Droughts affect plant communities, but their impacts may be mediated by soil biota. Soil communities may ameliorate drought stress, and droughts may leave legacies of altered soil communities that may affect future plant growth. However, it is not yet understood which groups of soil biota in particular affect plant performance under drought, nor which groups contribute to drought-legacy effects on future plant growth. We hypothesized that increasing soil-community complexity ameliorates drought stress and that drought-legacy effects are species-specific and soil-community-dependent. To test these hypotheses, we performed a two-phase experiment with six grassland species. In the first phase, we examined plant performance under drought and ambient conditions, in soils inoculated with a sterilized inoculum, or increasingly complex soil communities created by wet-sieving through 20-, 40- and 200-µm mesh sieves. In the second phase, we examined drought-legacy effects on conspecific plant performance. We separately analysed plant performance in both phases, and integrated data from both phases using structural equation models. Drought effects on first-phase root biomass depended on soil inoculum, and this interaction differed among plant species, while effects on shoot biomass differed among species, but did not depend on inoculum. Only one species experienced drought-stress-ameliorating effects of soil biota. Drought-legacy effects on plant performance were positive, but depended on soil inoculum in case of root biomass, and on soil inoculum and species identity in case of shoot biomass. Drought-legacy effects were often mediated by first-phase biomass. In some species this effect was independent of inoculum, suggesting an abiotic legacy effect. In others, low first-phase biomass corresponded with high second-phase performance in presence of the most complex soil community. We conclude that drought-legacy effects on plant performance were soil-community-dependent but positive, suggesting that plants establishing after drought may benefit from increased nutrient availability and more positive impacts of soil biota.

Keywords: drought-legacy effects, drought-stress amelioration, plant–soil feedbacks, soil-community modification, structural equation modelling
Introduction

Natural communities are increasingly more often exposed to droughts (Spinoni et al. 2014), which are also expected to intensify in terms of duration and severity in the coming years (Trenberth et al. 2003, Touma et al. 2015). Drought impacts on plant communities can be severe, and range from large-scale die-off (Breshears et al. 2005) and biodiversity loss (Tilman and El Haddi 1992) to transformations of entire ecosystems (Jiang et al. 2013). Droughts directly affect plants by reducing water and nutrient availability (Farooq et al. 2012). Additionally, drought poses an environmental stress on plants, which may change their interactions with pathogens (Lafferty and Holt 2003), and alter their dependency on mutualists (Singh et al. 2011). Understanding plant responses to drought therefore requires the examination of plants in the presence of natural communities of plant-associated biota. Indeed, recent research has shown that plant responses to drought partly depend on their interactions with plant-associated soil communities (Kim et al. 2012, Ulrich et al. 2019, de Vries et al. 2020). However, it is still largely unknown how such interactions vary across plant species and soil communities.

There are different ways in which interactions between plants and soil communities can affect plant responses to drought (Fig. 1A). Firstly, the soil microbiome provides essential ecosystem functions, such as mineralization and depolymerization, which may be impaired under drought (Rouphael et al. 2012). Secondly, droughts can decrease abundances of microbes and microfauna, e.g. nematodes (Schimel 2018, Wilschut and Geisen 2021), and shift soil community composition (Waldrop and Firestone 2006, Naylor and Coleman-Derr 2017, Jansson and Hofmockel 2020). For instance, abundances of pathogens (Garrett et al. 2006, Meisner and de Boer 2018) and root-feeding nematodes (Franco et al. 2019) may increase in soils exposed to drought, possibly with negative consequences for plant performance (Franco et al. 2020). Such shifts in soil community composition may partly be mediated by altered root-exudation patterns under drought (Williams and de Vries 2020). Thirdly, drought effects on plants can be mitigated by various mutualists (Marulanda et al. 2009, Revillini et al. 2016).

Figure 1. (A) Conceptual overview of immediate and legacy-mediated drought effects on plants. Firstly, drought stress has an immediate negative effect on plant performance through water limitation (1). Secondly, drought-induced changes in soil-community composition (2), or drought-induced changes in the plant’s susceptibility to belowground enemies or investment in belowground mutualisms (3), may result in altered impacts of soil communities on plant performance (4). In turn, changes in plant performance under drought may also affect soil-community composition (5). Together, changes in soil-community composition occurring under drought will cause legacy effects on future plant generations after the drought has ended (6). (B) Schematic overview of the experiment. In the first phase, we examine drought effects on the performance of six plant species, grown in soils inoculated with soil communities of different complexity, created using a wet-sieving approach. In the second phase, we examine drought-legacy effects by growing the same plant species in soils conditioned by conspecifics in the first phase.
For example, arbuscular mycorrhizal fungi (AMF) exhibit multiple mechanisms of ameliorating drought stress on plants (Ruiz-Lozano et al. 2012, Gholamhoseini et al. 2013, Revillini et al. 2016, Li et al. 2019), and many bacteria have been shown to improve plant performance under drought as well (Casanovas et al. 2002, Timmusk et al. 2014, Gagné-Bourqué et al. 2016, Niu et al. 2017, Rubin et al. 2017). Finally, drought stress can also increase plant susceptibility to pathogen infection (Garrett et al. 2006, Dikilitas et al. 2016, Meisner and de Boer 2018), for instance through weakening plant-defence mechanisms (Ramegowda and Senthilkumar 2015). Altogether, different groups of soil biota may either worsen (e.g. root-feeding nematodes) or alleviate (e.g. AMF) the negative effects of drought on plant performance. However, while knowledge of soil-community-mediated drought effects on plants is increasing, the relative contributions of different parts of the community to these effects remain largely unknown. Disentangling these contributions may therefore help to understand how plant responses to drought depend on soil-community composition.

In addition to immediate impacts on plant–soil interactions, droughts can also affect future plant–soil interactions, by leaving a legacy in the soil community (de Vries et al. 2012, Meisner et al. 2021) (Fig. 1A). These legacy effects occur when previously described drought-induced changes in soil-community composition persist after the drought has ended, and affect soil-feedback effects on plants (Lau and Lennon 2012, Meisner et al. 2013, Kaisermann et al. 2017). For example, droughts can increase the relative number of AMF in the soil (de Vries et al. 2018), which could positively impact long-term plant growth. Additionally, changes in soil community in response to altered plant-root exudation patterns can lead to increased nutrient availability after drought, possibly leading to improved future plant growth (Lau and Lennon 2012, de Vries et al. 2020, Williams and de Vries 2020). However, it is not yet understood how the strength of drought-legacy effects depends on soil-community composition and how drought-legacy effects vary among plant species.

Here, we performed a greenhouse experiment to study how soil-community composition affects immediate drought impacts on plant performance and drought-legacy effects on future plant performance (Fig. 1B). Using a wet-sieving approach, we created soil communities that differ in complexity, but also shifts the functional composition (Wagg et al. 2014). This method not only decreases the diversity of the community, but also shifts the functional composition (Wagg et al. 2014). For example, AMF are greatly reduced in abundance in communities sieved through 50-μm meshes, and when filtered through a 25-μm sieve, fungi are less abundant than bacteria and nematodes are largely absent (Wagg et al. 2014). Based on this, we assumed that 1) our 200-μm inoculum contained an intact microbial and nematode community, 2) AMF were strongly reduced in our 40-μm inoculum and 3) our 20-μm inoculum largely consisted of small-sized fungi and bacteria.

To prepare our inocula, on 5 June 2020, we collected 25 kg of field soil from a grassland patch within the botanical garden of the University of Konstanz (47°41′30.9″N, 9°10′44.4″E). In the four days prior to this date, there had been modest precipitation (13 mm in total). We sieved the collected soil through a 7-mm sieve to remove stones and other large particles. Per 1 kg of field soil, we added 2 l of tap water. This solution was sequentially sieved through sieves of decreasing mesh sizes. To remove large particles as well as soil insects and earthworms, the solution was first sieved through a 2-mm mesh. After 5 min, we filtered this solution through a 200-μm-mesh sieve, to create a soil community containing all micro-organisms as well as nematodes. The 40-μm sieving step was performed in the same way. Before the 20-μm sieving step, we let the solution set for 15 min. In total, we created 7 l of both the 20- and 40-μm inocula and 14 l of the 200-μm inoculum. We then sterilized half of the 200-μm inoculum by autoclaving for 60 min at 123°C. Inocula were stored at 4°C until the start of the experiment.

First phase: drought experiment

On 10 June, we filled 240 0.5-l pots with 400 g of a sand-vermiculite mixture (50:50 v:v), which we inoculated with 100 ml of one of the four inocula (200, 40, 20 μm or sterilized). During the inoculation process, we continuously homogenized the inocula. We then planted each pot with a single individual of one of the six plant species, establishing ten pots per plant species for each of the four inocula, resulting in a total of 240 pots (4 inocula × 6 species × 10 initial replicates). Pots were individually placed on plastic dishes in a climatized greenhouse (16 h light/8 h dark; 20/14°C), and arranged according to a replicate block design with five

Material and methods

Study species

We tested our hypotheses using the grass species Lolium perenne, Bromus hordeaceus and Alopecurus pratensis and the forb species Centaurea jacea, Diplotaxis tenuifolia and Prunella vulgaris. All six species are native to central-European grasslands. Seeds were ordered from Rieger-Hofmann GmbH, except for D. tenuifolia seeds, which were collected from a local population in Konstanz, Germany. Seeds of all species were germinated on potting soil. Due to low germination of A. pratensis in advance of the legacy experiment, we did not include this species in that part of the study.

Soil inocula

Analogous to the methods of König et al. (2016), we used a series of sieves with mesh sizes of 200, 40 and 20 μm to produce soil inocula containing organisms of different size classes. This method not only decreases the diversity of the community, but also shifts the functional composition (Wagg et al. 2014). For example, AMF are greatly reduced in abundance in communities sieved through 50-μm meshes, and when filtered through a 25-μm sieve, fungi are less abundant than bacteria and nematodes are largely absent (Wagg et al. 2014). Based on this, we assumed that 1) our 200-μm inoculum contained an intact microbial and nematode community, 2) AMF were strongly reduced in our 40-μm inoculum and 3) our 20-μm inoculum largely consisted of small-sized fungi and bacteria.

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blocks (containing two replicates for each species × inoculum combination). Non-viable seedlings were exchanged in the first week of the experiment. During this week, we also estimated initial plant size (leaf number × length biggest leaf × width biggest leaf).

During the first two weeks of the experiment, we maintained soil moisture of all pots at 20% by watering the pots to weight, and continued this watering treatment throughout the experiment for the non-drought control pots. In the 3rd and 5th week of the experiment, half of the pots (i.e. one of the two replicates for each species × inoculum combination in each block) did not receive any water. After each week-long drought period, we restored soil moisture to non-drought, control-level conditions. Throughout the experiment, we fertilized each pot with 150 ml of 1% diluted Universol Blue (18 − 11 − 18 + 2.5 MgO + TE; ICL Speciality Fertilizers), divided over three application events. After seven weeks, we harvested plant shoots and dried them at 70°C for 72 h. We then carefully separated soil and roots, after which we washed and dried the roots and stored the soil from each pot at 4°C until further use. Both root and shoot samples were weighed to determine plant biomass.

Second phase: legacy experiment

Using each individual soil from the drought experiment, except for the A. pratensis soils, we established 200 0.2-l pots filled with first-phase soil on 7 August. We planted each individual pot with a seedling of the same species that grew in the soil during the first phase. We estimated initial plant size as in the first phase. We arranged pots according to the same randomized block design in a non-climatized greenhouse with the same light conditions. Pots were watered to a common weight (~20% soil moisture) each week and regularly watered during the rest of the week to ensure well-moistened soils. After five weeks, we clipped shoots from each pot, carefully washed the roots and dried all plant parts at 70°C for 72 h, after which we weighed all samples to determine plant biomass.

Statistical analyses

All statistical analyses were performed using R ver. 4.1.0 (<www.r-project.org>). We analysed drought and soil-inoculum effects separately for shoot and root biomass, as soil-community effects on root biomass may be stronger than effects on shoot biomass (Kostenko et al. 2012). Additionally, we also analysed effects on total biomass. Biomass data of first-phase plants that died during the experiment (n = 3) or had no detectable root systems at the time of harvest (n = 2) were excluded from the analyses, and soils of these plants were not included in the second phase of the experiment. Additionally, a single dead second-phase plant was excluded from the analyses. Prior to the analyses, we In-transformed root, shoot and total biomass data of the first phase, and square-root-transformed root, shoot and total biomass of the second phase, in order to improve normality and homoscedasticity of the residuals. We modelled first- and second-phase root, shoot and total biomass data using mixed linear models (nlme package; Pinheiro et al. 2014) with the ‘block’ term as random effect, ‘plant species’, ‘drought treatment/legacy’ (i.e. drought treatment in first phase, and drought-legacy treatment in second phase) and ‘soil inoculum’ as fixed effects and ‘initial plant size’ as covariate. We subsequently performed post hoc analyses using least square means comparisons with Tukey-adjustments for multiple comparisons (emmeans package; Lenth et al. 2018).

To further examine drought-legacy effects on second-phase plant biomass of each species separately, we used structural equation modelling (SEM). Potential drought-legacy effects on second-phase biomass could principally be explained by 1) direct drought-mediated shifts in soil communities that affect second-phase plant performance, or by 2) first-phase biomass-mediated drought impacts on abiotic or biotic soil conditions that affect second-phase plant performance. For each plant species, we therefore constructed three SEMs using the package piecewiseSEM (Lefcheck 2016), in which we individually examined the potential legacy effects on second-phase root, shoot or total biomass. In each of these models, we modelled first-phase root biomass using the fixed effect ‘drought’ and the random effect ‘block’, and second-phase biomass using the fixed effects ‘first-phase root biomass’ and ‘drought’, the ‘block’ term as random effect and ‘initial plant size’ as covariate. The random ‘block’ term was excluded from the analyses of D. tenuifolia biomass to allow model convergence. We then used the ‘multigroup’ function to examine whether effects of explanatory factors on first- and second-phase biomass depended on inoculum type. In case one or more explanatory values interacted with inoculum type, SEMs are presented individually for each inoculum type, whereas a single model is presented when no interactions between inoculum type and other explanatory variables were found. Overall model fit was examined based on Fisher’s C, as implemented in piecewiseSEM.

Results

Drought and inoculum effects on first-phase biomass

Drought significantly reduced shoot biomass of all plant species except for Prunella vulgaris, which showed similar biomass under drought and ambient watering conditions (drought × species: χ² = 128.3, p < 0.001; Table 1, Fig. 2A). Shoot biomass depended on inoculum type (χ² = 14.4, p < 0.01; Table 1), and was significantly lower in soil inoculated with the 40-μm inoculum than in soil inoculated with the sterilized inoculum (Fig. 2B).

Drought and soil inoculum interactively determined root biomass in a plant species-specific way (drought × inoculum × species: χ² = 30.2, p < 0.05; Table 1). This interaction in part appeared to be driven by the difference between D. tenuifolia, of which root biomass was negatively affected by
Table 1. Effects of drought treatment, inoculum type and species identity and their interactions on first-phase shoot and root biomass. Significant results (p < 0.05), based on type-III Anova, are shown in bold.

|                          | Shoot biomass | Root biomass |
|--------------------------|---------------|--------------|
|                          | \( \chi^2 \) | df | p       | \( \chi^2 \) | df | p       |
| Fixed                    |               |    |         |               |    |         |
| Initial size (covariate) | 45.17         | 1  | \(< 0.001\) | 14.57         | 1  | \(< 0.001\) |
| Drought treatment (D)    | 183.85        | 1  | \(< 0.001\) | 269.25        | 1  | \(< 0.001\) |
| Inoculum (I)             | 14.45         | 3  | \(< 0.01\)  | 24.47         | 3  | \(< 0.001\) |
| Species (S)              | 573.71        | 5  | \(< 0.001\) | 282.32        | 5  | \(< 0.001\) |
| D \( \times \) I         | 0.72          | 3  | 0.87      | 5.25          | 3  | 0.15    |
| D \( \times \) S         | 128.31        | 5  | \(< 0.001\) | 244.33        | 5  | \(< 0.001\) |
| I \( \times \) S         | 18.90         | 15 | 0.22      | 36.81         | 15 | \(< 0.01\) |
| D \( \times \) I \( \times \) S | 16.22       | 15 | 0.37      | 30.25         | 15 | \(< 0.05\) |
| Random                   | SD            |    | SD       |               |    |         |
| Block                    | 0.04          |    | 0.12     |               |    |         |

Drought in all soils, and the other plant species, for which drought effects on root biomass appeared to be less strong and at least in part dependent on inoculum type (Fig. 3).

Total biomass, like shoot biomass, was affected by the interaction between the drought treatment and species identity (Supporting information). In contrast to effects on shoot biomass, inoculum effects on total biomass also depended on species identity, as the total biomass of only two species was significantly affected by inoculum (Supporting information).

Legacy effects on second-phase biomass

Legacy effects of drought and soil inoculum interactively affected shoot biomass, and these effects depended on plant-species identity (drought \( \times \) inoculum \( \times \) species: \( \chi^2 = 23.5 \), p < 0.05; Table 2, Fig. 4). Most notably, in the case of D. tenuifolia, the drought-legacy effects on shoot biomass appeared to be the most positive in soil inoculated with the sterilized inoculum, while in case of C. jacea and L. perenne, drought-legacy effects appeared to be the most positive in soil inoculated with the 200-\( \mu \)m inoculum (Fig. 4).

Drought-legacy effects on root biomass depended on soil inoculum (drought \( \times \) inoculum: \( \chi^2 = 8.7 \), p < 0.05; Table 2, Fig. 5). Root biomass was higher in soils that were exposed to first-phase drought when these soils had been inoculated with the 40- or 20-\( \mu \)m inoculum, but not when they had been inoculated with a 200-\( \mu \)m or sterilized inoculum (Fig. 5). Soil-inoculum effects on second-phase root biomass also depended on plant-species identity (inoculum \( \times \) species: \( \chi^2 = 38.9 \), p < 0.001; Table 2, Fig. 6): root biomass of B. hordeaceus was significantly lower in soil inoculated with the sterilized inoculum than in soil inoculated with the 20- or 40-\( \mu \)m inoculum (Fig. 5). Root biomass of C. jacea was significantly higher in soil with the 40-\( \mu \)m inoculum than in soils with the 20- or 200-\( \mu \)m inoculum, while root biomass of P. vulgaris was higher in soil with the 200-\( \mu \)m inoculum than in soil with the sterilized inoculum (Fig. 6).

Drought-legacy effects on total plant biomass were positive and independent of plant-species identity and soil inoculum (Supporting information). As was the case for second-phase shoot biomass, inoculum effects on second-phase total biomass depended on plant-species identity (Supporting information), and variation in second-phase total biomass largely reflected variation in second-phase shoot biomass (Supporting information).

Structural equation models

All structural equation models showed appropriate model fit (p-value Fisher’s C test > 0.05). Drought effects on first-phase biomass only significantly depended on inoculum type in the case of C. jacea (Fig. 7, Supporting information). In this species, negative effects of drought on first-phase root biomass were only significant in soils inoculated with the 200-\( \mu \)m or sterilized inoculum. In the case of B. hordeaceus, L. perenne and D. tenuifolia, drought effects on first-phase root biomass were significantly negative (Fig. 7, Supporting information), while in case of P. vulgaris drought effects on first-phase biomass were non-significant, but positive in direction (Fig. 7, Supporting information).

Direct effects of first-phase drought on second-phase root biomass were never significant and did not depend on inoculum type (Fig. 7). However, the relationship between first-phase root biomass and second-phase root biomass depended on soil-inoculum type in B. hordeaceus, C. jacea and P. vulgaris. In B. hordeaceus, the direction of this relationship was negative in soil inoculated with live soil communities, but positive in soil inoculated with the sterilized inoculum (Fig. 7), although the individual relationships were not significant in any of these soils. In C. jacea, first-phase root biomass had significantly negative effects on second-phase root biomass in soil with the 200-\( \mu \)m inoculum, while this relationship was not significant in other soils, and had a positive direction in the presence of the 40-\( \mu \)m and sterilized inoculum. In P. vulgaris, the direction of the relationship between first- and second-phase root biomass was positive in soil with the 40-\( \mu \)m inoculum, but negative in all other soils, although none of the individual relationships were significant.

The direct effects of first-phase drought on second-phase shoot biomass also were never significant nor dependent on inoculum type (Supporting information). Moreover, the relationship between first-phase root biomass and second-phase shoot biomass did not depend on soil inoculum, except in the case of C. jacea (Supporting information). In that species, first-phase root biomass significantly negatively corresponded with second-phase shoot biomass, while this relationship was non-significantly positive in control soil and non-significantly negative in soils with the 20- and 40-\( \mu \)m inocula. In the other plant species, the direction of the relationship between first-phase root biomass and second-phase shoot biomass was negative, but only significant in B. hordeaceus and L. perenne.

Structural equation models based on second-phase total biomass showed comparable indirect relationships between drought and second-phase total biomass (Supporting information). Notably, first-phase root biomass had a significantly
negative effect on second-phase total biomass of *B. hordeaceus* in soil inoculated with the 200-μm inoculum, but not in soils with the other inocula (Supporting information). Likewise, higher first-phase root biomass of *C. jacea* corresponded with significantly lower second-phase total biomass in soils inoculated with the 200- or 40-μm inocula (Supporting information).}

**Discussion**

Our results show that soil-community composition affected immediate impacts of drought on plant performance as well as drought-legacy effects on the performance of a next generation of plants in the same soil. However, the direction and strength of these effects differed among plant species. We hypothesized that soil communities with the highest structural complexity would most strongly ameliorate drought stress due to the presence of a diverse mutualist community, including mycorrhizal fungi (Augé 2001, Revillini et al. 2016). Drought negatively affected shoot and total biomass of all species except for *P. vulgaris*, but these effects did not depend on soil inoculum. Drought effects on belowground biomass were also generally negative, but the strength of root-biomass responses to drought was species-specific and indeed depended on soil-community composition (O’Brien et al. 2018, Xi et al. 2018). Moreover, we only found an indication of drought-stress amelioration in the case of *C. jacea*. Nevertheless, also for this species we did not find support for our hypothesis of enhanced drought-stress amelioration with increasing soil-community complexity, as negative impacts of drought on root biomass of this species were only weaker in soils with inocula sieved through 40- or 20-μm sieves (Fig. 2, 6). This suggests a drought-stress-ameliorating role for...
small-sized, non-mycorrhizal mutualists (Revillini et al. 2016, Niu et al. 2017, Rubin et al. 2017). It remains unknown why this potential stress-ameliorating effect disappeared when also larger-sized organisms (200-μm inoculum) were present, and why stress-amelioration by mycorrhizal fungi appeared to be limited. Possibly, densities of inoculated mycorrhizal fungi were too low, or mycorrhizal impacts on plant performance were negative rather than positive (Kempel et al. 2013, Sendek et al. 2019). It further remains unknown why we did not find drought-ameliorating effects of soil communities in the majority of the plant species, but we speculate that the relatively short length of the first phase (7 weeks) may have limited mutualistic plant–microbe associations. While environmental stressors such as drought may also aggravate negative impacts of pathogens and parasites (Lafferty and Holt 2003), and previous research has shown increased abundances of root-feeding nematodes and soil pathogens in response to drought (Garrett et al. 2006, Franco et al. 2019), we did not find clear evidence for increased negative impacts of soil biota under drought. Instead, drought appeared to reduce the negative impacts of soil biota in the 200-μm inoculum in the case of *A. pratensis* (Fig. 3), suggesting that drought negatively affected the abundances of plant-antagonists in this soil community. The inoculum- and species-dependent drought effects on root biomass nevertheless show that drought alters plant–soil interactions in species-specific ways (Fry et al. 2018, Koorem et al. 2021).

Figure 3. First-phase root biomass (g) of six grassland plant species in response to control (blue; left) and drought (red; right) conditions, in soils inoculated with either one of three live soil inocula, created by wet-sieving through 200-, 40- and 20-μm mesh sieves, or a sterilized inoculum. Large dots and error bars represent mean values ± standard deviations, while small dots represent individual data points. Note differences in y-axis scales.
suggests that the positive drought effects on root biomass are explained by increased positive – rather than reduced negative – effects of soil biota. Similar to the drought-ameliorating effects of these inocula when interacting with *C. jacea*, this legacy effect on root biomass across species may be best explained by the positive effects of small mutualistic microbes. The abundances of such microbes may have been promoted under drought, for example as a consequence of changed root-exudation patterns (Williams and de Vries 2020). These small microbes may also have contributed to a flush of nitrogen after rewetting, leading to increased nutrient availability in the second phase (Austin et al. 2004). These positive effects of small microbes on belowground plant performance in the second phase again appeared to be less strong in soils inoculated with the most complex soil community. This obscuring effect could possibly be explained by negative effects of mycorrhizal fungi, of which the relative abundances may have increased in response to first-phase drought (de Vries et al. 2018). Indeed, possible mycorrhizal effects

Table 2. Effects of drought legacy, inoculum type and species identity and their interactions on second-phase shoot and root plant biomass. Significant results (*p* < 0.05), based on type-III Anova, are shown in bold.

|                     | Shoot biomass | Root biomass |
|---------------------|---------------|--------------|
|                     | *χ²* | df | *p* | *χ²* | df | *p* |
| Fixed               |      |    |    |      |    |    |
| Initial size (covariate) | 22.80 | 1 | < 0.001 | 25.00 | 1 | < 0.001 |
| Drought legacy (L) | 18.74 | 1 | < 0.001 | 9.56 | 1 | < 0.01 |
| Inoculum (I)       | 6.06  | 3 | 0.11 | 12.50 | 3 | < 0.01 |
| Species (S)        | 45.81 | 4 | < 0.001 | 150.35 | 4 | < 0.001 |
| L × I              | 1.22  | 3 | 0.75 | 8.86  | 3 | < 0.05 |
| L × S              | 5.06  | 4 | 0.28 | 6.27  | 4 | 0.18 |
| I × S              | 13.79 | 12 | 0.31 | 38.85 | 12 | < 0.001 |
| L × I × S          | 23.49 | 12 | < 0.05 | 9.24  | 12 | 0.68 |
| Random SD          |      |    |    |      |    |    |
| Block              | < 0.001 |    | 0.007 |

Figure 4. Second-phase shoot biomass (g) of five grassland plant species in soils with a legacy of first-phase control (blue; left) or drought (red; right) conditions, and inoculated with either one of three live soil inocula, created by wet-sieving through 200-, 40- and 20-µm mesh sieves, or a sterilized inoculum. Large dots and error bars represent mean values ± standard deviations, while small dots represent individual data points. Note differences in y-axis scales.
on root biomass only appeared to be positive in the case of
Prunella vulgaris, which can strongly benefit from AMF pres-
ence (Van Der Heijden et al. 2003), but this effect did not
depend on the drought treatment. In the other species, there
was no indication that they benefitted from their potential
interactions with AMF in soils inoculated with the 200-µm
inoculum. As an alternative or additional explanation, the
200-µm inoculum may have contained soil organisms, e.g.
large omnivorous nematodes and micro-arthropods, that
restructured the microbial community by preferential feed-
ing on specific microbial groups (Thakur and Geisen 2019),
which may have altered soil-community responses to drought
and weakened drought-legacy effects on root biomass.

Drought-legacy effects on shoot biomass tended to be
positive, but also depended on soil inoculum, and this inter-
active effect varied among plant species. Understanding the
underlying mechanisms of such three-way interactions is par-
cularly difficult, but the results correspond with previous
research showing that drought-legacy effects on plant bio-
mass can be plant–species-specific (Kaisermann et al. 2017).
In contrast to root- and shoot-biomass responses to drought
legacies, drought-legacy effects on total biomass did not
depend on soil inoculum, and had generally a positive direc-
tion. This indicates that the inoculum-dependent drought-
legacy effects on root and shoot biomass were subtle and
partly differed in their direction. Moreover, these results sug-
gest that after a drought event, subsequent plant growth may
be promoted through changes in soil conditions, possibly
aiding plant-community recovery. Such positive drought-leg-
acy effects have also been observed in natural communities
and field experiments (Hofer et al. 2017, Griffin-Nolan et al.
2018). However, as drought-legacy effects can also be nega-
tive (Sala et al. 2012), future studies should aim to under-
stand their context-dependency, for example by elucidating
drivers of plant–soil feedback variation in drought-exposed
soils (Kaisermann et al. 2017).

We used structural equation modelling, separately for
each individual species, to further improve our understand-
ing of how drought-legacy effects on biomass depended on
soil inoculum in our experiment. These models firstly showed
that first-phase drought did not directly explain second-phase
shoot or root biomass, which would have been expected
when drought would have directly altered the community
composition of plant mutualists or antagonists, leading to
altered feedback effects on plant performance (as found in
Lau and Lennon 2012, Meisner et al. 2013). Instead, second-
phase root, shoot and total biomass were partly explained
by first-phase root biomass, which typically was negatively
affected by drought. The direction or strength of this rela-
tionship between first-phase root biomass and second-phase
root and total biomass depended on soil inoculum in three
of the five plant species. There were no clear indications that
higher first-phase root biomass resulted in stronger mutual-
istic effects on plant performance. Instead, in C. jacea and
B. bordeaceus, second-phase root and total biomass were
most negatively related to first-phase root biomass in soils

Figure 5. Second-phase root biomass (g) in soils previously exposed to control (blue; left) or drought (red; right) conditions, and inoculated
with either one of three live soil inocula, created by wet-sieving through 200-, 40- and 20-µm mesh sieves, or a sterilized inoculum. Large
dots and error bars represent mean values ± standard deviations, while small dots represent individual data points. Asterisks ‘*’ and ‘n.s.’
represent significant (p < 0.05) and non-significant differences according to post hoc comparisons of root-biomass differences between
drought and control conditions for each individual inoculum.
inoculated with the 200-μm inoculum. This suggests that in soils inoculated with this community, plant growth resulted in a more hostile soil community than in soils inoculated with the other soil communities. Also, *C. jacea* shoot biomass appeared to be most negatively related to first-phase root biomass in soils with the 200-μm inoculum. In all other species, the direction of the relationship between first-phase root biomass and second-phase shoot biomass was negative, but independent of soil inoculum. Likely, first-phase plant growth negatively affected soil nutrient availability and subsequent second-phase plant growth. As drought reduced first-phase plant growth, drought-legacy effects on plant performance in our study are thus likely explained by a combination of increased nutrient availability and species-specific alteration of soil communities, both mediated by first-phase biomass. Together, these effects increased plant performance in the second phase, suggesting that plants establishing after a drought event may benefit from increased nutrient availability and a reduction of belowground antagonists.

Whereas soil inocula differently affected plant performance in both the first and second phase of the experiment, the live soil-inoculum effects on plant biomass appeared to be subtle and did not always differ from the sterilized treatment. In the first phase, relatively minor effects were to be expected due to the use of soil suspensions that may not contain as many soil biota as regular unsuspended field-soil inocula (Wang et al. 2019). In this phase, root biomass was most negatively affected by the 40-μm inoculum, which possibly contained the highest relative abundance of pathogens and root-feeding nematodes (Wagg et al. 2014), perhaps due to a release from top to down control by large-sized soil organisms (Thakur and Geisen 2019). We expected that in the second phase, plants would show the strongest performance in sterilized soils, as many grassland plant species experience negative plant–soil feedbacks in response to their own soil communities (van der Putten et al. 2013, Wilschut et al. 2019). Strong negative plant–soil feedbacks appeared to be largely absent in our study. Possibly, differences in plant growth between

Figure 6. Second-phase root biomass (g) of five grassland plant species in soils inoculated with either one of three live soil inocula, created by wet-sieving through 200-, 40- and 20-μm mesh sieves, or a sterilized inoculum. Large dots and error bars represent mean values ± standard deviations, while small dots represent individual data points. Capital letters represent post hoc analysis of root biomass differences among soil inocula, examined for each individual plant species. Note differences in y-axis scales.
Figure 7. Structural equation modelling results of direct and indirect (through first-phase root biomass) drought-legacy effects on second-phase root biomass, analysed separately for each plant species. Panels for individual inocula are shown when drought effects on second-phase biomass depended on inoculum type; otherwise only a single panel with global effects is shown. In each panel, dashed arrows represent effects that are independent of inoculum type (global effects), while continuous lines indicate inoculum-dependent effects. Red and blue lines indicate negative and positive effects, respectively. Bright-coloured lines indicate significant effects, while faint lines indicate non-significant effects, and line thickness is proportional to standardized path coefficients. Details on model structure and model fit can be found in the methods and results sections.
the sterilized inoculum and the inocula containing live soil communities were partially masked due to reduced nutrient availability in sterilized soils, mediated by a high average first-phase plant biomass in this treatment (Fig. 1). Alternatively, sterilized soils may have been colonized by soil biota throughout the first phase, reducing the detectability of negative plant–soil feedbacks in the second phase (Geisen et al. 2017).

As we did not study soil-community composition in the different soil inocula, it is not possible to determine which soil biota drove variation in plant performance in this study. The used wet-sieving method likely resulted in the creation of three distinct communities, by stepwise exclusion of mycorrhizal fungi and microarthropods (40 µm), and nematodes (20 µm) (Wagg et al. 2014). However, it cannot be excluded that, for example, large nematodes were also sieved out through the 40 µm sieving, or that nematode eggs were still present in the community after the 20 µm sieving (Wurst et al. 2010, Wagg et al. 2014). Our study nevertheless shows that immediate drought effects and drought-legacy effects on plant performance appear to partly depend on the presence of soil biota from different size classes. This is in line with known drought responses of different groups of plant-associated soil biota that were likely present in the different soil inocula (e.g. AMF, nematodes and microbial pathogens; de Vries et al. 2018, Meisner and de Boer 2018, Franco et al. 2019, Wilschut and Geisen 2021). Understanding soil-mediated plant responses to drought therefore requires the examination of the full spectrum of soil biota.

We conclude that soil-mediated drought impacts on plant performance are plant-species-specific and depend on composition of the soil community. Moreover, we show that drought-legacy effects in our study were mostly mediated by root biomass, indicating that droughts directly alter plant–soil feedbacks through altered plant conditioning of the soil. Our results overall suggest that through varying impacts on individual plant species, drought and soil biota may interactively affect plant-community composition in natural systems. However, our understanding of which plant species may be most strongly affected remains limited. Possibly, the incorporation of root traits associated with positive interactions with soil biota will help to improve our understanding of how variation in plant species responses to drought depends on interactions with soil biota (Lozano et al. 2021, Weigelt et al. 2021). Such knowledge is crucial to understand how drought will structure plant communities that are increasingly exposed to drought events.

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Author contributions

Rutger A. Wilschut: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Supervision (lead); Visualization (lead); Writing – original draft (equal); Writing – review and editing (equal). Mark van Kleunen: Conceptualization (supporting); Resources (lead); Supervision (supporting); Writing – review and editing (equal). Nikolas Buchenau: Data curation (equal); Formal analysis (supporting); Investigation (equal); Methodology (equal); Visualization (supporting); Writing – original draft (equal); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.rbnzs7hc> (Buchenau et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

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