlings were then transplanted into 0.7 litre pots (10x10x11cm) containing either the sterilised substrate or the same substrate inoculated with AM fungi.

The soil used in our experiment (both for germination and the main experiment) were a mixture of 50% quartz sand (particle size = 0.3-0.7 mm), 40% universal potting soil (Terre Suisse AG, Switzerland) and 10% perlite (abiotic properties of the substrate: Ph = 6.7, organic matter = 3.4%, N = 0.004%, C = 0.034%, Pbioavailable = 96 mg/kg). The soil was hand-mixed after bigger particles – such as stones, clay and wood – were removed from the potting soil with a coarse-meshed sieve of 0.5 cm mesh. Soil mixtures were also sterilised in an autoclave twice at 121°C for 20 minutes, and the two cycles were separated by at least 48 hours exactly in the same way as the soil used for the germination of plants. The autoclaved soil was then distributed into the plant pots (height = 120 mm, diameter = 140 mm), with a total of 800 g of dry weight in each pot.

For the colonisation of plant roots with mycorrhizal fungi, we used MYC 800 (Andermatt Biocontrol, Switzerland), a powder that is commonly used as a solid fertiliser containing germinating spores of *R. irregularis*. As a supporting substrate, the MYC 800 powder consists of 80% kaolin and 20% diatomite. One gram of this product provides approximately 800 propagules (mainly spores). We inoculated AM fungi treatments with 2 g of this powder (i.e., ~1600 spores of AM fungi). The inoculum was mixed into the substrate before planting the seedlings in order to enable faster contact with the root surfaces of the plant. To control for AM fungi-associated microbes present in the inoculum, we collected a microbial wash by filtering the same amount of inoculum used for the AM fungi treatment with 6 L deionised water through a 25 µm mesh net. The size of the mesh was large enough to allow microbes to pass through and small enough to prevent contamination of mycorrhizal spores and hyphal fragments (Błaszkowski et al. 2008; Taktek et al. 2015). Each pot assigned to non-mycorrhizal (control) treatment received 50 mL (corresponding to the amount of inoculum added to mycorrhizal treatment pots) of the microbial wash when watered for the first time. Analysis of root mycorrhizal colonisation in plants that were grown in soils without AM fungi confirmed that our sterilised soils (added with AM fungi-associated microbes) were free of AM fungal spores.

Seedlings ranging between 1 – 2 cm in height were transplanted into pots with densities ranging from one to five, with and without AM fungal inoculation (Figure 1). Pots were then randomly allocated to four tables in two climate chambers with identical light and temperature settings. In both climate chambers, the plants were exposed to the following growing conditions: 16 hours of daytime at 20°C with a light intensity of ~13,500 lux and 8 hours of night-time at 16°C. The room's relative humidity (RH) was approximately 50% during the day and about 80% during the night. Temperature, light intensity and room's air RH were constantly monitored to account for differences on the four tables in the two climate chambers (Supplementary figure 1). We let the plants grow for a total of nine weeks, within which half the pots were exposed to an extreme drought event (Figure 1). When not subjected to drought, pots were continuously watered every four to five days with 100 ml of deionised water. For the drought treatment, the plants were watered with the same amount for the first three weeks. These three weeks were to enable plants to establish themselves in the soil, but also to facilitate the root colonisation by *R. irregularis,* which is usually well established after around the third or fourth week from the initial colonisation (Corkidi et al. 2004). After that, drought treatments received no water at all for the next three weeks, as shown in Figure 1. We withheld water for three weeks in these treatments to simulate an extreme drought event, pushing many plants to their wilting point as soil water content reached 0% (volumetric water content) (Figure 1). Following this drought period, plants were allowed to recover by rewetting the pots, which was carried out by a regular addition of deionised water (the same way for no drought treatments). Soil moisture was regularly checked with a Soil Moisture Meter TDR 150 (FieldScout, Spectrum Technologies Inc., USA) at two depths (3.8 and 7.6 cm) on 24 extra pots (one for each treatment combination) (Figure 1) in order to monitor soil water availability across treatments without disturbing the main treatment units. Each treatment combination was replicated six times, resulting in a total of 240 pots (2 plant species x 5 densities x 2 AM fungi treatments x 2 drought treatments x 6 replicates) and 720 plant individuals.

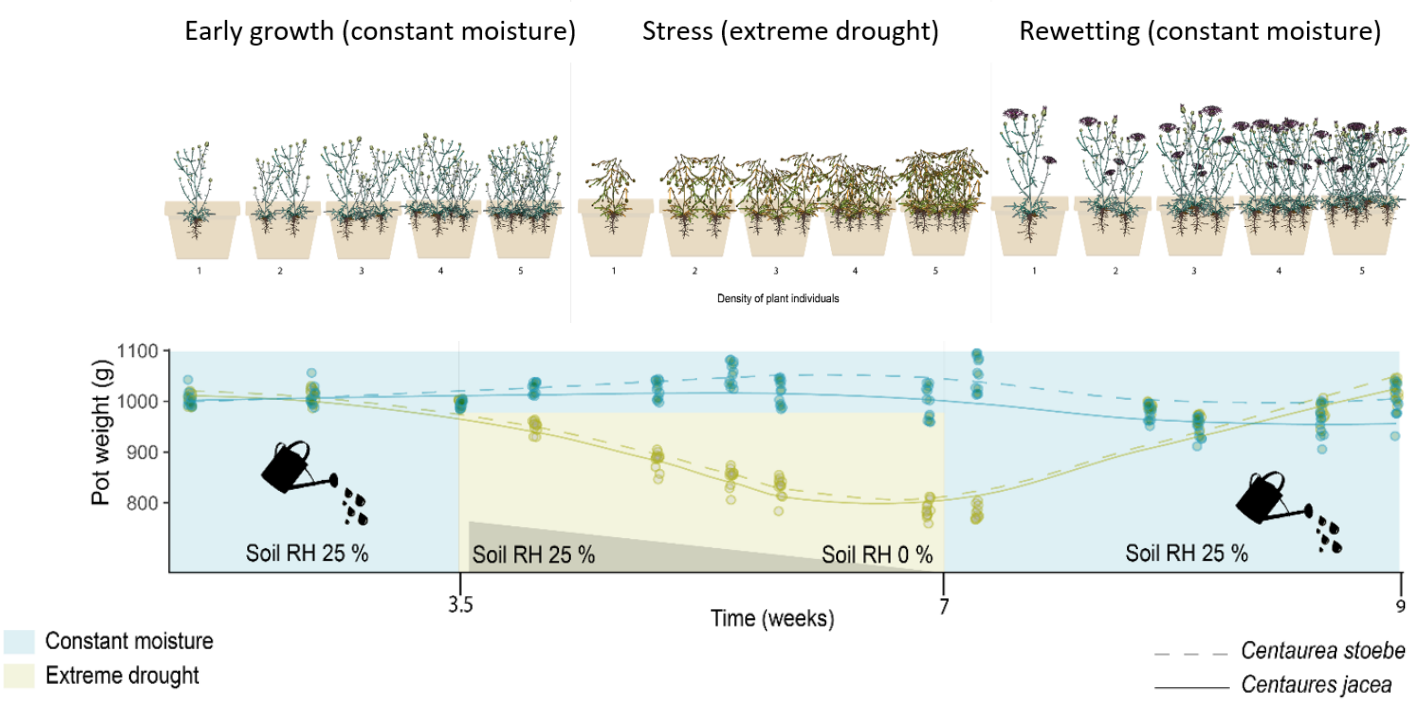

Figure 1 Schematic representation of experimental design with plant density and extreme drought as our main treatments. Temporal soil water content of the pot under drought and control treatments are indicated in the lower panel (data shown from extra pots, details in methods). Plants in different drought and density treatments were inoculated or left un-inoculated with the mycorrhizal fungus *Rhizophagus irregularis*.

Harvest and response variables

The height of each plant was recorded as the distance from the soil surface to the highest point of the upstretched longest leaves every week during the experimental period. Measurement of chlorophyll content was taken before and after the extreme drought of the two youngest fully expanded healthy leaves per plant using a SPAD-502 Chlorophyll Meter (Konica Minolta, Tokyo 100-7015, Japan). After two weeks of post-drought recovery and on the ninth week of the experiment, final measurements of the plant height and chlorophyll content were taken again, and plants were harvested.

During the harvest, the aboveground tissue of each plant was removed just above the soil level, and a single young fully expanded leaf from each plant was cut at the base of the petiole to later measure plant leaf traits. Roots were meticulously washed in order to remove attached substrates,

and root samples of about 1 g (fresh weight) were taken from each pot for mycorrhizal colonisation and root trait measurements. The fresh weight of the remaining root was weighed and then dried in an oven for 3 days at 40°C along with the plant shoot to measure the dry biomass of each plant. Due to the intertwining of roots, it was not possible to measure the dry biomass of each individual plant, as such, the total root biomass per pot was divided by the number of plant individuals to express the average plant root biomass per individual. The fresh leaf and root samples were weighed and scanned using an Epson Perfection V850 Pro Scanner, and were analysed using ImageJ and RhizoVision Explorer v2.0.3 (Rasband 1997; Seethepalli et al. 2021, respectively), to collect data on specific leaf area (SLA; leaf area divided by its dry weight), root diameter and specific root length (SRL; root area divided by root weight). Specific root length was estimated as the ratio of root length to its dry mass. Leaf samples were also dried as described above to calculate the leaf dry matter content (LDMC) as the leaf fresh weight divided by their dry biomass (Cornelissen et al. 2003).

We also estimated carbon, nitrogen and their ratio (C: N ratio) of belowground and aboveground plant organs by dry combustion of ground root and leaf material using a CN elemental analyser (CNS-Analyzer: Elementar vario EL cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) following the Micro-Dumas combustion method (Stewart et al. 1963). Sample preparation, prior to C and N analyses, consisted of grounding of one young fully expanded dry leaf (for density treatment with more than 1 individual, only a leaf from a random individual was chosen) and root samples (for density treatment with more than 1 individual the pool of root of each pot) material using tissue lyser machine (QIAGEN Tissue Lyser II Retsch MM400, Düsseldorf, Germany) and record the exact weight of the tissue powder (around 2 mg).

Finally, we measured the percentage of total root AM fungal colonisation and specific AM fungal structures by staining roots with dye (Pelikan 4001 ink) using techniques modified from Philips and Hayman (1970). This allowed us to visualise colonisation of mycorrhizal structures within the roots. Once stained, root samples were immersed in a mixture of water, glycerin and lactic acid (v:v:v) and were inspected under a Leica S9i Microscope (55x magnification)(Leica Microsystems, Wetzlar, Germany). To measure the percentage of mycorrhizal colonisation, we used the modified gridline intersect method from Giovannetti & Mosse (1980). Root length

colonisation (%) was calculated as a measure of all mycorrhizal structures present in the root, also using the equation presented in Giovannetti & Mosse (1980).

Statistical analysis

For non-temporally measured response variables (i.e., only at the end of the experiment), we used linear mixed-effects models to test the effects of plant density (as a continuous variable), AM fungi and drought treatment on plant responses, while using pot placement (i.e., two different tables used in each climate room-thus, four tables in total) as a random intercept (to account for any variability in light intensity among the four tables). For the temporal data collected for plant height, plant leaf production and chlorophyll content throughout the study, we used linear mixed-effect models: fixed effects in these models were the same as for the previous models, whereas the random effects were the week of measurement and pot placement (e.g., following the model structure of the lme4 package in R: biomass~plant density*AMF treatment*drought treatment + (1|random effect1) + (1|random effect2)). Mixed-effects models were run using the lme4 package (Bates 2015) for R statistical software v4.0.3 (R Core Team 2020). The treatment effects in mixed models were evaluated with a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for the estimation of degrees of freedom, using the lmerTest package v4.0.3 (Kuznetsova et al. 2017). Model assumptions (e.g., homogeneity of variance and normality of residuals) were inspected visually for each linear model. To meet the model assumptions, some response variables were log-transformed (indicated in Table 1). We ran all mixed-models separately for *C. jacea* and *C. stoebe*, given that we expected the effects of all treatments to be general across the two species and to further reduce the complexity of models. Conditional R^2 values were taken as the proportion of total variance explained through both fixed and random effects of the linear models and their statistical significance was obtained from the r2glmm package v4.0.3 (Nakagawa & Schielzeth 2012; Jaeger 2017). To explore the strength of relationships between two variables for understanding of potential mechanisms, we tested correlations between response variables such as, root N content and root mycorrhizal colonisation, using major axis regression models (RMA) with the lmodel2 package v4.0.3 (Legendre 2018). We further ran a multivariate statistical test (PERMANOVA) with 999 permutations via the adonis2 function and carried out a principle component analysis (PCA) using the vegan package v4.0.3 (Oksanen et al. 2020). The PCA allowed us to analyse variation

in multiple plant trait responses including those of specific leaf area (SLA), leaf dry matter content (LDMC), leaf number, leaf chlorophyll content (measured as SPAD), root diameter and specific root length (SRL). For the PCA, we only chose two extremes of density treatments (density=1 and density= 5) to understand how plant traits at these two ends may help explain post-drought recovery in two plants. We ran PCA in the vegan package (Oksanen et al. 2020) and used the scores of the first and second PCA axes (as they two explained most of the variation) to represent overall variation in response to drought and intraspecific competition. A multivariate statistical test (PERMANOVA) was run using the adonis2 function in the vegan package. All (data) figures were created using the ggplot 2 package v4.0.3 (Wickham 2016).

Results

Plant biomass responses

Increasing plant density consistently decreased shoot and root biomass of individual plants in both *C. jacea and C. stoebe* (shoot: $F_{1,111} = 173.49$, $P < 0.001$ and $F_{1,111} = 202.54$, $P < 0.001$; root: $F_{1,111} = 156.74$, $P < 0.001$ and $F_{1,111} = 162.56$, $P < 0.001$, for both *C. jacea* and *C. stoebe* respectively; Table 1 & Figure 2), leading to a 76% decrease in total biomass (shoot + root) of plant individuals at the highest plant density in *C. jacea* and a 71% decrease in *C. stoebe*, compared to the lowest plant density treatments ($F_{1,111} = 180.73$, $P < 0.001$ for *C. jacea* and $F_{1,111} = 191.20$, $P < 0.001$ for *C. stoebe*; Table 1 & Figure 2). Increasing plant density further increased the root: shoot ratio of *C. stoebe* ($F_{1,109} = 6.06$, $P < 0.05$; Table 1 & Figure 2), while *C. jacea* was unaffected ($F_{1,107} = 1.72$, $P = 0.19$). Recovery from the extreme drought event exacerbated these negative plant density effects on plant biomass at the end of the experiment, specifically by reducing root biomass in *C. jacea* (significant interaction between drought and density, $F_{1,109} = 11{,}28$, $P < 0.01$) but not in *C. stoebe* ($F_{1,110} = 0.63$, $P = 0.43$; Table 1 & Figure 2).

The presence of AM fungal species increased shoot biomass of both plants ($F_{1,110} = 17.71$ and $F_{1,108} = 28.16$, $P < 0.001$, for *C. jacea* and *C. stoebe* respectively; Table 1 & Figure 2). These biomass responses to AM fungi shifted depending on the drought treatment and plant density. For example, *C. jacea* individuals subjected to extreme drought responded negatively to AM fungi resulting in decreased root and total biomass (Root: $F_{1,111} = 6.61$, $P < 0.05$; Total Biomass:

$F_{1,111} = 6.58$, $P < 0.05$; Table 1 & Figure 2); whereas, in *C. stoebe* these conditions led to a shift in root: shoot ratio with reduced root biomass allocation ($F_{1,107} = 4.26$, $P < 0.05$; Figure 2). At high plant density, *C. jacea* and *C. stoebe* plants also responded negatively to AM fungi with further reductions in their shoot biomass ($F_{1,111} = 9.93$, $P < 0.01$ for *C. jacea*; $F_{1,110} = 14.82$, $P < 0.001$ for *C. stoebe*; Table 1 & Figure 2). However, in *C. stoebe* individuals recovering from drought, the presence of AM fungi at high plant densities resulted in a significant increase in the root: shoot ratio ($F_{1,105} = 4.96$, $P < 0.05$; Table 1 & Figure 2).

Temporal plant responses

We found that extreme drought induced a complete mortality of the plants within three pots (no recovery after rewetting), all of which were *C. jacea* at the highest population density in our experiment, with two of them inoculated with AM fungi and one without. Apart from these plants, recovery was visible for most plants, with a 34% increase in height for *C. jacea* and a 5% increase for *C. stoebe* during the recovery period after rewetting of pots (Temporal height data: Supplementary figure 2; Temporal SPAD data: Supplementary figure 3).

Table 1 Results from linear mixed-effect models testing the effects of extreme drought and rewetting (DR), intraspecific competition intensity (DEN), and AM fungi presence (AM fungi) for *C. jacea* and *C. stoebe* (with table number as a random effect). Bold values are statistically significant ($P < 0.05$). Green upward arrows indicate a significant increase, whereas red downward arrows indicate a significant decrease in a given response variable. Biomass was calculated as an average per individual. Conditional R^2 represents the combined effects of fixed and random effects used in our models. We also provide overall model R^2 for all mixed-effect models used in our study. df stands for degrees of freedom.

	Response Type	Response Variable	Drought (DR)			AMF			Density (D)			DR x AMF			DR x DEN			AMF x DEN			DR x AMF x DEN			Random intercept	Model
			$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	$F\text{-value}_{df}$	p-value	R^2 (conditional)	Variance	R^2
C. jacea	Biomass	Average total biomass	$1.5_{1,109}$	0.22	0.01	$1.28_{1,111}$	0.26	0.01	$180.73_{1,111}$	**<0.001** ↓	0.62	$6.61_{1,111}$	**<0.05** ↓	0.06	$0.26_{1,109}$	0.61	0.00	$0.99_{1,111}$	0.32	0.01	$3.34_{1,111}$	0.07	0.03	0.00	0.65
		Average shoot biomass	$0.40_{1,109}$	0.53	0	$17.71_{1,110}$	**<0.001** ↑	0.14	$173.49_{1,111}$	**<0.001** ↓	0.61	$1.43_{1,110}$	0.24	0.01	$2.40_{1,109}$	0.12	0.02	$9.93_{1,111}$	**<0.01** ↓	0.08	$0.43_{1,111}$	0.51	0.00	79.81	0.65
		Average root biomass (log-transformed)	$0.26_{1,109}$	0.61	0	$<0.01_{1,111}$	1.00	0.00	$156.74_{1,111}$	**<0.001** ↓	0.59	$6.58_{1,111}$	**<0.05** ↓	0.06	$11.28_{1,109}$	**<0.01** ↓	0.09	$0.19_{1,111}$	0.66	0.00	$2.58_{1,111}$	0.11	0.02	0.00	0.66
		Root:shoot	$1.51_{1,104}$	0.22	0.01	$9.24_{1,107}$	**<0.01** ↓	0.08	$1.72_{1,107}$	0.19	0.02	$1.98_{1,107}$	0.16	0.02	$0.02_{1,104}$	0.88	0.00	$0.61_{1,107}$	0.43	0.01	$1.55_{1,107}$	0.22	0.02	0.00	0.30
	Leaf Traits	LDMC	$<0.01_{1,105}$	0.93	0	$0.31_{1,106}$	0.58	0.00	$3.92_{1,107}$	0.05	0.04	$4.12_{1,106}$	**<0.05** ↓	0.04	$11.38_{1,105}$	**<0.01** ↓	0.10	$3.71_{1,106}$	0.06	0.03	$0.05_{1,106}$	0.83	0.00	58.78	0.52
		SLA (log-transformed)	$0.09_{1,106}$	0.77	0.03	$0.09_{1,108}$	0.76	0.03	$8.34_{1,108}$	**<0.01** ↓	0.01	$5.75_{1,108}$	**<0.05** ↑	0.05	$9.10_{1,106}$	**<0.01** ↑	0.04	$2.58_{1,108}$	0.11	0.01	$<0.01_{1,108}$	0.98	0.00	0.00	0.48
	Root Traits	Root Diameter	$0.04_{1,103}$	0.84	0.00	$1.72_{1,105}$	0.19	0.02	$2.93_{1,105}$	0.09	0.03	$0.16_{1,104}$	0.69	0.00	$0.74_{1,102}$	0.39	0.01	$0.21_{1,105}$	0.65	0.00	$<0.01_{1,103}$	0.96	0.00	0.00	0.21
		SRL	$0.38_{1,102}$	0.54	0.00	$<0.01_{1,103}$	0.97	0.00	$1.73_{1,105}$	0.19	0.02	$0.37_{1,102}$	0.55	0.00	$0.57_{1,102}$	0.45	0.01	$0.30_{1,104}$	0.58	0.00	$0.58_{1,102}$	0.45	0.01	20.35	0.12
	Nutrient	Leaf N% (log-transformed)	$0.06_{1,105}$	0.81	0.00	$0.15_{1,107}$	0.70	0.00	$1.01_{1,108}$	0.32	0.01	$8.26_{1,107}$	**<0.01** ↑	0.07	$10.59_{1,106}$	**<0.01** ↑	0.09	$2.63_{1,107}$	0.11	0.02	$0.79_{1,107}$	0.38	0.01	0.00	0.48
		Leaf C:N (log-transformed)	$0.03_{1,106}$	0.86	0.00	$0.34_{1,108}$	0.56	0.00	$1.07_{1,108}$	0.30	0.01	$8.21_{1,107}$	**<0.01** ↓	0.07	$10.66_{1,106}$	**<0.01** ↓	0.09	$2.83_{1,108}$	0.10	0.03	$0.89_{1,108}$	0.34	0.01	0.00	0.47
		Root N% (log-transformed)	$0.32_{1,109}$	0.57	0.00	$0.02_{1,111}$	0.89	0.00	$1.38_{1,111}$	0.24	0.01	$7.58_{1,111}$	**<0.01** ↑	0.07	$7.61_{1,109}$	**<0.01** ↑	0.06	$0.53_{1,111}$	0.47	0.01	$1.95_{1,111}$	0.16	0.02	0.00	0.41
		Root C:N	$0.59_{1,109}$	0.44	0.01	$0.17_{1,111}$	0.68	0.00	$4.15_{1,111}$	**<0.05** ↑	0.04	$8.69_{1,111}$	**<0.01** ↓	0.07	$11.14_{1,109}$	**<0.01** ↓	0.09	$1.09_{1,111}$	0.30	0.01	$2.23_{1,111}$	0.14	0.02	0.00	0.50
C. stoebe	Biomass	Average total biomass	$0.68_{1,109}$	0.41	0.01	$4.64_{1,107}$	**<0.05** ↑	0.04	$191.20_{1,111}$	**<0.001** ↓	0.64	$1.18_{1,108}$	0.28	0.01	$0.23_{1,111}$	0.63	0.00	$3.95_{1,109}$	**<0.05** ↓	0.04	$1.14_{1,108}$	0.29	0.01	964.40	0.64
		Average shoot biomass	$0.08_{1,110}$	0.78	0.00	$28.16_{1,108}$	**<0.001** ↑	0.20	$202.54_{1,112}$	**<0.001** ↓	0.65	$0.23_{1,109}$	0.63	0.00	$0.07_{1,111}$	0.79	0.00	$14.82_{1,110}$	**<0.001** ↓	0.12	$0.13_{1,109}$	0.72	0.00	0.00	0.68
		Average root biomass	$1.61_{1,108}$	0.21	0.02	$0.42_{1,107}$	0.52	0.00	$162.56_{1,111}$	**<0.001** ↓	0.60	$2.96_{1,108}$	0.09	0.03	$0.63_{1,110}$	0.43	0.01	$1.07_{1,108}$	0.30	0.01	$2.62_{1,108}$	0.11	0.02	1079.00	0.61
		Root:shoot	$0.85_{1,106}$	0.36	0.01	$17.60_{1,106}$	**<0.001** ↓	0.14	$6.06_{1,109}$	**<0.05** ↑	0.05	$4.26_{1,107}$	**<0.05** ↓	0.04	$0.15_{1,108}$	0.70	0.00	$0.64_{1,107}$	0.42	0.01	$4.48_{1,106}$	**<0.05** ↑	0.04	0.04	0.42
	Leaf Traits	LDMC	$0.44_{1,110}$	0.51	0.00	$1.28_{1,108}$	0.26	0.01	$13.46_{1,112}$	**<0.001** ↓	0.11	$0.02_{1,109}$	0.88	0.00	$1.22_{1,111}$	0.27	0.01	$0.13_{1,110}$	0.72	0.00	$0.13_{1,109}$	0.71	0.00	0.00	0.16
		SLA	$0.97_{1,110}$	0.33	0.01	$1.42_{1,108}$	0.24	0.01	$0.04_{1,112}$	0.84	0.00	$0.07_{1,109}$	0.79	0.00	$0.74_{1,111}$	0.39	0.01	$0.68_{1,110}$	0.41	0.01	$0.37_{1,109}$	0.54	0.00	0.00	0.03
	Root Traits	Root Diameter	$0.64_{1,106}$	0.43	0.01	$12.94_{1,105}$	**<0.001** ↑	0.11	$0.78_{1,107}$	0.38	0.01	$5.53_{1,105}$	**<0.05** ↓	0.05	$1.13_{1,107}$	0.29	0.01	$0.53_{1,106}$	0.47	0.01	$4.96_{1,105}$	**<0.05** ↑	0.04	0.00	0.34
		SRL	$0.01_{1,104}$	0.93	0.00	$0.45_{1,104}$	0.50	0.00	$1.48_{1,107}$	0.23	0.01	$0.29_{1,104}$	0.59	0.00	$<0.01_{1,107}$	0.95	0.00	$0.03_{1,105}$	0.87	0.00	$0.28_{1,104}$	0.60	0.00	0.00	0.05
	Nutrient	Leaf N%	$4.23_{1,110}$	**<0.05** ↑	0.04	$0.16_{1,108}$	0.69	0.00	$5.45_{1,112}$	**<0.05** ↓	0.05	$1.41_{1,109}$	0.24	0.01	$0.34_{1,111}$	0.56	0.00	$0.03_{1,110}$	0.86	0.00	$0.38_{1,109}$	0.54	0.00	0.00	0.16
		Leaf C:N	$3.57_{1,108}$	0.06	0.04	$0.03_{1,107}$	0.87	0.00	$7.10_{1,111}$	**<0.01** ↑	0.06	$3.03_{1,108}$	0.08	0.03	$<0.01_{1,111}$	1.00	0.00	$<0.01_{1,108}$	0.96	0.00	$1.87_{1,108}$	0.17	0.02	0.41	0.22
		Root N%	$8.73_{1,110}$	**<0.01** ↑	0.07	$0.06_{1,108}$	0.81	0.00	$28.64_{1,112}$	**<0.001** ↓	0.21	$0.01_{1,109}$	0.93	0.00	$1.07_{1,111}$	0.30	0.01	$0.22_{1,110}$	0.64	0.00	$<0.01_{1,109}$	0.97	0.00	0.00	0.33
		Root C:N	$4.24_{1,109}$	**<0.05** ↓	0.04	$0.06_{1,107}$	0.80	0.00	$30.48_{1,111}$	**<0.001** ↑	0.22	$0.04_{1,108}$	0.85	0.00	$0.04_{1,111}$	0.84	0.00	$0.12_{1,109}$	0.72	0.00	$0.01_{1,108}$	0.94	0.00	2.37	0.35

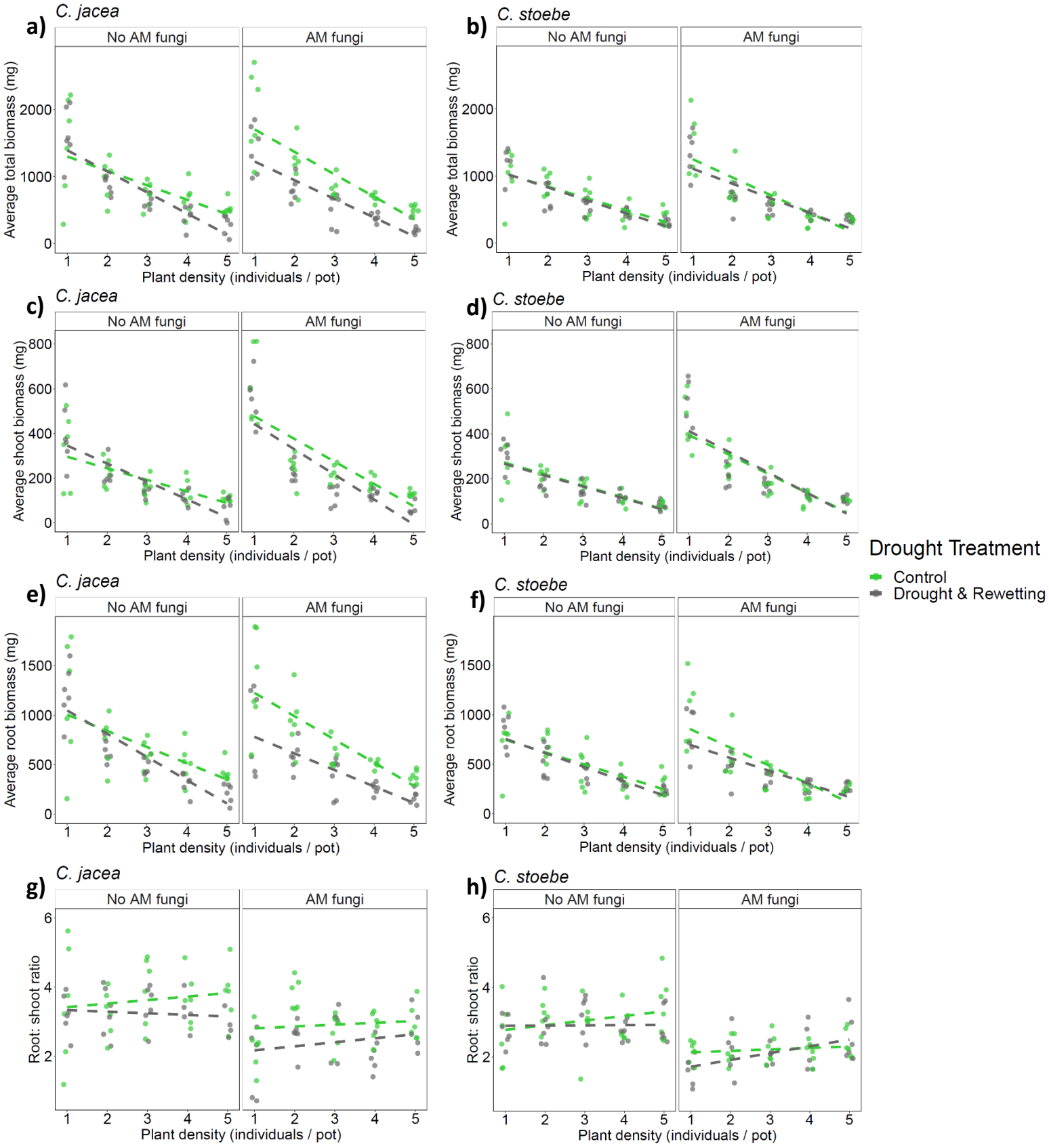

Figure 2 Plant biomass responses of *C. jacea* (left: a, c and e) and *C. stoebe* (right: b, d and f) to drought, AM fungi and plant density. Average plant total biomass: a, b; Average aboveground

biomass: c, d; Average belowground biomass: e, f; Root to shoot ratio. Raw data are shown as points, whereas dashed lines are based on linear regressions.

Plant Trait Responses

Leaf morphological traits – Leaf trait were lesser responsive to AM fungi or to drought treatment compared to plant density in both species (Table 1). Increasing plant density reduced leaf trait values in both *C. jacea* and *C. stoebe*, such as declines in LDMC in *C. stoebe* ($F_{1,112} = 13.46$, $P < 0.001$; Table 1 & Supplementary figure 4) and SLA in *C. jacea* ($F_{1,108} = 8.34$, $P < 0.01$; Table. 1). Leaf trait responses to AM fungi in *C. jacea* were, however, dependent on other treatments, such as drought, in which AM fungi induced a greater decline in LDMC ($F_{1,106} = 4.12$, $P < 0.05$; Table 1 & Supplementary figure 4) and an increased SLA, although only in *C. jacea* ($F_{1,108} = 5.75$, $P < 0.05$; Table 1).

Root morphological traits – Root trait responses to the experimental treatments were species dependent and only evident in *C. stoebe*, not in *C. jacea* (Table 1; Figure 3). In *C. stoebe*, the presence of AM fungi increased root diameter ($F_{1,105} = 12.94$, $P < 0.001$; Table 1 & Figure 3), however, when subjected to extreme drought, the presence of AM fungi decreased root diameter ($F_{1,105} = 5.53$, $P < 0.05$; Table 1). By contrast, we found increase in root diameter among *C. stoebe* individuals after the extreme drought event in the presence of AM fungi when grown at high densities ($F_{1,105} = 4.96$, $P < 0.05$; Table 1 & Figure 3).

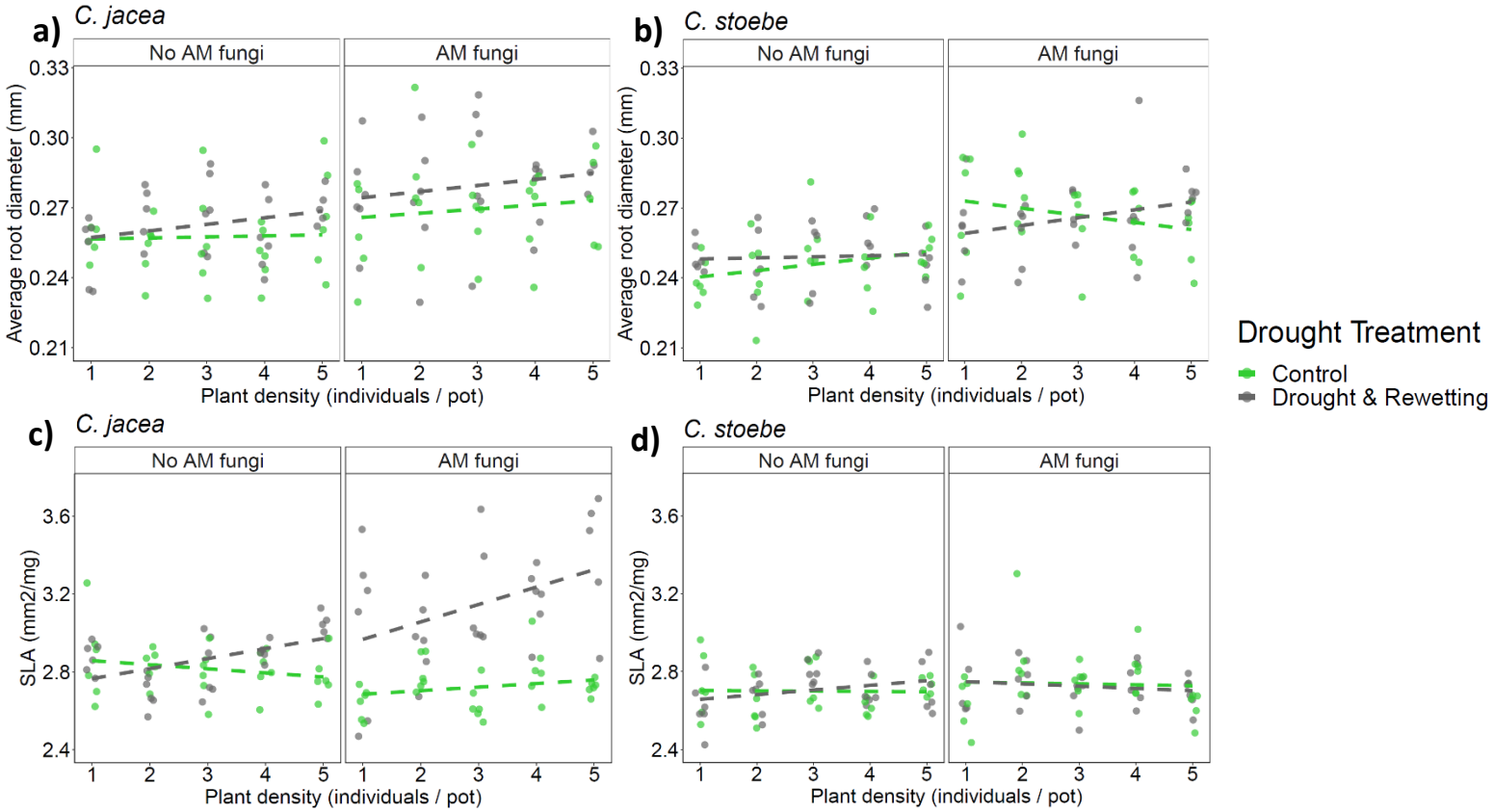

Figure 3 Plant average root diameter responses of *C. jacea* (a) and *C. stoebe* (b) and specific leaf area (log-transformed) of *C. jacea* (c) and *C. stoebe* (d) to drought, AM fungi and plant density. Drought treatments are indicated in grey, control indicated in green. Raw data are shown as points, whereas dashed lines are based on linear regressions.

Plant nutrient content:

Centaurea stoebe plants showed a significant increase in leaf and root nitrogen (N) content to extreme drought ($F_{1,110} = 4.23$, $P < 0.05$; $F_{1,110} = 8.73$, $P < 0.01$, for leaf and root N, respectively). Increasing plant density had the opposite effect in *C. stoebe* resulting in declines in leaf and root N content ($F_{1,112} = 5.45$, $P < 0.05$; $F_{1,112} = 28.64$, $P < 0.001$, for leaf and root N, respectively; Table 1 & Figure 4) and subsequent increases in leaf and root C: N ratio ($F_{1,111} = 7.10$, $P < 0.01$; $F_{1,111} = 30.48$, $P < 0.001$, for leaf and root C: N, respectively; Figure 4). Changes to these responses in combination with other treatment were not statistically significant (Table 1). In *C. jacea,* plants subjected to the extreme drought event only substantially increased their leaf and root N content the presence of AM fungi (leaf: $F_{1,107} = 8.26$, $P < 0.01$ and root: $F_{1,111} = 7.58$, $P < 0.01$) or when grown at high plant densities (leaf: $F_{1,106} = 10.59$, $P < 0.01$ and root: $F_{1,109} = 7.61$, $P < 0.01$; Table 1 & Figure 4).

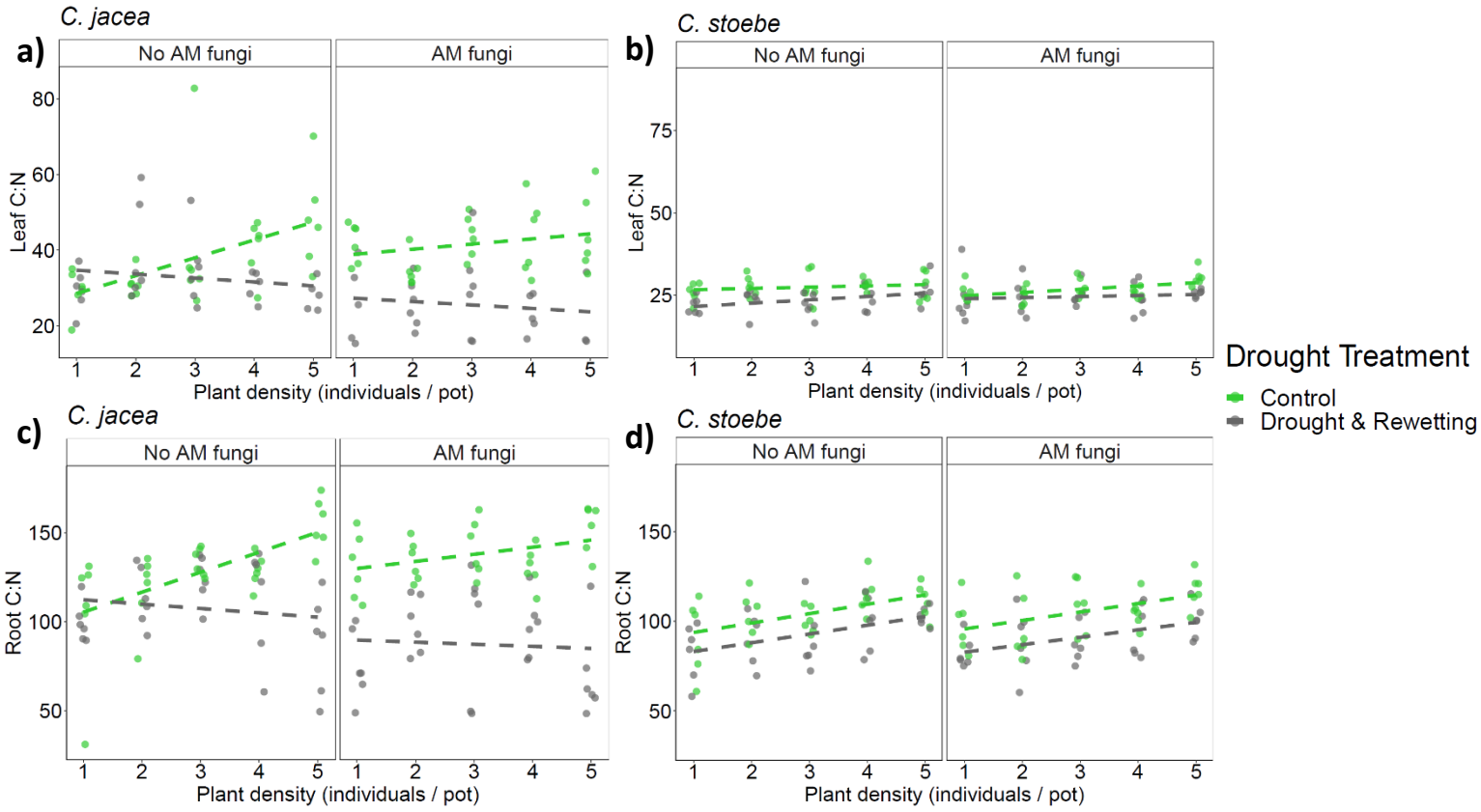

Figure 4 Plant nutrient content responses of *C. jacea* (left) and *C. stoebe* (right) to drought, AM fungi presence and plant density. Drought treatment indicated in grey, control indicated in green. Leaf C: N ratio: a, b; Root C: N ratio: c, d. Raw data are shown as points, whereas dashed lines are based on linear regressions.

Mycorrhizal colonisation responses

Root colonisation in the AM fungal treatment averaged 13.7% (standard deviation (sd) = 7.9, min = 1.7%, max = 32.9%) and 21.3% (sd = 11.5, min = 0.6%, max = 54%) for *C. jacea* and *C. stoebe*, respectively, while all plants not grown in soil inoculated with AM fungi showed no root fungal colonisation. Root colonisation by AM fungi in *C. jacea* plants declined due to extreme drought or due to plant density ($F_{1,47} = 8.96$, $P < 0.01$ and $F_{1,47} = 4.20$, $P < 0.05$, for drought and plant density, respectively; Supplementary figure 5). *Centaurea stoebe* plants did not show any variation in their root mycorrhizal colonisation in response to either extreme drought or to increased plant density. We found no interactive effects of extreme drought and plant density on the root colonisation by AM fungi in both plants (Supplementary figure 5). Root colonisation by

AM fungi associated with other response variables, but only in *C. jacea*. For instance, root colonisation was positively associated with root biomass (R^2=0.12, P <0.05) but negatively associated with SLA (R^2=0.14, P <0.01 and root N (R^2 = 0.15, P <0.05; Supplementary figure 6).

Principle component analysis

We found a significant variation in trait responses to density in both plant species (pseudo $F_{1,63}$ = 13.34, P <0.001 and pseudo $F_{1,96}$ = 18.79, P <0.001; for *C. jacea* and *C. stoebe* respectively, Figure 5) as well as significant variation in trait responses to drought but only for *C. jacea* (pseudo $F_{1,34}$ = 7.23, P <0.001; Figure 5). This suggests that trait responses in *C. jacea* were influenced by both drought and plant density treatments, while in *C. stoebe*, trait variability was primarily driven by plant densities (Figure 5).

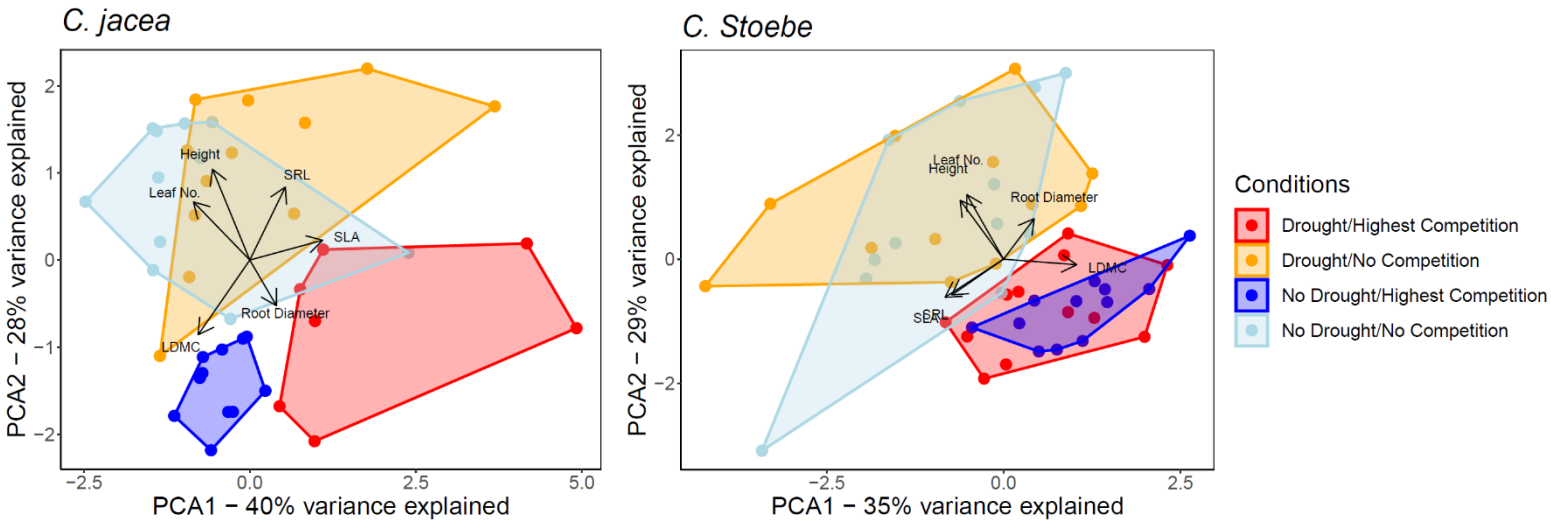

Figure 5 Principle component analysis (PCA) of plant responses to drought (red and orange colours = extreme drought, blue and azure = constant moisture) and intraspecific competition ((Highest Competition = 5 individuals competing, No Competition = 1 individual alone)) using plant trait variables. Arrows generated based on loading values.

Discussion

With increasing drought frequency and severity, it is important to investigate how biotic interactions influence plant drought tolerance and recovery, as this has important repercussions on the plant community composition and functioning (Walter 2018). In this study, we investigated how increasing plant densities (as a gradient of intraspecific plant competition) impacted the post-drought recovery of *C. jacea* and *C. stoebe*. We found that the biomass recovery of plants after rewetting following an extreme drought was constrained by intraspecific plant competition, although only true for the native resident *C. jacea* (Table 1; Figure. 2). For instance, increasing plant densities induced a strong negative effect on root biomass of *C. jacea* especially when subjected to drought, indicating how intraspecific interactions can modulate post-drought recovery (Table 1; Figure 2). Moreover, density-constrained plant recovery of the resident native *C. jacea* was not ameliorated in the presence of AM fungi. Root trait responses, namely root diameter, provide insight into the underlying mechanisms contributing to the reduced drought recovery under high intraspecific competition within *C. jacea* compared to *C. stoebe.*

Intraspecific competition can intensify drought effects by restricting both the biomass accumulation in plants and their ability to spread in the soil, hindering their capacity to obtain sufficient water supply (Casper & Jackson 1997; Foxx & Fort 2019; Postma 2021; Rehling et al. 2021). Our results highlighted this, as one major difference between *C. stoebe* and *C. jacea* was that *C. stoebe* allocated substantially more biomass into root growth when grown in high densities at the expense of shoot growth (as indicated by increased root: shoot ratio; Table 1; Figure 2). This shift in biomass allocation allows *C. stoebe* to still meet its nutrient and water requirements even in populations with high intraspecific competition. Such shifts in biomass allocation strategies at high plant densities have been reported previously (Ravenek et al. 2016; Rehling et al. 2021), and we suspect that this may have allowed *C. stoebe* to persist and recover after drought in their high density treatments (Table 1; Figure 2).

We further suspect that *C. stoebe* utilized AM fungal symbiosis more effectively to recover after drought event compared to *C. jacea*, particularly at high intraspecific competition. For instance, in the presence of AM fungi alone, *C. stoebe* produced thicker roots (higher diameter), possibly to optimise the symbiosis with AM fungi as thicker roots are usually associated with high AM

fungi colonisation (Table 1; Figure 3; Wen et al. 2019; Bergmann et al. 2020). By contrast, in the presence of AM fungi and drought, *C. stoebe* produced thinner roots (low diameter) in soils. This effect of drought on root diameter of *C. stoebe* was, however, overturned at high plant density, where *C. stoebe* again had thicker roots (Table 1; Figure 3). This demonstrates how plasticity in root trait responses allows *C. stoebe* to tolerate different biotic and abiotic stressors. Such plasticity in trait responses may enable range-expanding plants, such as *C. stoebe* to adapt to local conditions and thus promote their establishment even under drought stress (Usui et al. 2023).

The lack of root trait plasticity in *C. jacea* may have contributed to its greater vulnerability to extreme drought event in our study, particularly when intraspecific competition was high. This is further illustrated in our PCA, which showed variability in trait responses of *C. jacea* to both drought event and plant density, while trait variability in *C. stoebe* were mainly driven by plant densities (Figure 5). These findings highlight the importance of a plants' trait plasticity in adapting to changing environmental conditions and help us to better understand variability in plant recovery after extreme drought events (Berg & Ellers; Thakur et al. 2022).

Despite some evidence for AM fungal-mediated benefits to *C. stoebe* after the drought in high intraspecific competition (Table 1), our results are less conclusive on the positive roles of AM fungi to foster plant recovery after extreme drought, at least with a single AM fungal species used in our study. Indeed, AM fungi has been well-studied for ameliorating the effects of drought on their host plants (Delavaux et al. 2017; Jayne & Quigley 2014). By contrast, in both plants we found indications that plants in symbiosis with AM fungi may have a disadvantage when subjected to adverse conditions, particularly high intraspecific competition. Both *C. stoebe* and *C. jacea* produced more aboveground biomass in AM fungal soils (Table 1; Figure 1). However, for both plant species, these responses shifted from positive to negative in high plant density treatments, indicating that the negative impact of intraspecific competition topples any positive AM fungal effects (Table 1; Figure 2). Such density-dependent reduction in AM fungal benefits for plants could be due to an increase in the cost: benefit ratio of mycorrhizal colonisation with plant roots, as competition for light increases with increasing plant density and photosynthetic ability declines (Koide & Dickie 2002). As such, plants may become more carbon limited than P or N limited and benefit less for mutualistic exchange of nutrients with AM fungi

(Koide & Dickie 2002; Pérez & Urcelay 2009; Werner et al. 2018). Furthermore, this (plant) density dependent decline in mutualistic interactions between plants and AM fungi may have further implications on the ability of AM fungi to mitigate negative plant responses to droughts.

Although intraspecific competition strongly constrained the post-drought recovery of *C. jacea* (Table 1), their nutrient values, such as N content (leaf and root), showed a substantial increase after the drought event, particularly in the presence of AM fungi at high plant densities. Whether such shifts in N content were costly for their biomass recovery or if they would have fostered recovery, in the long run, remains to be tested. Both drought and plant density also negatively influenced mycorrhizal root colonisation in *C. jacea* (Supplementary figure 5). Reduced soil moisture can instigate the dieback of other beneficial microorganisms than AM fungi (Preece et al. 2019; Xu et al. 2020), and the accumulation of fresh litter (leaves and roots) from drought-stressed plants. Subsequent rewetting of the soil during the recovery period may have initiated rehydration and lysis of the dead microbial cells, as well as a boost in microbial activity (Borken & Matzner 2009; Brangari et al. 2021; Leitner et al. 2017). Rewetting of the soil can thereby create a temporary pulse of soil nitrogen due to increased accessibility of N through diffusive transportation and accelerated microbial activity and N mineralisation (Gao et al. 2020; Rennenberg et al. 2009). This increase in nutrient availability upon rewetting has been well established in other studies (Gao et al. 2020). Indeed, in *C. stoebe*, rewetting led to an increase in root nitrogen (Table 1; Figure 4); however, in *C. jacea*, the benefits from rewetting appeared to be contingent on the extent of intraspecific competition and the presence of arbuscular mycorrhizal fungi (Table 1; Figure 4; Supplementary figure 6).

In conclusion, we highlight how two closely related plants might have different strategies to recover from the drought when growing under intraspecific competition combined with interactions with mycorrhizal fungi. The results of our study are indeed limited in their application as we used only two plant species with a single species of AM fungi. Nevertheless, the AM fungi used in our study is one of the most studied mycorrhizal fungi, and both *Centaurea* species are commonly found in temperate grasslands, which makes our findings relevant for highlighting the importance of intraspecific root trait variation for understanding the recovery of grassland plants after extreme drought. Moreover, although our study found that AM fungi or

intraspecific plant competition impacted the outcome of the drought treatment on both plant species, we did not differentiate if these plant responses were a direct response to the low soil moisture content or an indirect response via the shifts in soil microbial activity and nutrient availability during the rewetting period. We encourage future studies to consider indirect pathways, such as the co-response of other soil microorganisms in presence of AM fungi to be able to better explain how rewetting after the drought in presence of symbiotic and competitive interactions impact plant performance in grasslands.

Statements & Declarations

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Competing interests

The authors declare that they have no conflict of interest.

Author contributions

MPT conceived the study. LF, MF and SKDS performed the experiment. SKDS and LF analysed the data with inputs from MPT. SKDS wrote the manuscript with substantial contributions from LF and MPT.

Data availability

Data supporting this study are openly available from DRYAD.

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Supporting Information

Figure 1. Climatic conditions measured throughout the experiment.

Figure 2. Temporal changes in plant height throughout the experimental period.

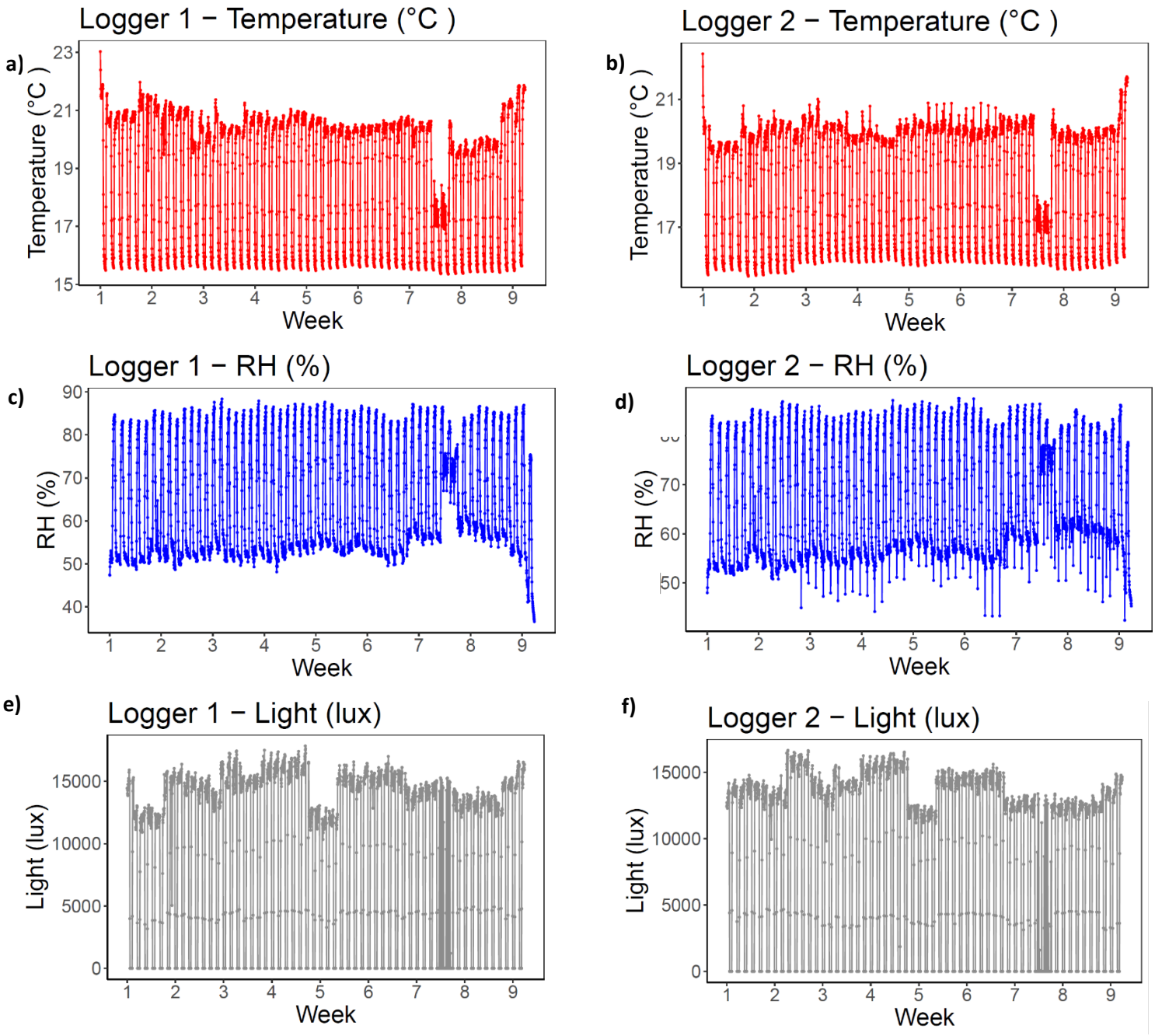
Figure 3. Temporal changes in chlorophyll content (SPAD) throughout the experimental period.

Figure 4. Plant trait responses measured after recovery.

Figure 5. Proportion of root length colonised by AM fungi.

Figure 6. Correlation between root colonisation by AM fungi and root N content.

Supplementary material

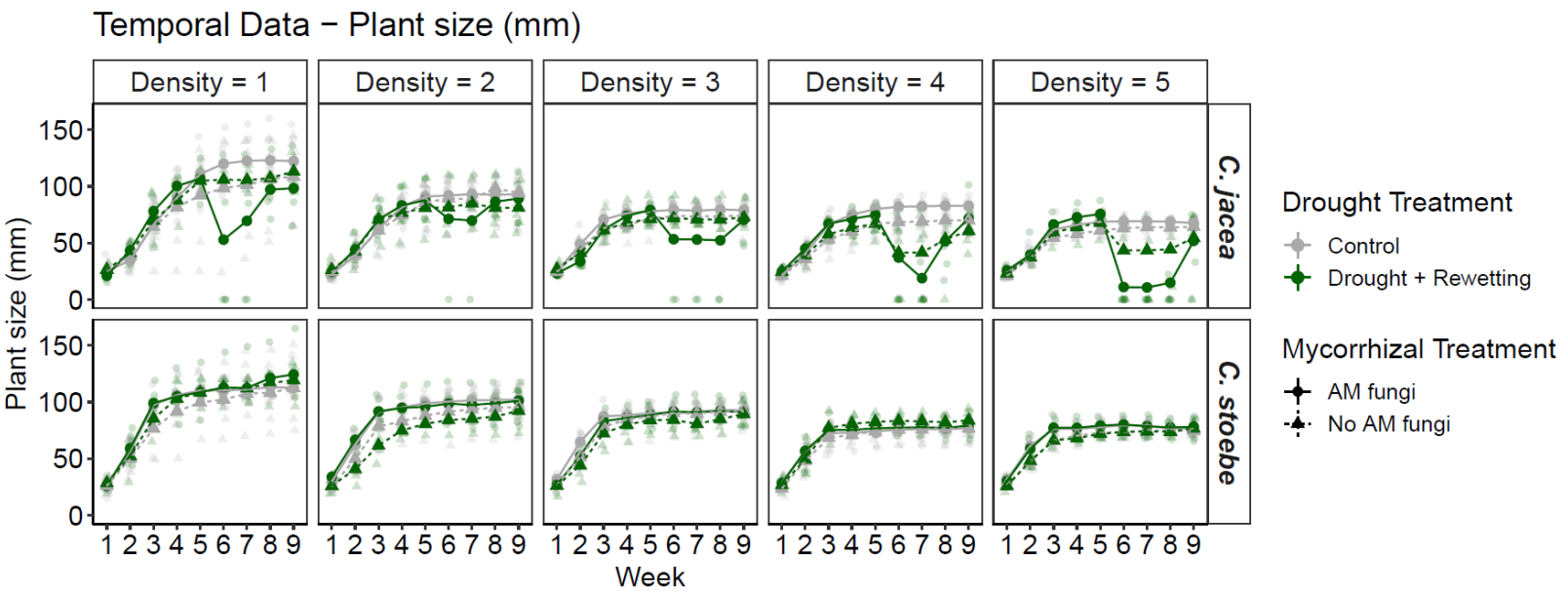


Supplementary figure 1. Climatic conditions measured in both climate chambers throughout the experiment. Logger 1 was placed in room D321 and logger 2 in room D322 (Baltzerstrasse 6, 3012, Bern). Temperature (a, b), relative humidity (RH) (c, d) and light (e, f) recorded from logger one (left) and two (right). Due to a malfunction error of the lighting system between week

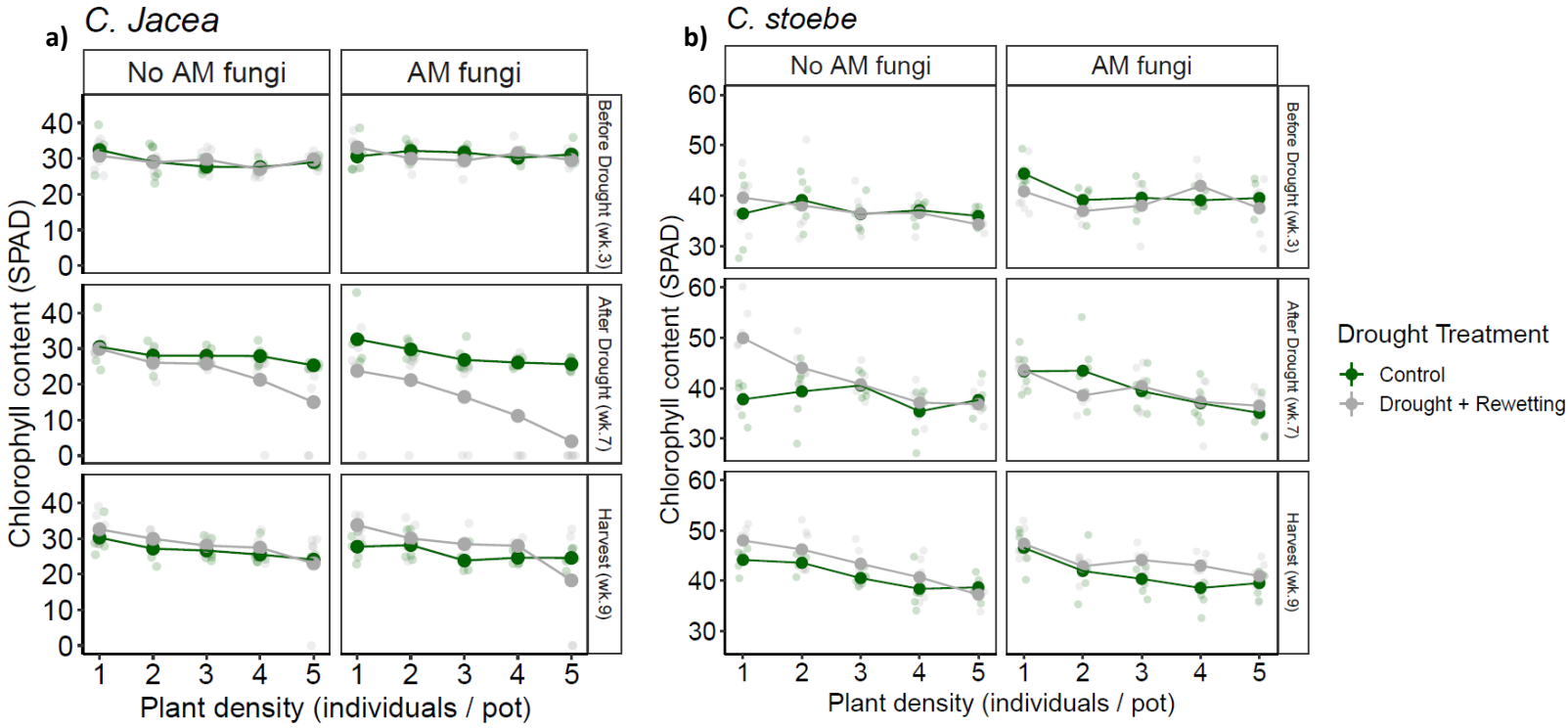
7 to 8, lux values overly fluctuated in both room for 48 hours (2/12/20 7.00 am – 4/12/20 6.30 am) resulting in a decrease in temperature (°C) and increase in RH (%).

Temporal plant responses

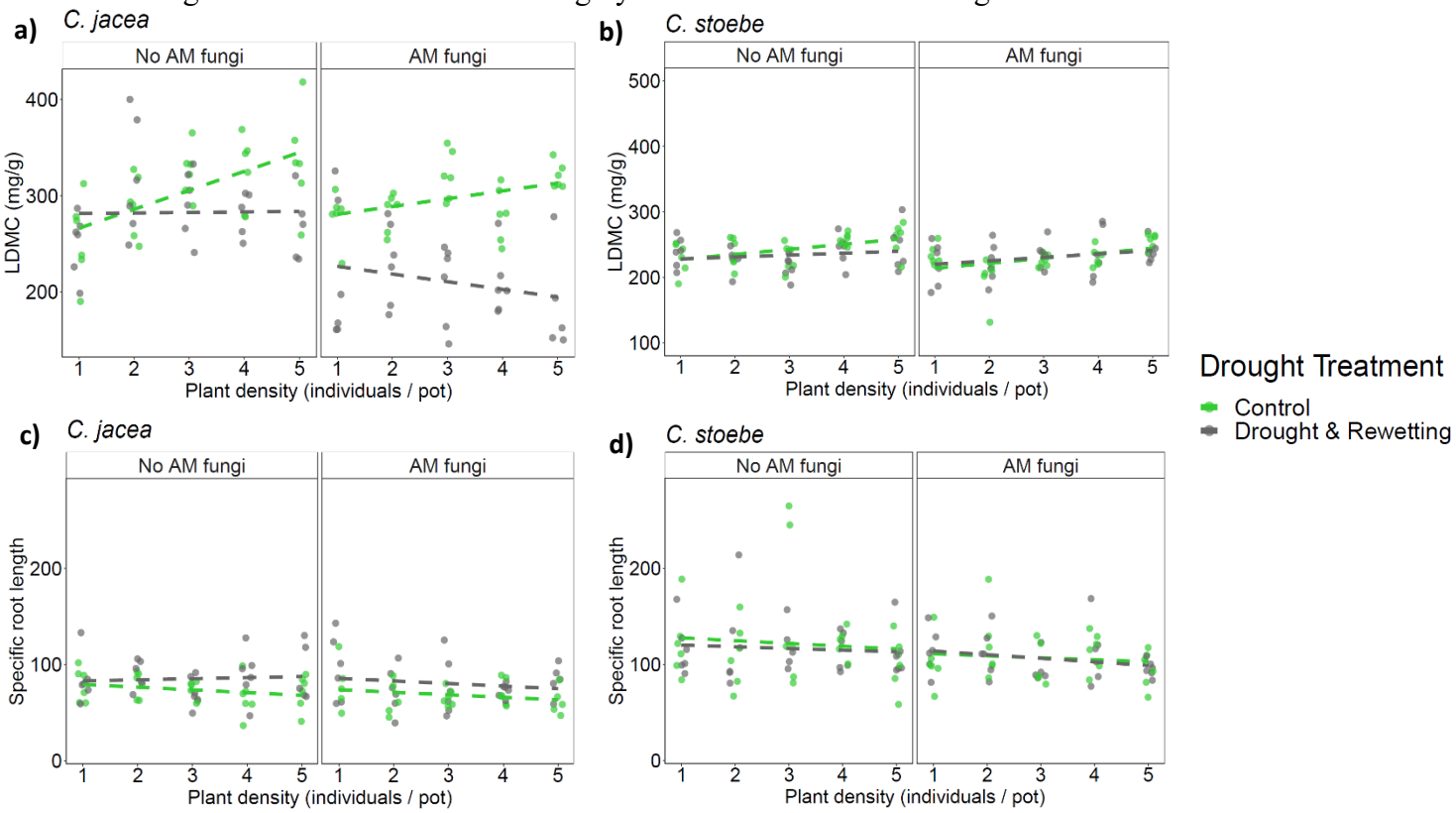
High plant density decreased plant height over time in both species ($F\text{-value}_{df} = 114.21_{1,341}$, p-value <0.001 for *C. jacea* and $F\text{-value}_{df} = 246.87_{1,340}$, p-value <0.001 for *C. stoebe*), while drought had negligible effect (Supplementary figure 2). In *C. stoebe*, AM fungi had a positive influence on plant height over time, ($F\text{-value}_{df} = 13.49_{1,332}$, p-value <0.001). However, at higher plant densities this positive effect of AM fungi on plant height was dampened ($F\text{-value}_{df} = 4.23_{1,334}$, p-value <0.05; Supplementary figure 2). SPAD was negatively affected by high plant density over time in both species ($F\text{-value}_{df} = 45.72_{1,337}$, p-value <0.001 for *C. jacea* and F-valuedf= 104.201,340, p-value <0.001, for C. stoebe). In *C. stoebe*, drought led to a reduction in SPAD ($F\text{-value}_{df} = 6.38_{1,331}$, p-value <0.5), however, the presence of AM fungi helped to ameliorate the reduction in chlorophyll content due to drought ($F\text{-value}_{df} = 22.13_{1,335}$, p-value <0.001; Supplementary figure 3). This positive effect of AM fungi on plant chlorophyll content was exclusive to plant individuals subjected to drought but not to control (watered) treatments. Indeed, in watered conditions AM fungi actually negatively impacted SPAD in plant communities at low densities, however, this effect disappeared at higher densities resulting in a three-way interaction ($F\text{-value}_{df} = 16.03_{1,333}$, p-value <0.001; Supplementary figure 3).



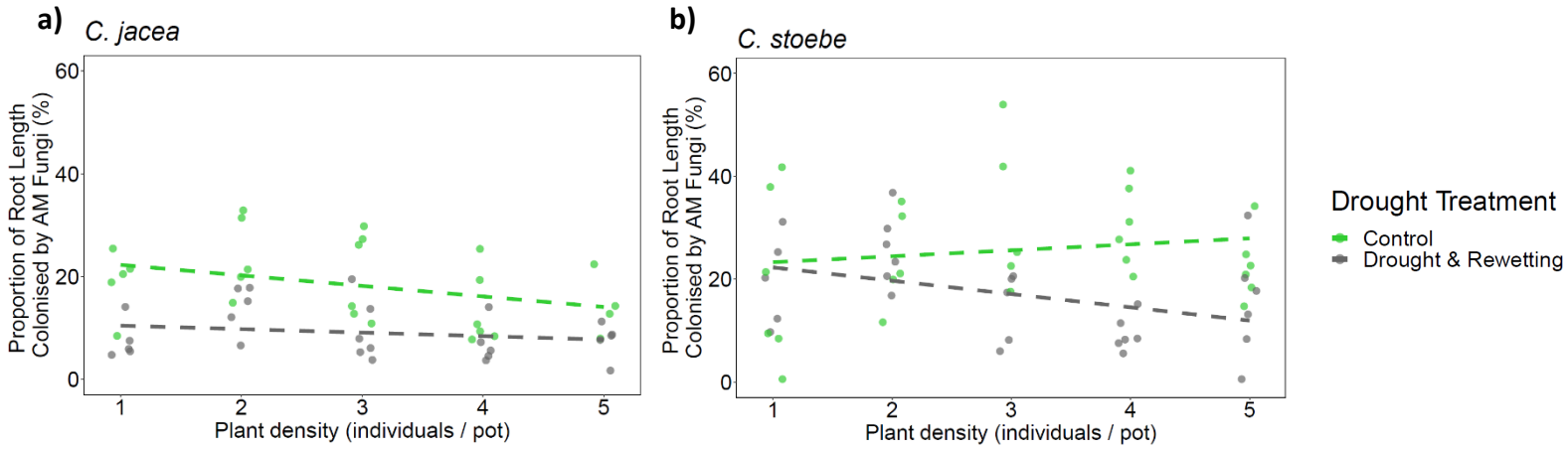
Supplementary figure 2. Temporal changes in plant height throughout the experimental period. Drought treatment indicated in grey and watered conditions indicated in green. Plants grown in the presence of AM fungi shown with triangle symbols and control conditions shown with square symbols. Plants with severe wilting were not measured and marked as 0cm to prevent damaging the plant.



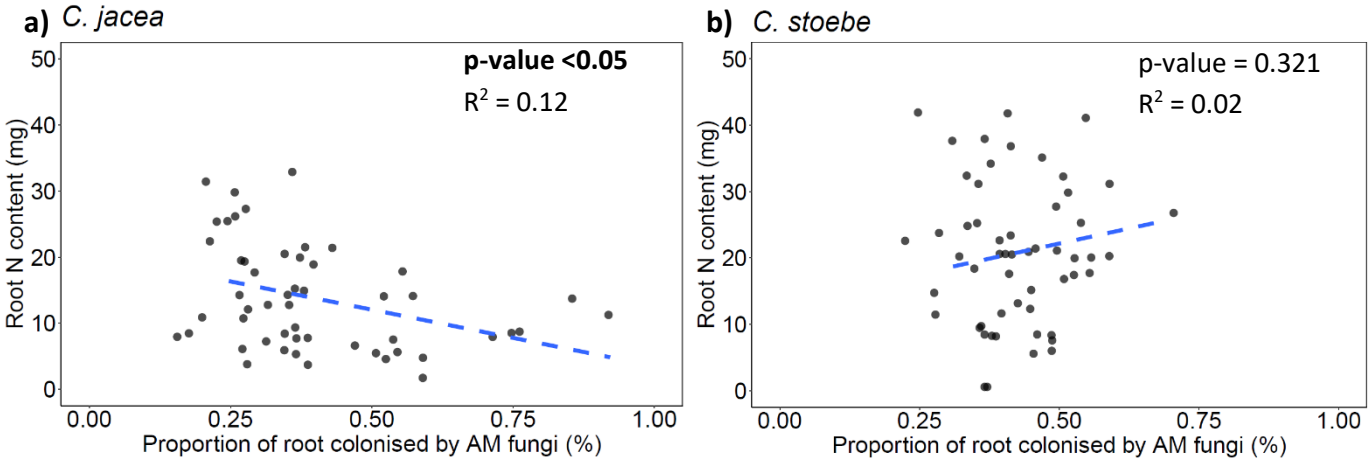
Supplementary figure 3. Chlorophyll content of *C. jacea* (a) and *C. stoebe* (b) measured before and directly after the drought event (before re-wetting) as well as at the end of the experiment. Drought conditions are indicated in grey and watered conditions in green.



Supplementary figure 4. Responses of plant traits of *C. jacea* (left) and *C. stoebe* (right) to drought, AM fungi and plant density measured after recovery. Drought treatments are indicated in grey, control indicated in green. Leaf dry matter content: a, b; specific root length: c, d.



Supplementary figure 5. Proportion of root length colonised by AM fungi of *C. jacea* (a) and *C. stoebe* (b) in response to drought, AM fungi and plant density.



Supplementary figure 6: Correlation between root colonisation by AM fungi and root N content in C. jacea (a) and C. stoebe (b). The shaded area represents the 95% confidence interval of the fit, p-value and R2 values calculated from ranged major axis (RMA) models.

Chapter 3

Drought and mycorrhizal-mediated soil legacies alter the invasion success of *Solidago canadensis* in non-native soils

Shareen K.D. Sanders, Ludovico Formenti, Akshay Bharadwaj, Eric Allan, Madhav P. Thakur

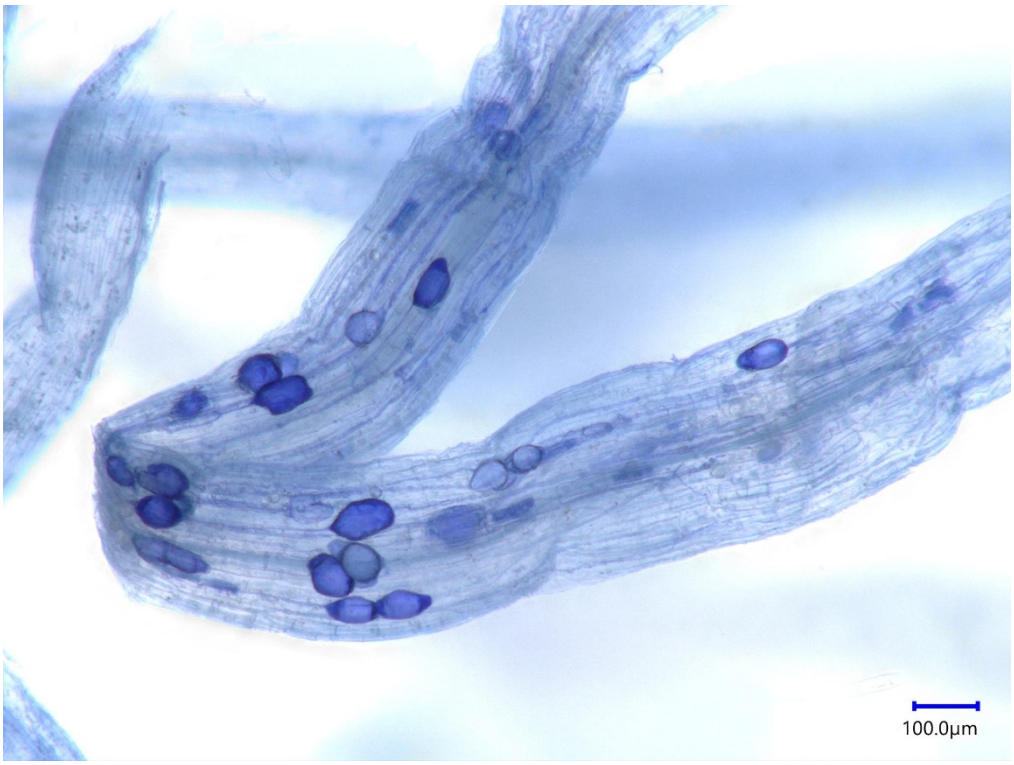


Photo credit: Ludovico Formenti

Manuscript in preparation

Abstract

The susceptibility of native ecosystems to invasion is likely shaped by the biotic and abiotic characteristics of the soil that influence the performance of an invasive plant species. Plant-soil feedbacks (PSFs) driven by the trait composition of native plant communities can alter soil nutrient and microbial dynamics, influencing invasion success. Extreme drought may further influence this PSF by disrupting plant performance and interactions, creating conditions favourable for invasive species. However, the role of beneficial soil microorganisms, such as arbuscular mycorrhizal (AM) fungi, in mitigating or enhancing these PSF effects remains poorly understood. In this study, we employed a PSF approach to investigate how the presence of AM fungi alters the plant-soil feedback effect from native plant communities, differing in traits and diversity, and from soil drought legacies on *Solidago canadensis*, a wide-spread invasive plant species in Europe. Our results suggest that the presence of AM fungi interacts with the abiotic conditions and the diversity of native plant communities to influence the performance of *S. canadensis*. Specifically, AM fungi enhanced the biomass accumulation of S. canadensis in soils conditioned by monocultures but reduced its growth in more diverse mixed plant communities, particularly those with a drought legacy. These findings illustrate the context-dependent role of AM fungi and drought in shaping plant-soil feedbacks and emphasise the importance of interactive effect between biotic and abiotic factors on the invasibility of native ecosystems.

Keywords: Invasion, plant-soil feedback, soil microbial diversity, arbuscular mycorrhizal fungi, Extreme drought

Introduction

The spread of invasive plant species is a major threat for native biodiversity in grasslands (1). Detection of native communities with high invasibility, where management strategies are most effective, has remained a challenge within invasion biology (2,3). To predict whether native communities are vulnerable to successful invasion, it is vital to establish which biotic and abiotic characteristics that contribute to the invasion success of non-native plants within a community (3). Among these factors, the role of soil biota in facilitating or hindering invasion has garnered increasing research attention in recent years (4–6). The roles of soil microorganisms in driving plant performance became apparent to ecologists mainly through plant-soil feedback (PSF) experiments (7). PSFs are soil legacies from the growth and interactions of soil occupying plants (conditioning plants), which shape the nutritional and microbial composition of the soil, on which the performance of secondary plants (feedback or test plants) is measured. As such, PSFs play an important role in determining the biotic and abiotic properties of soil, which may help explain the patterns of invasibility in a native community (4,8,9).

In this vein, plant traits are a key driver of PSFs (10), as they strongly regulate the composition of soil microorganisms during the conditioning phase (11). For instance, a plant's position along the fast–slow resource economic spectrum has implications not only for nutrient acquisition but also for their responses to environmental stresses, defensive mechanisms against pathogens and symbiotic interactions with mycorrhizal fungi (10–14). In particular, slow-growing plants with conservative traits tend to promote fungal-dominated soil microbial communities; while fast-growing plants with more exploitative traits promote bacterial-dominated soil microbial communities (11,15,16). As such, native plant traits impact the invasibility of the community by determining the soil nutrient availability and balance between generalist mutualistic and pathogenic soil micro-organisms (10,11). In mixed communities that contain both slow- and fast-growing plants, the soil microbial community composition may differ substantially from communities dominated solely by either strategy. Such mixed plant community could balance soil nutrient availability and modulate interactions between different microbial species, diluting the dominance of certain microbes and fostering a more diverse, stable microbial community (17). Non-native plants entering a new range often experience

positive to neutral feedback due to enemy release from antagonistic interactions (9,18). However, these positive feedbacks may be mitigated in communities with a greater diversity of soil microbes where the likelihood of encountering effective enemies is increased (biotic resistance: 17–19) or strengthen by the dilution of species-specific pathogens and the increased functionality of soil microbes (22–24). Whether such feedback effects contribute to the invasibility of a native ecosystem remains uncertain, particularly as invasive species often seem less affected by soil microbes and more adept at exploiting mutualistic relationships than native plants in their home ranges (6,18).

One plant-microbial interaction which has received a lot of attention is the symbiotic relations between plants and arbuscular mycorrhizal (AM) fungi (25–27). AM fungi play a crucial role in shaping plant-soil feedbacks (PSFs) by modulating nutrient acquisition and driving the microbial community composition (28,29). However, the effects of plant–AM fungal symbiosis on PSFs can vary depending on the identity of the host plant, as well as on nutrient availability and abiotic stressors (27,30). Plant traits are thought to influence a plant's dependency on AM fungi; for instance, plants with slower, more conservative traits are likely to invest more in their mycorrhizal partnerships compared to fast-growing, resource-acquisitive plants (12,31). However, this can likewise depend on a plants position along the collaboration gradient (32). As such, the dominant plant species within a native community may significantly affect the extent to which mycorrhizal fungi influence PSF dynamics (23). In addition to AM fungi, the role of AM-associated microbes has emerged as a significant area of research. These microbes, which inhabit the mycorrhizosphere, can interact with both AM fungi and plant roots, potentially enhancing the benefits provided by the fungal symbionts (33). Within mixed plant communities the increased availability of different hosts, with different AM fungal preferences, supports a wider range of AM fungal and their associated microbial species (34). These diverse microbial communities likely generate more positive PSF, by enhancing the selection of the most profitable mycorrhizal partnerships (23,35). Likewise, an increase in functional variation within the microbial community broadens the range of biotic stressors, such as soil-borne pathogens, and abiotic stressors, such as drought, to which the existing AM fungi can offer benefits (36,37).

Invasive plants also benefit from the presence of AM fungi and have demonstrated a greater ability to exploit beneficial soil microbes in their non-native habitats compared to their native ranges (enhanced mutualism hypothesis; (18)). Research indicates that one mechanism driving this enhanced mutualism is the diverse range of AM fungi that invasive plants associate with in their non-native range. This diversity of AM fungi can lead to improved nutrient acquisition, and enhanced protection against pathogens, which collectively contribute to the increased growth and reproductive success of the invasive plant (38,39). Consequently, in mixed plant communities, a rise in AM fungal diversity and their associated microbes may lead to more positive plant-soil feedback effects for invasive species.

Environmental stressors such as drought, impacts both the performance of plants and their interactions with soil microbes; the soil legacy of such drought events can further influence PSF and the invasibility of the community (8,40). Drought-induced decline in plant performance reduces nutrient uptake (41), which can further lead to a decoupling of interactions between host plants and mycorrhizal fungi as plants invest less carbon to their roots and their symbiotic partners (42,43). However, studies examining the direct effects of drought on soil microbes frequently observe that soil fungi are less negatively affected than soil bacteria (44,45), leading to significant shifts in community composition and declines in overall richness of both microbial groups (46). These drought-mediated declines in soil microbial richness can create a biotic legacy in the soil that either favours the growth of invasive plants, as they encounter a less diverse presence of soil pathogens (47), or hinders their performance through a loss of soil functional diversity (24). Furthermore, numerous studies have reported a temporary pulse of soil nitrogen after drought, particularly of extreme intensity, during re-wetting due to increased accessibility of N through diffusive transportation and accelerated microbial activity and N mineralisation (48,49). These increases in soil nutrients are particularly beneficial to invasive plants that can often utilize resources better than native plants, enabling them to better exploit such fluctuations (50,51).

An often overlooked aspect of drought-mediated PSF is how the timing of drought events in reference to plant's growth stage affects the soil biotic legacy. The impact a plant has on the soil microbial composition continuously fluctuates over time as the plant ages (52), where older plants often accumulate greater abundances of mutualistic and pathogenic microbes than younger plants (53–55). As plant drought responses are partially governed by their interactions with mutualistic and pathogenic microbes (56,57), the timing of drought at different plant growth stages may well impact how the plant respond to the drought with further implication on the nutritional and microbial composition in the soil. When droughts occur at critical plant growth stages, it can disrupt plant-microbe interactions and the formation of plant-AM fungal symbiosis, leaving native communities more susceptible to pathogenic microbes (58). In mixed plant communities, such disruptions in plant-microbial interactions can lead to less diverse, drought-tolerant soil microbial communities, potentially weakening biotic resistance (46). As such the timing of drought may be crucial to understanding how drought mediated soil legacies and impacts the vulnerability of native communities to invasion.

In this study, we aim to investigate how the presence of AM fungi and soil legacy of plant-mycorrhizal interactions influence the PSF effect on the growth of the invasive species, *Solidago canadensis.* Specifically, we predicted that the PSF effect on *S. canadensis* performance will differ between soil from difference plant communities due to the effect that plant traits and diversity has on soil nutritional and microbial composition. The presence of AM fungi may mitigate some PSF to facilitate the growth of *S. canadensis*. However, the effect of plant community driven PSF and AM fungi may likewise differ in soils with different drought legacies due to disrupted plant-microbial interactions and increased availability of soil nutrients.

Methods

To evaluate the soil-mediated biotic resistance of slow- and fast-growing native plants to drought, and their subsequent impact on an invasive plant, we conducted a plant-soil feedback experiment consisting of two phases. In the conditioning phase, native plants conditioned the

soil, creating a biotic soil legacy influenced by their traits, diversity, the presence of AM fungi, and the effects of different drought treatments. In the subsequent feedback phase, we tested this soil legacy effect by assessing its impact on the performance of the invasive plant, *Solidago canadensis*, thereby evaluating the soil-mediated biotic resistance (e.g., 12,68).

Study species

Plants – Four native *Asteraceae* species were chosen for the soil conditioning phase of the experiment based on previous research to incorporate two plant species with slow growth traits (*Achillea millefolium* and *Centaurea jacea*) and two plant species with fast growth traits (*Crepis biennis* and *Taraxacum officinale*) (60). All four native species are perennial plants, which are widespread in northern Europe and grow predominately within grasslands and pastures. *Achillea millefolium* and *C. jacea* both typically grow in moderately dry to fresh soils, which are lightly acidic to neutral, and medium-poor to medium rich in nutrients. Both *C. biennis* and *T. officinale* typically grow in soils which are moderately damp, lightly acidic to neutral and rich in nutrients (61) *Solidago canadensis* was used as the invasive plant, which is a perennial Asteraceae originating from North America, wild populations of which were first observed in Europe from around 1850 (62). Since then, *S. canadensis* has become widely distributed within northern Europe causing ecological, economic and health concerns, and is on the "black list" of harmful neophytes in many European countries, such as Switzerland (63), where our study took place.

Germination

Seeds of all native plant species (*A. millefolium*, *C. jacea*, *C. biennis* and *T. officinale*) were obtained from a seed company (UFA Samen, Switzerland) while seeds of *S. canadensis* were obtained from a different seed company (B and T world seeds, France). All seeds were stored at 4°C before germination. For surface sterilisation, seeds were bleached for 15 minutes in a 30% bleach solution (commercial bleach with sodium hypochlorite) and rinsed with deionised water. Seeds were placed on germinating soil containing peat, peat substitute, compost, sand and organic fertiliser (Landi, Switzerland), using black containers (18 cm x 14 cm x 5 cm). The

germinating soil was prepared by sieving out large particles using a 5 mm mesh and sterilising the soil twice in an autoclave (Systec VX-150, Systec GmbH & Co., Germany) at 121°C for 20 minutes. The two cycles were separated by at least 48 hours to target more resistant fungal species that opportunistically spread in the soil. Seeds were moistened using deionised water, and moisture was retained by partially placing a lid on the container. The seedlings grew in climate chambers at 20°C for 16 hours (i.e., with light) and 16°C for 8 hours (i.e., dark). Seedlings were grown for around three weeks. To ensure consistency in plant height across species at the beginning of the experiment, some fast-growing seedlings were placed at 10°C (with a 16 hr light/ 8 hr dark cycle) to slow down their growth during the germination phase.

Experimental soils

The soil used in our experiment was a mixture of 50% quartz sand (particle size = 0.3-0.7 mm), and 50% universal potting soil (abiotic properties of the substrate: pH = 6.7, SOM = 0.68 kg kg^{-1}, NO_3 = 83 ppm). The soil was hand-mixed after bigger particles – such as stones, clay and wood – were removed from the potting soil with a coarse-meshed sieve of 5 mm mesh. The mixed soil was dried for around 48 hours at 70°C to ensure 0% water content before being sterilised in an autoclave at 121°C for 20 minutes. The soil was sterilised twice with two cycles separated by at least 48 hours exactly in the same way as the soil used for the germination of plants. The autoclaved soil was then distributed into the plant pots (width: 10.5 cm, depth: 10.5 cm, height: 22 cm, 1.8 Ltr), with a total of 1.8 kg of dry soil weight in each pot.

AM fungi

Soils were inoculated with four species of AM fungi to create an AM fungal community in the conditioning phase soil: *Rhizoglomus irregular*, *Funneliformis mosseae*, *Funneliformis caledonium*, *Funneliformis geosporum*. All AM fungal species belong to Glomeraceae family and have been well-researched for the ecosystem services they provide such as nutrient cycling, modification of the soil's physical properties, and influencing plant's interactions with other organisms (35). For the colonisation of plant roots with the four AM fungal species, we used a pre-prepared mixture of vermiculite (chemical composition) containing mycorrhizal units

(spores and hyphae; 155 mycorrhizal units per ml). The mixture was provided by INOQ GmbH (Schnega, Germany) and was stored at 4ºC before inoculation. For the control treatments, we created a microbial wash by diluting the vermiculite-mycorrhizal mixture with distilled water (1:1) and filtering the runoff using a 90 and 25 µm mesh. A runoff sample after the 25 µm was centrifuged for 5 minutes at 1800 rpm and controlled for the presence of spores using the sucrose gradient/filtering technique (64). The 25 µm mesh was selected to allow AM-associated microbes to pass through while filtering out mycorrhizal spores and hyphal fragments (65,66). The remaining microbial wash was stored at 4ºC while the vermiculite-mycorrhizal mixture was dried in an oven at 70ºC for ~48 hours and sterilised once in an autoclave (Systec VX-150, Systec GmbH & Co., Germany) at 121°C for 20 minutes.

In the AM-fungi treatments, we added 100 ml of non-sterilised AM fungal vermiculite (~5 g) along with 400 ml of distilled water to each pot of 1.8 kg of soil and mixed together to keep homogeneity. The inoculum was mixed into the soil before transplanting the seedlings into the soil, to enable faster contact with the root surfaces of the plant. In the control treatment, 5 g of sterilised vermiculite, 100 ml of microbial wash and 400 ml of distilled water were added to soil and were thoroughly mixed. Analysis of root mycorrhizal colonisation in plants grown in soils without AM fungi confirmed that our sterilised soils (added with AM fungi-associated microbes) were free of AM fungal structures.

Conditioning phase

Four individual seedlings ranging between 5 – 7 cm in height were transplanted into pots, with and without inoculation of the AM fungal community. Monocultures of each native plant species were created as well as a mixed community containing one of each of the native plant species (i.e., two levels of diversity: monocultures and four species mixtures). Pots were then randomly allocated to four tables in two climate chambers (i.e., each climate room containing two tables) with identical light and temperature settings. In both climate chambers, the plants were exposed to the following growing conditions: 16 hours of daytime at 20°C with a light intensity of ~13,500 lux and 8 hours of night-time at 16°C. The room's relative humidity (RH)

was approximately 50% during the day and about 80% at night. Temperature, light intensity and the room's air RH were constantly monitored to account for differences on the four tables in the two climate chambers (Supplementary Fig. S1), and given the slight variability, we fitted random intercepts for the four tables in our statistical models (details below). We let the plants condition the soil for a total of eleven weeks, within which pots were exposed to two separate extreme drought treatments. One extreme drought treatment occurred after 5 five weeks (early drought) of plant growth, and the other after 7 weeks (late drought) of plant growth. When not subjected to drought, pots were continuously watered every four to five days with 100 ml of deionised water (Supplementary Fig. S2). For the drought treatment, no water was added for three weeks, which made soil water content close to 0 % for several days. Following this drought period, plants were allowed to recover by rewetting the pots, which was carried out by adding deionised water (the same way for regularly watered treatments). Soil moisture was regularly checked with a soil moisture meter TDR 150 (FieldScout, Spectrum Technologies Inc., USA) at a depth of 3.8 cm on 30 extra pots (one for each treatment combination) in order to monitor soil water availability across treatments without disturbing the main treatment units (Supplementary Fig. S2). Each treatment combination was replicated seven times, resulting in a total of 210 pots (5 plant communities x 2 AM fungi treatments x 3 drought treatments x 7 replicates) and 840 plant individuals.

Harvest and response variables of the conditioning phase

The height of each plant was recorded as the distance from the soil surface to the highest point of the upstretched longest leaves four times during the experimental period (before and after each drought treatment and before the harvest). Measurement of chlorophyll content (SPAD) was taken at the same time as the height measurements. To measure SPAD, we non-destructively sampled the two youngest fully expanded healthy leaves per plant and repeated three times using a SPAD-502 Chlorophyll Meter (Konica Minolta, Tokyo 100-7015, Japan). After four (early drought) and two (late drought) weeks of post-drought recovery and on the eleventh week of the experiment, final measurements were taken, and plants were harvested.

Soils (containing roots) were removed from the pots and loosened within separate plastic bags; once thoroughly loosened, roots were removed from the loosened soil and meticulously washed in order to remove attached substrates. Equipment used while handling soils from different pots, including gloves, were thoroughly sterilised using a 70% ethanol solution. Fine root samples of about 1 g (fresh weight) were taken from each pot for mycorrhizal colonisation and root trait measurements. Soil samples were analysed for nitrogen (NO_3) and potassium (K) content (ppm) by diluting 1 g of soil with Milli-Q water (1:1) and analysing the solution using L'Aqua Twin meters (HORIBA, UK).

Soil samples were further taken for 16S and ITS genomic sequencing, in which DNA was extracted from 0.2 g of frozen soil using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's instructions. Extracted soil DNA was sent to the Next Generation Sequencing (NGS) platform (Bern, Switzerland). Phased primers from QIAseq 16S/ITS Smart Control (Qiagen, Germany) targeted the 16S (V1-V9) & ITS (5.8S) regions with 0–11 additional bases to the 5'-end of the 16S rRNA and ITS amplicon primers (Supplementary material S3). Full-length Illumina sequencing was performed, with DNA quantification and dilutions managed by Qiagen (Germany) to complete the library preparation. Quality control was conducted on control samples, and custom sequencing was carried out using the MiSeq v3 600 cycle platform. Sequencing reads were processed using the Dada2 pipeline with paired-end reads and trimmed to remove 16 bases from the left and 24 bases from the right. Taxonomic classification was performed using the SILVA and UNITE databases for 16S rRNA and ITS sequences, respectively.

During the harvest, the aboveground shoot tissue of each plant was removed just above the soil level and a single young fully expanded leaf from each plant was cut at the base of the petiole to later measure plant leaf traits. The fresh weight of the remaining roots was measured and then roots were dried in an oven for 3 days at 40°C, along with the plant shoot, to measure the total dry biomass of each plant. Due to the intertwining of roots, it was not possible to measure the dry biomass of each individual plant, as such, the total root biomass per pot was measured.

The fresh leaf and root samples were weighed and scanned using an Epson Perfection V850 Pro Scanner, and were analysed using ImageJ and RhizoVision Explorer v2.0.3 (67,68) to collect data on specific leaf area (SLA), root diameter and specific root length (SLR). Specific root length was estimated as the ratio of root length to its dry mass. Leaf samples were also oven-dried to calculate the leaf dry matter content (LDMC) as the leaf fresh weight divided by their dry biomass (69).

Finally, we measured the percentage of total root AM fungi colonisation and colonisation by specific AM fungal structures by staining roots blue using techniques modified from Philips and Hayman (1970) (70). This allowed us to estimate the colonisation of mycorrhizal structures within the roots. Once stained, root samples were immersed in a mixture of water, glycerin and lactic acid (v:v:v) and were inspected under a Leica S8i Microscope (Leica Microsystems, Wetzlar, Germany). To measure the percentage of mycorrhizal colonisation, we used the modified gridline intersect method from Giovannetti & Mosse (1980) (71). Root length colonisation (%) was calculated as a measure of all mycorrhizal structures present in the root, also using the equation presented in Giovannetti & Mosse (1980) (71).

Feedback phase

Conditioned homogenised soils containing root fragments from each pot in the conditioning phase were transferred to new 0.7 litre pots (width: 10 cm, depth: 10 cm height: 11 cm) and weighed 600 g (fresh weight) using sterilised equipment. One seedling of *S. canadensis,* with a height of around 1 cm, was transplanted within each pot and then randomly allocated among the four tables in two climate chambers with the same replication as in the conditioning phase. Temperature, light and humidity settings matched those used in the conditioning phase and were constantly monitored to account for differences across the four tables in the two climate chambers (Supplementary Fig. S1). Plants were left to grow for five weeks during this feedback phase and were regularly watered using extra pots to measure water content, similar to the conditioning phase. After five weeks of growth, all *S. canadensis* plants were harvested and leaf

traits such as specific leaf area (SLA), leaf dry matter content (LDMC) and plant height were measured along with shoot and root biomass. All feedback data was measured in the same way as in the conditioning phase.

Data analysis

All data were analysed in R statistical software v4.0.3 (72). All figures were created using the ggplot 2 package (73), except those of piecewise structural equation models. Microbial DNA sequence data was processed to eliminate errors and artefacts and reads were aligned to a reference microbial database. A table containing Operational Taxonomic Units (otu's) was generated, representing the abundance of different microbial taxa in each sample. The data was imported into Phyloseq (74), an R package for microbiome data analysis. Low abundance OTUs (lower than 10 for the ITS dataset and 15 for the 16S dataset) were filtered out to reduce noise in the dataset and taxonomic data was reformatted using the "psmelt" function. The different hypervariable regions within the 16S data were compared by phylogenetic resolution and diversity indices, through which the hypervariable regions (combination of V3 and V4) were chosen as the best representation of the bacterial communities. Using the package FUNGuildR fungal guilds were assigned to various fungal OTU's (75). Shannon (index) diversity, simpson diversity, richness and evenness within microbial communities was estimated using the vegan package (76).

We used non-metric multidimensional scaling to perform the ordination of the microbial communities based on the absolute abundances of taxa after the conditioning phase. We created Principal Coordinate Analysis (PCoA) using the Bray-Curtis dissimilarity index for the community data pertaining to both 16S and ITS microbial community data. The ordination analysis was conducted using the vegan package in R (76). First, pairwise dissimilarities were computed using the Bray-Curtis method (*vegdist* function). Then, a Principal Coordinate Analysis (PCoA) was performed on the dissimilarity matrix using the *wcmdscale* function to obtain eigenvalues and scores. The explained variance for each axis was calculated by dividing

the eigenvalue of each axis by the sum of all eigenvalues. Multivariate statistical tests (PERMANOVA) were run to investigate differences in microbial communities due to drought treatment, mycorrhizal treatment and plant communities using the adonis2 function in the vegan package (76).

Mixed-effects linear models were run on all response variables measured in the conditioning and feedback phase using the lme4 package (77). The fixed effects of these models were the drought treatment, AM fungal treatment and the native plant community during the conditioning phase. The random intercepts used in the models were the bench on which pots were placed (e.g., following the model structure of the lme4 package in R: response variable~Native plant conditioning community*AM treatment*Drought treatment + (1|Bench)). The treatment effects in mixed models were evaluated with a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for the estimation of degrees of freedom, using the lmerTest package (78). Model assumptions (e.g. homogeneity of variance and normality of residuals) were inspected visually for each linear model. To meet the model assumptions, some response variables were log-transformed (indicated in Table 1). Conditional R^2 values were taken as the proportion of total variance explained through both fixed and random effects of the linear models and their statistical significance was obtained from the r2glmm package (79). To explore the strength of relationships between response variables in the conditioning and feedback phase we tested correlations using major axis regression models (RMA) with the lmodel2 package, allowing for greater understanding of potential mechanisms driving feedback on *S. canadensis*, (80).

To explore how the response variables from the native plants of conditioning phase influenced the soil legacy effect on *S. canadensis* in the feedback phase, several RMA models were run. Results from RMA models were utilized to inform on interactions between variables in the conditioning and feedback phase and subsequently guide the pathways used in structural equation models. Structural equation models (SEM) were run with the piecewiseSEM package

(81), with each component model ran using linear models (72). Categorical variables (AM fungal treatment and drought treatment) were converted to numeric factors to ensure appropriate model input. We subset the data into the three different plant conditioning communities (Slow, Fast, and Slow + Fast) to explore how the drought and AM fungal mediated shifts in plant-microbial interactions differ in the context of the plant community composition. For each plant community, we conducted linear models to explore the relationships between soil bacterial (16S) richness, root-to-shoot ratio, AM fungi, drought treatment and the interaction effect of AM fungi and drought treatment. Justification of this pathway is based on evidence from previous studies illustrating the impact of the soil microbial community on root biomass allocation and traits (82–84). The component models were assessed for multicollinearity using variance inflation factors (VIFs) and for the fit of the SEM using the Directed Separation (d-sep) test (85). The d-sep test involves examining the conditional independencies implied by the model and computing the Fisher's C statistic. This statistic is compared to a chi-square distribution with degrees of freedom corresponding to the number of conditional independence claims tested. A non-significant p-value (> 0.05) from Fisher's C test indicates that the model adequately fits the data, confirming the hypothesized relationships.

Results

Conditioning Phase: Microbial and nutrient responses

The Shannon diversity, species richness and evenness of fungal communities significantly increased in the presence of AM fungi (Shannon diversity: F-value$_{df}$ = 56.79$_{1,185}$, p-value <0.001; Richness: F-value$_{df}$ = 77.62$_{1,185}$, p-value <0.001; Evenness: F-value$_{df}$ = 48.59$_{1,185}$, p-value <0.001; Fig. 1; Supplementary Table S1 & Fig. S4). AM fungal-mediated increases in fungal species richness differed significantly between soils conditioned by different plant communities (F-value$_{df}$ = 3.10$_{1,185}$, p-value <0.05). Notably, within soils from mixed plant communities, these increases were substantially hampered compared to monoculture soils. The Shannon diversity and richness of soil bacterial (16S) communities decreased in AM fungal soils (Shannon diversity: F-value$_{df}$ = 35.35$_{1,165}$, p-value <0.001; Richness: F-value$_{df}$ = 6.40$_{1,185}$, p-value <0.05; Fig.

1; Supplementary Table S1); however, for bacterial richness the extent of this AM fungal mediated decrease differed with soils conditioned by different plant communities (F-value$_{df}$ = 10.57$_{1,185}$, p-value <0.001; Fig 1; Supplementary Table S1). Bacterial species richness was further influenced by the soil's drought legacy, resulting in a significant three-way interaction (F-value$_{df}$ = 4.09$_{1,185}$, p-value <0.01). In particular, within soils conditioned by slow + fast mixed communities, the presence of AM fungi mediated larger declines in bacterial diversity than in soils conditioned by monocultures. Soil NO_3 content (ppm) in the soil differed significantly depending on the drought treatment, with increased NO_3 content in the soils subjected to the early and late drought treatments compared to the no drought treatment (F-value$_{df}$ = 5.90$_{2,163}$, p-value <0.01; Supplementary Table S1 & Fig. S5).

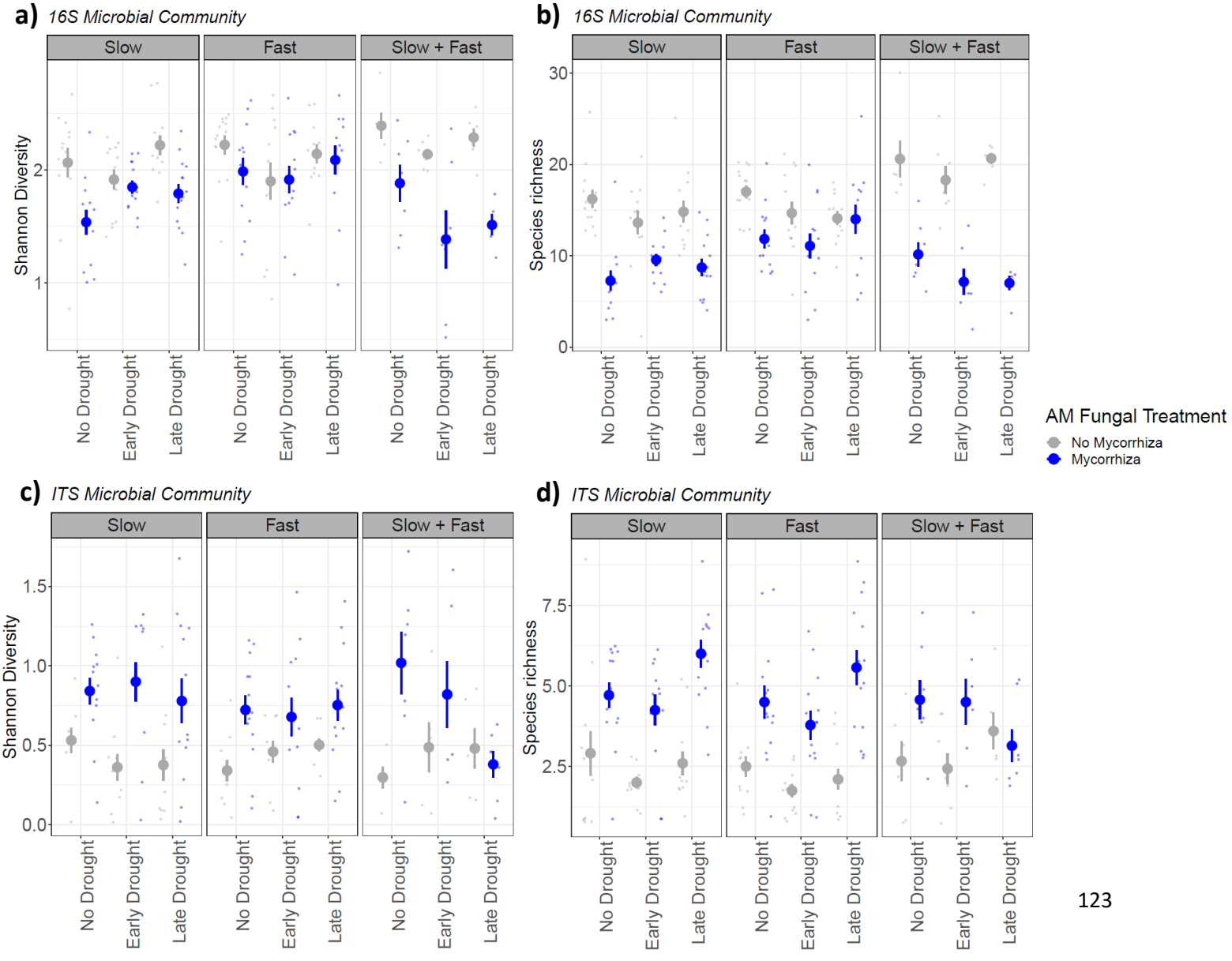


Figure 1. Bacterial Shannon diversity (a), bacterial species richness (b), fungal Shannon diversity (c), and fungal species richness (c) of conditioned soil. Soils conditioned by slow-growing (Slow), fast-growing (Fast) and mixed (Slow + Fast) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis within each the presence of AM fungi is shown using the colours grey (control) or blue (mycorrhizal). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).

Multivariate analysis of variance using dissimilarity distances among the entire soil microbial communities (16S & ITS) indicated that the presence of AM fungi was a strong driver of microbial community composition (F-value = 24.49, R^2 = 0.11, p-value <0.001). This impact of AM fungi on the microbial community was further influenced by the drought legacy of the conditioning phase (F-value = 1.77, R^2 = 0.02, p-value <0.05; Fig. 3). Plant conditioning communities also had a significant effect on the microbial community composition (F-value = 2.51, R^2 = 0.02, p-value <0.01). A separate analysis of variance among bacterial (16S) communities similarly indicated that the presence of AM fungi was a significant driver of differences in bacterial community composition (F-value = 4.61, R^2 = 0.02, p-value <0.001). Likewise, the impact of drought legacy from the conditioning phase also had an influence on bacterial community composition (F-value = 1.63, R^2 = 0.02, p-value = <0.01; Supplementary Fig. S6). In contrast, analysis of the variance among fungal (ITS) communities showed the presence of mycorrhizal fungi to be the only experimental variable driving fungal community composition (F-value = 8.28, R^2 = 0.04, p-value <0.001; Supplementary Fig. S6).

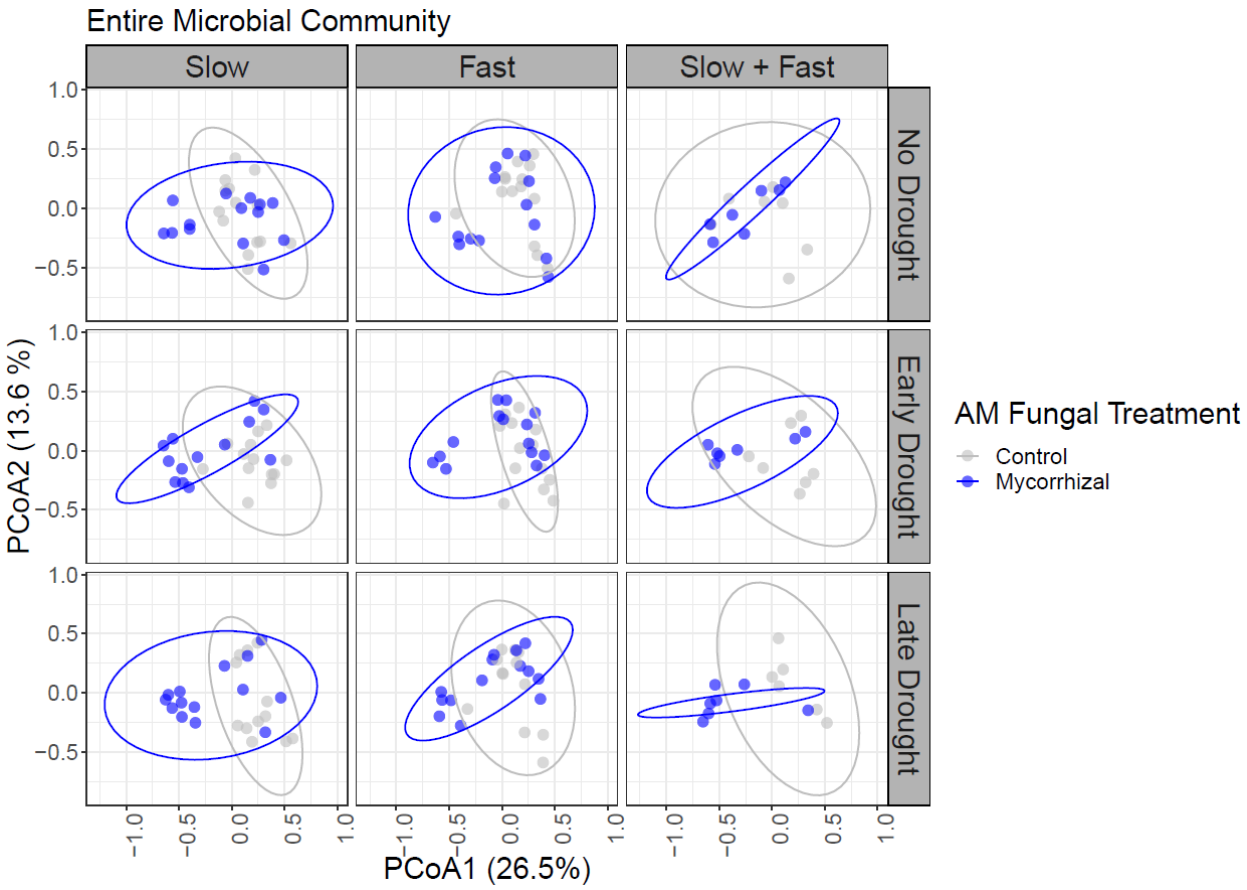


Figure 2. Principal Coordinate Analysis (PCoA) plots illustrating the differences in microbial community composition under various mycorrhizal treatments (No mycorrhiza: grey, Mycorrhiza: blue) and drought treatments. The plots are divided into three columns representing different native plant conditioning communities' groups: slow-growing (Slow; left), fast-growing (Fast; middle), and mixed (Slow + Fast; right). Each point represents a sample, and the ellipses indicate 95% confidence intervals for the respective treatment groups. PCoA1 and PCoA2 explain 26.5% and 13.6% of the variation in microbial community composition, respectively.

Conditioning Phase: plant responses

Community composition (monocultures versus mixed community) of the native conditioning plants greatly impacted total plant biomass during the conditioning phase ($F\text{-value}_{df} = 31.45_{2,33}$,

p-value <0.001; Supplementary Table S1 & Fig. S7), with the slow + fast mixed plant treatment group accumulating 56% more biomass than the monoculture of slow-growing plants and 70% more biomass than that of fast-growing plants (Supplementary Fig. S7). Shoot and root biomass significantly decreased with early and late drought treatments compared to treatments with no drought (Shoot: $F\text{-value}_{df} = 6.68_{2,154}$, p-value <0.01; Root: $F\text{-value}_{df} = 57.28_{2,179}$, p-value <0.001; Supplementary Table S1 & Fig. S7). The shoot biomass within each conditioning plant community differed in response to AM fungi ($F\text{-value}_{df} = 17.31_{2,154}$; p-value <0.001). In fast growing plants, the shoot biomass in AM fungal treatments was substantially higher, while slow growing and slow + fast mixed communities showed no difference between AM fungal and non-AM fungal treatments (Supplementary Fig. S7). However, the root biomass responses exhibited a three-way interaction, varying significantly across different conditioning plant communities in response to the combined effects of AM fungi and drought treatments. ($F\text{-value}_{df} = 3.59_{4,179}$, p-value <0.01; Supplementary Table S1 & Fig. S7). For example, in slow + fast plant communities, the presence of AM fungi decreased root biomass in the non-drought treatment but increased root biomass in both early and late drought treatments. In contrast, within fast-growing monocultures, AM fungi increased root biomass in the non-drought treatment, but this effect was dampened in early and late drought treatments (Supplementary Fig. S7).

The variation of root: shoot ratio within different conditioning plant communities differed in response to AM fungi and drought treatment ($F\text{-value}_{df} = 7.48_{4,146}$, p-value <0.001; Supplementary Table S1 & Fig. S7). In particular, in the fast community, the late drought treatment drastically increased the root: shoot ratio, especially in non-AM fungal treatments (Supplementary Fig. S7). Other root traits such as root diameter and specific root length significantly differed in response to AM fungi depending on the plant conditioning community ($F\text{-value}_{df} = 5.78_{2,186}$, p-value <0.01 & $F\text{-value}_{df} = 4.34_{2,178}$, p-value <0.05, respectively; Supplementary Table S1 & Fig. S8). In this case, slow communities significantly decreased root diameter and increased specific root length in the presence of AM fungi, while slow + fast communities showed a marginally decreased root diameter and significantly increased specific root length in the presence of AM fungi (Supplementary Fig. S8).

Feedback phase response

Biomass accumulated by *S. canadensis* during the feedback phase significantly differed among conditioning phase treatments. In particular, soils with a drought legacy (both early and late) increased shoot biomass compared to non-drought soils ($F\text{-value}_{df} = 6.30_{1,189}$; p-value <0.01; Fig. 3; Table. 1). Furthermore, the impact of AM-fungi on shoot biomass differed across soils from different conditioning plant communities ($F\text{-value}_{df} = 4.40_{2,190}$; p-value <0.05; Table. 1). For instance, while the presence of AM fungi increased shoot biomass within soils from plant monocultures, this effect was diminished in soils conditioned by mixed plant communities. In these mixed soils, the effect of AM fungi on shoot biomass was not only reduced but also reversed, with the presence of AM fungi leading to a slight decrease in shoot biomass (especially in soils exposed to extreme drought events at later growth stages) (Fig. 3).

Plant community of the conditioning phase significantly affected the root biomass of *S. canadensis* ($F\text{-value}_{df} = 13.67_{2,190}$; p-value <0.001; Table. 1), with soils from fast-growing conditioning communities leading to substantially more root biomass compared to soils from slow-growing or mixed plant communities (Fig. 3). However, the impact of conditioning communities on the root biomass of *S. canadensis* was not uniform but was significantly influenced by a three-way interaction between the community type, the legacy of drought conditions, and the presence of AM fungi ($F\text{-value}_{df} = 3.89_{4,190}$; p-value = <0.01; Table. 1). For example, in soils conditioned by slow-growing plants, root biomass of *S. canadensis* was lowest in soils with no history of drought and without AM fungi. In contrast, slow community soils with a drought legacy yielded significantly higher biomass, especially in the presence of AM fungi. Conversely, in soils conditioned by fast-growing plants, root biomass was highest in soils without a drought legacy and without AM fungi, while fast community soils without AM fungi yielded significantly lower root biomass in soils with a late drought legacy (Fig. 3).

The root: shoot ratio of *S. canadensis* exhibited significant variation in biomass allocation due to the interaction between the conditioning plant communities and the presence of AM fungi ($F\text{-value}_{df} = 5.82_{,190}$; p-value = <0.01; Table. 1). Specifically, in soil conditioned by slow-growing or fast-growing plant communities root allocation was significantly higher in the absence of AM

fungi compared to soils where AM fungi was present. However, in soils conditioned by the slow + fast mixed plant communities, this mycorrhizal mediated biomass allocation shifted in direction with *S. canadensis* allocating marginally more biomass to the root in the presence of mycorrhizal fungi (Fig. 3).

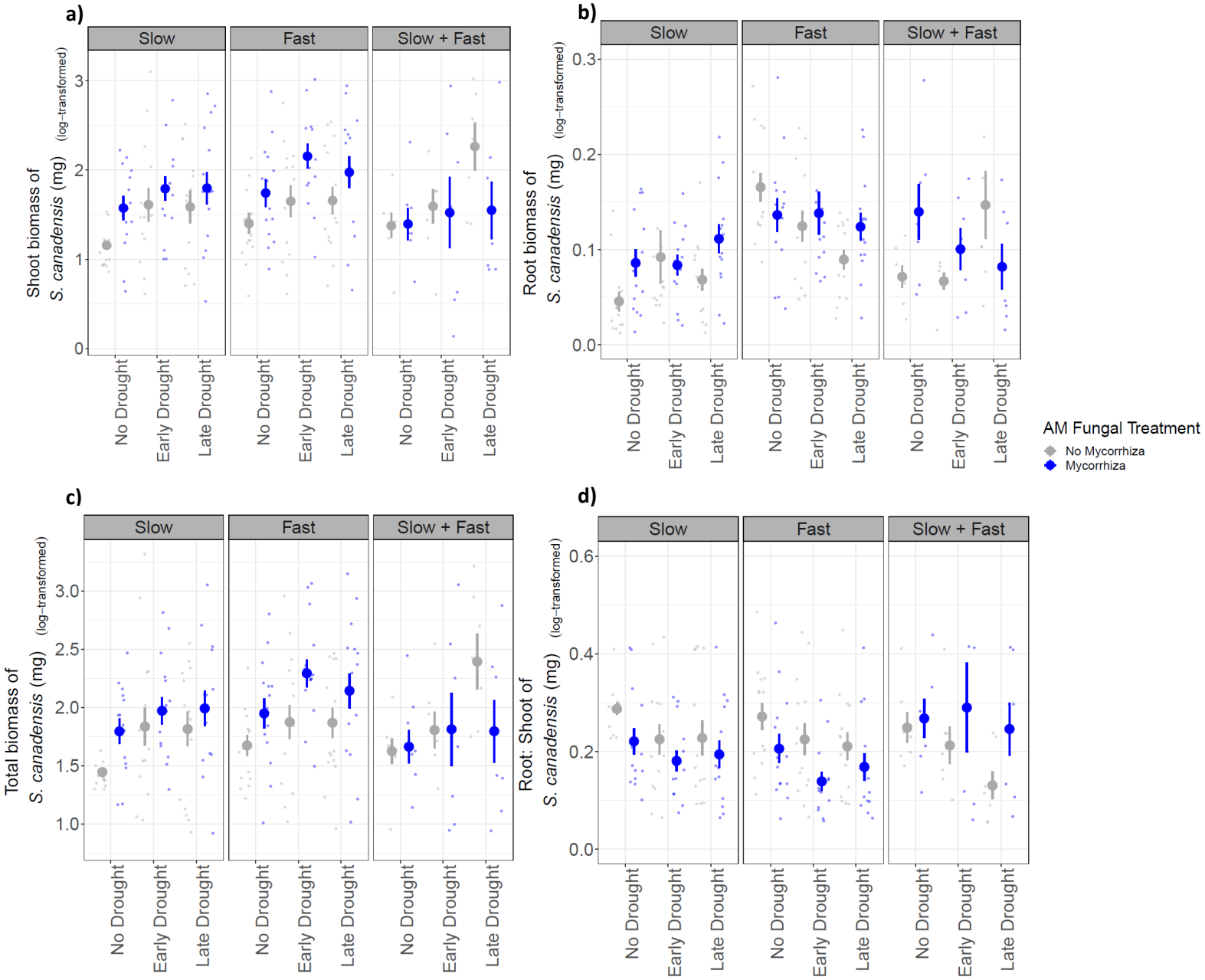


Figure 3. Plant shoot biomass (a), root biomass (b), total biomass (c) and root: shoot ratio (d) responses of *S. canadensis* in the feedback phase. Feedback responses of *S. canadensis* to

soils conditioned by slow-growing (Slow), fast-growing (Fast) and mixed (Fast + Slow) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis, within each the presence of AM fungi is shown using the colours grey (no mycorrhiza) or blue (mycorrhiza). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).

Table 1. Results from linear mixed-effect models testing the effects AM fungi presence (AM), extreme drought treatment (Dr) and native conditioning community (Com) for the dependent variables in the feedback phase. Bold values are statistically significant (p-value < 0.05). Conditional R^2 represents the combined effects of fixed and random effects used in our models. The random intercept of SLA had a variance of 0 across all dependent variables. df stands for degrees of freedom.

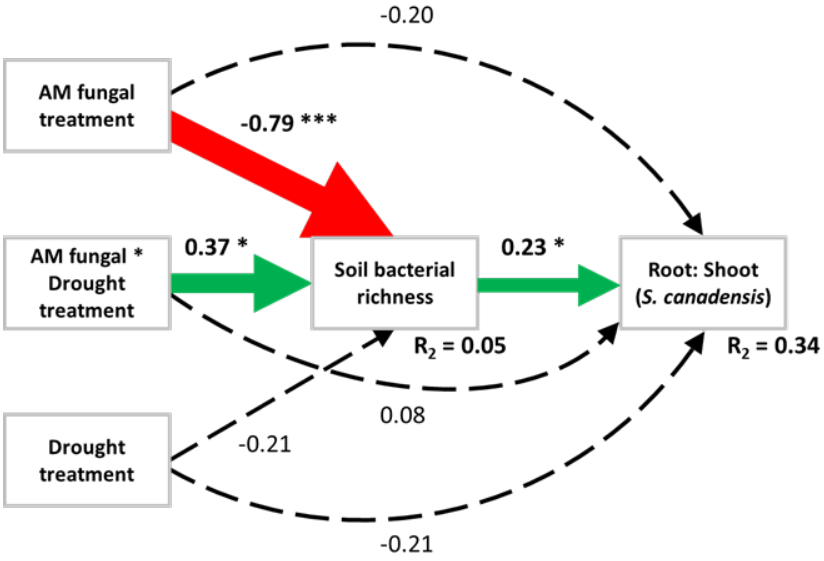
Response Variable	AM Fungi (AM)		Drought (Dr)		Community (Com)		DR x AM		AM x Com		Dr x Com		Dr x AM x Com		Random intercept (Bench)	Model
	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	$F\text{-value}_{DF}$	p-value	Variance	R-squared (conditional)
Total biomass (log-transformed)	$2.69_{1,189}$	0.10	$6.71_{2,189}$	**<0.01 ****	$2.37_{2,190}$	0.09	$1.40_{2,189}$	0.25	$4.01_{2,190}$	**<0.05 ***	$0.68_{4,189}$	0.61	$0.79_{4,190}$	0.53	0.01	0.18
Aboveground biomass (log-transformed)	$2.30_{1,189}$	0.13	$6.30_{2,189}$	**<0.01 ****	$2.10_{2,190}$	0.13	$1.31_{2,189}$	0.27	$4.40_{2,190}$	**<0.05 ***	$0.65_{4,189}$	0.62	$0.64_{4,190}$	0.64	0.01	0.17
Belowground biomass (log-transformed)	$2.63_{1,189}$	0.11	$0.18_{2,189}$	0.83	$13.67_{2,190}$	**<0.001 *****	$0.56_{2,189}$	0.57	$0.50_{2,190}$	0.61	$2.90_{4,189}$	**<0.05 ***	$3.89_{4,190}$	**<0.01 ****	0.00	0.23
Root:Shoot (log-transformed)	$0.77_{1,189}$	0.38	$4.04_{2,189}$	**<0.05 ***	$1.19_{2,190}$	0.31	$0.87_{2,189}$	0.42	$5.82_{2,190}$	**<0.01 ****	$0.61_{4,190}$	0.65	$0.23_{4,190}$	0.92	0.00	0.13

Structural equation modelling

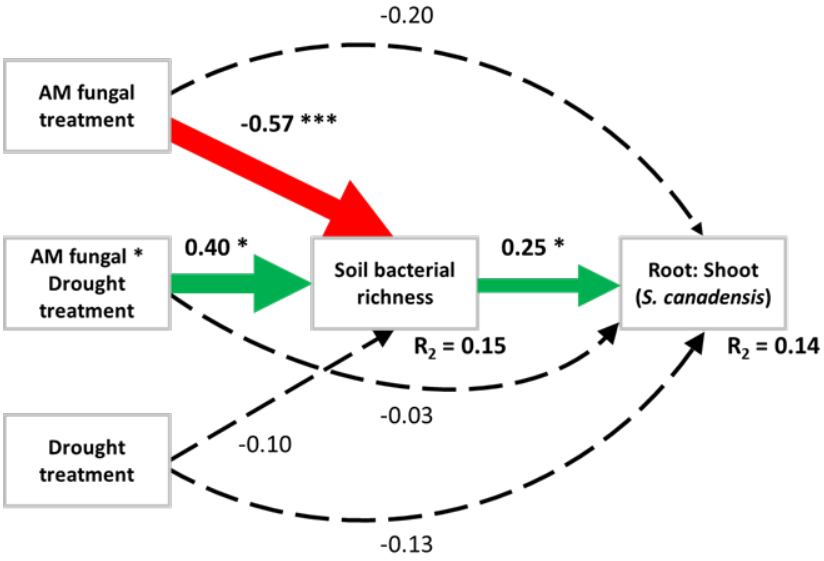
All three final structural equation models provided good fits to the data (Slow plants: chi-squared statistic was χ^2(degrees of freedom=2) = 3.77, p-value= 0.15; Fast plants: chi-squared statistic was χ^2(degrees of freedom= 1) = 2.09, p-value = 0.15; Slow + Fast plants: chi-squared statistic was χ^2(degrees of freedom= 2) = 2.09, p-value = 0.15). The models illustrate that specifically within soils conditioned by mixed (slow + fast) communities the interactive effects of drought legacy and AM fungi had a significant negative influence on soil bacterial species richness (Fig. 4). In contrast, within soils from monocultures of slow or fast growing

conditioning communities, while the presence of AM fungi had a negative influence on bacterial richness, the interactive influence of AM fungi and drought legacy increased the soil bacterial richness. The models also show that bacterial richness drives the allocation of root and shoot biomass in *S. canadensis* (Fig. 4). Within the soil from slow + fast communities, bacterial richness negatively correlated with root: shoot ratio whereas in the two monocultures (the slow and the fast plant monocultures), soil bacterial richness was positively correlated with root: shoot ratio. This shift in correlation between bacterial richness and root: shoot with the slow + fast communities may be indirectly mediated by the soil legacy effects of drought and AM fungi (Fig. 4).

a) Slow



b) Fast



c) Slow + Fast

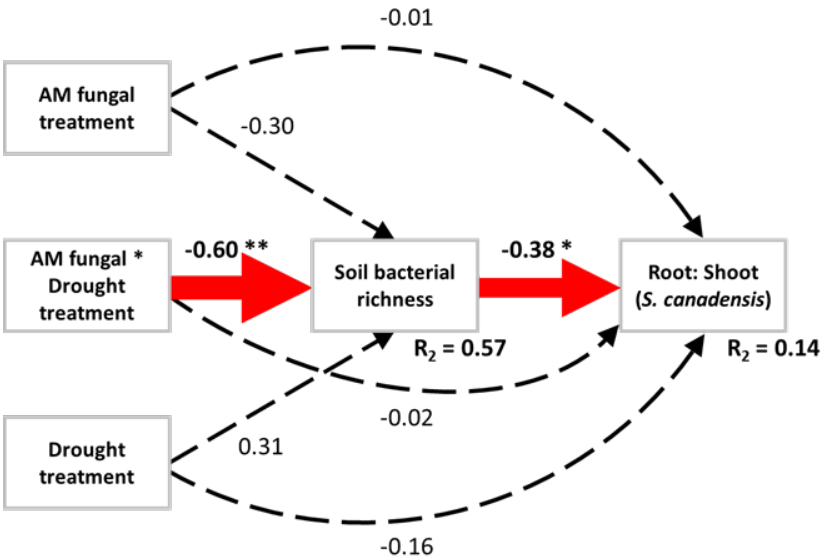


Figure 4. Structural equation models illustrating the relationships among mycorrhizal treatment, drought treatment, bacterial species richness, and the root: shoot ratio of *Solidago canadensis* in soils conditioned by separate native plant communities: a) Slow-growing monocultures, b) Fast-growing monocultures, and c) Fast + Slow (Mixed). Solid arrows represent significant relationships, with red arrows indicating negative effects and green arrows indicating positive effects. Dashed arrows represent non-significant relationships. Standardized path coefficients are shown alongside each arrow, with asterisks denoting significance levels (*p < 0.05, **p < 0.01). The R^2 values for each endogenous variable are indicated below their respective boxes.

Discussion

Elucidating how biotic and abiotic factors influence the success of an invasive species holds promise in identifying the invasibility of native communities and informing management strategies to control the spread of invasive species. (86). Our results demonstrate that the influence of AM fungi on the success of an invasive plant may be dependent on the abiotic and biotic contexts of the native community soils (Fig. 3). For example, the effects of the native conditioning community strongly modulated the effects of AM fungi on *S. canadensis*. Within soils from monocultures, *S. canadensis* benefitted from the presence of AM fungi by accumulating more biomass, while within soils from mixed (fast + slow) communities AM fungi appeared to lower the biomass of *S. canadensis* compared to soils without AM fungi. This AM fungal mediated decline in biomass accumulation in mixed soils was especially prominent within soils that have were subjected to drought in later plants growth stages (late drought). As such, our study suggests that the interactive context of drought legacy and AM fungi are key mediators of biotic resistance, as their influence differs within the context of native community diversity (Fig. 3).

The soil drought legacy played a major role in the PSF through influencing the microbial diversity (Figure 4) and the availability of nutrients in the soil (Supplementary Fig. S5). Drought

has been well documented to increase microbial activity and nutrient availability within the soil, particularly after rewetting. Decreased soil moisture can lead to reduced microbial activity (45,87,88), however, rewetting of the soil can initiate rehydration and lysis of the dead microbial cells, as well as create a surge in microbial activity (87–90). Rewetting of the soil can thereby create a temporary pulse of soil nitrogen due to increased accessibility of N through diffusive transportation and accelerated microbial activity and N mineralisation (87,91,92). Such increased resource availability can boost the success of invasive plants, particularly as these plants are adept at exploiting these transient nutrient surges (50,51). Indeed, the biomass accumulated by *S. canadensis* positively correlated with the concentration of NO_3 measured in the soil, highlighting its ability to capitalize on these conditions (Supplementary Fig. S9). As such, the overall performance of *S. canadensis* was greater within soils with a legacy of drought (both late and early) compared to soils from well-watered communities. However, the extent to which *S. canadensis* benefited from drought-mediated nutrient pulses was moderated by the biotic context of the soil including the legacy of the conditioning plant trait composition and the presence of AM fungi (Fig. 3). For example, within soil from mixed communities, the presence of AM fungi reduced both the shoot and root biomass accumulated by *S. canadensis* particularly in soils with a late drought legacy (resulting in three-way interactions between treatments for root biomass). As soil mutualists, the presence of AM fungi plays a major role in driving PSF not only by providing nutrients to their plant host but also by mediating shifts in the soil microbial community (93,94). Across all treatments the presence of AM fungi reduced the diversity and richness of soil microbes, particularly for bacterial communities (Figure 2). Such mycorrhizal mediated reductions in soil microbial diversity have been well documented in other studies (95), including declines in the abundance of pathogenic microbes in the soil, which benefits host plants (94). In our experiment this reduction in soil bacterial diversity and richness was especially distinct within soils conditioned by the mixed native community with drought legacies which may help elucidate the contrasting response of *S. canadensis* to AM fungi in these treatments.

Within the soils conditioned by mixed plant communities, we did find that increased bacterial species richness resulted in lower root biomass allocation within *S. canadensis*, and greater shoot biomass allocation (Figure 4 C). Root to shoot allocation in plants is a crucial and highly plastic trait response that reflects plant adaptations to various local environmental conditions (96). Higher root: shoot ratio indicates a need for greater root investment to compensate for a lower efficiency of resource uptake or utilization (97). Indeed, for *S. canadensis* root: shoot ratio negatively correlated with total biomass of the plant (Supplementary figure S10), suggesting that *S. canadensis* invests less to root growth when conditions are more favourable. These adaptive responses in biomass allocation may obscure the impact of microbial richness on the biomass accumulation of *S. canadensis*, leading to less definitive microbial driven PSF on plant biomass in our findings (98). However, from the biomass allocation response of *S. canadensis*, it could be assumed that increasing the richness of bacteria in soils conditioned by mixed plant communities may benefit *S. canadensis* in acquiring nutrients, therefore *S. canadensis* is able to allocate relatively more biomass to its shoot. In contrast, within soils conditioned by monocultures of slow or fast-growing plants, increased bacterial species richness led to greater root biomass allocation in *S. canadensis* (Figures 4 A and B).

Examining the indirect effects of soil drought legacy and AM fungi presence may elucidate why increased bacterial species richness led to reduced root biomass allocation in soils conditioned by mixed plant communities but not in monocultures. For instance, drought soil legacy led to increased bacterial richness only within soils conditioned by mixed plant communities, though not significantly (Fig. 4 C). In soils conditioned by mixed plant communities, where a wider array of plant-microbial interactions fosters a richer microbial community, the legacy of drought can enhance bacterial richness by enabling less dominant species to increase in abundance (45). However, the presence of AM fungi significantly modifies this relationship between drought and bacterial richness; more specifically, the positive effect of drought on bacterial richness was reduced when AM fungi was present. However, increased bacterial richness in soil with a drought legacy and a lack of AM fungi may promote *S. canadensis* directly through the increased functionality of soil microbes (24,84), or indirectly by diluting the impact of pathogenic microbes (99). All soils in the experiment were inoculated with AM-associated

microbes either in the presence of absence of AM fungi. These AM-associated microbes are reported to positively influence plant growth and facilitate plant-mycorrhizal interactions (33). As such, our findings suggest that *S. canadensis* may receive greater benefit from AM-associated microbes in the absence of AM fungi, particularly where the diversity of these microbes is heightened. This could facilitate greater nutrient uptake in soils with a drought legacy, where increased nutrient availability and enhanced microbial functionality promote more efficient resource acquisition.

Our study has several limitations, mainly that we kept the study design as simple as possible due to its inherent complexity with multiple treatments, focusing on just one invasive species and four native plants. This approach may limit the generalizability of our findings, although our findings of drought and AM fungi mediated effects on invader performance via AM-associated bacterial communities can be tested with many other contexts. We acknowledge that at the four-species level, we did not have true replicates, as all mixed communities contained the same species composition. While ideally, species composition would vary to create true replicates at this diversity level, it was not feasible within the scope of this study to include more than four native species due to resource constraints and logistical considerations. Despite this limitation, we believe the inclusion of both monocultures and four-species mixtures provides useful insights into the role of diversity in plant-soil feedbacks. We also did not use natural soils, which might have introduced greater variety in the soil microbial community due to previous plant-soil feedback effects. While, the primary aim of our study was to rule out or identify specific factors that may drive biotic resistance, rather than providing a comprehensive analysis of all possible influences, these constraints should be taken into account when interpreting our results. Natural systems are far more complex than the simplified study systems used to investigate biotic resistance. While the biodiversity of native ecosystems may contribute to biotic resistance, other factors such as abiotic conditions, invasive species traits, and community network characteristics introduce significant complexity and context dependency (100,101). Future research on biotic resistance should aim to disentangle this

complexity by incorporating a broader range of ecological factors, as at least indicated in our results (e.g., drought and plant's symbiosis with AM fungi)

In conclusion, our study contributes to a broad understanding of the complex factors influencing invasive success from a belowground perspective. The influence of soil drought legacy and AM fungi presence played major roles in modulating soil microbial diversity, richness and community composition (Fig. 1 & 2) which had consequences on the performance of *S. canadensis*. Soils from mixed native plant communities increased bacterial species richness, and subsequently led to reduced root biomass allocation and increased shoot biomass allocation in *S. canadensis*, possibly due to improved nutrient acquisition efficiency. However, in soils conditioned by monoculture, *S. canadensis* showed opposite trends of increased root biomass allocation with increasing bacterial species richness (Fig. 4). Our findings highlight both biotic and abiotic contexts associated with mechanisms influencing invasive success and underscore the need for further research incorporating a broader range of ecological factors to better understand and manage invasive species in diverse ecosystems.

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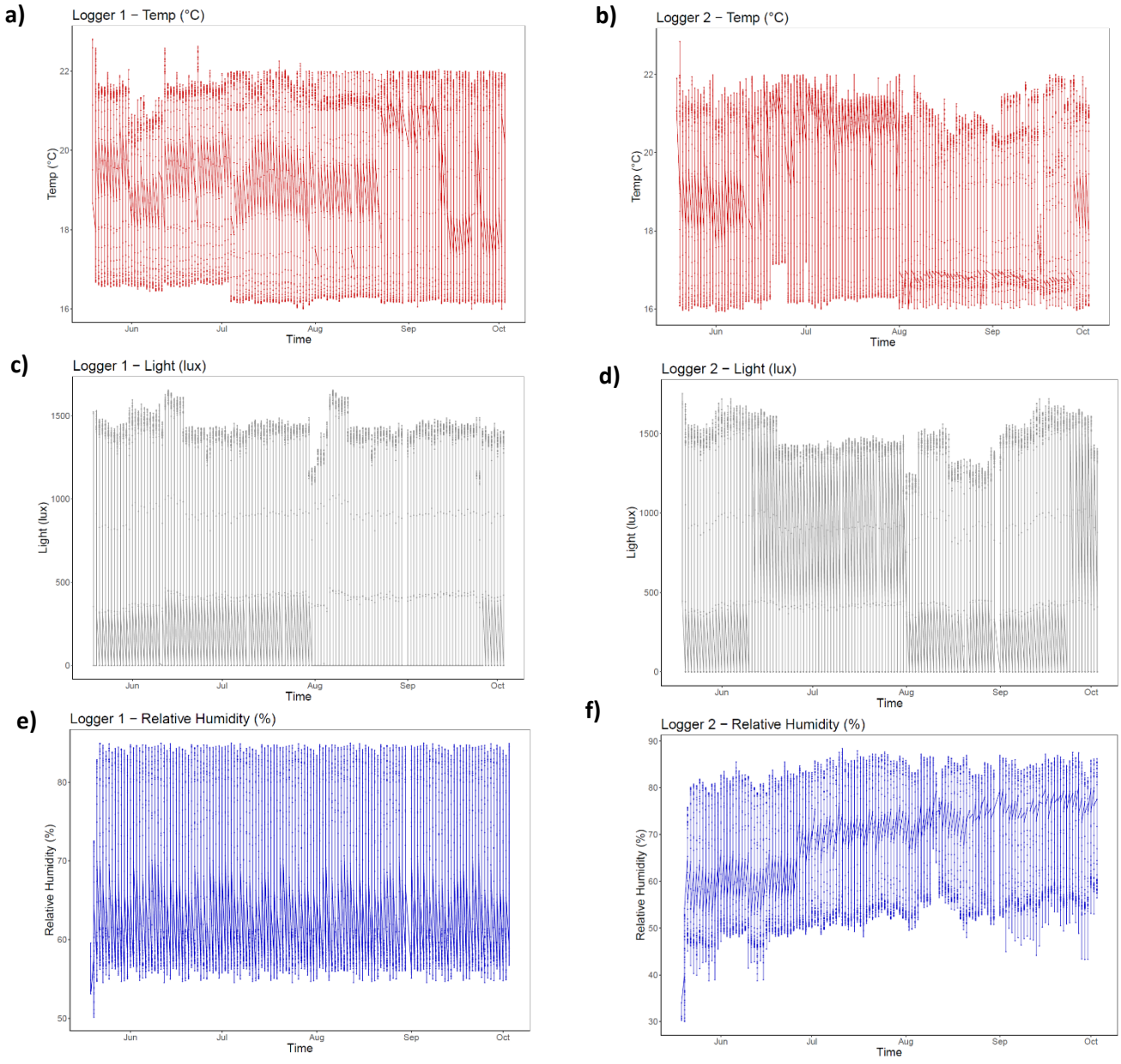
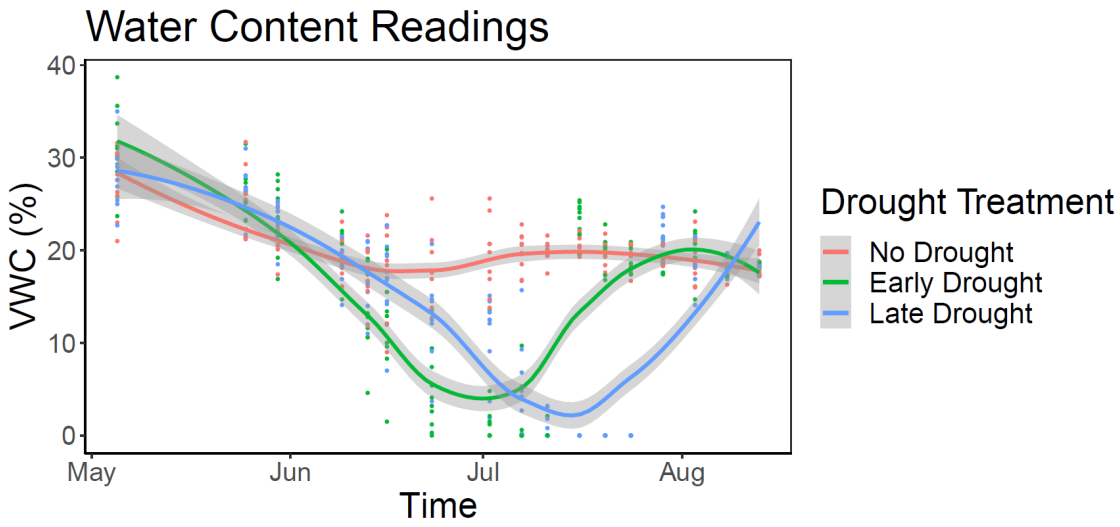
Supplementary material



Supplementary figure 1. Climatic conditions measured in both climate chambers throughout the experiment (both conditioning and feedback phase). Logger 1 was placed in room D321 and logger 2 in room D322 (Baltzerstrasse 6, 52 3012, Bern). Temperature (a, b), relative humidity (RH) (c, d) and light (e, f) recorded from logger one (left) and two (right).



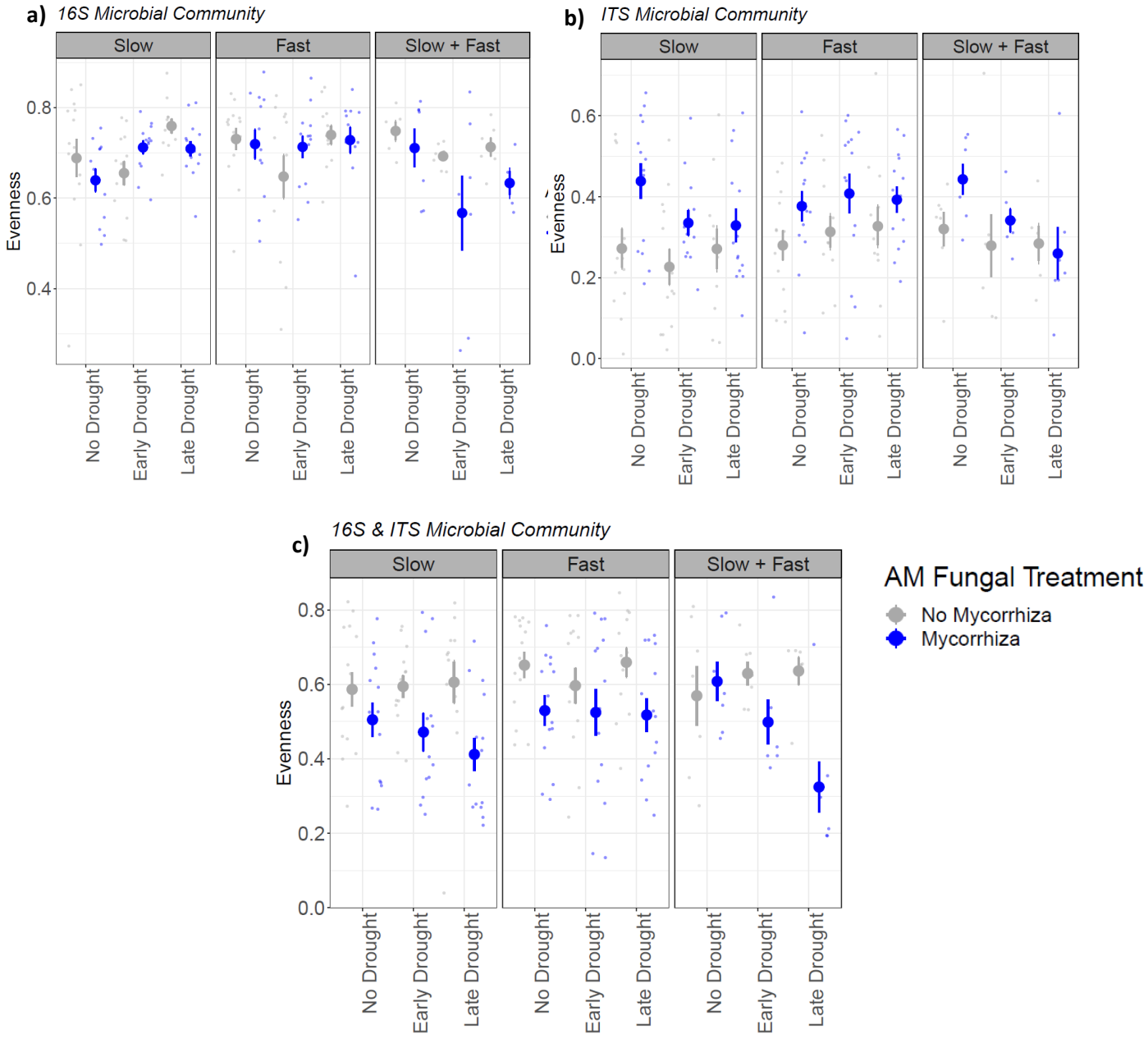
Supplementary figure 2. Water content reading throughout the experiment measured using a soil moisture meter TDR 150 (FieldScout, Spectrum Technologies Inc., USA) on 30 extra pots (one for each treatment combination).

Supplementary material 3. Phased primers from QIAseq 16S/ITS Smart Control (Qiagen, Germany) targeting the 16S (V1-V9) & ITS (5.8S) regions with 0–11 additional bases to the 5'-end of the 16S rRNA and ITS amplicon primers:

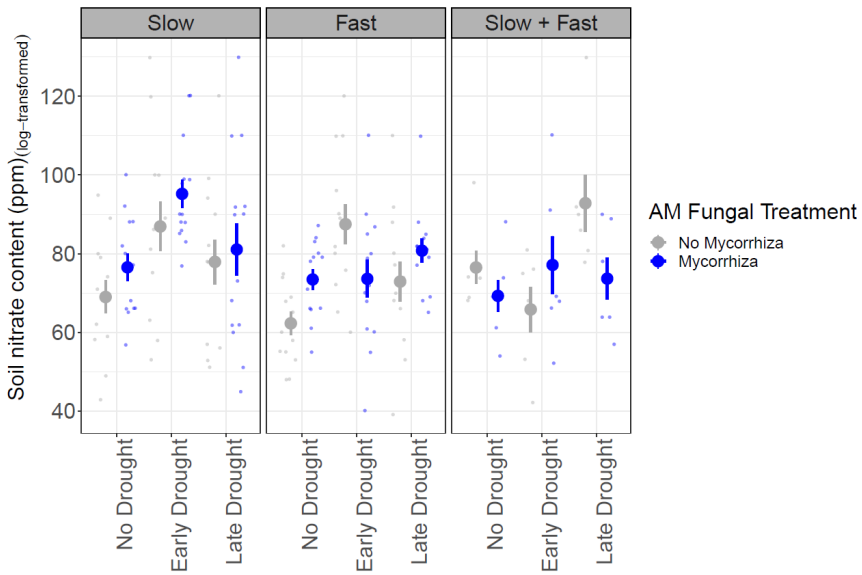
Qiagen ITS primers:
ITS forward primer: 'CTTGGTCATTTAGAGGAAGTAA'
ITS reverse compliment of forward primer: 'TTACTTCCTCTAAATGACCAAG'
ITS reverse primer: 'GCTGCGTTCTTCATCGATGC'
ITS reverse compliment of reverse primer: 'GCATCGATGAAGAACGCAGC'
Qiagen 16S primers
16S forward primer for V1V2 region: 'AGRGTTTGATYMTGGCTC'
16S reverse primer for V1V2 region: 'CTGCTGCCTYCCGTA'
16S forward primer for V2V3 region: 'GGCGNACGGGTGAGTAA'
16S reverse primer for V2V3 region: 'WTTACCGCGGCTGCTGG
16S forward primer for V3V4 region: 'CCTACGGGNGGCWGCAG'
16S reverse primer for V3V4 region: 'GACTACHVGGGTATCTAATCC'
16S forward primer for V4V5 region: 'GTGYCAGCMGCCGCGGTAA'
16S reverse primer for V4V5 region: 'CCGYCAATTYMTTTRAGTT'
16S forward primer for V5V7 region: 'GGATTAGATACCCBRGTAGTC'
16S reverse primer for V5V7 region: 'ACGTCRTCCCCDCCTTCCTC'
16S forward primer for V7V9 region: ' YAACGAGCGMRACCC'
16S reverse primer for V7V9 region: 'TACGGYTACCTTGTTAYGACTT'

Supplementary Table 1. Results from linear mixed-effect models testing the effects AM fungi presence (AM), extreme drought treatment (Dr) and native conditioning community (Com) for the dependent variables in the conditioning and feedback phase. Bold values are statistically significant (p-value < 0.05). Conditional R2 represents the combined effects of fixed and random effects used in our models. We also provide overall model R2 for all mixed-effect models used in our study. df stands for degrees of freedom.

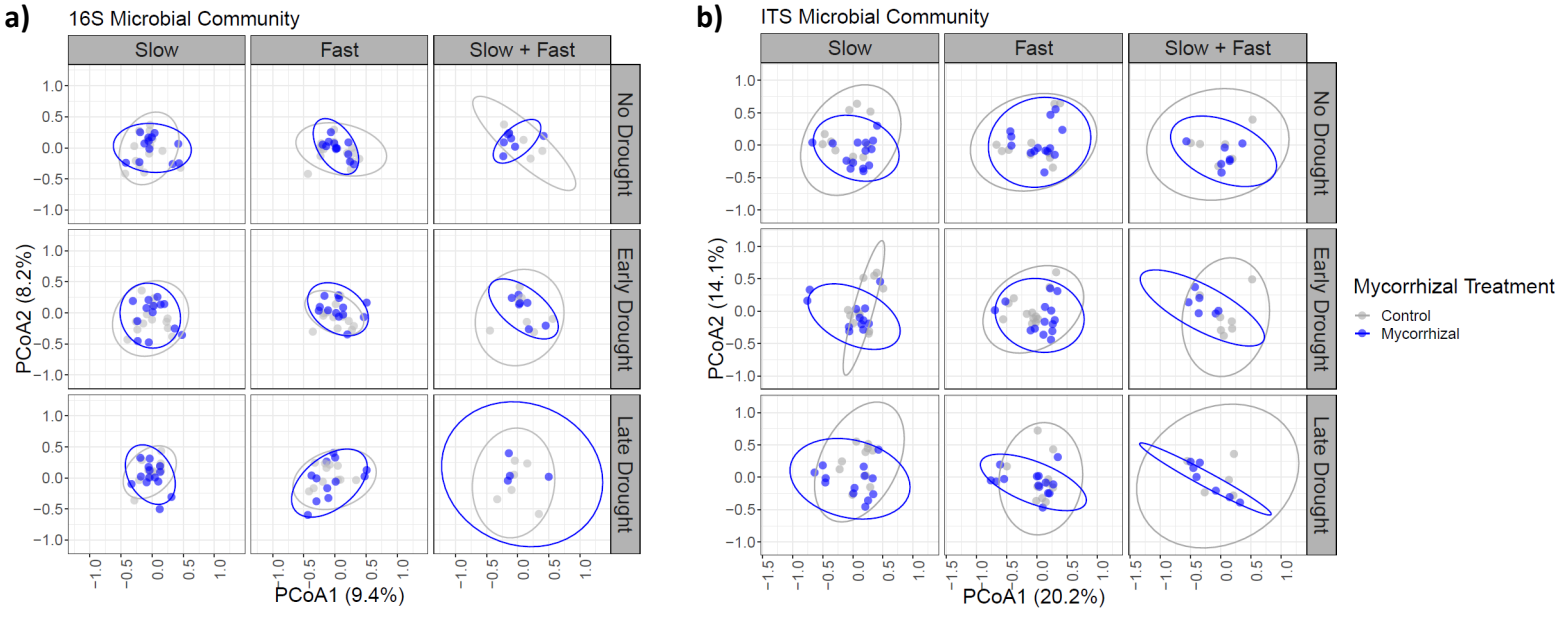
	Response Type	Response Variable	AM Fungi (AM)		Drought (Dr)		Community (Com)		DR x AM		AM x Com		Dr x Com		Dr x AM x Com		Random intercept (Bench)	Model
			F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	F-value$_{DF}$	p-value	Variance	R-squared (conditional)
Feedback	**Leaf Traits**	Height (log-transformed)	$15.46_{1,189}$	**<0.001** ***	$9.77_{2,189}$	**<0.001** ***	$1.20_{2,189}$	0.30	$1.76_{2,189}$	0.17	$2.91_{2,189}$	0.06	$0.59_{4,189}$	0.67	$1.23_{4,189}$	0.30	0.01	0.26
		LDMC (log-transformed+1)	$12.42_{1,189}$	**<0.001** ***	$15.07_{2,189}$	**<0.001** ***	$1.03_{2,189}$	0.36	$1.74_{2,189}$	0.18	$0.67_{2,189}$	0.52	$0.94_{4,189}$	0.44	$1.85_{4,189}$	0.12	0.00	0.25
		SLA (log-transformed)	$0.22_{1,189}$	0.64	$1.85_{2,189}$	0.16	$1.04_{2,190}$	0.36	$0.17_{2,189}$	0.84	$1.91_{2,190}$	0.15	$1.34_{4,189}$	0.26	$0.63_{4,190}$	0.64	0.02	0.10
Conditioning	**Biomass**	Average total biomass (log-transformed)	$1.52_{1,144}$	0.22	$3.50_{2,144}$	**<0.05** *	$65.74_{2,144}$	**<0.001** ***	$0.30_{2,144}$	0.74	$7.74_{2,144}$	**<0.001** ***	$1.58_{4,144}$	0.18	$1.35_{4,144}$	0.25	0.00	0.53
		Average aboveground biomass (log-transformed)	$0.00_{1,154}$	0.97	$6.68_{2,154}$	**<0.01** **	$32.58_{2,154}$	**<0.001** ***	$0.50_{2,154}$	0.61	$17.31_{2,154}$	**<0.001** ***	$2.37_{4,154}$	0.06	$1.09_{4,154}$	0.36	0.00	0.42
		Average belowground biomass	$2.54_{1,180}$	0.11	$57.28_{2,179}$	**<0.001** ***	$1.74_{2,178}$	0.18	$0.73_{2,178}$	0.48	$2.96_{2,179}$	0.05	$0.92_{4,179}$	0.45	$3.59_{4,179}$	**<0.01** **	0.00	0.45
		Root:Shoot	$0.47_{1,146}$	0.49	$1.05_{2,146}$	0.35	$23.83_{2,145}$	**<0.001** ***	$2.81_{2,146}$	0.06	$27.37_{2,143}$	**<0.001** ***	$4.55_{4,145}$	**<0.01** **	$7.48_{4,146}$	**<0.001** ***	0.00	0.44
	Leaf Traits	LDMC (log-transformed)	$0.34_{1,152}$	0.56	$1.01_{2,151}$	0.37	$1.08_{2,151}$	0.34	$0.30_{2,152}$	0.74	$2.67_{2,152}$	0.07	$1.86_{4,152}$	0.12	$1.08_{4,152}$	0.37	0.00	0.17
		SLA	$3.23_{1,154}$	0.07	$0.19_{2,153}$	0.82	$10.45_{2,153}$	**<0.001** ***	$0.65_{2,154}$	0.53	$17.82_{2,153}$	**<0.001** ***	$1.13_{4,153}$	0.35	$0.54_{4,153}$	0.71	0.74	0.29
	Root Traits	Root diameter	$13.92_{1,186}$	**<0.001** ***	$0.52_{2,186}$	0.59	$0.05_{2,186}$	0.95	$0.94_{2,186}$	0.39	$5.78_{2,186}$	**<0.01** **	$0.23_{4,186}$	0.92	$1.64_{4,186}$	0.17	0.00	0.13
		SRL (log-transformed)	$2.43_{1,178}$	0.12	$0.71_{2,178}$	0.49	$5.49_{2,178}$	**<0.01** **	$0.15_{2,178}$	0.86	$4.34_{2,178}$	**<0.05** *	$0.72_{4,178}$	0.58	$0.90_{4,178}$	0.47	0.00	0.14
		Proportion colonisation			$1.07_{2,92}$	0.35	$0.14_{2,91}$	0.90					$0.31_{4,90}$	0.87			0.00	0.06
	Nutrient	NO_3	$0.15_{1,165}$	0.70	$5.90_{2,163}$	**<0.01** **	$2.51_{2,20}$	0.11	$0.57_{2,163}$	0.57	$1.45_{2,165}$	0.24	$2.39_{4,164}$	0.05	$3.17_{4,163}$	**<0.05** *	0.00	0.21
	Microbial Diversity	16S Shannon diversity	$35.35_{1,165}$	**<0.001** ***	$3.17_{2,165}$	**<0.05** *	$2.39_{2,165}$	0.09	$0.76_{2,166}$	0.47	$6.67_{2,166}$	**<0.01** **	$1.98_{4,165}$	0.10	$0.98_{4,165}$	0.42	0.01	0.29
		16S Simpson diversity	$2.83_{1,182}$	0.09	$5.78_{2,182}$	**<0.01** **	$1.13_{2,183}$	0.32	$0.19_{2,182}$	0.83	$2.73_{2,183}$	0.07	$0.97_{4,182}$	0.42	$1.90_{4,182}$	0.11	0.00	0.17
		16S richness	$6.40_{1,185}$	**<0.05** *	$0.57_{2,182}$	0.57	$0.57_{2,184}$	0.57	$0.06_{2,183}$	0.94	$10.57_{2,184}$	**<0.001** ***	$0.11_{4,183}$	0.98	$4.09_{4,183}$	**<0.01** **	0.00	0.18
		16S evenness	$2.50_{1,166}$	0.12	$3.57_{2,165}$	**<0.05** *	$1.39_{2,165}$	0.25	$0.67_{2,166}$	0.52	$2.34_{2,166}$	0.10	$1.98_{4,165}$	0.10	$1.04_{4,165}$	0.39	0.01	0.29
		ITS Shannon diversity	$56.79_{1,185}$	**<0.001** ***	$0.26_{2,185}$	0.77	$0.33_{2,185}$	0.72	$2.75_{2,185}$	0.07	$0.19_{2,185}$	0.83	$0.62_{4,185}$	0.65	$1.58_{4,185}$	0.18	0.00	0.28
		ITS Simpson diversity	$3.73_{1,185}$	0.05	$0.32_{2,185}$	0.73	$0.55_{2,185}$	0.58	$0.93_{2,185}$	0.40	$0.53_{2,185}$	0.59	$0.39_{4,185}$	0.81	$1.17_{4,185}$	0.32	0.00	0.06
		ITS richness	$77.62_{1,185}$	**<0.001** ***	$2.34_{2,185}$	0.10	$0.28_{2,185}$	0.75	$0.38_{2,185}$	0.68	$3.10_{2,185}$	**<0.05** *	$0.61_{4,185}$	0.65	$1.28_{4,185}$	0.28	0.01	0.39
		ITS evenness	$48.59_{1,185}$	**<0.001** ***	$0.20_{2,185}$	0.82	$0.28_{2,185}$	0.75	$3.20_{2,185}$	**<0.05** *	$0.09_{2,185}$	0.91	$0.48_{4,185}$	0.75	$1.37_{4,185}$	0.25	0.00	0.25
		Entire microbial Shannon diversity	$26.04_{1,182}$	**<0.001** ***	$1.38_{2,182}$	0.25	$2.29_{2,183}$	0.10	$2.96_{2,183}$	0.05	$1.03_{2,184}$	0.36	$0.49_{4,183}$	0.74	$1.85_{4,183}$	0.12	0.00	0.19
		Entire microbial Simpson diversity	$29.24_{1,185}$	**<0.001** ***	$1.70_{2,185}$	0.19	$1.59_{2,185}$	0.21	$3.49_{2,185}$	**<0.05** *	$0.56_{2,185}$	0.57	$0.87_{4,185}$	0.48	$1.78_{4,185}$	0.13	0.00	0.21
		Entire microbial richness	$6.40_{1,185}$	**<0.05** *	$0.57_{2,185}$	0.57	$0.57_{2,185}$	0.57	$0.06_{2,185}$	0.94	$10.57_{2,185}$	**<0.001** ***	$0.11_{4,185}$	0.98	$4.09_{4,185}$	**<0.01** **	0.00	0.18
		Entire microbial evenness	$22.68_{1,185}$	**<0.001** ***	$1.25_{2,185}$	0.29	$2.29_{2,185}$	0.10	$3.51_{2,185}$	**<0.05** *	$0.06_{2,185}$	0.94	$0.59_{4,185}$	0.67	$1.08_{4,185}$	0.37	0.00	0.17



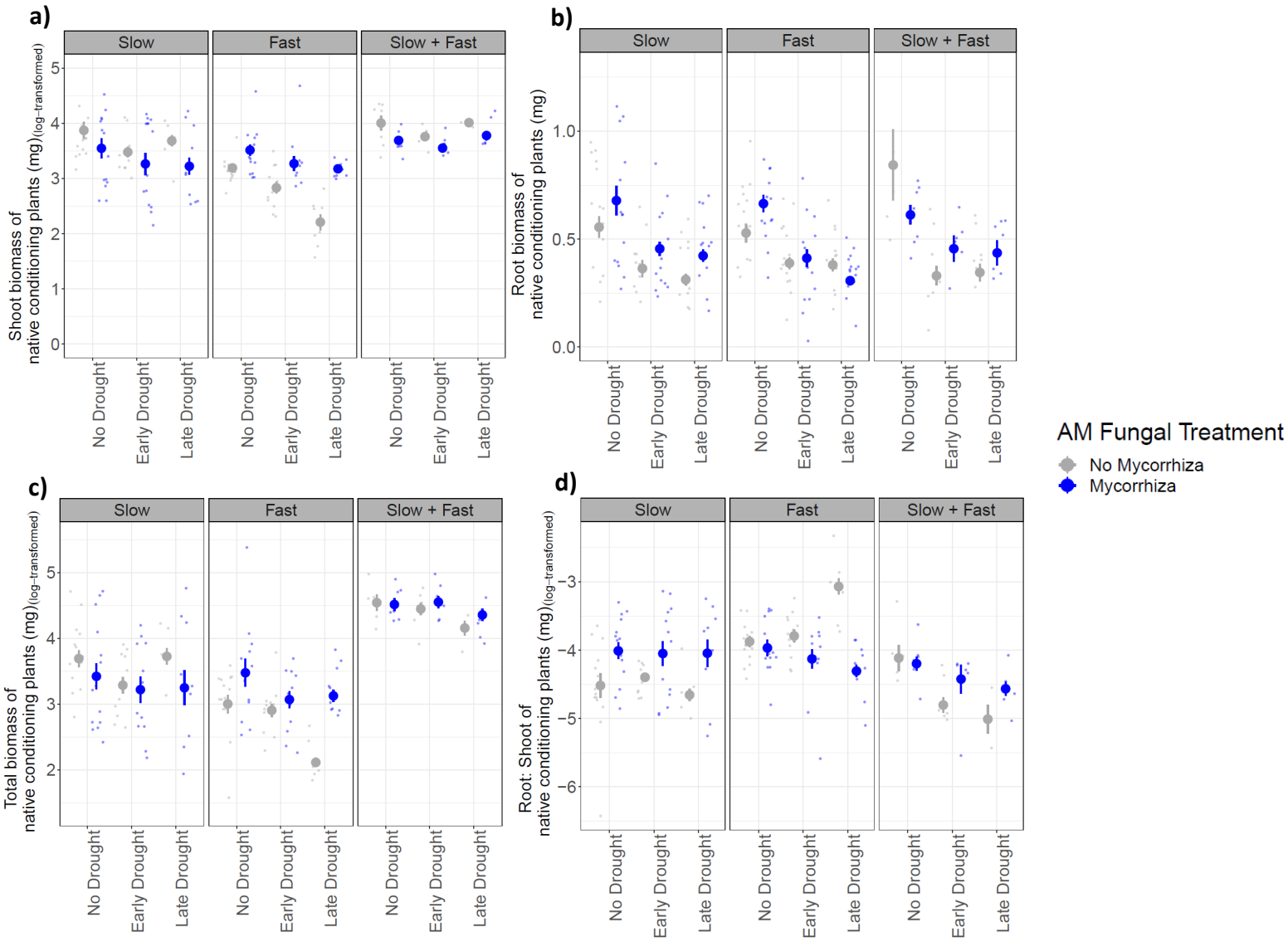
Supplementary Figure 4. Bacterial community evenness (a), fungal community evenness (b), and total community evenness (c) within conditioned soil. Soils conditioned by slow-growing (Slow), fast-growing (Fast) and diverse (Slow + Fast) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis within each the presence of AM fungi is shown using the colours grey (control) or blue (mycorrhizal). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



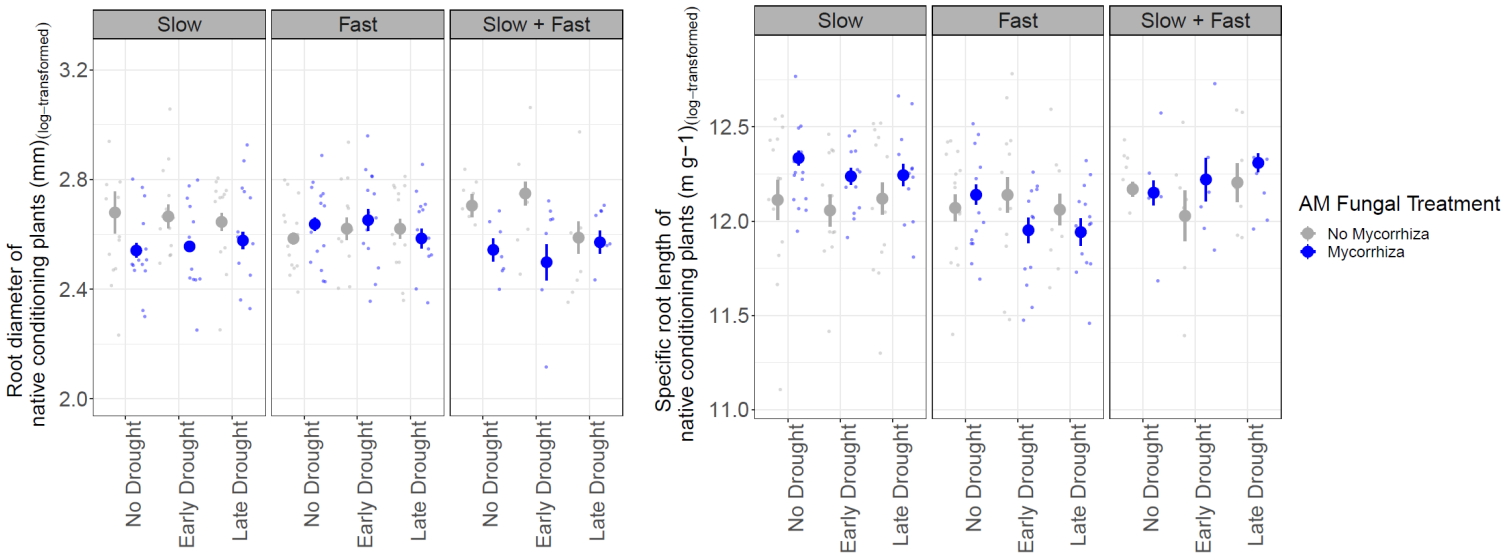
Supplementary Figure 5. Variation in soil nitrate (NO_3) after the conditioning phase. Nitrate concentrations in soils conditioned by slow-growing (Slow), fast-growing (Fast) and diverse (Fast + Slow) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis, within each the presence of AM fungi is shown using the colours grey (no mycorrhiza) or blue (mycorrhiza). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



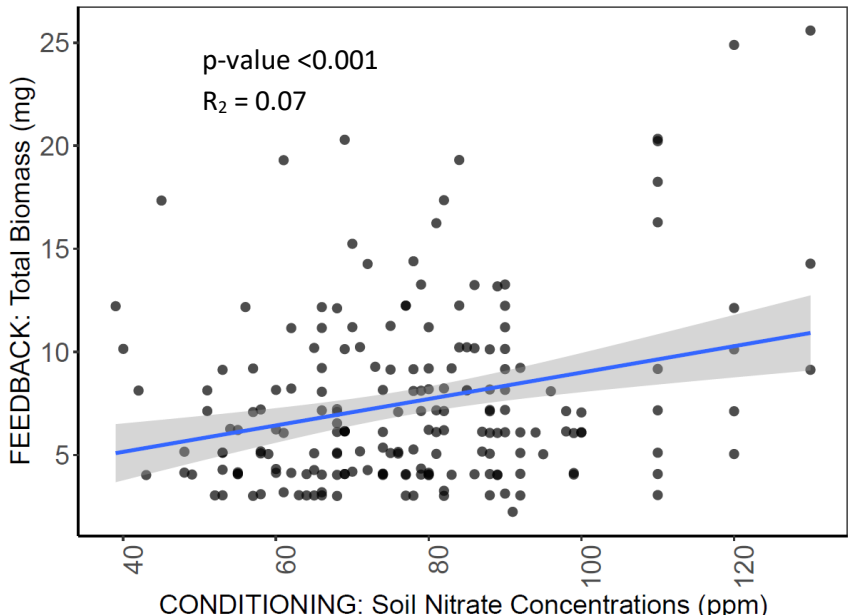
Supplementary Figure 6. Principal Coordinate Analysis (PCoA) plots illustrating the differences in a) bacterial (16S) and b) fungal (ITS) community composition under various mycorrhizal treatments (No mycorrhiza: grey, Mycorrhiza: blue) and drought treatments. The plots are divided into three columns representing different native plant conditioning communities' groups: slow-growing (Slow; left), fast-growing (Fast; middle), and mixed (Slow + Fast; right). Each point represents a sample, and the ellipses indicate 95% confidence intervals for the respective treatment groups.



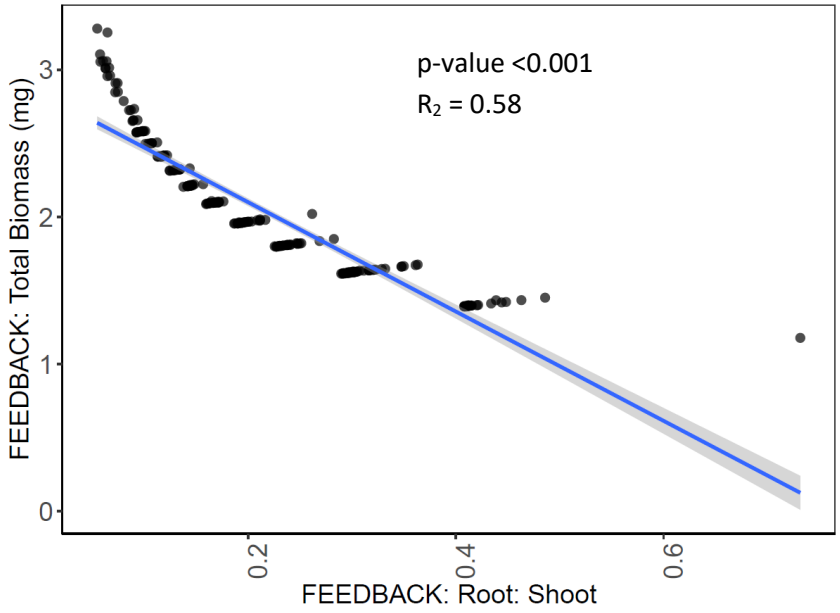
Supplementary Figure 7. Plant shoot biomass (a), root biomass (b), total biomass (c) and root: shoot ratio (d) responses of native conditioning plants in the conditioning phase. Biomass responses of slow-growing (Slow), fast-growing (Fast) and diverse (Fast + Slow) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis, within each the presence of AM fungi is shown using the colours grey (no mycorrhiza) or blue (mycorrhiza). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary Figure 8. Root diameter (left) and Specific root length (right) responses of native conditioning plants in the conditioning phase. Biomass responses of slow-growing (Slow), fast-growing (Fast) and diverse (Fast + Slow) native plant communities are shown within separate facets. Drought treatments are shown on the x-axis, within each the presence of AM fungi is shown using the colours grey (no mycorrhiza) or blue (mycorrhiza). Raw data are shown as fine points, while the horizontal error bars represent the mean ± standard error (SE).



Supplementary Figure 9. Correlation between total plant biomass of *S. canadensis* in the feedback phase by soil nitrate concentrations. The shaded area represents the 95% confidence interval of the fit, p-value and R2 values calculated from ranged major axis (RMA) models.



Supplementary Figure 10. Correlation between total plant biomass by the root: shoot ratio of *S. canadensis* in the feedback phase. The shaded area represents the 95% confidence interval of the fit, p-value and R2 values calculated from ranged major axis (RMA) models.

Chapter 4

Effects of extreme drought on the invasion dynamics of non-native plants

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Keywords : ecological responses; invasive stages; invasion windows; biotic resistance; invasion meltdown

Abstract

The increasing frequency of extreme droughts poses significant challenges for predicting the invasion success (or failure) of non-native plant species. While current frameworks are primarily based on seasonal droughts, the unique characteristics of extreme droughts necessitate re-evaluating our understanding of plant invasion during and after extreme droughts. Here, using core principles of community assembly and invasion biology, we discuss how the invasibility of non-native plants during and after extreme droughts differs due to: 1) differences in the ecological response of the native community, 2) barriers at different invasive stages and 3) the traits of non-native plants. We incorporate ideas from current ecological theories of invasive success and suggest how drought-mediated invasion is influenced by biotic interactions in the native community.

Seasonal variation in precipitation and temperature cause temporal, usually cyclical, variation in the water available for plant growth. As plants have adapted to these alternating wetter and drier periods, average climatic variables (e.g. average annual temperature, average annual precipitation, seasonality) can predict the potential distributions of species quite well [1]. However, with continued climate change, the frequency of extreme climatic events, both their amplitudes and durations, is increasing and will continue to increase [I]. From a purely climatic perspective, extremes are defined

as the tails of the distribution of climatic events [I]. Common metrics of climate extremes include counts, percentages, or days when climatic variables fall below certain percentiles, usually within specific time frames, relative to the 1961-1990 reference period [II]. So, in case of **extreme drought** (see Glossary), this would be periods with much more or much less precipitation than usual during a certain reference period, where 'usual' is location specific. However, as pointed out by Smith (2011) [2], a more synthetic definition would not only consider the extremeness of the driver (e.g. precipitation), but also the extremeness of the ecological response. An extreme ecological response, however, can be difficult to define, as responses vary significantly depending on the specific system under consideration and the duration being examined [3]. Gutschick & Bassirirad (2003) [4] characterise an extreme event as one which surpasses the capacity of organisms to acclimate, often leading to persistent effects with long-term impacts on fitness. Subsequently, this extreme response at the individual and population level can lead to significant changes in ecosystem structure and function, such as the re-ordering of key species in the community, widespread species loss, or invasion by novel species [2].

1. Invasion success and ecological responses to extreme drought events

Climate extremes may play an important role in facilitating non-native species by creating "invasion windows" [5]. This concept suggests that climate extremes may create favourable conditions for non-native species by reducing abundances of the native species and inducing extreme ecological responses. As after a physical

disturbance event, such extreme ecological responses in the native community both increase the availability of nutrients and space, and decrease **biotic resistance** through declines in species richness [5, 6] (Box 1); thereby influencing the successful **establishment** of non-native plant species. The study by Jiménez et al. (2011) [7] observed that an extreme drought in 1998 facilitated the establishment of non-native species within native annual plant communities in northern Chile. This extreme drought event had a significant impact on the plant community, disrupting its dynamics, altering recovery processes, and reshaping species composition. While the cover of non-native annual plants was low and stable prior to 1998, it increased notably after the extreme drought event in 1998. Invasibility in this case was driven by the extreme ecological responses of the native community. This clearly shows that the creation of an invasion window is dependent on whether a climate extreme also induces an extreme ecological response (Fig 1).

In native communities with a legacy of past drought stress, native plants may possess stressor-induced “memory” or “legacy” that allows for enhanced drought resilience [8]. Plastic and evolutionary shifts in plant traits, epigenetic changes, shifts in community composition and soil biotic legacies [9, 10] may **climatically condition** plant communities that do not respond strongly to climatically extreme droughts. Where extreme drought does not induce ecological extreme responses in native plant communities, invasion by non-native species may not be facilitated (Fig. 1). For example, a study by Pérez-Navarro et al. (2021) [11], found that extreme drought

promoted the abundance of more drought tolerant species through reductions in poor performing drought intolerant plant species in semiarid shrublands. This reduction in climatic disequilibrium (i.e. the occurrence of species whose climatic preferences do not match the climatic conditions) suggests that extreme climatic events, such as drought, can act as environmental filters that shift species dominances and result in a more arid-adapted plant community [11]. More arid adapted native plant communities are therefore generally more tolerant to recurrent extreme drought events and invasion windows are less likely to widen in such drought-resistant communities. However, even these drought-resistant ecosystems can experience extreme ecological responses under prolonged periods of extreme drought [12]. The responses of these ecosystems are also highly context-dependent, and the influence of other environmental stressors may generate more extreme ecological responses in response to drought [13].

Moreover, ecologically extreme responses can also occur in response to **moderate droughts**. For example, the legacy of an extreme drought may cause **maladaptive conditioning** through an “overshoot phenomenon”, whereby, increased nutrient availability stimulates over-recovery of the ecosystem [3, 14]. This can lead to a depletion of other potentially limiting resources such as soil moisture, leaving the ecosystem vulnerable to subsequent drought events [14]. For example, a study by Valliere et al. (2017) found that even drought-adapted species in California's semi-arid shrublands experienced substantial dieback during an extended multi-year drought [15]. Notably, the severity of these dieback responses was intensified in areas subjected to

experimental nitrogen deposition. The increased nutrient availability led to greater plant productivity, but also heightened the ecosystem's vulnerability to subsequent drought [15]. As a result, the combination of drought-induced plant mortality and elevated resource availability increased the likelihood of non-native species establishing and thriving in these environments [15, 16]. This is particularly true as non-native plants can exploit resources better than native species. [17, 18].

Anthropogenic changes, including agricultural intensification, land-use change, eutrophication, and nitrogen deposition, further exacerbate the vulnerability of drought afflicted ecosystems to invasion [19 - 21]. Drought together with some of these global change drivers can significantly alter resource availability and community structures, providing windows of opportunity for non-native plants to become invasive [20]. Indeed, multiple stressors can interact in complex ways, often exhibiting synergistic effects that exacerbate plant decline beyond the sum of their individual impacts [13, 22]. For example, extreme heat combined with drought can cause irreversible damage to plant functions like hydraulic conductance and photosynthesis, while also heightening the production of reactive oxygen species, ultimately prolonging recovery and reducing carbon uptake and widening invasion windows [22, 23]. This increase in invasibility may also be magnified in the presence of already established non-native plants due to the influence of **invasional meltdown** on the native community [24] (Box 1).

Knowledge of these ecological responses helps form strategies for controlling invasive species, restoring native habitats, and mitigating the impacts of drought on vulnerable ecosystems. However, while invasibility may increase with the severity of ecological drought responses within the native community, this is related more to conditions after the drought event (i.e. during rewetting) while plant communities (consisting of non-natives as well) are recovering (Fig. 1). Indeed, during the drought event itself, non-native species likewise suffer and respond negatively to the impact of reduced water availability with further impacts on growth and reproductive success [25, 26]. As such, it is likely that the impact of extreme drought on invasion varies during and after the extreme drought event.

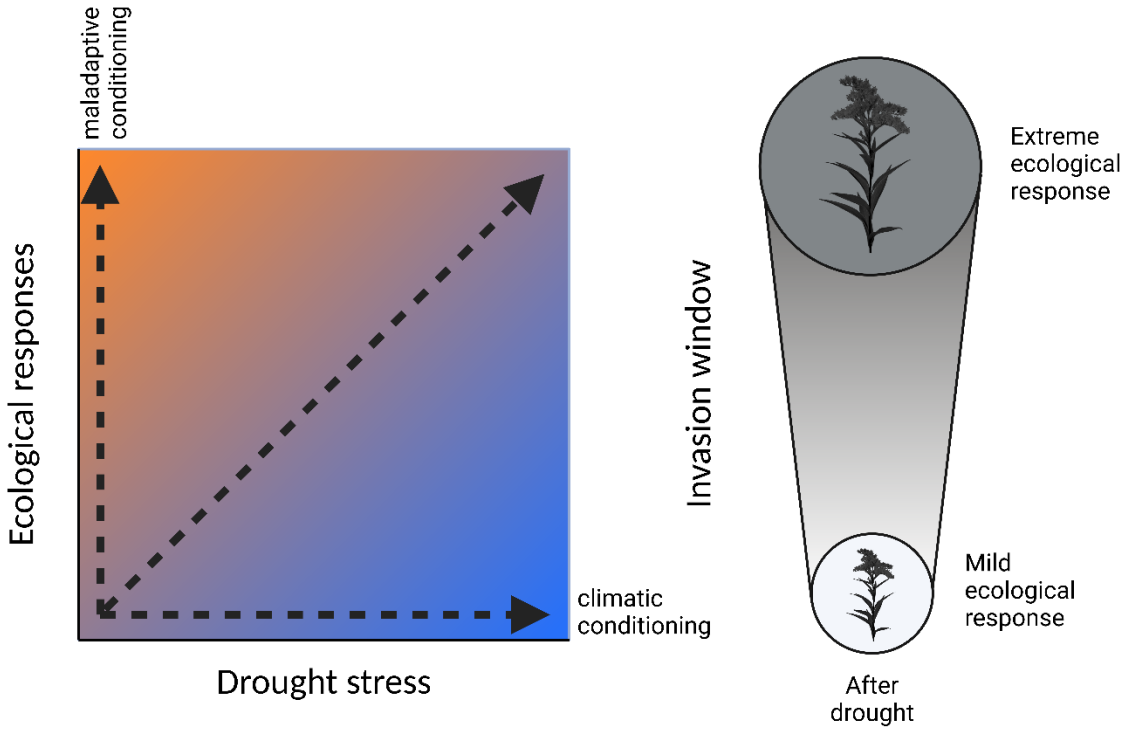

Figure 1. A conceptual representation of the relationship between extreme ecological responses of the native ecosystem and the severity of drought events (represented by the black dashed arrows). Scenarios where extreme drought conditions do not illicit extreme ecological responses can occur through climatic conditioning (blue colour gradient), whereby, pre-exposure to a stressor event drives the community to cope better with recurrent drought due shifts to a more arid-adapted native plant community. However, extreme ecological responses, such as, extreme declines in primary productivity, to moderate or seasonal drought represents maladaptive conditioning whereby, a compound stressor event or pre-exposure to a stressor drives the community to cope worse with drought (orange colour gradient). The width of the invasion window within an ecosystem is positively related to the ecological response during the recovery period (right: white to grey coloured circles). For the sake of simplicity, maladaptive and climatic conditioning of plant communities are exclusively meant for native plants here (but see section 2). Created with BioRender.com and Canva.com.

Box 1: Current ecological theories on the influence of the native community on invasion success

Multiple ecological theories have been developed, attempting to explain invasion success. Among them, two have often received attention to understand the invasibility from the context of the native ecosystem: the biotic resistance hypothesis [6] and the invasion meltdown hypothesis [24].

The biotic resistance hypothesis centres on the influence of native biodiversity in preventing or reducing non-native establishment. Native communities with high functional diversity can weaken the establishment success of an invader by possessing a greater variety of complex interactions, such as herbivory, predation, disease and

competition [6]. The hypothesis poses that more diverse plant communities limit the available niche space to non-native species and have a greater likelihood of hosting competitive neighbours and enemies [6].

The invasion meltdown hypothesis suggests that the presences of one invasive plant species modifies the ecosystem to such an extent that it facilitates the invasion of other non-native species [24]. Invasive plants can directly facilitate the invasion of another non-native plant, for example, a nitrogen-fixing invasive plant species which increases the nutrient availability to other non-native species [24]. Indirectly, invasive species can also modify the native community composition to facilitate further invasion by suppressing enemies and promoting mutualists [27, 28]. Furthermore, separate alien plant species from different origins are distant in their phylogenetic history, perhaps even more so than some aliens to native plant species in the novel community [28]. This lack of similarity can increase the invasion of a non-native plant species as there is less overlap in shared enemies than with members of the native community [29, 30].

2. How do extreme droughts alter invasibility at different invasive stages?

Extreme drought can substantially reduce native plant performance and increases mortality [31, 32]. A recent global study indicates that the magnitude of aboveground net primary productivity (ANPP) loss during extreme droughts is 60% greater compared to mild and moderate droughts [32]. As such, when compared to moderate drought, extreme drought acts like a disturbance that substantially reduces the net competitive

effects of native species and increases potential nutrient availability to non-native species [33] (Fig. 2). Following the extreme drought, during the recovery period, there is, therefore, an invasion window, and this persists for longer after extreme droughts compared to moderate droughts [5, 31] (Fig. 1). However, it is well recognized that successful invasion occurs across multiple stages, each representing different hurdles that have to be overcome by a non-native species in the new environment [34, 35] (Fig. 3). While extreme drought may facilitate crossing some hurdles in the invasion process, drought may have neutral or even detrimental effects on invasion at other stages (Fig. 3).

After successful **introduction** of non-native propagules into the native environment, drought first impacts the **spontaneous establishment** success of the non-native species by influencing its germination success and growth [34] (Fig. 3). Several studies have suggested that, compared to native plants, non-native plants may suffer more under drought as they tend to possess more ruderal rather than stress-tolerant plant traits [26, 36, 37]. In fact, some of these plant traits increase the water use of non-native plants, which amplify the effects of extreme drought further [37, 38]. As such, an extreme drought might initially hinder the establishment success of many non-native plants with high water use. However, after an extreme drought, the establishment of a non-native plant is facilitated during the recovery phase [5, 7] (Fig. 3). Extreme drought also induces shifts in the community structure of neighbouring plants as well as pathogenic and mutualistic species of other trophic levels, with further impacts on

invasibility (Box 2). Plant community reshuffling following an extreme drought can occur through the senescence of drought intolerant species and the increase of stress tolerant and opportunistic species [39, 40]. Consequently, this species turnover can result in a decline in species richness and reordering of dominance hierarchies within the native plant community [39, 41]. After exposing plant communities for three years to various intensities of drought, a study performed by Zhang et al. (2019), found that plant species richness was reduced by 25.7% under the most extreme drought condition, while less extreme drought treatments (e.g., mild or moderate) had no effects [41]. Such a decrease in species richness reduces the biotic resistance of the native community by opening niches and reducing the likelihood of interactions with competitive neighbours and enemies in the native habitat [6] (Box 1; Fig. 2).

Non-native plants that have already arrived in the native community and passed the establishment hurdle, are subjected to the extreme drought stress together with their native neighbours. As such, the benefits received by an individual non-native plant after the drought event depends on its own post-drought recovery [5]. Indeed, some studies have suggested that invasive plants may utilize soil microbes more effectively than native plants to enhance their recovery after drought [42]. However, extreme drought can still detrimentally impact a non-native's ability to **proliferate** and expand its population by reducing growth, seed production and germination success [26, 36, 37] (Fig. 3). On the other hand, seeds produced prior-drought by the successfully established non-native plants and stored in the seedbank, will likely profit during the rewetting. Though this may equally be dependent on the phenology of the non-native

plant and the timing of the drought event [20]. Seed banks play a crucial role in promoting the proliferation of non-native plant species by providing a reservoir of seeds that can remain dormant for extended periods, allowing invasive plants to persist and spread even under unfavourable conditions such as extreme drought [43]. This aligns with the concept of 'invasion debt,' whereby populations of non-native species that are too similar to native species may persist in small numbers until native populations crash, at which point they can proliferate and spread [44, 45]. It has long been postulated that invasive species benefit from an increased amount of unused resources (fluctuating resource hypothesis: [17, 18]). During rewetting, the benefits invasive seedlings receive from surges in nutrient availability, reduced functioning of native plant species, and decreased species richness of the native community are all amplified after extreme droughts compared to moderate droughts, thereby enhancing the proliferation of non-native plants [5, 31, 46] (Fig. 3).

Seed production is a critically important trait for the proliferation and **landscape spread** of non-native species as higher reproductive outputs enable them to efficiently colonize new habitats, ensuring constant dispersal and establishment [47]. In a study by Mojzes et al. (2020) [48], moderate drought induced increased seed production and higher abundance compared to watered plots. This was attributed to the enhanced phenotypic plasticity of the invasive, which allowed it to exploit unused nutrients. However, this response was dampened under adverse extreme drought conditions [48]. Likewise, in a study by Valliere et al. (2019) [37], extreme droughts were shown to impair the reproductive traits of invasive plants substantially more than those of native plants.

Despite this, the invasive species in the study still produced more seeds than native species under both drought and non-drought conditions and may still be the "winners" in the long run [37]. In some cases, impairment of reproductive traits (e.g. flowering and seed count; [37]) during extreme drought could pose a significant obstacle to the invasive species' proliferation and spread across the landscape (Fig. 3). A similar study by LaForgia et al. (2018) also found that the abundance of seeds of non-native species in a belowground seedbank fell by 52% following an extreme drought event. In this case, native seed production increased and dominated over non-native seed abundance, subsequently leading to a decline in the non-native plant cover [25]. This reduction in propagule pressure can be particularly detrimental for dispersal into communities unaffected by extreme drought, where no “invasion window” is present and where biotic resistance is high (Fig. 3).

In contrast to the other stages, the invasion window during landscape spread is dependent on both the non-natives own ecological responses to drought and the ecological responses of the native community which it is dispersing into during or after drought (Fig. 3). These ecological responses can differ depending on the legacy of past disturbance events and the conditioning effect of the native community, with impacts on invasibility (Fig. 1; Fig. 3). Native communities responding less severely (climatic conditioning, see Fig. 1) during moderate drought may facilitate invasion through retention of soil moisture and humidity under the plant canopy [49], reducing the negative impact of moderate drought on the germination success and seedling growth of the range expanding invasive plants. However, this facilitative effect may disappear

during extreme drought or within communities with maladaptive conditioning [50] (Fig. 1). It has been shown that the beneficial impact of shrubs on soil moisture levels underwent a significant reduction and ultimately ceased entirely when subjected to the most extreme drought conditions [50]. As such, landscape spread in extreme drought conditions is facilitated more during recovery and in the absence of a drought stress, particularly within maladaptive conditioned native communities where severe native drought responses create a wider invasion window (Fig. 3).

Lastly, the intentional or unintentional transport of non-native species through global trade and agricultural imports may increase the frequency of new invasion episodes, especially during periods of extreme drought. As extreme droughts increasingly disrupt local agriculture and destroys harvests, there will be more imports of agricultural products, including potential seed contaminants [51]. Greater reliance on drought-tolerant species, often introduced for agriculture, gardening, or forestry, may inadvertently outcompete native species and exacerbate the issue. The relationship between drought and invasion emphasises the need for integrated management strategies that account for the complex effects of climate change and human activities on ecosystem dynamics.

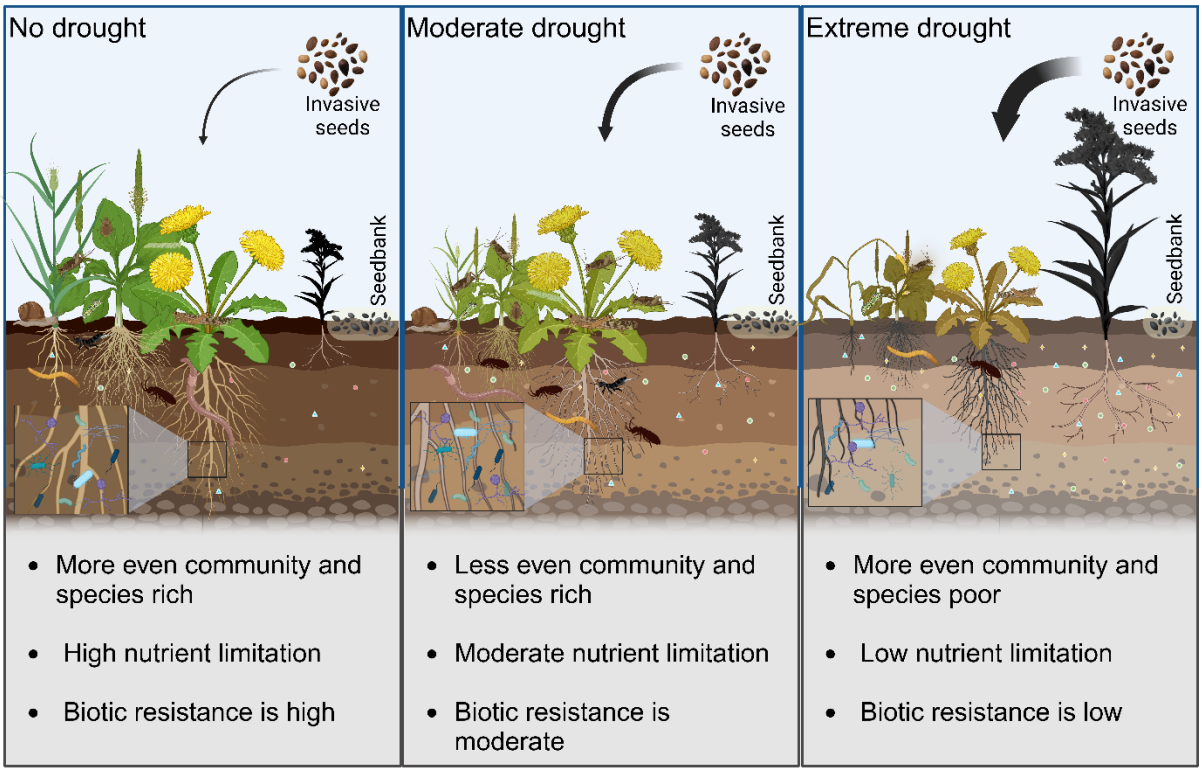

Figure 2. Changes in the properties of recipient ecosystems during moderate and extreme droughts compared to non-drought periods. Some changes in recipient ecosystems after extreme drought enhance or hinder the success of non-native plants (shown by size of the seedbank and arrow drawn from the invasive seed pool). Moderate drought conditions often favour certain species adapted to limited water availability, leading to their dominance and reduced diversity in communities. Conversely, extreme drought creates harsh conditions where only the most resilient species survive, resulting in a more even distribution of species but overall reduced diversity due to the loss of sensitive or less drought-tolerant species. The accumulation of dead biomass, reduced nutrient uptake and reduced washing out of nutrients, increases the nutrient availability in the extreme drought scenario. This increases the chance for opportunistic non-native plants to invade in these ecosystem. Created with BioRender.com and Canva.com.

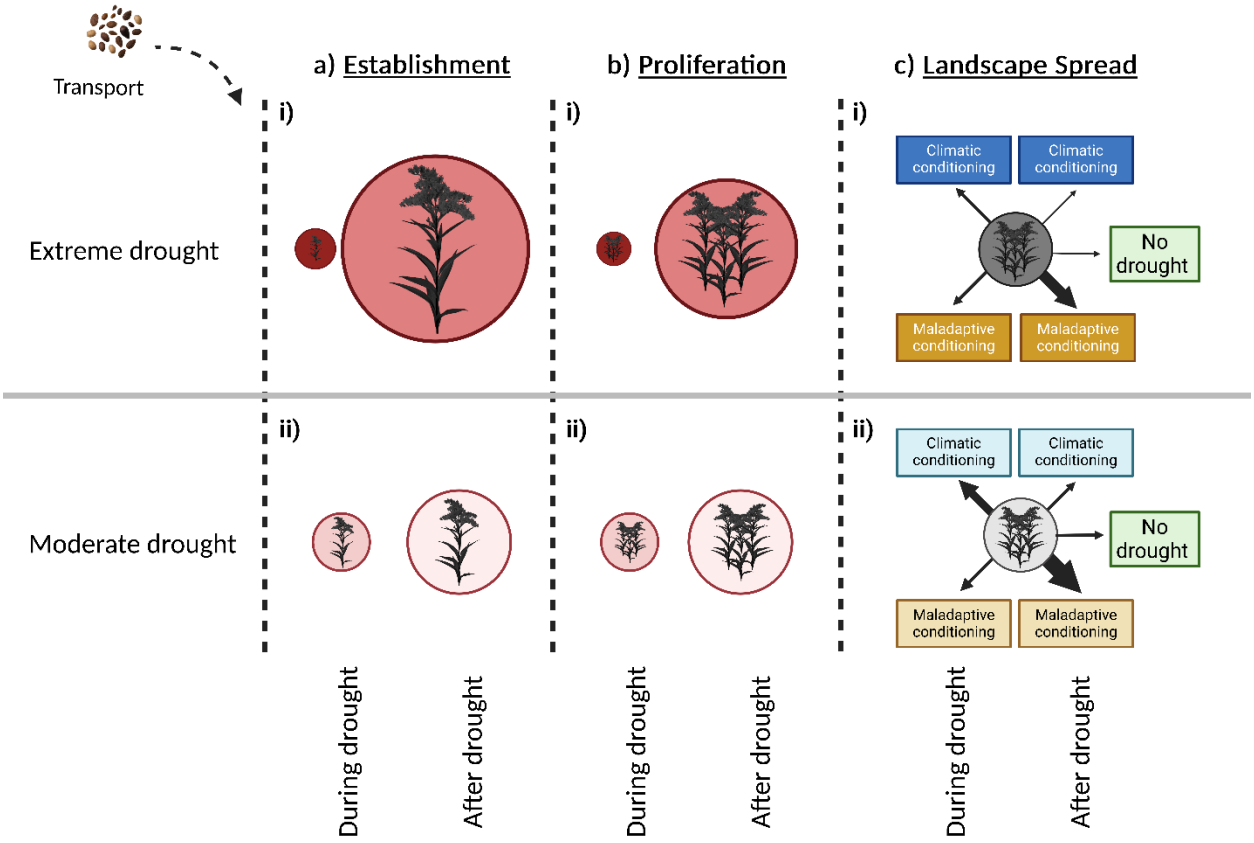

Figure 3. Representation of three invasive stages and how the strength of drought mediated invisibility differs between each stage. The dotted lines represent the hurdles that need to be overcome for the invasion success of non-native plants within each stage [34, 35]. The size of the circles in the establishment (a) and proliferation (b) phase represent the impact of extreme drought (i) and moderate drought (ii) on facilitating each invasive stage, both during (left side; dark coloured colours) and after (right side; light coloured circles) the drought event. Within the landscape spread phase, invasibility of the new habitat during (left-side) and after (right-side) extreme (i) and moderate (ii) drought or in the absence of a drought event (green box) is represented by the size of the arrows. Here, invasibility is shown as being dependent on whether the ecological responses of the new habitat have been climatically (blue boxes) and maladaptively conditioned (orange/brown boxes) (See figure 1). Created with BioRender.com and Canva.com.

Box 2. Relative importance of above- and belowground interactions affecting non-native plants

Aboveground processes

Extreme drought substantially compromises the ability of plants to defend themselves against herbivores [52]. A meta-analysis found that extreme drought stress results in higher levels of damage by secondary agents living in woody organs compared to moderate stress [53]. Despite this, many studies report negative responses in herbivores to extreme drought, though this is species dependent, generating "winners" and "losers" among invertebrate herbivores [3, 54, 55]. Where extreme drought reduces the species richness of aboveground invertebrate herbivores, this could have a negative impact on the biotic resistance of the native community (Fig. 2). For pollinators, extreme drought can shift the phenology of host plants and cause mismatches [56]. Nonetheless, non-native plants may be less sensitive to shifts in abundances of native herbivores and pollinators [57, 58]. Non-native plants lack a shared co-evolutionary history with native herbivores and pollinators, and as such, non-native plants will be mostly affected by generalist herbivores and pollinators [59]. However, very few non-native plants are restricted by the lack of species-specific pollinators [57], partly because many non-native plants can self-fertilize [60].

Belowground processes

In contrast to aboveground subsystems, belowground subsystems with drought legacies can greatly influence the success of a non-native plant [61, 62]. Extreme drought directly affects soil organisms through decreases in soil moisture content and indirectly

through the decreased functioning of plants [63]. Studies investigating the effects of extreme drought on soil microbes often find less negative effects on soil fungi than on soil bacteria [64, 65]. However, by decreasing root biomass, extreme drought also reduces fungi inhabiting the rhizosphere, both symbiotic and pathogenic [66]. The benefits of decreased pathogenic fungi may outweigh the drawbacks associated with reduced mycorrhizal fungi, as invasive plants may utilize mycorrhizal fungi more effectively than native plants [67]. These changes in the microbial community may persist for some time after the drought, and this biotic legacy provides an advantage to the growth and competitive ability of non-native plants [61, 62].

3. Differences in functional traits between native and non-native plants predict the drought-invasion success relationship

Many invasive plants possess specific traits and characteristics, such as fast growth and high propagule production, that contribute to their successful growth and establishment [68, 69]. Propagule pressure, which is the number of seeds reaching a site, greatly influences the success of non-native plant species in colonizing and thriving in new habitats. Invasive species often have a higher reproductive output, producing more and larger seeds, which helps them successfully invade new environments [47]. The ability of invasive plants to form persistent seed banks with dormant seeds further enhances their establishment potential in new environments (Fig. 2). Seed banks serve as reservoirs of viable seeds that can germinate when conditions become favourable such as after a drought event [43]. Moreover, the autonomous self-fertilization capability

of invasive plants allows for seed production without the need for mating partners and external pollination [60]. This trait provides reproductive assurance and facilitates the constant dispersal, particularly under drought conditions where pollinators may be limited [60].

Biotic interactions within the native ecosystem further shape how invasive plants are affected during and after an extreme drought event (see Box 2). Invasive plants and native organisms lack shared co-evolutionary history, as such, invasive plants arriving into native ecosystems may possess novel weapons that give them an upper hand with competitive, grazing and parasitic interactions [70]. Furthermore, as invasive plants disperse to new habitats, they often experience “enemy-release” as they leave behind their species specific pathogens and herbivores [71, 72]. Consequently, invasive plants may experience diminished effects from biotic interactions and this may allow them to invest more in growth and competitive ability [73], but possibly also in resisting and recovering from the extreme drought event. Moreover, the enhanced mutualism hypothesis proposes that invasive plants utilize soil microbes in their non-native habitats more effectively than in their native range, potentially facilitating their growth and competitive advantage [67]. In times of drought, these enhanced mutualistic associations could assist invasive plants in thriving amidst water scarcity, granting them an edge over native species [42].

The fluctuating resource hypothesis proposes that increases in resource availability can facilitate the success of non-native plants [17, 18]. The hypothesis proposes that invasive plants utilize increases in resources better than native species, enabling them to exploit fluctuations (like the nutrient pulse following a drought [46]), and enhancing their competitiveness in new environments [17, 18]. Indeed, nutrient availability within the ecosystem can impact on not only the ecological responses of the native community [15], but also the competitive strength, as well as, the drought resilience and recovery of an invasive plant [15, 16, 74]. Such increased nutrient availability tends to favour invasive plant species under drought conditions due to their unique life history and functional traits. Studies have shown that invasive annuals often exhibit a combination of high relative growth rates (RGR) and superior water-use efficiency (WUE), particularly under conditions of elevated nitrogen, allowing them to rapidly accumulate biomass while conserving water. In contrast, native perennials frequently experience physiological trade-offs, such as between RGR and WUE, which limit their competitiveness under similar conditions [75, 76]. In drought afflicted ecosystems, invasive species are therefore able to emerge earlier and outcompete native species by capitalizing on increased nutrients, resulting in reduced native recruitment and overall biodiversity [76].

3. Concluding remarks and future outlook

Extreme droughts induce substantial shifts in biotic and abiotic features, impacting soil stability, nutrient cycling, and plant-plant and trophic interactions, which in turn influence

plant productivity, community assembly, and ecosystem functioning. We outlined key differences in how the invasibility of a native community changes during and after extreme droughts compared to seasonal/moderate droughts (Fig 3). In particular, we provide insights into how invasion is driven by extreme drought responses of the native community, highlighting that invasibility increases with the severity of native ecological responses (Fig 1). However, we also postulate that the benefits of extreme drought on invasibility varies at different invasive stages (Fig 3).

While extreme droughts likely play a huge role in driving invasion, this remains difficult to quantify at the landscape scale. The integration of technological innovations for invasion monitoring such as satellite and drone imaging in combination with weather data holds promise for advancing our understanding of drought responses and invasibility of both natural and managed ecosystems. Earth observation data can provide valuable insights into the establishment and spread of non-native plant species in response to extreme drought events. In particular, this technology could be implemented to see how an “invasion window” may differ temporally between an extreme and moderate drought event (see Outstanding Questions). By monitoring changes in vegetation cover and composition at a landscape scale, satellite imaging can also help elucidate how shifts in community diversity impact invasibility (see Outstanding Questions).

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Glossary

Biotic resistance: Biotic resistance refers to the ability of native biodiversity, particularly in highly functionally diverse communities, to impede the establishment success of invasive species through complex interactions such as herbivory, predation, disease, and competition, thereby limiting the available niche space for non-native species

Climatic conditioning: Climatic conditioning is where previous drought events results in more arid-adapted native plant communities that are resilient to recurrent extreme drought events.

Establishment (invasive stage): The successful integration of a non-native species into a new environment, where it forms self-sustaining populations. It can be split into two phases:

- **Spontaneous establishment:** The initial phase of the establishment process in biological invasions, where a non-native species successfully develops in a new habitat. This phase involves the species adapting to the environmental

conditions, finding suitable resources, and overcoming initial barriers to survival and reproduction.

- **Permanent establishment:** Permanent establishment occurs when a non-native species achieves sustained reproduction and population growth in the new environment, leading to the establishment of self-sustaining populations over an extended period.

Extreme drought: Extreme droughts are defined as statistically rare reductions in soil moisture content; below the fifth - tenth percentile of the historically norm for the given ecosystem

Introduction (invasive stage): The initial stage of the invasion process where a non-native species is brought into a new environment, either intentionally or accidentally, and may or may not establish viable populations

Invasion meltdown: Invasional meltdown hypothesizes that the presence of one invasive plant species can modify the native ecosystem in ways that facilitate the invasion of other non-native species.

Landscape spread (invasive stage): The expansion of an invasive species across a broader geographical area within a specific landscape, involving the colonization of new habitats and the establishment of populations in diverse environmental conditions.

Maladaptive conditioning: Maladaptive conditioning refers to a situation where the legacy of an extreme environmental event leads to changes in ecosystem dynamics that render it more vulnerable to future drought events.

Moderate drought: Moderate droughts are less severe reductions in soil moisture content than extreme droughts; above the fifth - tenth percentile of the historically norm for the given ecosystem

Proliferation (invasive stage): The widespread growth and increase in the population size of the non-native species, often leading to high densities and extensive spatial coverage in the native community area.

Transport (invasive stage): The process of moving individuals or propagules of a non-native species, often facilitated by natural means (such as wind, water currents, or animal dispersal) or human activities (such as trade, transportation, or intentional introductions)

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Synthesis and Concluding Remarks

The chapters presented in this thesis touch upon some key factors that influence plant performance in a changing world. Throughout the thesis, I show that plant-soil interactions, shaped by the legacy of plant communities, climatic extreme and the presence of AM fungi, have an influence on the performance of all plant species (native, range-expanding and non-native; Chapter 1, 2 & 3). In particular, I show that the soil legacy of an extreme drought facilitates the growth of plant species through more positive PSF's (Chapter 1 & 3), however, these responses are dependent on the traits, diversity and origin of the feedback and conditioning plants (Chapter 1 & 3). Specifically, for non-native and range expanding plants, their unique physiology (Chapter 2) and soil legacy (Chapter 1) allow for greater facilitation (Chapter 1) and drought recovery (Chapter 2) than native plant species. I also show that while the mutualist interactions with AM fungi, or its legacy, generally enhance plant performance, these benefits may disappear under drought conditions (Chapter 2) or even negatively impact plant growth, depending on the soil legacy of the plant communities (Chapter 3). Lastly, I discuss that while drought is often suggested to disrupt native communities and leave open niche spaces for non-native invasive plants, this ultimately depends on the extent to which the native community responses and recover from the extreme drought event and the distinctive ecological barriers at different invasive stages (Chapter 4). Below I will elaborate on these

findings in more detail and conclude this chapter by suggesting promising directions for future research on plant-soil interactions and invasion in the context of a changing world.

Effects of drought-induced soil legacies

In chapter 1, the PSF of extreme drought, extreme flood and sequential extreme drought and flood events were investigated. Within this chapter I show that the drought soil legacy had the strongest influence on the performance of plants, thereby setting the rational of focusing on drought effects in later chapters. In contrast, the soil legacy of the extreme flood event and the sequential drought and flood event yielded feedbacks close to that of the control treatments. Reduced soil moisture can instigate the dieback of soil microbes (Preece *et al.*, 2019; Xu *et al.*, 2020) as well as the accumulation of fresh litter (leaves and roots) from drought stressed plants. The subsequent rewetting of the soil during the recovery period can initiate a rehydration and lysis of the dead microbial cells, as well as a boost in microbial activity (Borken and Matzner, 2009; Leitner *et al.*, 2017; Brangarí, Manzoni and Rousk, 2021). Rewetting of the soil can thereby create a temporary pulse of soil nutrients due to an increased accessibility through diffusive transportation and accelerated microbial activity and nutrient cycling (Rennenberg *et al.*, 2009; Gao *et al.*, 2020). Indeed, a recent meta-analysis by Gao et al. (2020) illustrates that across a wide range of studies, soil rewetting consistently resulted in a significant increase in N mineralization. Throughout all chapters I frequently attribute drought facilitated plant performance as a result of such temporary increases in soil nutrient availability and microbial activity. This increase in nutrient availability plays an important role in the facilitation of invasive plant species, which are highly capable of exploiting such nutrient pulses, allowing for greater trait plasticity, water use-efficiency and competitive ability (Davis, Grime and Thompson, 2000; Daehler, 2003; Davidson, Jennions and Nicotra, 2011; Valliere *et al.*, 2022). Furthermore, in Chapters 1 and 3, I speculate that while surges in microbial activity upon rewetting may benefit invasive plant species due to increases in soil functionality, these benefits may be limited to invasive plants that are less impacted by negative plant-soil interactions, as discussed specifically in Chapter 1.

Influence of plant origin on responses to drought

Despite drought mediated increases in plant performance, within chapter 1 I also show that the legacy of extreme drought disproportionately influenced the performance of non-native and native plant species due to limiting similarity. In particular, native plants experienced reduced drought mediated facilitation in their own (conspecific) soil, while invasive plant species grew comparably well in all soil without being limited by conspecific negative feedback. Soils which have been subjected to extreme drought, and undergo rewetting tend to trigger a surge in microbial activity (Iovieno and Bååth, 2008; Brangarí, Manzoni and Rousk, 2021). This increase in microbial activity may magnify microbial driven PSF effects, which thereby influences the extent to which plants can benefit from the PSF of drought soil legacy. In particular, invasive non-native plants that experience less negative PSF in novel habitats (Reinhart and Callaway, 2006; Maron *et al.*, 2014) can exploit increases in soil nutrient availability more effectively than many native plant species. For example, invasive non-native plants often associate with a richer community of AM fungi in these new environments, selectively promoting fungal taxa that offer mutualistic benefits (Zhang *et al.*, 2010; Sheng *et al.*, 2022) which could promote rather than inhibit invasive plants in their own soil. This lack of negative conspecific feedback could allow invasive plants form denser populations and increase in dominance in native communities recovering from drought, thereby causing declines in native biodiversity.

In chapter 2 I extend on this by investigating how conspecific plant density, and thereby the level of intraspecific competition, influences the direct plant responses to an extreme drought event. The impact of intraspecific competition is an important factor that can determine the success of a plant species that is expanding into a new environment as it influences the plants ability to form self-sustaining populations (Z. Zhang *et al.*, 2019). This is likewise relevant for range-expanding plant species that expand into new habitats as a strategy to mitigate the impacts of climate change (Corlett and Westcott, 2013). Within this chapter I therefore explore whether native and range-expanding plant species differ in their recovery to an extreme

drought event under increasing intraspecific competition. Unlike chapter 1 I am here also exploring the direct impact of drought on plant performance and recovery rather than the indirect PSF effects. The recovery dynamics following an extreme drought are crucial in shaping the composition of the native plant community, as species with faster recovery rates are better equipped to exploit the increased availability of nutrients and vacant spaces (Luo *et al.*, 2019; Ploughe *et al.*, 2019).

In chapter 2 I indeed show that increasing intraspecific competition negatively influenced the performance of both a native plant species and it's range-expanding congener. However, a notable distinction between the two species emerged whereby only the native species experienced a significant hindrance in its drought recovery trajectories as a result of increased competition. In contrast, the range-expanding species, exhibited a more robust response to increasing intraspecific competition and a remarkable capacity for adaptation. Specifically, the range-expanding species, *Centaurea stoebe*, demonstrated a high degree of trait plasticity, particularly in its root trait and biomass allocation strategies. When faced with heightened competition, *C. stoebe* was able to adjust its growth patterns by investing more resources into root development rather than shoot growth. This strategic shift allowed *C. stoebe* to enhance its ability to access water and nutrients from the soil, even in densely populated conditions where competition for these resources are intense. It is further suspected that *C. stoebe* may have utilized AM fungal symbiosis more effectively to recover after drought event compared to its native congener, *Centaurea jacea*, particularly at high intraspecific competition. For instance, *C. stoebe* was able to adjust its root thickness in response to the presence of AM fungi, plant density and drought condition. The influence of these root traits on the interactions between plants and AM fungi has recently attracted significant research interest (Bergmann *et al.*, 2020; Rutten and Allan, 2023). This research emphasizes that the collaboration gradient between plants and AM fungi allows for a range of resource acquisition strategies, where plants can either invest in thicker roots for efficient symbiosis or maintain thinner roots for direct soil exploration (Bergmann *et al.*, 2020). By optimizing root thickness*, C. stoebe* may enhance its ability to outsource nutrient acquisition through AM fungi, thereby improving its resilience and

recovery in drought-affected environments. In contrast, *C. jacea* did not show the same level of adaptability in its root traits, which may have limited its ability to adjust to the biotic and abiotic conditions within community. This difference in plasticity between the two species emphasizes the importance of adaptive traits in determining plant performance and survival (Z. Zhang et al. 2019), particularly in the context of climate change and shifting ecological dynamics. In part this allows us to further speculate on the mechanisms that allow some non-native plant species to succeed in non-native ranges and to take advantage of drought legacies in the community, while other non-native fail. In this case especially, such responsive root traits allowed for adaptation to mycorrhizal fungi and increasing intraspecific competition to optimise plant growth under adverse climatic conditions. These plant characteristics may increase drought resilience and recovery of non-native plants, allowing them to form denser, self-sustaining populations where adverse drought conditions are not intensified with increasing plant density. Furthermore, this ability to sustain high plant density under drought conditions may also stem from a reduction of negative plant-soil interactions in conspecific soil for non-native species (as shown in chapter 1). The ability of non-native species to form such self-sustaining populations is crucial barrier within the invasion within the invasion process, as it enables them to establish a foothold in new environments (see chapter 4).

Context-dependency of plant mycorrhizal interaction

Although in chapter 2 I suggest that the effective utilization of AM fungi is believed to provide significant advantages to *C. stoebe* under varying biotic and abiotic conditions, the benefits of this symbiosis are not always straightforward, particularly after drought periods. In some instances, the presence of AM fungi did not lead to clear positive outcomes for the plants recovery. Instead, the interaction between *C. stoebe* and AM fungi were shifted by influence of plant density and competition, which sometimes diminished the expected benefits. This complexity highlights the need to further investigate the conditions under which AM fungi can truly enhance plant resilience and recovery in challenging environments.

In chapter 3, I further investigated how the presence of AM fungi and soil legacy of plant-mycorrhizal interactions influences the PSF's on the growth of the invasive species, *Solidago canadensis*. Specifically, I examined whether AM fungi shifts the impact of PSF from native plant communities that differ in their plant traits and diversity. This is particularly relevant as the presence of AM fungi can shift or enhance such PSF's from native communities by supporting more efficient nutrient uptake and shifting the soil microbial composition (Veresoglou and Rillig, 2012; Bennett and Klironomos, 2019). Moreover, AM fungi can impact the mechanisms behind drought-induced soil facilitation by shaping both the response and recovery of native plants to drought events, as well as influencing the composition of soil microbial communities that surge in activity during rewetting (Iovieno and Bååth, 2008; Brangarí, Manzoni and Rousk, 2021). As I suggested in chapter 1, the composition of these microbial communities can significantly affect the performance of plants in drought legacy soils, and influence the dominance of invasive plant species. In chapter 3, I likewise show that *S. canadensis* performed better in soils with a legacy of extreme drought. However, I also show that the extent to which *S. canadensis* benefited from these drought-mediated nutrient pulses was dependent on the biotic context of the soil including the conditioning plant community legacy and the presence of AM fungi. These context-dependent plant-mycorrhizal responses were likewise shown chapter 2, where the observed benefits of AM fungal symbiosis diminished at higher plant densities. In chapter 3 I show that, when *S. canadensis* was grown in soils from monocultures of slow or fast growing native species, the presence of AM fungi mediated greater plant growth in soils with a legacy of drought. However, when grown in soils from mixed plant communities with a legacy of drought and the presence of AM fungi constrained the performance of *S. canadensis* compared to soils where AM fungi was absent. In soils from mixed plant communities, the greater diversity of plant hosts likely leads to a higher diversity of mycorrhizal species (Johnson *et al.*, 2004), which theoretically should benefit invasive plant species by providing a broader range of beneficial plant-mycorrhizal partnerships (Sheng *et al.*, 2022). Instead, I show that where AM fungi reduced the diversity of soil microbes, this resulted in a reduction in the benefits received from soils with drought legacies. As such, I suggest that interaction effect of *S.*

canadensis and AM fungi is dependent on the soil microbial diversity, possibly as a result of decreased soil functional diversity. This is particularly relevant because plant-mycorrhizal interactions are often thought to be key drivers of the success of invasive plants, as they enhance reproductive success, nutrient acquisition, and overall adaptability in new environments, allowing these species to thrive and spread rapidly (Richardson, Allsopp, *et al.*, 2000). However, this chapter allows for a boarder understanding of how these mutualistic plant-soil interactions may shift, or rather depend on, the overall diversity of soil microbes.

Context-dependency of drought facilitation

Lastly, in chapter 4 I further explored the relationship between extreme drought and the invasion of non-native species. This chapter suggests that the impact of extreme drought on invasion success is not uniform; rather, it varies significantly depending on the ecological context and the specific barriers that non-native species encounter at various stages of invasion. For example, I first discuss that drought facilitated invasion is ultimately determined by the ecological responses of the native community, thereby more negative responses and slower recovery generate wider "invasional windows" (Diez *et al.*, 2012). However, the ecological responses of the native communities can be further influence by various factors such as, historical drought exposure, community composition, and compounding stressors (F. Zhang *et al.*, 2019; Zandalinas and Mittler, 2022). Additionally, the chapter discusses how the potential for drought facilitated invasion varies in different invasional stages (such as introduction, establishment, and spread (Richardson, Pyšek, *et al.*, 2000; Blackburn *et al.*, 2011)). These stages present unique challenges for non-native species whereby the benefits of extreme drought are not always as clear, especially when the non-native plant is directly exposed the impacts of extreme drought and not just exploiting the benefits associated with drought legacies within the native community.

This chapter along with chapter 1, 2 and 3 have allowed me to form a comprehensive

exploration into the various aspects influencing drought-mediated invasion, including the direct and indirect effects of drought, the context dependency of the native community, and the traits and invasional stages of the non-native species. Understanding these dynamics is crucial, as the spread of invasive plants species is a major component of ongoing anthropogenic global change threating the diversity and resilience of native communities (Vetter *et al.*, 2020; Carboni *et al.*, 2021). Consequently, this has further implications on the ecosystem services such as water purification, pollination, and climate regulation, which are vital for human well-being and the sustainability of natural resources (Pejchar and Mooney, 2009).

Opportunities for further research exploration

The findings presented in this thesis highlight key factors influencing plant-soil interactions under climate change, but there remain several areas that warrant further exploration. One key area for future research involves expanding the investigation of plant-soil interactions to more complex field conditions, where the interaction between plants and their environment can be influenced by a multitude of biotic and abiotic factors not fully captured in controlled experiments. Field studies can provide valuable insights into how these interactions unfold in real-world conditions, particularly as plants encounter variable soil types, microbial communities, and competing species. While this thesis primarily focused on drought as a single abiotic factor, future research could also explore how other stressors, such as temperature extremes, nutrient limitation, or anthropogenic disturbances, interact with drought to shape plant-soil feedbacks. In particular future research could aim to answer: how does a wide range of biotic interactions over various trophic groups influence the success of a non-native plant species under drought?; how does the influence of other abiotic factors, such as nitrogen deposition or warming, affect plant-soil interactions and invasion dynamics?; and last, how do the benefits of extreme drought on invasibility vary across different stages of invasion, and what factors contribute to these variations?

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Declaration of consent

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Title of the thesis: Understanding plant-soil interactions of native and non-native plants under climatic extremes

Supervisor: Madhav P. Thakur
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