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Research

Legacy effects of drought on plant–soil feedbacks and plant–plant interactions

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Summary

- Interactions between aboveground and belowground biota have the potential to modify ecosystem responses to climate change, yet little is known about how drought influences plant–soil feedbacks with respect to microbial mediation of plant community dynamics.
- We tested the hypothesis that drought modifies plant–soil feedback with consequences for plant competition. We measured net pairwise plant–soil feedbacks for two grassland plant species grown in monoculture and competition in soils that had or had not been subjected to a previous drought; these were then exposed to a subsequent drought. To investigate the mechanisms involved, we assessed treatment responses of soil microbial communities and nutrient availability.
- We found that previous drought had a legacy effect on bacterial and fungal community composition that decreased plant growth in conspecific soils and had knock-on effects for plant competitive interactions. Moreover, plant and microbial responses to subsequent drought were dependent on a legacy effect of the previous drought on plant–soil interactions.
- We show that drought has lasting effects on belowground communities with consequences for plant–soil feedbacks and plant–plant interactions. This suggests that drought, which is predicted to increase in frequency with climate change, may change soil functioning and plant community composition via the modification of plant–soil feedbacks.

Introduction

Ecologists have long sought to understand how plant communities assemble and respond to environmental change. The importance of plant–plant interactions for community dynamics is well documented (Connell, 1983; Schoener, 1983; Hunter & Aarssen, 1988; Callaway, 1995), but evidence is growing that plant–soil feedbacks also influence various plant community attributes, including plant species coexistence, invasion and rarity (van der Putten *et al.*, 2013). Plant–soil feedback describes the relative growth of a plant in its own conspecific soil, compared with heterospecific soil conditioned by other plant species (Bever *et al.*, 1997; Ehrenfeld *et al.*, 2005), and is thought to arise through biotic changes in specific plant-associated microbial communities, but also through abiotic changes, such as soil chemical modification (e.g. nutrient depletion). As such, plant responses to plant–soil feedback can be negative, mostly via the promotion of pathogens or reductions in nutrient availability, or positive through the promotion of symbionts and/or soil nutrient availability (Bever *et al.*, 1997; Klironomos, 2002; Bever, 2003; van der Putten *et al.*, 2013). There is also evidence that plant–soil feedbacks can mediate plant–plant interactions (van der Putten *et al.*, 2013; Baxendale *et al.*, 2014); for instance, when two species compete in soil conditioned by one species, the feedback effect of that one plant species can influence the performance of itself (intraspecific feedback) or the competing species (interspecific feedback) (Jing *et al.*, 2015). By influencing plant–plant interactions in such a way, plant–soil feedbacks can have consequences for the outcome of plant competition (van der Putten & Peters, 1997).

There is currently much debate about the potential consequences of ongoing climate change for both the structure and functioning of terrestrial ecosystems (Zhao & Running, 2010; Reichstein *et al.*, 2013). Much recent research has focused on extreme climatic events, such as drought, which is predicted to increase in frequency and intensity, and can have significant impacts on belowground processes with potential consequences for plant community dynamics (Davidson *et al.*, 2008; Kardol *et al.*, 2010; Wu *et al.*, 2011; Classen *et al.*, 2015). For instance, periods of drought have been shown to change the composition and activity of soil microbial communities (Fierer *et al.*, 2003; Hawkes *et al.*, 2011; Sheik *et al.*, 2011; Barnard *et al.*, 2013) and to influence related processes of nutrient cycling and primary production (Sardans & Penuelas, 2005). Moreover, studies have shown that drought can have long-lasting legacy effects on ecosystem processes and plant growth. For instance, negative

impacts of drought on primary productivity and soil respiration were detected 2 yr after the event (Arnone *et al.*, 2008), and the adaptation of soil microbial communities to recurrent droughts has been shown to improve plant fitness and the ability of plants to withstand subsequent drought (Marulanda *et al.*, 2009; Lau & Lennon, 2012; Meisner *et al.*, 2013). There is also evidence that plants regulate carbon (C) allocation belowground in response to drought (Hasibeder *et al.*, 2015) and that the C released is differently allocated into the soil microbial community (Fuchslueger *et al.*, 2014), which could, in turn, select for microbial populations (Jones *et al.*, 2004; Berg & Smalla, 2009) that enable plants to cope with water stress (Preece & Peñuelas, 2016). This suggests that plants growing in conspecific soil with a history of drought might be better adapted to a subsequent drought than plants growing in heterospecific soil, thereby influencing the response of plant–soil feedback to subsequent droughts. This also suggests that the drought-induced changes in plant–soil feedback of one plant species could affect the interspecific feedback of a second plant species, as well as directly influencing plant–plant interaction, for example through competition for growth-limiting nutrients. However, to our knowledge, the relative roles of intraspecific and interspecific plant–soil feedbacks in plant competition and plant responses to drought have not been tested. Further, despite the potential for drought to have legacy effects on plant–soil feedbacks, our understanding of the mechanism involved is incomplete, which weakens our ability to quantify and predict the contribution of plant–soil feedback to ecosystem responses to extreme climate events (van der Putten *et al.*, 2016).

The aim of this study was to investigate how drought modifies plant–soil feedback, plant–plant interactions and their responses to a subsequent drought. Specifically, we tested three hypotheses: first, we hypothesized that drought influences the strength and direction of plant–soil feedback as a result of its impact on the composition of the soil microbial community; second, we hypothesized that drought-driven changes in plant–soil feedback have consequences for plant competitive interactions (through intraspecific and interspecific feedbacks); and third, we hypothesized that the response of plants to subsequent drought events depends on the legacy effect of previous drought on plant–soil interactions. We tested these hypotheses using a two-phase, pairwise plant–soil feedback experiment with two co-existing, widely distributed temperate grassland plant species: *Dactylis glomerata* and *Leontodon hispidus*. The first phase of the experiment was designed as a classic plant–soil feedback experiment, which involved the conditioning of soil by plant communities dominated by either *D. glomerata* or *L. hispidus* with or without drought, and then a second generation of each plant species was grown in monoculture (hypothesis 1) or in competition (hypothesis 2) in conditioned soils. During the second phase of the experiment, the second plant generation was exposed to a new drought. The resistance and recovery of plant and microbial communities to this drought were measured to assess whether a soil biotic legacy of a previous drought influences plant–soil feedback and plant competition during a subsequent drought.

Materials and Methods

Experimental setup

Soil and plants Two common grassland plant species were used in this experiment, namely *Dactylis glomerata* L. and *Leontodon hispidus* L. These two species were selected because they naturally co-exist and are widely distributed across European grasslands, but have contrasting life history characteristics: *L. hispidus* is a slow-growing forb with a tap root system that helps to sustain water supply in dry habitats, and which performs well in nutrient-poor situations, whereas *D. glomerata* is an exploitative, fast-growing grass with a high maximal relative growth rate because of its ability to efficiently capture resources (Poorter & Remkes, 1990; Ryser & Lambers, 1995). Seeds of *D. glomerata* and *L. hispidus* were obtained from a seed company (Emorsgate Seeds, Norfolk, UK) and the first 20 cm of a local soil for the experiment were collected from a permanent grassland at Hazelrigg Field Station, Lancaster University, UK (54°1′N, 2°46′W, 94 m above sea level (asl)), where the conditioning phase of the experiment was performed in field-based mesocosms (Fig. 1). The soil was a silt loam (Brickfield 2 Association; Avis & Harrop, 1983) of pH 6.2, and had a C and nitrogen (N) content of 3.13 and 0.25 g kg^{-1}, respectively. Soil was homogenized manually and large stones and roots were removed before planting.

Phase 1: plant–soil feedback phase The plant–soil feedback experiment consisted of an initial conditioning stage to obtain soils with plant species-specific soil communities that had been subject to drought or not, which were then used in a feedback stage to compare the growth of plant species in differently conditioned soils (Fig. 1).

Conditioning stage. The soil was conditioned in field mesocosms by mixed plant communities dominated by either *D. glomerata* or *L. hispidus*. Briefly, each mesocosm of 42 l (38 × 38 cm^2, 40 cm depth) was filled with soil in May 2012 and planted with 36 seedlings. These pots were part of a larger experiment designed to test how differences in plant community evenness and dominant species identity affect belowground response to drought (F. T. De Vries *et al.*, unpublished). The first plant community was dominated by *D. glomerata* (30 seedlings) in association with two seedlings each of *L. hispidus*, *Anthoxanthum odoratum* L. and *Rumex acetosa* L. The second plant community was built with the same four species, but dominated by *L. hispidus* (30 seedlings). Plant communities were left for two growing seasons and, during the second, half of the mesocosms were subjected to a simulated drought, whereas the other half remained under ambient climatic conditions. The drought, designed to simulate a 100-yr drought event, was simulated by covering mesocosms with transparent rain shelters from May to July 2013, following a similar design to Bloor & Bardgett (2012). Local weather data (1967–2008) were used to fit a Gumbel I distribution to the annual extremes of drought duration for the local growing period. The 100-yr drought corresponded to 34 consecutive days with < 1 mm of rainfall. Two months after ending the

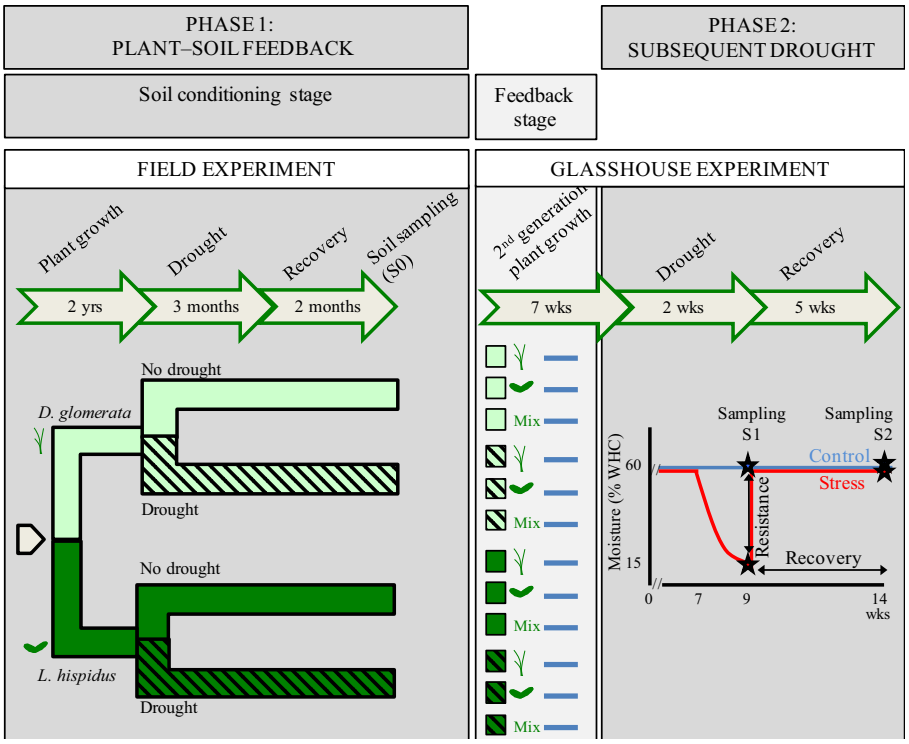

Fig. 1 Experimental framework to study the influence of drought on plant–soil interactions. WHC, water holding capacity.

drought, soil was sampled from drought and non-drought mesocosms for use in the feedback phase of the experiment. For this, soils were collected from four treatments, replicated four times, representing soils conditioned by two plant communities dominated by *D. glomerata* or *L. hispidus,* each with a drought and non-drought treatment (Fig. 1). Treatment effects on soil microbial community composition and a suite of soil physicochemical properties were analysed as detailed below (sampling S0).

Feedback stage. The soils were brought to the glasshouse at Firs Experimental Grounds, The University of Manchester, to carry out a pot experiment designed to test whether: (a) drought altered plant–soil feedback responses of the two plant species *D. glomerata* and *L. hispidus* (hypothesis 1) and their competitive interactions (hypothesis 2). Seeds of *D. glomerata* and *L. hispidus* were germinated in trays on a 1 : 1 sand and compost mixture (John Innes no. 3 mature plant compost, Reading, UK) in the glasshouse. Seedlings of similar size (~ 15 d after germination) were transplanted into pots (8.7 cm diameter × 9 cm depth) filled with field moist soil (equivalent to 180 g of dry soil) sieved at 4 mm. In each pot, two seedlings were planted in monoculture or in competition, meaning that some seedlings grew in conspecific soil (i.e. in their own soil) and others in heterospecific soil (i.e. in soil conditioned by the other species). This design resulted in 12 treatments (*D. glomerata* and *L. hispidus* grown in monoculture, and in mixture – named 'Mix' – in the four soil types), each replicated in the four blocks of the field experiment. Plants were grown for 14 wk with the temperature varied between 14.8 and 22.8°C with an average of 18.5°C. Moisture contents were monitored gravimetrically throughout the incubation and were maintained at 60% water holding capacity (WHC) by the addition of tap water. Microcosms were destructively sampled 9 wk after the beginning of the feedback period (sampling S1).

Phase 2: effects of subsequent drought on plant–soil feedback and plant–plant interaction The goal here was to assess how a biotic legacy of a previous drought influences the ecosystem response to a subsequent drought and rewetting event (hypothesis 3). For this purpose, all microcosms of phase 1 of the plant–soil feedback experiment were duplicated. From the seventh week, duplicated microcosms were subjected to a drought for 2 wk by stopping watering until the soil water content reached, on average, 0.09 g g^{-1} DW and up to 85% of plant leaves were senescent. After 2 wk of drought, microcosms were rewetted by adding 85 g of water to bring the soil moisture back to *c.* 60% WHC, whilst simulating a rainfall event of identical intensity (equal to 14 mm), and the recovery was followed for 5 wk (Fig. 1). Drought microcosms were destructively sampled at the end of the drought period (sampling S1) and 5 wk after rewetting (sampling S2). Microcosms of phase 1 (kept at constant moisture) were sampled on the same days and were used as controls for phase 2 of the experiment. In total, this resulted in 192 soil microcosms comprising 12 treatments (cf. feedback stage above), each replicated in four blocks of the field experiment, incubated with or without subsequent drought, and destructively sampled at two dates. At each of the two sampling dates, plants were removed from soil and roots were washed before subsequent biomass quantification.

Plant and soil analyses

Total leaf and root biomass were measured across all treatments as the dry weight after oven drying for 48 h at 70°C. In addition,

to estimate plant resistance to subsequent drought (phase 2), the biomass of detached leaves at the end of the drying period (sampling S1) was weighed in order to calculate leaf biomass before the drying period. For all sampling times (S0, S1, S2) and treatments, total genomic DNA was extracted from 0.35 g equivalent dry soil using a PowerSoil kit (MoBio, Carlsbad, CA, USA). The composition of bacterial and fungal communities was assessed by terminal restriction fragment length polymorphism (T-RFLP) analysis, as detailed by Griffiths *et al.* (2011) and Plassart *et al.* (2012). For bacteria, 16S DNA was PCR amplified using the primer couple 63F/530R. For fungi, the internal transcribed spacer (ITS) region of DNA was amplified using the primers ITS1/ITS4. The relative abundances of the different microbial units were calculated as the ratio between the fluorescence of each terminal restriction fragment (T-RF) and the total integrated fluorescence of all T-RFs, and bacterial and fungal diversity were estimated using Shannon and evenness indices (Hill *et al.*, 2003).

At the end of the conditioning stage (sampling S0), a suite of soil properties was measured. Total C and N were measured using a CN analyser (Elementar Vario El Cube, Hanau, Germany) after grinding in a ball-mill and using acetanilide for internal calibration; pH was measured using a 1 : 5 soil–water ratio; the maximum soil WHC was measured as detailed by Haney & Haney (2010). For the three sampling times, we measured water-extractable C and N in soil (10 g soil + 70 ml MilliQ water, shaken for 20 min). In these extracts, total dissolved organic C (TOC) was measured with a TOC analyser (Shimadzu, Tokyo, Japan) and dissolved inorganic N (NH_4^+ and NO_3^-) was assessed with an Auto Analyser (Seal Analytical, Mequon, WI, USA). In addition, soil respiration was assessed 2 h after rewetting the microcosms: fluxes of CO_2 were measured by placing the microcosms in a dark chamber and measuring the accumulation of CO_2 for 2 min with an infrared gas analyser (IRGA) (EGM-4 PP-System).

Statistical analyses

Phase 1: plant–soil feedback All statistical analyses were performed with R software v.3.1.3 (R Core Team, 2015) and all mixed effect linear models were performed using LME in the NLME package (Pinheiro *et al.*, 2015) with block as a random effect. For phase 1 of the experiment, effects of conditioning treatments on soil properties and microbial diversity (conditioning stage, sampling S0) were analysed using lme, with plant species and drought and their interaction as fixed effects. We assessed T-RFLP data using ordination by nonmetric multidimensional scaling (NMDS) and Adonis tests to determine the dissimilarity of the bacterial and fungal communities at sampling S0. For the feedback stage of phase 1, which was designed to test whether previous drought influenced plant–soil feedback (hypothesis 1), we calculated feedback responses using total plant biomass (sampling S1). For plants in monoculture, we calculated the average weight of the two plants in a pot in order to use an equal number of plants for the statistical analyses for monoculture and competition treatments. We calculated the plant–soil feedback in pairwise comparisons for the two subgroups non-drought and drought conditioning as in Brinkman *et al.* (2010):

$$\mathrm{PSF}_k = (O_k - F_k)/F_k \quad \text{Eqn 1}$$

where O is the total plant biomass in its own soil and F is the biomass in the foreign soil for the k replicates. lme models were constructed, with plant species identity (*D. glomerata* or *L. hispidus*), drought (without or with drought), plant community (monoculture or competition) and their interactions as fixed factors. To test whether drought-driven changes in plant–soil feedback have a knock-on effect on plant competitive interactions (hypothesis 2), the competitiveness of the two plant species in mixed communities was calculated as:

$$\mathrm{Competitiveness}_k = (C_k - M_k)/M_k \quad \text{Eqn 2}$$

where C is the total plant biomass of a species in competition and M is the biomass in monoculture for the k replicates. Competitiveness was analysed with lme, with previous drought, previous plant conditioning and growing plant species (*D. glomerata* or *L. hispidus*) as fixed factors. When interactions were significant, Tukey *post hoc* tests were performed.

To test whether the influence of previous drought on plant–soil feedback and plant competitiveness was related to an altered soil microbial community composition or soil nutrient availability (hypotheses 1 and 2), we assessed the influence of the 12 treatments on the concentrations of dissolved organic C and inorganic N during phase 1 (sampling S1). We constructed lme models, with previous drought, previous plant and growing plant species (*D. glomerata* in monoculture, *L. hispidus* in monoculture, the two plants in competition), and their interactions as fixed factors. Next, we examined the effects of treatments on the microbial community composition with two successive tests. First, an Adonis test was performed on T-RFLP data to evaluate whether soil conditioning by plant and drought, and plant species identity, influenced soil bacterial and fungal community composition. Then, we selected the T-RFLP fragments (T-RF) that significantly varied with these factors (ANOVA, $P < 0.05$). The relative abundances of each of these T-RFs within communities in different treatments were used for the generation of cluster plots created by the HEATMAP2 function of the GPLOTS package in R; the double dendrogram allows the clustering of the microbial communities according to the similarity of their composition (horn similarity index) and a comparison of the distribution of the abundance of T-RFs within the different treatments.

Phase 2: response to subsequent drought We assessed whether the biotic legacy effects of previous drought modified plant responses to a subsequent drought (hypothesis 3). First, we calculated plant–soil feedback and competitiveness as above for control and drought microcosms at the end of the experiment (sampling S2). Then, to test whether an adaptation of microbial community to previous drought prevents changes in drivers of plant–soil feedbacks and plant–plant interaction, the responses to a subsequent drought of plant growth, microbial community

composition, soil respiration and soil nutrient availability were assessed. At sampling S1, the soil compaction at the end of the drying period restricted the harvest of the entire root system; therefore, the plant growth response was assessed with leaf biomass only. Plant resistance to drought was assessed as the leaf biomass lost during the drought, and plant recovery as the increase in leaf biomass between samplings S1 and S2. Two microbial responses to the subsequent drought were measured: soil respiration 2 h after rewetting and the intensity of changes in microbial community composition at the end of the drought (sampling S1). For this, the similarity of microbial community composition between control and drought microcosms (horn index in the 'VEGAN' R package; Oksanen *et al.*, 2015) was calculated for bacterial and fungal T-RFs (sampling S1). The smaller the horn similarity index, the more drought changed the microbial community composition compared with a control. Plant–soil feedback, competitiveness, plant resistance and recovery, horn index, soil respiration and the concentration of dissolved organic carbon (DOC), ammonium and nitrate (sampling 1) were all analysed with lme, with previous drought, previous plant, growing plant species (*D. glomerata* in monoculture, *L. hispidus* in monoculture, the two plants in mixture) and 'subsequent drought effect' as fixed factors.

Results

Phase 1: plant–soil feedback phase

Conditioning stage Conditioning of soils with plant communities dominated by the two different plant species had limited effects on soil microbial community composition and physicochemical properties (Supporting Information Table S1), apart from soil extractable nitrate, which was greater when *D. glomerata* was the dominant plant species, irrespective of the drought treatment. However, the drought treatment, which was imposed after 2 yr of soil conditioning (sampling S0), significantly changed the bacterial and fungal community composition (Adonis tests, $P = 0.012$ and $P = 0.016$, respectively), albeit in different ways: drought increased fungal diversity (increased evenness; $P_{ANOVA} = 0.02$), but decreased bacterial diversity (decreased evenness; $P_{ANOVA} = 0.01$). The drought treatment had no detectable impact on soil physicochemical properties, except for soil water retention capacity, which was higher in the drought treatment (Fig. S1).

Feedback stage When grown in monoculture and in non-drought soils, the plant–soil feedback responses of the two plant species differed: the growth of *D. glomerata* did not differ when it was grown in conspecific (i.e. home) or heterospecific (i.e. away) soil, whereas *L. hispidus* grew better in conspecific soil, indicating a positive plant–soil feedback for this species (Fig. 2a; Table 1a). However, when grown in soil that had been subjected to drought, the direction of plant–soil feedback changed (Table 1a, $P = 0.04$): both plant species performed worse in conspecific than heterospecific soil, indicating that a previous drought caused both species to display negative feedback. When grown in competition, both species displayed negative plant–soil feedback in both drought and non-drought soils (Table 1a, $P = 0.47$).

Drought had a legacy effect on plant competitive interactions, although effects differed for the two plant species and were dependent on soil conditioning (Fig. 2b and Table 1a). There was a significant legacy effect of drought on *D. glomerata* and *L. hispidus* competitiveness when soils were conditioned by *L. hispidus* (Soil L; Tukey tests, $P = 0.06$ and $P < 0.001$, respectively), whereas there was no effect when soils were conditioned by *D. glomerata* (Soil D; Tukey tests, $P = 1.00$ and $P = 0.35$, respectively). The competitiveness of *D. glomerata* was slightly negative (-0.2 ± 0.1) when grown in non-drought soil that had been conditioned by *L. hispidus*, whereas the competitiveness of *L. hispidus* was neutral in this soil (-0.04 ± 0.19). However, the competitiveness of *L. hispidus* was positive (0.64 ± 0.09) when grown in conspecific soil that had been subjected to drought, meaning that this species grew better in competition than in monoculture under such conditions (Tukey test, $P < 0.001$). By contrast, the competitiveness of *D. glomerata* decreased in heterospecific soil that had been subjected to drought (-0.47 ± 0.1, $P = 0.06$) because of a lower growth in competition than in monoculture. Thus, in soil conditioned by *L. hispidus*, previous drought increased the competitive ability of *L. hispidus*, whereas it decreased that of *D. glomerata*.

During the feedback experiment (sampling S1), bacterial community composition was significantly influenced by previous drought (Table S2), but not by plant species identity. A total of 34 of the 150 bacterial T-RFs decreased in abundance in soils that had been subjected to drought (Fig. 3a), which was in line with the decrease in bacterial diversity (Shannon index) detected at sampling S0, i.e. after the drought and before the growth of plants of the second generation. Despite weak effects of plant species on fungal communities in the conditioning phase at sampling S0 (Fig. S1), we detected significant effects of previous plant species on fungal community composition during the feedback phase (Fig. 3b; Table S2). The previous drought also had a significant legacy effect on fungal community composition during the feedback phase in soils conditioned by *L. hispidus* (Table S2, $P = 0.029$). Indeed, the abundance of 11 of the 183 fungal T-RFs was very high only in soil conditioned with *L. hispidus* and subjected to previous drought, whereas the abundance of 12 others was very high only in non-drought soils conditioned with *L. hispidus* (Fig. 3b). Thus, *L. hispidus* was associated with different fungal populations during previous drought and non-drought soils, and, during the feedback phase, the previous drought effect was still the most important driver of fungal community composition, whereas the later-growing plants had no effect.

Previous drought had no detectable influence on soil chemical properties during the feedback period (Table S3). By contrast, soil chemical properties were strongly influenced by the identity of the growing plant species, although the effect was dependent on the conditioning species. First, soil concentrations of ammonium and nitrate were higher when *D. glomerata* was grown in monoculture in conspecific soil than in all other treatments (sampling S1). Second, between sampling S1 and S2, the growth of *D. glomerata* in monoculture and in heterospecific soil increased

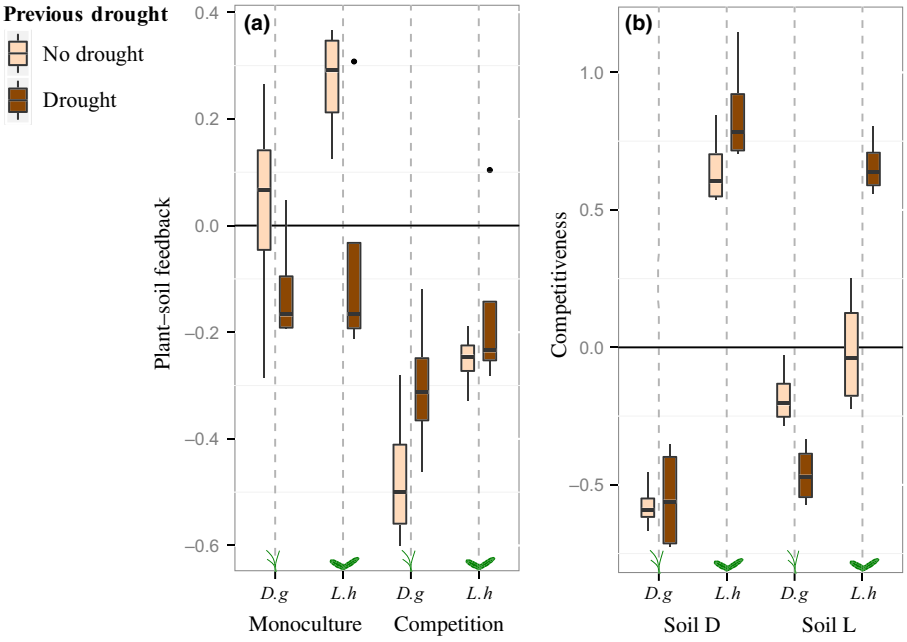

Fig. 2 Boxplot diagrams depicting the influence of previous drought on plant performance during the feedback experiment (phase 1). (a) Plant–soil feedback of *Dactylis glomerata* (*D.g*) and *Leontodon hispidus* (*L.h*) growing in monoculture and in competition ($n = 4$) and (b) competitiveness of *D. glomerata* (*D.g*) and *L. hispidus* (*L.h*) growing in soil previously planted with *D. glomerata* (Soil D) and *L. hispidus* (Soil L) ($n = 4$) calculated with plant biomass. The box in each boxplot shows the lower quartile, median and upper quartile values, and the whiskers show the range of the variation; horizontal black lines indicate zero; points indicate extreme values.

the soil concentration of nitrate, whereas the growth of both plants in a mixture decreased soil nitrate (Fig. S2). Thus, *D. glomerata* increased, and *L. hispidus* decreased, soil nitrate concentrations.

Phase 2: response to subsequent drought

The effectiveness of the second, glasshouse-based drought was similar across all treatments, with soil moisture contents being similar across treatments at the end of the drying period (0.09 ± 0.02 g g^{-1} DW) and after the rewetting period (0.39 ± 0.03 g g^{-1} DW) (Fig. S3). This second drought decreased leaf biomass across all treatments ($P < 0.001$), and the response was proportional to leaf biomass before the drying period (Fig. S4). The detected increases in leaf biomass over the 5-wk recovery period following drought were also proportional to the leaf biomass at the end of the drying period. As a consequence, the competitiveness values after drought recovery (sampling S2) were similar to those observed during the feedback experiment (Table 1a,b), as well as the plant–soil feedbacks of *L. hispidus* (Table 1b; $P < 0.001$). Therefore, our results showed a persistent legacy effect of previous drought on plant–soil feedback, especially for *L. hispidus*, and plant competitive interactions during a subsequent drought.

At the end of the second drought (phase 2, sampling S1), bacterial and fungal community composition differed significantly between control and drought microcosms (Adonis tests, $P = 0.034$ and $P = 0.001$, respectively; Table S2). The intensity of the changes in bacterial and fungal communities was assessed by calculating the similarity of their composition (with the horn index) for each treatment between control and second-drought microcosms at sampling S1 (Fig. 4a,b). No significant previous drought effect was observed on the horn similarity index (Fig. 4a, b); therefore, the intensity of the change in bacterial and fungal community composition in response to the second drought was similar in previous drought and non-drought soils, i.e. irrespective of previous drought history. By contrast, the previous drought had a strong legacy effect on soil functioning: CO_2 respiration (Fig. 4c) and DOC concentrations (Fig. 4d) after rewetting, and ammonium concentrations at the end of the new drought (Fig. 4e), were significantly lower when soils had been subjected to previous drought (Fig. 4; Table S4), except for CO_2 respiration from soils conditioned with *L. hispidus* when plants were grown in competition.

The plant species present previously or during the second drought influenced the effects of the second drought on soil properties, although the effects varied for different soil properties (Fig. 4). For instance, for plants in monoculture, bacterial community composition showed a greater change when plants were grown in conspecific than in heterospecific soils (Fig. 4a, $P = 0.01$), and this was associated with lower soil respiration (Fig. 4c, $P = 0.008$) and DOC concentration (Fig. 4d, $P = 0.047$). The flush of CO_2 (Fig. 4c), DOC (Fig. 4d) and ammonium (Fig. 4e) was also greater when *L. hispidus* was grown in monoculture than with *D. glomerata* ($P = 0.023$, $P = 0.0006$ and $P = 0.045$, respectively). Fungal community composition changed less in response to drought in soils conditioned with *L. hispidus* than in soils conditioned with *D. glomerata* (Fig. 4b, $P = 0.011$). For plants growing in competition, bacterial community composition showed a greater change in response to drought in soil conditioned with *D. glomerata* than with *L. hispidus* (Fig. 4a; $P = 0.047$). Altogether, these results showed that the soil response to second drought was dependent on plant–soil feedback and plant competition effects.

Discussion

The first aim of this study was to evaluate whether a previous drought affects plant–soil feedback. This was tested using an experiment that involved an initial stage of soil conditioning by

Table 1 Analysis of variance of mixed linear models for plant performance (i.e. plant–soil feedback and competitiveness) (a) during the feedback experiment (phase 1, sampling S1), and (b) after the subsequent drought (phase 2, sampling S2)

	Plant–soil feedback			Competitiveness	
(a)	*F*-value	*P*-value		*F*-value	*P*-value
Previous drought (A)	0.91	0.35	Previous drought (A)	11.06	0.003**
Growing species (B)	8.43	0.01**	Growing species (B)	436.60	< 0.0001**
Community (C)	32.93	< 0.0001***	Previous plant (C)	3.88	0.06
A : B	1.28	0.27	A : B	36.62	< 0.0001***
A : C	10.48	0.00***	A : C	0.73	0.40
B : C	0.06	0.80	B : C	50.92	< 0.0001***
A : B : C	0.20	0.66	A : B : C	16.93	0.00***

Tukey test	*z*-value	*P*-value	Tukey test	*z*-value	*P*-value
In monoculture Nondrought vs previous drought	−2.66	0.04*	*D. glomerata* in soil D Nondrought vs previous drought	0.27	1.00
In competition Nondrought vs previous drought	1.45	0.47	*D. glomerata* in soil L Nondrought vs previous drought	−2.99	0.06
			L. hispidus in soil D Nondrought vs previous drought	2.20	0.35
			L. hispidus in soil L Nondrought vs previous drought	7.17	< 0.001***

	Plant–soil feedback			Competitiveness	
(b)	*F*-value	*P*-value		*F*-value	*P*-value
Previous drought (A)	2.59	0.11	Previous drought (A)	2.97	0.09
Growing species (B)	26.46	< 0.0001***	Growing species (B)	260.76	< 0.0001**
Community (C)	79.25	< 0.0001***	Previous plant (C)	2.19	0.15
Subsequent drought (D)	1.35	0.25	Subsequent drought (D)	1.21	0.28
A : B	10.74	0.002**	A : B	21.66	< 0.0001***
A : C	6.90	0.01*	A : C	1.96	0.17
B : C	0.12	0.73	B : C	31.55	< 0.0001***
A : D	0.12	0.73	A : D	0.02	0.89
B : D	0.76	0.39	B : D	3.07	0.09
C : D	0.66	0.42	C : D	0.10	0.75
A : B : C	4.73	0.04*	A : B : C	5.87	0.02*
A : B : D	2.62	0.11	A : B : D	0.25	0.62
A : C : D	3.91	0.05	A : C : D	0.51	0.48
B : C : D	0.00	0.96	B : C : D	0.39	0.54
A : B : C : D	2.26	0.14	A : B : C : D	0.22	0.64

Tukey test	*z*-value	*P*-value	Tukey test	*z*-value	*P*-value
D. glomerata in monoculture Nondrought vs previous drought	0.51	1.00	*D. glomerata* in soil D Nondrought vs previous drought	−0.73	1.00
D. glomerata in competition Nondrought vs previous drought	0.99	0.98	*D. glomerata* in soil L Nondrought vs previous drought	−1.88	0.56
L. hispidus in monoculture Nondrought vs previous drought	−4.74	< 0.001***	*L. hispidus* in soil D Nondrought vs previous drought	1.46	0.83
L. hispidus in competition Nondrought vs previous drought	0.04	1.00	*L. hispidus* in soil L Nondrought vs previous drought	5.48	< 0.001***

Asterisks indicate a statistically significant effect tested with a mixed linear model: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

plant communities dominated by two plant species, which were then subjected to drought, followed by a feedback stage whereby the two plant species were grown in monoculture in these soils. Plant–soil feedback depends on the balance between positive and negative feedbacks occurring in conspecific and heterospecific soils (van de Voorde *et al.*, 2011). Positive feedback is facilitated by high nutrient availability (nutrient-mediated feedback) and the abundance of mutualistic microorganisms (microbial-mediated feedback), whereas negative feedback is driven by nutrient limitation or an accumulation of pathogens. We found that, under non-drought conditions, *D. glomerata* grew equally well in conspecific and heterospecific soil, suggesting a balance of

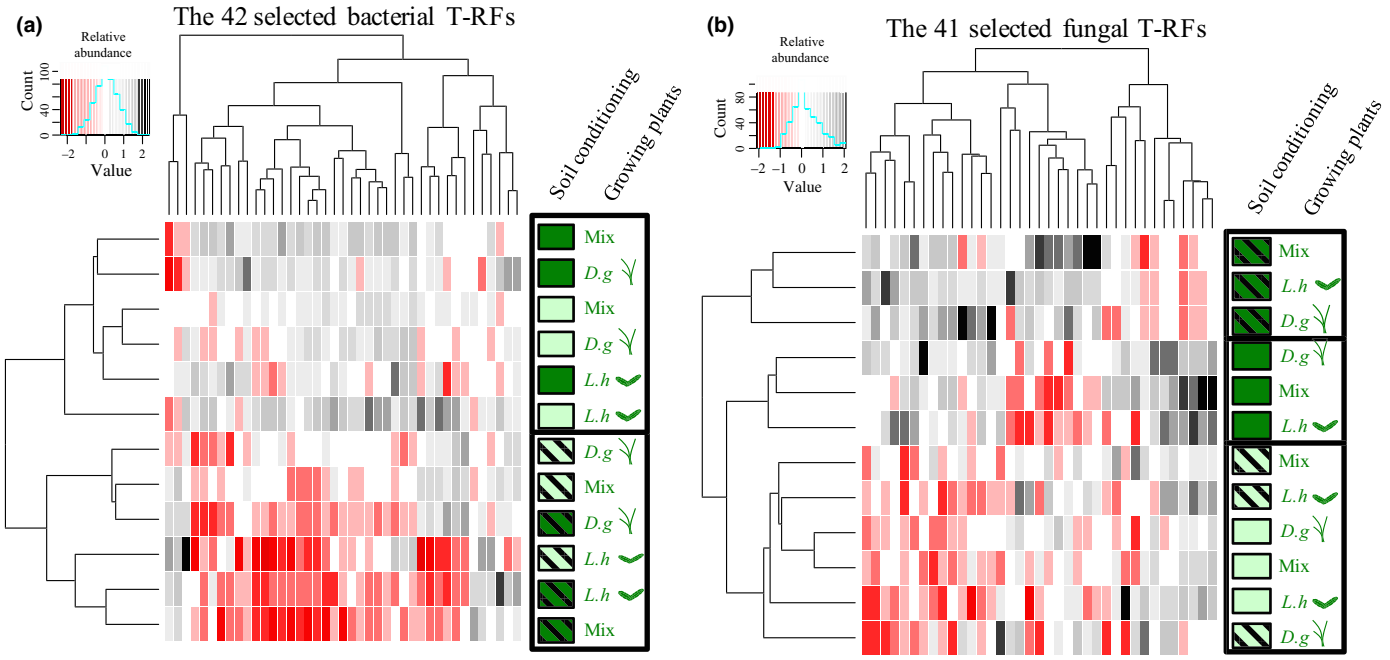

Fig. 3 Cluster of (a) bacterial and (b) fungal communities based on the relative abundance of terminal restriction fragments (T-RFs) during the feedback experiment (phase 1, sampling S1). Heatmaps were based on the hierarchical clustering solution (horn similarity) distance metric. Rows represent the mean ($n = 4$) of the 12 treatments: *Dactylis glomerata* (*D.g*) and *Leontodon hispidus* (*L.h*) grown in monoculture, and in mixture (Mix), in the four soil types, i.e. soils conditioned by *D. glomerata* (light green square) or *L. hispidus* (dark green square), each with a drought (dashed) and non-drought (without dash) treatment. Columns represent the selected T-RFs that varied significantly with at least one treatment (ANOVA, $P < 0.05$; drought conditioning, plant conditioning, growing plant species or their interactions). The colours in the heatmaps represent the relative abundance of each T-RF, as indicated in the upper left corner of each panel.

positive and negative feedback. By contrast, maximal growth of *L. hispidus* occurred in non-drought conspecific soil, despite this soil having a lower nutrient availability than soil conditioned with *D. glomerata*. This positive feedback was found to be associated with a specific fungal community (Fig. 3b), which probably optimized plant nutrient acquisition, possibly via the formation of mycorrhizal associations (Jackson *et al.*, 2008; Smith & Smith, 2011). This mechanism is supported by the knowledge that *L. hispidus* is strongly dependent on mycorrhizal fungi (Tawaraya, 2003), and suggests that plant–soil feedback of *L. hispidus* is microbially mediated with positive feedback from mutualistic microorganisms.

We found that drought altered the direction of plant–soil feedback: both plant species displayed negative feedback in soil that had been subjected to drought. We do not know the precise mechanism explaining the reduced performance of both plant species in conspecific soil with a history of drought, but it is probably a result of drought-induced changes in microbial community composition, rather than changes in nutrient availability. This view is supported by our finding that drought had no detectable legacy effect on soil nutrient availability, but significantly altered the composition of the microbial community: drought reduced bacterial diversity and the abundance of several T-RFs, as also shown by others (Bérard *et al.*, 2011; Barnard *et al.*, 2013), and changed the composition of the fungal community in soil conditioned by *L. hispidus*, causing a change in the dominance of some fungal taxa. This finding is consistent with the knowledge that certain plant species select for different fungal communities during drought (Compant *et al.*, 2010), and demonstrates that drought effects on soil fungal communities vary across plant species, most probably as a result of differences in rhizodeposition (Preece & Peñuelas, 2016). In addition, our results support the view that long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community composition (Kulmatiski & Beard, 2011). An alternative explanation for the change in soil microbial community composition is related to drought-induced changes in soil structure: drought is known to promote soil aggregate breakdown and alter soil wettability (Denef *et al.*, 2001), which might create heterogeneous penetration of water through soil and create new ecological niches for microorganisms (Ruamps *et al.*, 2011). Together, these findings indicate that the reduced growth of both plant species in conspecific soil subjected to drought might be a result of a combined effect of decreased abundance of beneficial soil microbes (Cavagnaro, 2016) and increased abundance of less beneficial microbes, i.e. pathogenic microbes, following drought. Further, these results support our hypothesis that drought impacts the direction and strength of plant–soil feedback as a result of a legacy effect on soil microbial communities.

We also tested whether soil conditioning and drought-driven changes in plant–soil feedback influenced plant–plant interactions. To address this, we compared the growth of the two plant species in monoculture and in mixtures in soils with different histories of conditioning and drought. As hypothesized, we found that previous drought influenced plant competitive interactions, but only in soil conditioned by *L. hispidus*: previous drought

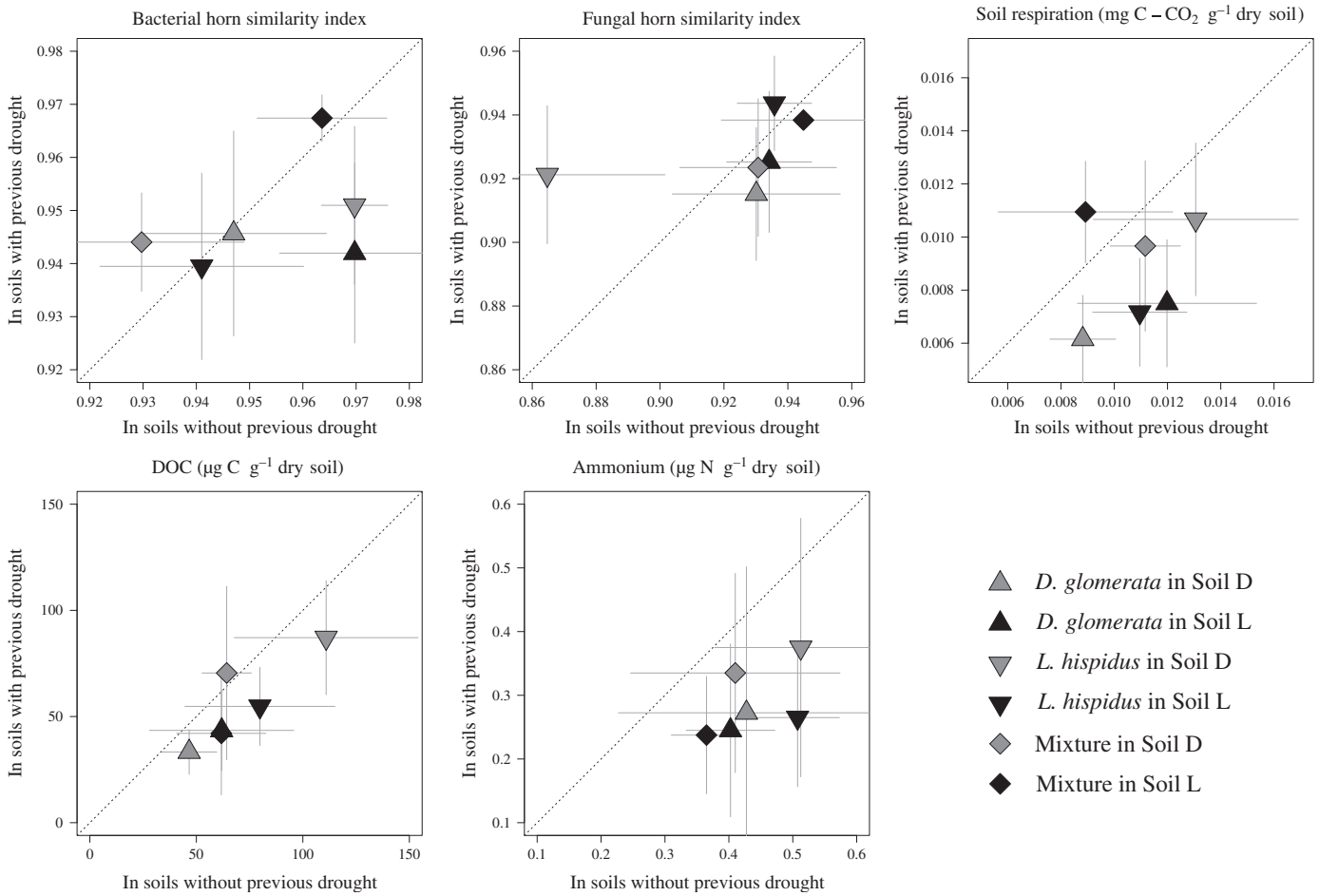

Fig. 4 Influence of subsequent drought on soil properties (phase 2, sampling S1). The influence of subsequent drought was determined at the end of the drying period for soil bacterial and fungal communities by measurement of the similarity of the community composition between control and drought microcosms, dissolved organic carbon (DOC) and ammonium available in soils and soil respiration, measured 2 h after the rewetting of dried soils. The plots represent the measures in soils without previous drought against those in soils with previous drought for soils previously conditioned with *Dactylis glomerata* (Soil D, grey) and *Leontodon hispidus* (Soil L, black) and planted with *D. glomerata* in monoculture, *L. hispidus* in monoculture and both in a mixture. Data are means ± SD ($n = 4$).

increased the competitive ability of *L. hispidus* in conspecific soil, but decreased the competitiveness of *D. glomerata* in this soil compared with non-drought soil. This is consistent with studies showing that plant–soil feedback influences plant competition (van der Putten & Peters, 1997; Kardol *et al.*, 2007; Baxendale *et al.*, 2014; Jing *et al.*, 2015), but also demonstrates that drought strongly modifies the outcome of plant–soil feedbacks for plant competitive interactions, and responses are species specific.

We propose that the opposite response of the two plant species to drought is related to their different resource acquisition strategies and nutrient supply to the plants. We found that, under non-drought conditions, *L. hispidus* and *D. glomerata* grew equally well in monoculture and mixtures, suggesting that competition for nutrients was low and, potentially, that both species could benefit from nutrients provided by their own microbial community. By contrast, in drought soils, improved growth of *L. hispidus* and reduced growth of *D. glomerata* occurred in mixtures compared with monoculture, despite no detectable effect of mixtures on soil microbial community composition. This suggests that drought changed the outcome of plant–soil feedbacks for plant competitive interactions because of drought-induced changes in nutrient competition and nutrient supply by microbial-mediated mechanisms. Indeed, the two plant species differed in their nutrient use strategies: *D. glomerata* increased soil nitrate concentrations (Fig. S2), which was probably a result of a positive influence of this species on the rates of nitrification (Bremer *et al.*, 2009; Legay *et al.*, 2016), whereas *L. hispidus* is known to have a high demand for nitrate, as shown by Onipchenko *et al.* (2001). As such, nitrate provided by the soil microbial community associated with *D. glomerata* could provide a more accessible N source for *L. hispidus,* but only when its own microbial community becomes less efficient in nitrate supply. This could be the case when *L. hispidus* is grown in conspecific drought soil, as indicated by its low growth in monoculture.

The above results suggest that drought weakens the strength of plant–microbe interactions for nutrient acquisition of *L. hispidus*; the microbial community associated with *L. hispidus* in drought soils is less efficient at supplying N to *L. hispidus* than that

associated with *L. hispidus* in non-drought soils. However, we acknowledge that we are uncertain about the effects of drought on soil N dynamics, given that we did not measure nitrifier abundance or rates of N mineralization/immobilization to confirm that the soil microbial community associated with *L. hispidus* in drought soil is making less N available. Nevertheless, our results indicate that drought has the potential to create shifts in soil N availability resulting from a change in soil microbial community composition, with consequences for plant–plant competition. This supports the notion that microbial control of plant productivity (Hendriks *et al.*, 2013) could evolve with drought. By contrast, the growth of *D. glomerata* in mixtures decreased in heterospecific drought soil, but not in monoculture or in mixtures in conspecific soil. Therefore, *D. glomerata* showed a lower growth only when *L. hispidus* was present with its conspecific drought microbial community: this indicates a negative interspecific feedback of *L. hispidus* on *D. glomerata*. These results support the view that interspecific plant–soil feedback can influence plant–plant competition (van de Voorde *et al.*, 2011; Jing *et al.*, 2015), which can evolve with drought as a result of a change in nutrient availability related to biotic change (Meisner *et al.*, 2013). Further, these results support our second hypothesis that drought influences plant competitive interactions depending on plant–soil feedbacks, probably because of a desynchronization of the plant–microbial partnership related to nutrient acquisition. Therefore, species-specific responses suggest that drought could be a particular threat to plant species with a high dependence on mycorrhizal fungi.

The final aim of this study was to investigate the influence of drought-induced changes in plant–soil feedback on plant responses to a subsequent drought. For this purpose, a second drought was applied to microcosms. We found that plant resistance to, and recovery from, a subsequent drought was proportional to plant biomass (shoot and root) before the event, resulting in persistent differences in plant–soil feedback and plant competitiveness. Our findings are broadly consistent with other studies that have detected a strong legacy effect of the initial drought on plant responses to a subsequent drought (Marulanda *et al.*, 2009; Lau & Lennon, 2012; Meisner *et al.*, 2013). One possible reason for this response is that a larger root biomass before a drought allows faster and more efficient water and nutrient uptake during drying and also on rewetting. Therefore, the advantage conferred to plants by the initial drought could have had implications for the ability of the plants to withstand the subsequent drought. We also observed a drought legacy effect on the drought response of several soil parameters, which supports our hypothesis that previous drought can influence plant response to drought because of drought legacy effects on nutrient and microbial-mediated drivers of plant–soil feedback and plant–plant interactions.

We found that the commonly observed flush of C and N following the second drought (Birch, 1958) was lower in soils that had previously been subjected to drought than in soils that had not. The hypothesized mechanisms explaining the Birch effect generally involve physical and biotic effects (rewetting can cause aggregate slaking, which releases previously protected soil C (Denef *et al.*, 2001) and microbial C following cell death) or microbial mechanisms of tolerance (accumulation of osmolytes during drought; Schimel *et al.*, 2007). With consecutive droughts, it is also possible that the physical disruption releases less C from a reduced quantity of easily disruptable aggregates; however, opposite responses have also been shown (Miller *et al.*, 2005). The second explanation might be a result of the adaptation to drought of microbial communities involved in the C and N cycles. We expected that previous drought would prevent large changes in microbial community composition during a subsequent drought because of the selection of microbial taxa able to tolerate the perturbation (Wallenstein & Hall, 2012; Bouskill *et al.*, 2013; Hawkes & Keitt, 2015). By contrast, we found that changes in microbial community composition in response to the second drought were of the same magnitude irrespective of the drought history, as also observed by Fuchslueger *et al.* (2016). However, it is possible that only a small proportion of active microorganisms can adapt to drought, and that the resuscitation of rare taxa after a drought event has a disproportionate influence on soil functioning (Aanderud *et al.*, 2015). Other adaptive mechanisms for coping with repeated drought could involve 'anticipatory regulation', an evolutionary process known to occur within species of microorganisms when adapting to fluctuating environmental conditions (Mitchell *et al.*, 2009) Therefore, the biotic legacy of drought could alter the expected microbial functional responses to drought (Hawkes & Keitt, 2015) with consequences for C and N turnover in the context of recurrent drought (Fuchslueger *et al.*, 2016).

Despite the weak effects of plant species on soil microbial communities in the field conditioning and subsequent laboratory conditioning phase, we did detect significant plant species effects (past and present) on soil microbial community composition and functioning following the subsequent drought. This finding indicates that plants influence the response of soil microbial communities to drought, probably through root exudation (Fuchslueger *et al.*, 2014), which is consistent with previous studies showing species-specific, drought-induced changes in rhizodeposition and soil microbial communities (Preece & Peñuelas, 2016). Our results also suggest that the drought-induced changes in rhizodeposition are dependent on plant–soil feedback. Collectively, our study supports our hypothesis that drought impacts on soil microbial communities have consequences for soil functioning during a subsequent drought, and that these effects depend on plant–soil feedbacks and impact plant responses to drought.

In conclusion, our results indicate that drought can alter the direction of plant–soil feedback as a result of long-lasting effects on soil microbial communities, and that this has consequences for plant–plant interactions and plant responses to subsequent drought. Moreover, we provide evidence that legacy effects of drought on soil microbial communities alter their functional capabilities when faced with subsequent drought, which supports the notion that the biotic legacy of drought causes divergence from the expected functional responses to drought (Hawkes & Keitt, 2015). These findings are of importance given the predicted increase in the frequency and intensity of drought events, and the demonstrated potential for drought history to shape microbial-mediated plant–soil feedbacks with consequences for

plant community dynamics and ecosystem functioning, and future plant and microbial responses to drought.

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Author contributions

R.D.B. initiated and gained funding for the study, which was planned and designed by A.K., F.T.d.V. and R.D.B. A.K. and F.T.d.V. performed the experiments, and A.K. analysed the resulting data. A.K., F.T.d.V., R.I.G. and R.D.B. wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Soil properties at the end of the conditioning period (phase 1, sampling S0) – soil water content, nitrate and microbial community composition.

Fig. S2 Effect of previous drought, previous plant and growing plant species on soil properties during the feedback experiment (phase 1) – ammonium and nitrate contents.

Fig. S3 Soil moisture in microcosms.

Fig. S4 Effect of subsequent drought on leaf biomass (phase 2).

Table S1 Soil properties at the end of the conditioning period (phase 1, sampling S0) – range of values and statistical analysis

Table S2 Tables of Adonis tests on the bacterial and fungal community composition

Table S3 Effect of previous drought, previous plant and growing plant species on soil properties during the feedback experiment (phase 1) – table of ANOVA

Table S4 Effect of subsequent drought on soil properties

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