Plant Soil
https://doi.org/10.1007/s11104-024-06485-1

RESEARCH ARTICLE

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

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Received: 7 August 2023 / Accepted: 8 January 2024

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 and 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 and 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.

Responsible Editor: Michael Luke McCormack.

Shareen K. D. Sanders and Ludovico Formenti shared first authorship.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06485-1.

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Published online: 23 January 2024

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 and 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 and Fort 2019; Guo et al. 2020) or facilitation through improved drought tolerance (Wang and 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 and 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 and 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. 2006; 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 and 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 et al 2008) and several meta-analyses have consistently shown that AM fungi can ameliorate the drought stress on plant performance (Delavaux et al. 2017; Hawkins and Crawford 2018; Jayne and 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 and 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 and 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 and 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 and 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).

Materials 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 min 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 min, and the two cycles separated by at least 48 h 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 h day (i.e., with light) and 8 h night (i.e., dark) conditions. One-week-old seedlings were then transplanted into 0.7 L pots (10×10×11 cm) 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 min, and the two cycles were separated by at least 48 h 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 and 2 cm in height were transplanted into pots with densities ranging from one to five, with and without AM fungal inoculation (Fig. 1). Pots were then randomly allocated to four tables in two climate chambers with identical light and temperature settings. In both climate

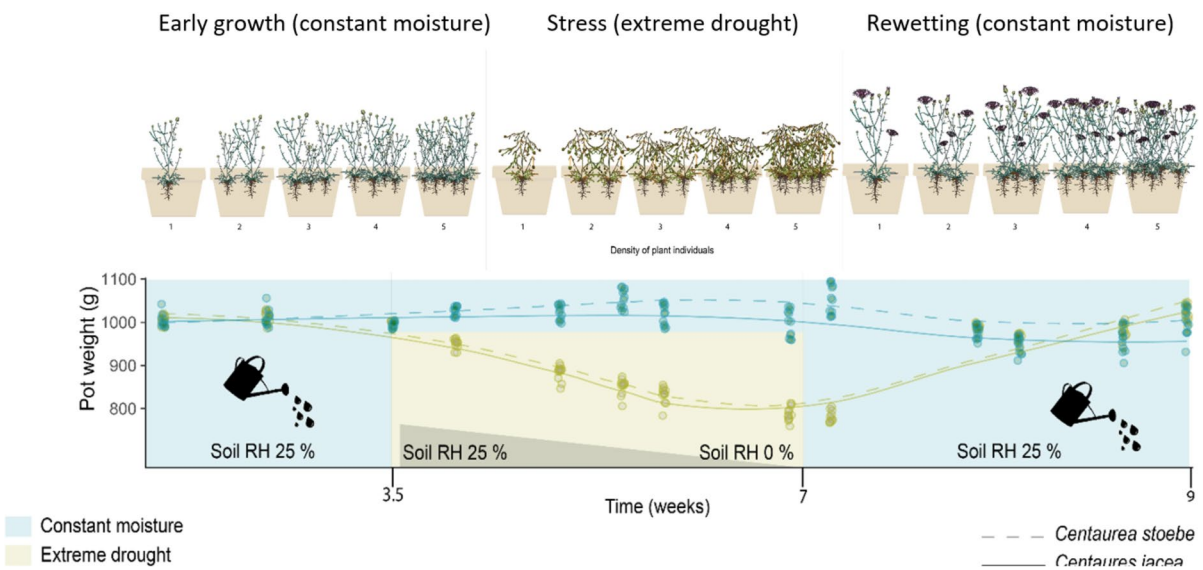

Fig. 1 Schematic representation of experimental design with plant density and extreme drought as our main treatments. Temporal soil water content of the pot under drought and control treatments are indicated in the lower panel (data shown from extra pots, details in methods). Plants in different drought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

chambers, the plants were exposed to the following growing conditions: 16 h of daytime at 20 °C with a light intensity of ~ 13,500 lx and 8 h 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 Fig. 1). We let the plants grow for a total of nine weeks, within which half the pots were exposed to an extreme drought event (Fig. 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 Fig. 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) (Fig. 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) (Fig. 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.

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 and 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 and 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

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)

	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

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 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

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 and 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; Fig. 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; Fig. 2). Increasing plant density further increased the root: shoot ratio of *C. stoebe* ($F_{1,109} = 6.06$, $P < 0.05$; Table 1; Fig. 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; Fig. 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; Fig. 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; Fig. 2); whereas, in *C. stoebe* these conditions led to a shift in root: shoot ratio with reduced root biomass allocation ($F_{1,107} = 4.26$, $P < 0.05$; Fig. 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; Fig. 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; Fig. 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 Fig. 2; Temporal SPAD data: Supplementary Fig. 3).

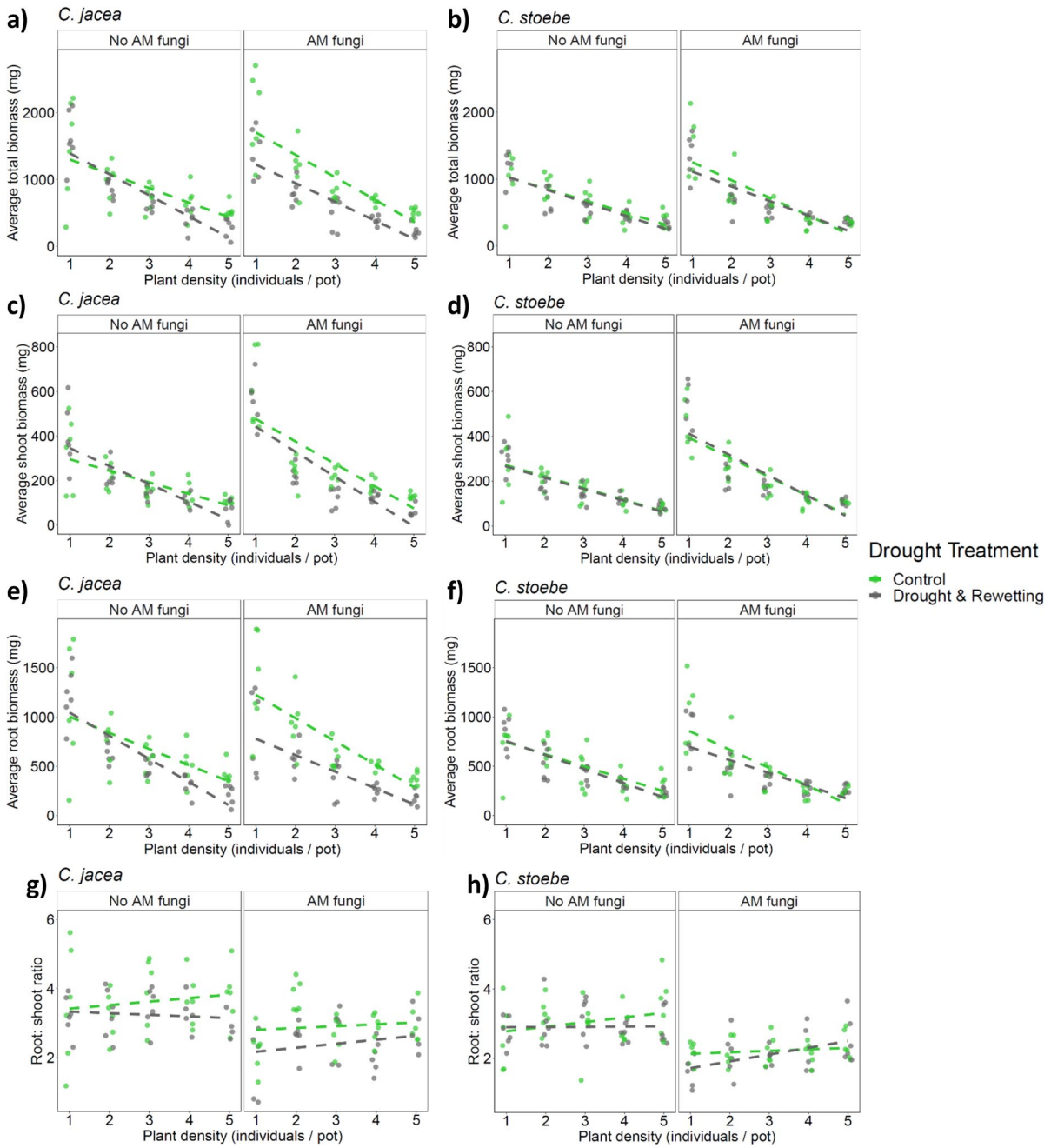

Fig. 2 Plant biomass responses of *C. jacea* (left: a, c and e) and *C. stoebe* (right: b, d and f) to drought, AM fungi and plant density. Average plant total biomass: a, b; Average aboveground biomass: c, d; Average belowground biomass: e, f; Root to shoot ratio. Raw data are shown as points, whereas 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 Fig. 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 Fig. 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; Fig. 3). In *C. stoebe*, the presence of AM fungi increased root diameter ($F_{1,105} = 12.94$, $P < 0.001$; Table 1 & Fig. 3), however, when subjected to extreme drought, the presence of AM fungi decreased root diameter ($F_{1,105} = 5.53$, $P < 0.05$; Table1). 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 & Fig. 3).

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; Fig. 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; Fig. 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; Fig. 4).

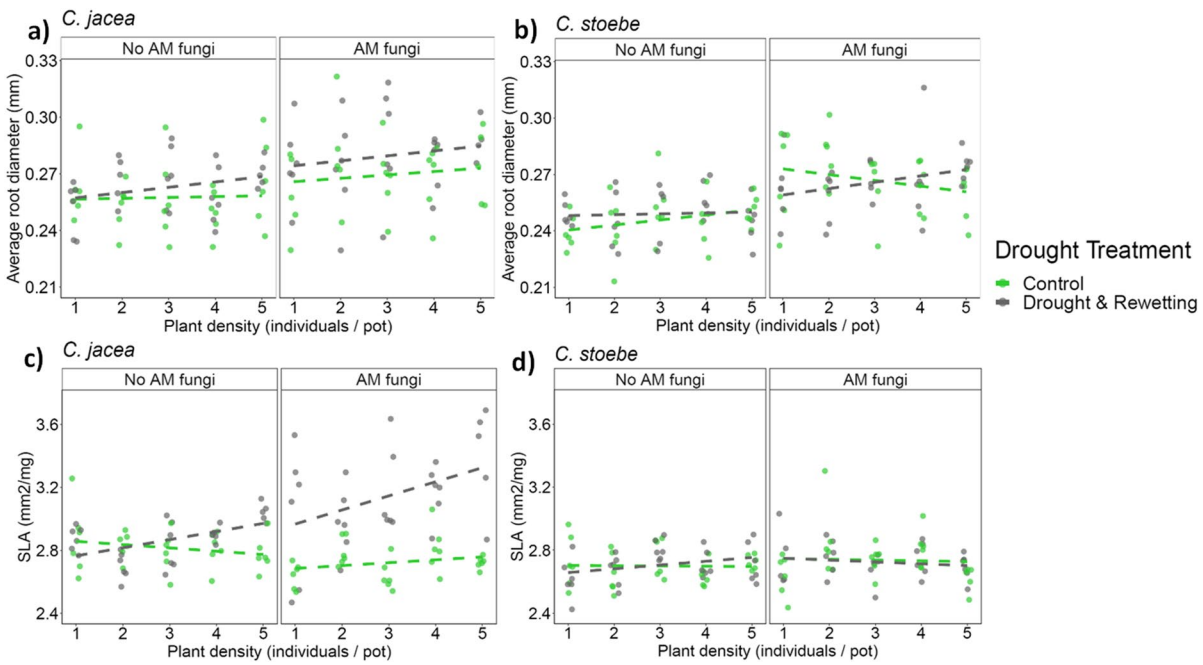

Fig. 3 Plant average root diameter responses of *C. jacea* (**a**) and *C. stoebe* (**b**) and specific leaf area (log-transformed) of *C. jacea* (**c**) and *C. stoebe* (**d**) to drought, AM fungi and plant density. Drought treatments are indicated in grey, control indicated in green. Raw data are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

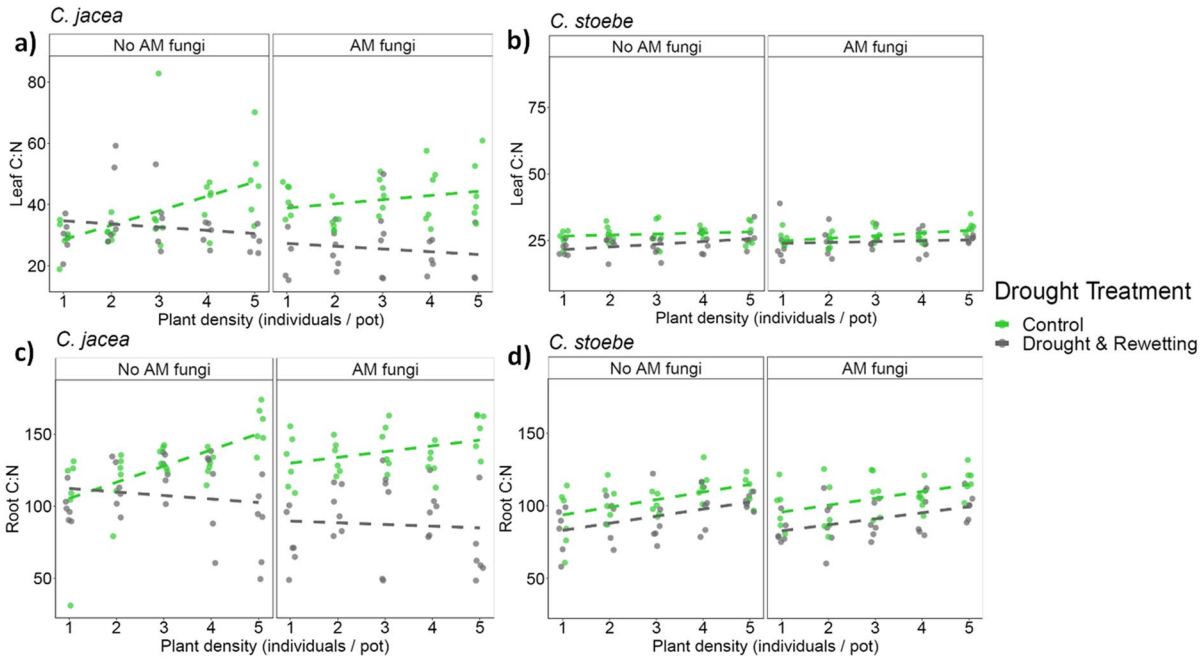

Fig. 4 Plant nutrient content responses of *C. jacea* (left) and *C. stoebe* (right) to drought, AM fungi presence and plant density. Drought treatment indicated in grey, control indicated in green. Leaf C: N ratio: a, b; Root C: N ratio: c, d. Raw data are shown as points, whereas dashed lines are based 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 Fig. 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 Fig. 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 Fig. 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, Fig. 5) as well as significant variation in trait responses to drought but only for *C. jacea* (pseudo $F_{1,34} = 7.23$, $P < 0.001$; Fig. 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 (Fig. 5).

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

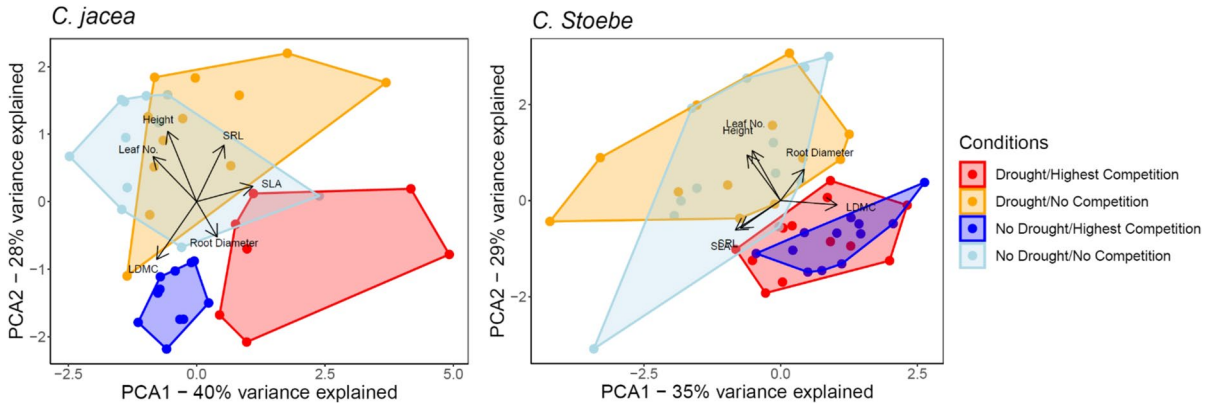

Fig. 5 Principle component analysis (PCA) of plant responses to drought (red and orange colours = extreme drought, blue and azure = constant moisture) and intraspecific competition ((Highest Competition = 5 individuals competing, No Competition = 1 individual alone)) using plant trait variables. Arrows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

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; Fig. 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; Fig. 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 and Jackson 1997; Foxx and 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; Fig. 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; Fig. 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; Fig. 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; Fig. 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 (Fig. 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 and Ellers 2010; 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 and 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; Fig. 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; Fig. 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 and 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 and Dickie 2002; Pérez and 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 Fig. 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 and 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; Fig. 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; Fig. 4; Supplementary Fig. 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.

Acknowledgements We are grateful to two anonymous reviewers for their constructive suggestions on our manuscript. We thank Maarika Bischoff from the GIUB Laboratory (University of Bern) for the precious help in conducting nutrient and soil analysis.

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.

Funding Open access funding provided by University of Bern MPT acknowledges the funding from the Swiss State Secretariat for Education, Research and lnnovation (SERI) under contract number M822.00029.

Data availability The data associated with this publication is available in DRYAD (https://doi.org/10.5061/dryad.1g1jwsv2d).

Declarations

Competing interests The authors declare that they have no conflict of interest.

References

Adler PB, Smull D, Beard KH, Choi RT, Furniss T, Kulmatiski A, Meiners JM, Tredennick AT, Veblen KE (2018) Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. Ecol Lett 21:1319–1329. https://doi.org/10.1111/ele.13098

Anderson JT (2015) Plant fitness in a rapidly changing world. New Phytol 210:81–87. https://doi.org/10.1111/nph.13693

Augé R (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42. https://doi.org/10.1007/s005720100097

Ayres RL, Gange AC, Aplin DM (2006) Interactions between arbuscular mycorrhizal fungi and intraspecific competition affect size, and size inequality, of *Plantago lanceolate* L. J Ecol 94:285–294. https://doi.org/10.1111/j.1365-2745.2006.01103.x

Basiru S, Mwanza HP, Hijri M (2021) Analysis of arbuscular mycorrhizal fungal inoculant benchmarks. Microorganisms 9:81. https://doi.org/10.3390/microorganisms9010081

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Berg MP, Ellers J (2010) Trait plasticity in species interactions: a driving force of community dynamics. Evol Ecol 24:617–629. https://doi.org/10.1007/s10682-009-9347-8

Bergmann J, Weigelt A, van der Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruelheide H, Freschet GT, Iversen CM, Kattge J, McCormack ML, Meier IC, Rillig MC, Roumet C, Semchenko M, Sweeney CJ, van Ruijven J, York LM, Mommer L (2020) The fungal collaboration gradient dominates the root economics space in plants. Sci Adv 6. https://doi.org/10.1101/2020.01.17.908905

Birhane E, Sterck FJ, Bongers F, Kuyper TW (2014) Arbuscular mycorrhizal impacts on competitive interactions between *Acacia Etbaica* and *Boswellia papyrifera* seedlings under drought stress. J Plant Ecol 7:298–308. https://doi.org/10.1093/jpe/rtt031

Błaszkowski J, Czerniawska B, Wubet T, Schäfer T, Buscot F, Renker C (2008) Glomus Irregulare, a new arbuscular mycorrhizal fungus in the Glomeromycota. Mycotaxon 106:247–267. https://doi.org/10.1016/j.mycres.2006.02.006

Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob Change Biol 15:808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x

Brangarí AC, Manzoni S, Rousk J (2021) The mechanisms underpinning microbial resilience to drying and rewetting – a model analysis. Soil Biol Biochem 162:108400. https://doi.org/10.1016/j.soilbio.2021.108400

Broennimann O, Mráz P, Petitpierre B, Guisan A, Müller-Schärer H (2014) Constrasting spatio-temporal climatic niche dynamics during the eastern and western invasions of spotted knapweed in North America. J Biogeogr 41:1126–1136. https://doi.org/10.1111/jbi.12274

Bunn RA, Lekberg Y, Gallagher C, Rosendahl S, Ramsey PW (2014) Grassland invaders and their mycorrhizal symbionts: a study across climate and invasion gradients. Ecol Evol 4:794–805. https://doi.org/10.1002/ece3.917

Cadotte MW, Tucker CM (2017) Should environmental filtering be abandoned? Trends Ecol Evol 32:429–437. https://doi.org/10.1016/j.tree.2017.03.004

Casper BB, Jackson RB (1997) Plant competition underground. Ann Rev Ecol Syst 28:545–570. https://doi.org/10.1146/annurev.ecolsys.28.1.545

Cleland EE, Collins SL, Dickson TL, Farrer EC, Gross KL, Gherardi LA, Hallett LM, Hobbs RJ, Hsu JS, Turnbull

L, Suding KN (2013) Sensitivity of grassland plant community composition to spatial vs. temporal variation in precipitation. Ecology 94:1687–1696. https://doi.org/10.1890/12-1006.1

Corkidi L, Allen EB, Merhaut D, Allen MF, Downer J, Bohn J, Evans M (2004) Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions. J Environ Hortic 3:149–154. https://doi.org/10.24266/0738-2898-22.3.149

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Ter Steege H, Morgan HD, van der Heijden MGA, Pausas JG (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51:335–380. https://doi.org/10.1071/bt02124

Delavaux CS, Smith-Ramesh LM, Kuebbing SE (2017) Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98:2111–2119. https://doi.org/10.1002/ecy.1892

Duan H, Luo C, Zhu S, Naseer M, Xiong Y (2021) Density- and moisture-dependent effects of arbuscular mycorrhizal fungus on drought acclimation in wheat. Ecol Appl 31. https://doi.org/10.1002/eap.2444

Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. Nature 447:80–82. https://doi.org/10.1038/nature05747

Foxx AJ, Fort F (2019) Root and shoot competition lead to contrasting competitive outcomes under water stress: a systematic review and meta-analysis. PLoS One. https://doi.org/10.1371/journal.pone.0220674

Gao D, Bai E, Li M, Zhao C, Yu K, Hagedorn F (2020) Responses of soil nitrogen and phosphorous cycling to drying and rewetting cycles: a meta-analysis. Soil Biol Biochem 148. https://doi.org/10.1016/j.soilbio.2020.107896

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Goltapeh EM, Danesh YR, Prasad R, Varma A (2008) Mycorrhizal fungi: what we know and what should we know? Mycorrhiza: state of the art, Genetics and Molecular Biology, Eco-function, Biotechnology, Eco-physiology, structure and Systematics. Springer, Berlin Heidelberg, pp 3–27. https://doi.org/10.1007/978-3-540-78826-3

Guo Y, He Y, Wu P, Wu B, Lin Y, He M, Han X, Xia T, Shen K, Kang L, Tan Q, Ren W, Sun Y, Li Q (2022) The interspecific competition presents greater nutrient facilitation compared with intraspecific competition through AM fungi interacting with litter for two host plants in karst soil. J Plant Ecol 15:399–412. https://doi.org/10.1093/jpe/rtab110

Guo Q, Wu X, Korpelainen H, Li C (2020) Stronger intra-specific competition aggravates negative effects of drought on the growth of Cunninghamia lanceolate. Environ Exp Bot 175:104042. https://doi.org/10.1016/j.envexpbot.2020.104042

Hart MM, Reader RJ, Klironomos JN (2003) Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends Ecol Evol 18:418–423. https://doi.org/10.1016/s0169-5347(03)00127-7

Hawkins AP, Crawford KM (2018) Interactions between plants and soil microbes may alter the relative importance of intraspecific and interspecific plant competition in a changing climate. AoB PLANTS 10:ply039. https://doi.org/10.1093/aobpla/ply039

IPCC (2023) Summary for policymakers. In: Lee, H Romero J(ed) Climate change 2023: Synthesis report. Contribution of working groups I, II and III to the sixth assessment report of the intergovernmental panel on climate change. Geneva pp 1–34. https://doi.org/10.59327/IPCC/AR6-9789291691647.001

Jaeger B (2017) R2glmm: computes R squared for mixed (multilevel) models. R Package Version 2:1–12

Jayne B, Quigley M (2014) Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: a meta-analysis. Mycorrhiza 24:109–119. https://doi.org/10.1007/s00572-013-0515-x

Jongen M, Albadran B, Beyschlag W, Unger S (2022) Can arbuscular mycorrhizal fungi mitigate drought stress in annual pasture legume? Plant Soil 472:295–310. https://doi.org/10.1007/s11104-021-05233-z

Kivlin SN, Emery SM, Rudgers JA (2013) Fungal symbionts alter plant responses to global change. Am J Bot 100:1445–1457. https://doi.org/10.3732/ajb.1200558

Koide RT (1991) Density-dependent response to mycorrhizal infection in *Abutilon theophrasti* Medic. Oecologia 85:389–395. https://doi.org/10.1007/bf00320615

Koide RT, Dickie IA (2002) Effects of mycorrhizal fungi on plant populations. Plant Soil 244:307–317. https://doi.org/10.1007/978-94-017-1284-2_30

Koorem K, Wilschut RA, Weser C, van der Putten WH (2021) Disentangling nematode and arbuscular mycorrhizal fungal community effect on the growth of range-expanding Centaurea stoebe in original and new range soil. Plant Soil 466:207–221. https://doi.org/10.1007/s11104-021-05020-w

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM (2014) Community assembly, coexistence and the environmental filtering metaphor. Funct Ecol 29:592–599. https://doi.org/10.1111/1365-2435.12345

Krüger M, Krüger C, Walker C, Stockinger H, Schüβler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984. https://doi.org/10.1111/j.1469-8137.2011.03962.x

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) {lmerTest} Package: tests in Linear mixed effects models. J Stat Softw 82:1–26. https://doi.org/10.18637/jss.v082.i13

Lange S, Volkholz J, Geiger T, Zhao F, Vega I, Jägermeyr J, Schewe J, Bresch DN, Büchner M (2020) Projecting exposure to extreme climate impact events across six event categories and three spatial scales. Earth's Future 8. https://doi.org/10.1029/2020EF001616

Lauber K, Wagner G, Gygax A (2018) Flora Helvetica: Illustrierte Flora der Schweiz. Auflage. Haupt Verlag 6. https://doi.org/10.1002/fedr.19971080522

Legendre P, Oksanen MJ (2018) Package 'lmodel2'. https://CRAN.R-project.org/package lmodel2 .

Leitner S, Homyak PM, Blankinship JC, Eberwein J, Jenerette GD, Zechmeister-Boltenstern S, Schimel JP (2017) Linking NO and N2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. Soil Biol Biochem 115:461–466. https://doi.org/10.1016/j.soilbio.2017.09.005

Liu W, Sun F, Lim WH, Zhang J, Wang H, Shiogama H, Zhang Y (2018) Global drought and severe drought-affected populations in 15 and 2°C warmer worlds. Earth Sys Dyn 9:267–283. https://doi.org/10.5194/esd-9-267-2018

Luo W, Zuo X, Griffin-Nolan RJ, Xu C, Ma W, Song L, Helsen K, Lin Y, Cai J, Yu Q, Wang Z, Smith MD, Han X, Knapp AK (2019) Long term experimental drought alters community plant trait variation, not trait means, across three semiarid grasslands. Plant Soil 442:343–353. https://doi.org/10.1007/s11104-019-04176-w

Marjanović Ž, Nehls U (2008) Ectomycorrhiza and water transport. Mycorrhiza: state of the art, Genetics and Molecular Biology, Eco-function, Biotechnology, Eco-physiology, structure and Systematics. Springer, Berlin Heidelberg, pp 3–27. https://doi.org/10.1007/978-3-540-78826-3

Meisner A, Deyn GBD, de Boer W, van der Putten WH (2013) Soil biotic legacy effects of extreme weather events influence plant invasiveness. Proc Natl Acad Sci 110:9835–9838. https://doi.org/10.1073/pnas.1300922110

Moeslund JE, Arge L, Bøcher PK, Dalgaard T, Ejrnæs R, Odgaard MV, Svenning J (2013) Topographically controlled soil moisture drives plant diversity patterns within grasslands. Biodivers Conserv 22:2151–2166. https://doi.org/10.1007/s10531-013-0442-3

Morriën E, Engelkes T, Macel M, Meisner A, van der Putten WM (2010) Climate change and invasion by intracontinental range-expanding exotic plants: the role of biotic interactions. Ann Bot 105:843–848. https://doi.org/10.1093/aob/mcq064

Nakagawa S, Schielzeth H (2012) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Oksanen J, Blanchet FG, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette M, Cunha ER, Smith T, Stier A, Ter Braak CJF, Weedon J (2020) Community Ecology Package. Rpackage version 2.5-7 https://doi.org/10.1556/comec.4.2003.2.11

Pérez M, Urcelay C (2009) Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plant belonging to contrasting functional types. Mycorrhiza 19:517–523. https://doi.org/10.1007/s00572-009-0254-1

Philips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161. https://doi.org/10.1016/s0007-1536(70)80110-3

Ploughe LW, Jacobs EM, Frank GS, Greenler SM, Smith MD, Dukes JS (2018) Community response to Extreme Drought (CRED): a framework for drought-induced shifts in plant-plant interactions. New Phytol 222:52–69. https://doi.org/10.1111/nph.15595

Postma JA, Hecht VL, Hikosaka K, Nord EA, Pons TL, Poorter H (2021) Dividing the pie: a quantitative review on plant density responses. Plant Cell Environ 44:1072–1094. https://doi.org/10.1111/pce.13968

Preece C, Verbruggen E, Liu L, Weedon JT, Peñuelas J (2019) Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol Biochem 131:28–39. https://doi.org/10.1016/j.soilbio.2018.12.022

Quist CW, van der Putten WH, Thakur MP (2020) Soil predator loss alters aboveground stoichmetry in a native but not in a related range-expanding plant when exposed to periodic heat waves. Soil Biol Biochem 150:107999. https://doi.org/10.1016/j.soilbio.2020.107999

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL https://www.R-project.org/. Accessed 10 Mar 2021.

Rasband WS (1997-2018) ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA

Ravenek JM, Mommer L, Visser EJW, van Ruijven J, van der Paauw JW, Smit-Tiekstra A, de Caluwe H, de Kroon H (2016) Linking root traits and competitive success in grassland species. Plant Soil 407:39–53. https://doi.org/10.1007/s11104-016-2843-z

Rehling F, Sandner TM, Matthies D (2021) Biomass partitioning in response to intraspecific competition depends on nutrients and species characteristics: a study of 43 plant species. J Ecol 109:2219–2233. https://doi.org/10.1111/1365-2745.13635

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen balance in forest soils: nutritional limitations of plants under climate change stresses. Plant Biol 11:4–23. https://doi.org/10.1111/j.1438-8677.2009.00241.x

Savary R, Masclaux FG, Wyss T, Droh G, Corella JC, Machado AP, Morton JB, Sanders IR (2018) A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus *Rhizophagus Irregularis*. ISME J 12:17–30. https://doi.org/10.1111/oik.06138

Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM (2021) RhizoVision Explorer: open-source software for root image analysis and measurement standardization. AoB Plants 56. https://doi.org/10.1093/aobpla/plab056

Spence AR, Tingley MW (2020) The challenge of novel abiotic conditions for species undergoing climate-induced range shifts. Ecography 43:1571–1590. https://doi.org/10.1111/ecog.05170

Stampfli A, Zeiter M (2004) Plant regeneration directs changes in grassland composition after extreme drought: a 13-year study in southern Switzerland. J Ecol 92:568–576. https://doi.org/10.1111/j.0022-0477.2004.00900.x

Stewart BA, Porter LK, Clark FE (1963) The reliability of a Micro-dumas Procedure for determining total Nitrogen in Soil. Soil Sci Am J 27:377–380. https://doi.org/10.2136/sssaj1963.03615995002700040008x

Stockinger H, Walker C, Schüßler A (2009) *Glomus intraradices* DAOM19798, a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytol 183:1176–1187. https://doi.org/10.1111/j.1469-8137.2009.02874.x

Taktek S, Trépanier M, Servin PM, St-Arnaud M, Piché Y, Fortin JA, Antoun H (2015) Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus Irregularis DAOM 197198. Soil Biol Biochem 90:1–9. https://doi.org/10.1016/j.soilbio.2015.07.016

Tedersoo L, Bahram M, Zobel M (2020) How mycorrhizal associations drive plant population and community biology. Science 367:6480. https://doi.org/10.1126/science.aba1223

Thakur MP, Quast V, van Dam NM, Eisenhauer N, Roscher C, Biere A, Martinez-Medina A (2019) Interactions between functionally diverse fungal mutualists inconsistently affect plant performance and competition. Oikos 128:1136–1146

Thakur MP, Risch AC, van der Putten WH (2022) Biotic responses to climatic extremes in terrestrial ecosystems. iScience 25:104559. https://doi.org/10.1016/j.isci.2022.104559

Tilman D, El Haddi A (1992) Drought and biodiversity in grasslands. Oecologia 89:257–264. https://doi.org/10.1007/bf00317159

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V et al (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci USA 110:20117–20122. https://doi.org/10.1073/pnas.1313452110

Usui T, Lerner D, Eckert I, Angert AL, Garroway CJ, Hargreaves A, Lancaster LT, Lessard J-P, Riva F, Schmidt C, van der Burg K, Marshall KE (2023) The evolution of plasticity at geographic range edges. Trends Ecol Evol 38:831–842. https://doi.org/10.1016/j.tree.2023.04.004

van der Heijden MGA, Bardgett RD, Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Wagg C, Jansa J, Stadler M, Schmid B, van der Heijden MGA (2011) Mycorrhizal fungal identity and diversity relaxes plant-plant competition. Ecology 92:1303–1313. https://doi.org/10.1890/10-1915.1

Walter J (2018) Effects of changes in soil moisture and precipitation patterns on plant-mediated biotic interactions in terrestrial ecosystems. Plant Ecol 219:1449–1462. https://doi.org/10.1007/s11258-018-0893-4

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416:389–395. https://doi.org/10.1038/416389a

Wang S, Callaway RM (2021) Plasticity in response to plant-plant interactions and water availability. Ecology 102:e03361. https://doi.org/10.1002/ecy.3361

Wang X, Ge Y, Wang J (2017) Positive effects of plant diversity on soil microbial biomass and activity are associated with more root biomass production. J Plant Interact 12. https://doi.org/10.1080/17429145.2017.1400123

Wen Z, Li H, Shen Q, Tang X, Xiong C, Li H, Pang J, Ryan MH, Lambers H, Shen J (2019) Trade-offs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. New Phytol 223:882–895. https://doi.org/10.1111/nph.15833

Werner GD, Cornelissen JH, Cornwell WK, Soudzilovskaia NA, Kattge J, West SA, Kiers ET (2018) Symbiont switching and alternative resource acquisition strategies drive mutualism breakdown. PNAS 115:5229–5234. https://doi.org/10.1101/242834

Wickham H (2016) Ggplot2: elegant graphics for data analysis. R package version 2.5-7. https://doi.org/10.1007/978-0-387-98141-3

Wilschut RA, Geisen S, Martens H, Kostenko O, de Hollander M, ten Hooven FC, Weser C, Snoek LB, Bloem J, Caković D, Čelik T, Koorem K, Krigas N, Manrubia M, Ramirez KS, Tsiafouli MA, Vreš B, van der Putten W (2019) Latitudinal variation in soil nematode communities under climate warming-related range-expanding and native plants. Glob Change Biol 25:2714–2726. https://doi.org/10.1111/gcb.14657

Worchel ER, Giauque HE, Kivlin SN (2013) Fungal symbionts alter plant drought response. Microb Ecol 65:671–678. https://doi.org/10.1007/s00248-012-0151-6

Xu S, Geng W, Sayer EJ, Zhou G, Zhou P, Liu C (2020) Soil microbial biomass and community responses to experimental precipitation change: a meta-analysis. Soil Ecol Lett 2:93–103. https://doi.org/10.1007/s42832-020-0033-7

Yang Q, Veen GF, Wagenaar R, Manrubia M, Ten Hooven FC, van der Putten WH (2022) Temporal dynamics of range expander and congeneric native plant responses during and after drought events. Ecol Monogr 92:e1529. https://doi.org/10.1002/ecm.1529

Zhang W, Jia X, Wang G (2017) Facilitation among plants can accelerate density-dependent mortality and steepen self-thinning lines in stressful environments. Oikos 126:1197–1207. https://doi.org/10.1111/oik.03983

Zhang Q, Xu L, Tang J, Bai M, Chen X (2011) Arbuscular mycorrhizal mediation of biomass-density relationship of *Medicago sativa* L. under two water conditions in a field experiment. Mycorrhiza 21:269–277. https://doi.org/10.1007/s00572-010-0331-5