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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 communities 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 depended 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 modification of plant-soil feedbacks.

Running head: Drought changes plant-soil feedbacks

Key words: Above-belowground interactions, biotic legacy, drought, plant-plant interaction, plant-soil feedback, resource competition, soil microbial communities.

Introduction

Ecologists have long sought to understanding 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 to 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 promoting 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 as 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 on-going 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 influence related processes of nutrient cycling and primary production (Sardans & Peñuelas, 2005). Moreover, studies show 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 two years after

the event (Arnone III *et al*., 2008), and 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 allocation belowground in response to drought (Hasibeder *et al.,* 2015) and that the carbon 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 plant 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 role of intraspecific and interspecific plant-soil feedback in plant competition and plant responses to drought has 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 due to 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 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 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 due to its ability to efficiently capture resource (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 20 first cm of a local soil for the experiment was collected from a permanent grassland at Hazelrigg Field Station, Lancaster University, UK (54°1'N, 2°46'W, 94 m a.s.l), where the conditioning phase of the experiment was done 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 N content of 3.13 and 0.25 g kg^{-1} respectively. Soil was homogenised manually and large stones and roots were removed prior to 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-

158 L (38 x 38 cm, 40 cm depth) was filled with soil in May 2012 and planted with 36 seedlings.
159 These pots were part of a larger experiment designed to test how differences in plant
160 community evenness and dominant species identity affect belowground response to drought
161 (De Vries *et al*., unpublished). The first plant community was dominated by *D. glomerata* (30
162 seedlings) in association with two seedlings each of *L. hispidus*, *Anthoxanthum odoratum* L.
163 and *Rumex acetosa* L. The second plant community was built with the same four species, but
164 dominated by *L. hispidus* (30 seedlings). Plant communities were left for two growing
165 seasons, and during the second, half of the mesocosms were subjected to a simulated drought,
166 whereas the other half remained under ambient climatic conditions. The drought, designed to
167 simulate 100-year drought event, was simulated by covering mesocosms with transparent rain
168 shelters from May to July 2013, following a similar design to Bloor and Bardgett (2012).
169 Local weather data (1967-2008) were used to fit a Gumbel I distribution to the annual
170 extremes of drought duration for the local growing period. The 100-year drought
171 corresponded to 34 consecutive days with less than 1 mm of rainfall. Two months after
172 ending the drought, soil was sampled from droughted and non-droughted mesocosms for use
173 in the feedback phase of the experiment. For this, soils were collected from four treatments,
174 replicated four times, representing soils conditioned by two plant communities dominated by
175 *D. glomerata* or *L. hispidus,* each with a droughted and non-droughted treatment (Fig. 1).
176 Treatment effects on soil microbial community composition and a suite of soil physico-
177 chemical properties were analysed as detailed below (Sampling S0).
178
179 ***Feedback stage.*** The soils were brought to the glasshouse at Firs Experimental Grounds, The
180 University of Manchester, to carry out a pot experiment designed to test whether: (a) drought
181 altered plant-soil feedback responses of the two plant species *D. glomerata* and *L. hispidus*
182 (hypothesis 1) and their competitive interactions (hypothesis 2). Seeds of *D. glomerata* and *L.*
183 *hispidus* were germinated in trays on 1:1 sand and compost mixture (John Innes no 3 mature
184 plant compost, Reading, UK) in the glasshouse. Seedlings of similar size (~ 15d after
185 germination) were transplanted into pots (8.7 cm diameter x 9 cm depth) filled with field
186 moist soil (equivalent to 180g of dry soil) sieved at 4mm. In each pot, two seedlings were
187 planted in monoculture or in competition, meaning that some seedlings grew in conspecific
188 soil (i.e., in their own soil) and others in heterospecific soil (i.e., in soil conditioned by the
189 other species). This design resulted in 12 treatments (*D. glomerata* and *L. hispidus* grown in
190 monoculture, and in mixture - named 'Mix' - in the four soil types), each replicated in the
191 four blocks of the field experiment. Plants were grown for 14 weeks and 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 adding tap water. Microcosms were destructively sampled nine weeks after the beginning of 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 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 weeks 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 two weeks of drought, microcosms were rewetted by adding 85 g of water to bring soil moisture back to about 60% WHC while simulating a rainfall event of identical intensity (equal to 14 mm), and the recovery was followed for 5 weeks (Fig. 1). Droughted microcosms were destructively sampled at the end of the drought period (Sampling S1) and 5 weeks after rewetting (Sampling S2). Microcosms of phase 1 (kept at constant moisture) were sampled at the same days and were used as control for phase 2 of the experiment. In total, this resulted in 192 soil microcosms comprising twelve 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 prior to subsequent biomass quantification.

PLANT AND SOIL ANALYSES

Total leaf and root biomass was measured across all treatments as the dry weight after oven-drying for 48h 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 PowerSoil kit (MoBio, Carlsbad, CA). The composition of bacterial and fungal communities was assessed by T-RFLP analysis, as detailed by Griffiths *et al.* (2011) and Plassart *et al.* (2012). For bacteria, 16S DNA were PCR-amplified using the couple of primers 63F/530R.

For fungi, the internal transcribe spacer (ITS) region of DNA was amplified using the primers ITS1/ITS4. 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 was estimated using Shannon and evenness indices (Hill *et al.*, 2003).

At the end of the conditioning stage (sampling S0) a suite of soil properties were measured. Total C and N was measured using a CN analyser (Elementar Vario El Cube, Germany) after grinding in a ball-mill and using acetanilide for internal calibration, pH was measured using a 1:5 soil-water ratio, and maximum soil water holding capacity was measured as detailed by Haney and Haney (2010). For the three sampling times, we measured water extractable carbon and nitrogen in soil (10 g soil + 70 ml MilliQ water, shaken for 20 min). In these extracts, total dissolved organic carbon (TOC) was measured with a TOC analyser (Shimadzu, Japan) and dissolved inorganic N (NH_4^+ and NO_3^-) was assessed with an Auto Analyser (Seal Analytical, Mequon, USA). Additionally, soil respiration was assessed two hours 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 two minutes with an IRGA (EGM-4 PP-System).

STATISTICAL ANALYSES

Phase 1: Plant-soil feedback

All statistical analyses were performed with R software version 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 sub-groups non-drought and drought conditioning as in Brinkman *et al*. (2010):

$$PSF_k = (O_k - F_k)/F_k$$

where O is the total plant biomass in its own soil and F 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 if drought-driven changes in plant-soil feedback have a knock-on effect on plant competitive interactions (hypothesis 2), the competitiveness of the two plants species in mixed communities was calculated as:

$$\text{Competitiveness}_k = (C_k - M_k)/M_k$$

where C is the total plant biomass of a species in competition and M 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's 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 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 if 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 abundance of each of these T-RFs within communities in different treatments were used for generation of cluster plots created by the heatmap2 function of the gplots package in R; the double dendrogram allows to cluster the microbial communities

according to the similarity of their composition (horn similarity index) and to compare the distribution of the abundance of T-RFs within the different treatments.

Phase 2: Response to subsequent drought

We assessed if biotic legacy effects of previous drought modified plant responses to a subsequent drought (hypotheses 3). First, we calculated plant-soil feedback and competitiveness as above for control and droughted 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 response 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 drying period restricted the harvest of the entire root system; therefore the plant growth response was assessed with leaves biomass only. Plant resistance to drought was assessed as the leaf biomass lost during the drought; plant recovery as the increase in leaf biomass between samplings S1 and S2. Two microbial responses to the subsequent drought were measured: soil respiration two hours 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 droughted microcosms (horn index in "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 microbial community composition compared to control. Plant-soil feedback, competitiveness, plant resistance and recovery, horn index, soil respiration, and the concentration of 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 physico-chemical 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 two years of soil conditioning (Sampling S0), significantly changed 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 physico-chemical properties, except soil water retention capacity, which was higher in drought treatment (Supporting Information Fig. S1).

Feedback stage.

When grown in monoculture and in non-droughted soils, the plant-soil feedback responses of the two plant species differed: the growth of *D. glomerat*a 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 and 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 droughted and non-droughted soils (Table 1a, *P=0.47*).

Drought had a legacy effect on plant competitive interactions, although effects differed for the two plant species and depended 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), while there was no effect when soils were conditioned by *D. glomerata* (Soil D; Tukey tests *P=1.00* and *P=0.35*). Competitiveness of *D. glomerata* was slightly negative (-0.2 ± 0.1) when grown in non-droughted soil that had been conditioned by *L. hispidus,* while competitiveness of *L. hispidus* was neutral in this soil (-0.04 ± 0.19). However, 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*). In contrast, the competitiveness of

360 *D. glomerata* decreased in heterospecific soil that had been subject to drought (-0.47 ±0.1,
361 *P=0.06*) because of a lower growth in competition than in monoculture. Thus, in soil
362 conditioned by *L. hispidus*, previous drought increased the competitive ability of *L. hispidus*,
363 while it decreased that of *D. glomerata*.
364
365 During the feedback experiment (Sampling S1), bacterial community composition was
366 significantly influenced by the previous drought (Supporting Information Table S2), but not
367 by plant species identity. A total of 34 of the 150 bacterial T-RFs decreased in abundance in
368 soils that had been subjected to drought (Fig. 3a), which was in line with the decrease in
369 bacterial diversity (Shannon Index) detected at sampling S0, *i.e.* after the drought and before
370 the growth of plants of second generation. Despite weak effects of plant species on fungal
371 communities in the conditioning phase at sampling S0 (Supporting Information Fig. S1), we
372 detected significant effects of previous plant species on fungal community composition
373 during the feedback phase (Fig. 3b and Supporting Information Table S2). The previous
374 drought also had a significant legacy effect on fungal community composition during the
375 feedback phase in soils conditioned by *L. hispidus* (Supporting Information Table S2, *P=*
376 *0.029*). Indeed, the abundance of 11 of the 183 fungal T-RFs was very high only in soil
377 conditioned with *L. hispidus* and subjected to previous drought, while the abundance of 12
378 others was very high only in non-droughted soils conditioned with *L. hispidus* (Fig. 3b).
379 Thus, *L. hispidus* was associated with different fungal populations during previous droughted
380 and non-droughted soils, and during the feedback phase the previous drought effect was still
381 the most important driver of fungal community composition while the later-growing plants
382 had no effect.
383
384 Previous drought had no detectable influence on soil chemical properties during the feedback
385 period (Supporting Information Table S3). In contrast, soil chemical properties were strongly
386 influenced by the identity of growing plant species, although the effect depended on the
387 conditioning species. First, soil concentrations of ammonium and nitrate were higher when *D.*
388 *glomerata* grew in monoculture in conspecific soil than in all other treatments (Sampling S1).
389 Second, between sampling S1 and S2, the growth of *D. glomerata* in monoculture and in
390 heterospecific soil increased soil concentrations of nitrate, while the growth of both plants in
391 mixture decreased soil nitrate (Supporting Information Fig. S2). Thus, *D. glomerata*
392 increased, and *L. hispidus* decreased, soil nitrate concentrations.
393

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 drying period (0.09 ± 0.02 g g^{-1} DW) and after the rewetting period (0.39 ± 0.03 g g^{-1} DW) (Supporting Information 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 (Supporting Information Fig. S4). Detected increases in leaf biomass over the five-week recovery period following drought were also proportional to leaf biomass at the end of drying period. As a consequence, the competitiveness values after the 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 droughted microcosms (Adonis *P=0.034* and *P=0.001, respectively;* Supporting Information Table S2). The intensity of changes in bacterial and fungal communities was assessed by calculating the similarity of their composition (with horn index) for each treatment between control and second-droughted microcosms at sampling S1 (Fig. 4a,b). No significant previous drought effect was observed on horn similarity index (Fig. 4 a,b), therefore the intensity of the change in bacterial and fungal community composition in response to the second drought was similar in previously droughted and non-droughted soils, i.e. irrespective to previous drought history. In contrast, the previous drought did have 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 new drought (Fig. 4e) were significantly lower when soils had been subject to previous drought (Fig. 4 and Supporting Information Table S4), except for CO_2 respiration from soils conditioned with *L. hispidus* when plants grew in competition.

The plant species present previously or during the second drought influenced effects of the second drought on soil properties, although effects varied for different soil properties (Fig. 4). For instance, for plants in monoculture, bacterial community composition changed more when plants grew in conspecific than in heterospecific soils (Fig. 4a, *P=0.01),* and this was

428 associated with lower soil respiration (Fig. 4c; *P= 0.008*) and DOC concentration (Fig. 4d,
429 *P=0.047*). The flush of CO_2 (Fig. 4c), DOC (Fig. 4d) and ammonium (Fig. 4e) was also
430 greater when *L. hispidus* was grown in monoculture than with *D. glomerata* (*P=0.023*,
431 *P=0.0006*, and *P=0.045*, respectively). Fungal community composition changed less in
432 response to drought in soils conditioned with *L. hispidus* compared to soils conditioned with
433 *D. glomerata* (Fig. 4b, *P=0.011*). And for plants growing in competition, bacterial
434 community composition changed more in response to drought in soil conditioned with *D.*
435 *glomerata* than with *L. hispidus* (Fig. 4a; *P=0.047*). Altogether, these results showed that the
436 soil response to second drought depended on plant-soil feedback and plant competition
437 effects.
438
439 **Discussion**
440
441 The first aim of this study was to evaluate whether a previous drought affects plant-soil
442 feedback. This was tested using an experiment that involved an initial stage of soil
443 conditioning by plant communities dominated by two plants species, which were then
444 subjected to drought, followed by a feedback stage whereby the two plant species were
445 grown in monoculture in these soils. Plant-soil feedback depends on the balance between
446 positive and negative feedbacks occurring in conspecific and heterospecific soils (van de
447 Voorde *et al.*, 2011). Positive feedback is facilitated by high nutrient availability (nutrient-
448 mediated feedback) and abundance of mutualistic microorganisms (microbial-mediated
449 feedback), while negative feedback is driven by nutrient limitation or an accumulation of
450 pathogens. We found that under non-droughted conditions, *D. glomerata* grew equally well
451 in conspecific and heterospecific soil, suggesting a balance of positive and negative feedback.
452 In contrast, maximal growth of *L. hispidus* occurred in non-droughted conspecific soil,
453 despite this soil having a lower nutrient availability than soil conditioned with *D. glomerata*.
454 This positive feedback was found to be associated with a specific fungal community (Fig.
455 3b), which likely optimised plant nutrient acquisition, possibly via the formation of
456 mycorrhizal associations (Jackson *et al.*, 2008; Smith & Smith, 2011). This mechanism is
457 supported by the knowledge that *L. hispidus* is strongly dependent to mycorrhiza fungi
458 (Tawaraya, 2003), and suggests that plant-soil feedback of *L. hispidus* is microbial-mediated
459 with positive feedback from mutualistic microorganisms.
460
461 We found that drought altered the direction of plant-soil feedback: both plant species

462 displayed negative feedback in soil that had been subject to drought. We do not know the
463 precise mechanism explaining the reduced performance of both plant species in conspecific
464 soil with a history of drought, but it is likely due to drought-induced changes in microbial
465 community composition, rather than changes in nutrient availability. This view is supported
466 by our finding that drought had no detectable legacy effect on soil nutrient availability, but it
467 significantly altered the composition of the microbial community: drought reduced bacterial
468 diversity and the abundance of several T-RFs, as also shown by others (Bérard *et al.*, 2011;
469 Barnard *et al.*, 2013), and changed the composition of the fungal community in soil
470 conditioned by *L. hispidus*, causing a change in dominance of some fungal taxa. This finding
471 is consistent with the knowledge that certain plant species select for different fungal
472 communities during drought (Compant *et al.,* 2010), and demonstrates that drought effects on
473 soil fungal communities vary across plant species, most likely due to differences in
474 rhizodeposition (Preece & Peñuelas, 2016). In addition, our results support the view that
475 long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial
476 community composition (Kulmatiski & Beard, 2011). An alternative explanation for the
477 change in soil microbial community composition is related to drought-induced changes in
478 soil structure: drought is known to promote soil aggregate breakdown and alter soil
479 wettability (Denef *et al.*, 2001), which might create heterogeneous penetration of water
480 through soil and create new ecological niches for microorganisms (Ruamps *et al.*, 2011).
481 Together, these findings indicate that the reduced growth of both plant species in conspecific
482 soil subject to drought might be due to a combined effect of decreased abundance of
483 beneficial soil microbes (Cavagnaro, 2016), and increased abundance of less beneficial
484 microbes, i.e. pathogenic microbes, following drought. Further, these results support our
485 hypothesis that drought impacts the direction and the strength of plant-soil feedback due to a
486 legacy effect on soil microbial communities.
487
488 We also tested whether soil conditioning and drought-driven changes in plant-soil feedback
489 influenced plant-plant interactions. To address this, we compared growth of the two plant
490 species in monoculture and in mixture in the soils with different histories of conditioning and
491 drought. As hypothesised, we found that previous drought influenced plant competitive
492 interactions, but only in soil conditioned by *L. hispidus*: previous drought increased the
493 competitive ability of *L. hispidus* in conspecific soil, while it decreased competitiveness of *D.*
494 *glomerata* in this soil compared to non-droughted soils. This is consistent with studies
495 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-droughted conditions, *L. hispidus* and *D. glomerata* grew equally well in monoculture and mixture, suggesting that competition for nutrients was low and, potentially, that both species could benefit from nutrients provided by their own microbial community. In contrast, in droughted soil, improved growth of *L. hispidus* and reduced growth of *D. glomerata* occurred in mixtures compared to 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 differ in their nutrient use strategies: *D. glomerata* increased soil nitrate concentrations (Supporting Information Fig. S2), which was likely due to a positive influence of this species on rates of nitrification (Bremer *et al.* 2009; Legay *et al*., 2016), whereas *L. hispidus* is known to have a high demand in 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 nitrogen source for *L. hispidus,* but only when its own microbial community became less efficient in nitrate supply. This could be the case when *L. hispidus* grew in conspecific droughted soil, as indicated by its low growth in monoculture.

The above results suggest that drought weakened the strength of plant-microbe interactions for nutrient acquisition of *L. hispidus*; the microbial community associated with *L. hispidus* in droughted soils being less efficient to supply nitrogen to *L. hispidus* than the one associated with *L. hispidus* in non-droughted soils. However, we acknowledge that we are uncertain about the effects of drought on soil nitrogen dynamics given that we did not measure nitrifier abundance or rates of nitrogen mineralisation/immobilisation to confirm that the soil microbial community associated with *L. hispidus* in droughted soil is making less nitrogen available. Nevertheless, our results do indicate that drought has the potential to create shifts in soil nitrogen availability resulting from a change in soil microbial community composition, with consequences for the plant-plant competition. This supports the notion that microbial

control of plant productivity (Hendriks *et al.*, 2013) could evolve with drought. In contrast, the growth of *D. glomerata* in mixture decreased in heterospecific droughted soil, but not in monoculture nor in mixture in its conspecific soil. Therefore, *D. glomerata* had a lower growth only when *L. hispidus* was present with its conspecific droughted 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 due to 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, likely because of a desynchronization of the plant-microbial partnership related to nutrient acquisition. So species-specific responses suggest that drought could be a particular threat to plant species with a high dependence of 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 plants ability to withstand to 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 carbon and nitrogen following the second drought (Birch, 1958) was less in soils that had previously been subjected to drought than in soils that hadn't. The hypothesized mechanisms explaining the Birch effect generally involves physical and biotic effects: rewetting can cause aggregate slaking, which releases

564 previously protected soil carbon (Denef *et al.*, 2001) and microbial carbon following cell
565 death, or microbial mechanisms of tolerance (accumulation of osmolytes during drought;
566 Schimel *et al.,* 2007). With consecutive droughts, it is also possible that the physical
567 disruption releases less C from a reduced quantity of easily disruptable aggregates; however,
568 opposite responses have also been shown (Miller *et al.,* 2005). The second explanation might
569 be due to the adaptation to drought of microbial communities involved in the carbon and
570 nitrogen cycles. We expected that previous drought would prevent large changes in microbial
571 community composition during a subsequent drought due to the selection of microbial taxa
572 able to tolerate the perturbation (Wallenstein & Hall, 2012; Bouskill *et al*., 2013; Hawkes &
573 Keitt, 2015). In contrast, we found that changes in microbial community composition in
574 response to the second drought were of the same magnitude irrespective of their drought
575 history, as also observed by Fuchslueger *et al.* (2016). However, it is possible that only a
576 small proportion of active microorganisms can adapt to drought, and that the resuscitation of
577 rare taxa after a drought event has a disproportionate influence on soil functioning (Aanderud
578 *et al.*, 2015). Other adaptive mechanisms for coping with repeated drought could involve
579 'anticipatory regulation', an evolutionary processes known to occur within species of
580 microorganisms in adapting to fluctuating environmental conditions (Mitchell *et al.,* 2009)
581 Therefore, biotic legacy of drought could alter expected microbial function responses to
582 drought (Hawkes & Keitt, 2015) with consequence for carbon and nitrogen turnover in the
583 context of recurrent drought (Fuchslueger *et al.,* 2016).
584
585 Despite weak effects of plant species on soil microbial communities in the field conditioning
586 and subsequent laboratory conditioning phase, we did detect significant plant species effects
587 (past and present) on soil microbial community composition and functioning following the
588 subsequent drought. This finding indicates that plants influence the response of soil microbial
589 communities to drought, likely through root exudation (Fuchslueger *et al.*, 2014), which is
590 consistent with previous studies showing species-specific drought-induced changes in
591 rhizodeposition and soil microbial communities (Preece & Peñuelas, 2016). Our results also
592 suggest that the drought-induced changes in rhizodeposition are dependent on plant-soil
593 feedback. Collectively, our study supports our hypothesis that drought impacts on soil
594 microbial communities have consequences for soil functioning during a subsequent drought,
595 and that these effects depend on plant-soil feedbacks and impact plant responses to drought.
596
597 In conclusion, our results indicate that drought can alter the direction of plant-soil feedback

598 due to long-lasting effects on soil microbial communities and that this has consequences for
599 plant-plant interactions and plant responses to subsequent drought. Moreover, we provide
600 evidence that legacy effects of drought on soil microbial communities alter their functional
601 capabilities when faced with subsequent drought, which supports the notion that biotic legacy
602 of drought cause divergence from expected functional responses to drought (Hawkes & Keitt,
603 2015). These findings are of importance given predicted increase in frequency and intensity
604 of drought events, and the demonstrated potential for drought history to shape microbial-
605 mediated plant-soil feedbacks with consequences for plant community dynamics and
606 ecosystem functioning, and future plant and microbial responses to drought.
607

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612

613 ## Author contributions

614 R.D.B initiated and gained funding for the study, which was planned and designed by A.K.,
615 F.T.D. and R.D.B. A.K. and F.T.D. performed experiments, and A.K. analysed the resulting
616 data. A.K., F.T.D., R.I.G. and R.D.B. wrote the manuscript.
617

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862 **LEGENDS FIGURES AND TABLE**

863

864 **Fig. 1: Experimental framework** to study the influence of drought on plant-soil interactions

865

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

874

875 **Fig. 3: Cluster of bacterial (a) and fungal (b) community based on terminal restriction**
876 **fragments (T-RFs) relative abundance during the feedback experiment (phase1,**
877 **sampling S1).** Heatmaps were based on the hierarchical clustering solution (horn similarity)
878 distance metric. Rows represent the mean (n=4) of the twelve treatments: *D. glomerata* (*D.g*)
879 and *L. hispidus (L.h)* grown in monoculture, and in mixture (Mix) in the four soil types that
880 are soils conditioned by *D. glomerata* (light green square) or *L. hispidus* (dark green square),
881 each with a droughted (dashed) and non-droughted (without dashed) treatment. Columns
882 represent the selected T-RFs that significantly varied with at least one treatment (ANOVA *P*
883 *<0.05*; drought conditioning, plant conditioning, growing plants species or their interactions).
884 The colors in the heatmaps represent the relative abundance of each T-RFs, as indicated in
885 the upper left corner of each panel.

886

887 **Fig. 4: Influence of subsequent drought on soil properties (phase 2, sampling S1).** The
888 influence of subsequent drought was determined at the end of drying period for soil bacterial
889 and fungal community in measuring the similarity of the community composition between
890 control and droughted microcosms, for dissolved organic carbon (DOC) and ammonium
891 available in soils and soil respiration was measured two hours after the rewetting of dried
892 soils. The plots represent the measures in soils without previous drought against the one in
893 soils with previous drought for soils previously conditioned with *D. glomerata* (Soil D, grey)
894 and *L. hispidus* (Soil L, black) and planted with *D. glomerata* in monoculture, *L. hispidus* in
895 monoculture and the both in mixture. Data are means ± sd (n=4).

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

902

903 *New Phytologist* Supporting Information

904 Article title: Legacy effect of drought on plant-soil feedbacks and plant-plant interactions
905 Authors: Aurore Kaisermann, Franciska T. de Vries, Robert I. Griffiths, Richard D. Bardgett
906 Article acceptance date: 17 April 2017
907

908 The following Supporting Information is available for this article:

909 **Table S1** Soil properties at the end of condition period (Phase 1, Sampling S0) – Range of
910 values and statistical analysis

911 **Fig. S1** Soil properties at the end of condition period (Phase 1, Sampling S0) – Soil water
912 content, nitrate and microbial community composition

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

914 **Table S3** Effect of previous drought, previous plant and growing plant species on soil
915 properties during the feedback experiment (Phase 1) – Table of ANOVA

916 **Fig. S2** Effect of previous drought, previous plant and growing plant species on soil
917 properties during the feedback experiment (Phase 1) – Ammonium and nitrate contents

918 **Fig. S3** Effect of subsequent drought on leaf biomass (Phase2)

919 **Table S4** Effect of subsequent drought on soil properties

920 **Fig. S4** Soil moisture in microcosms

PHASE 1: PLANT-SOIL FEEDBACK

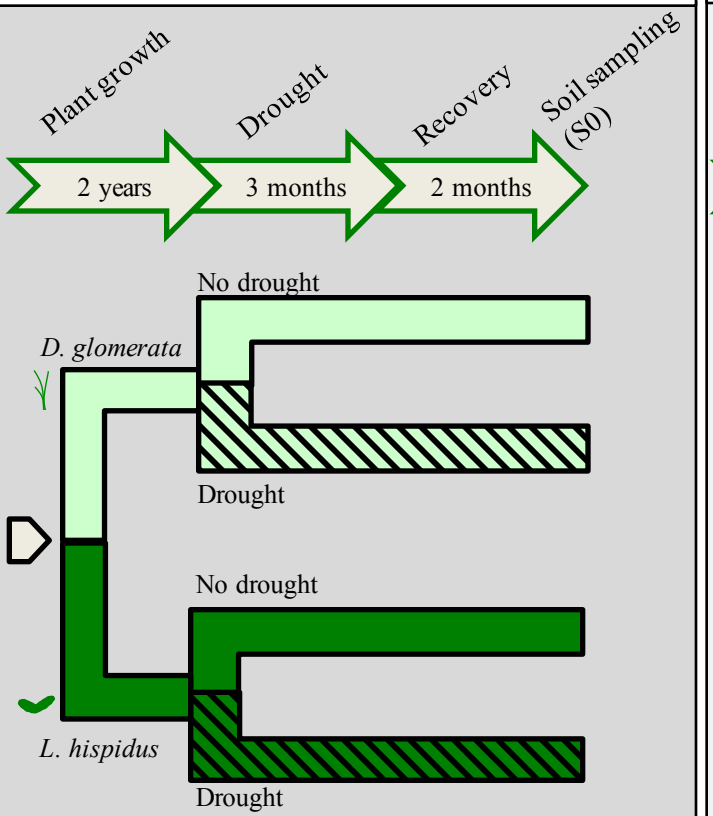
PHASE 2: SUBSEQUENT DROUGHT

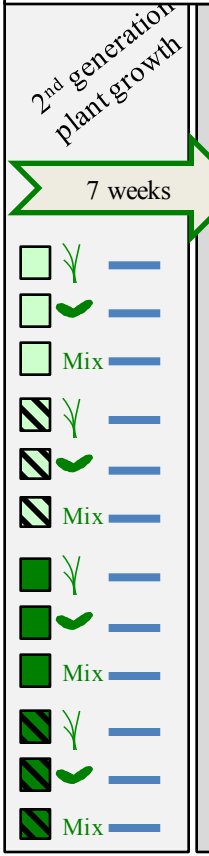
Soil conditioning stage

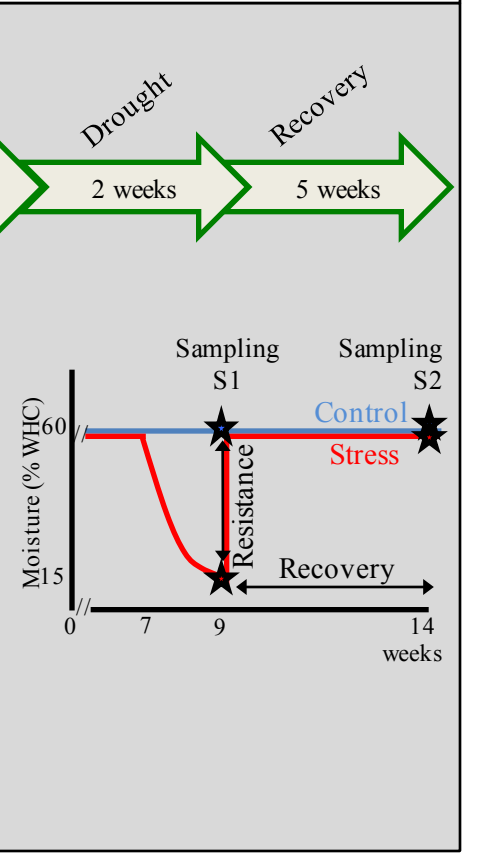
Feedback stage

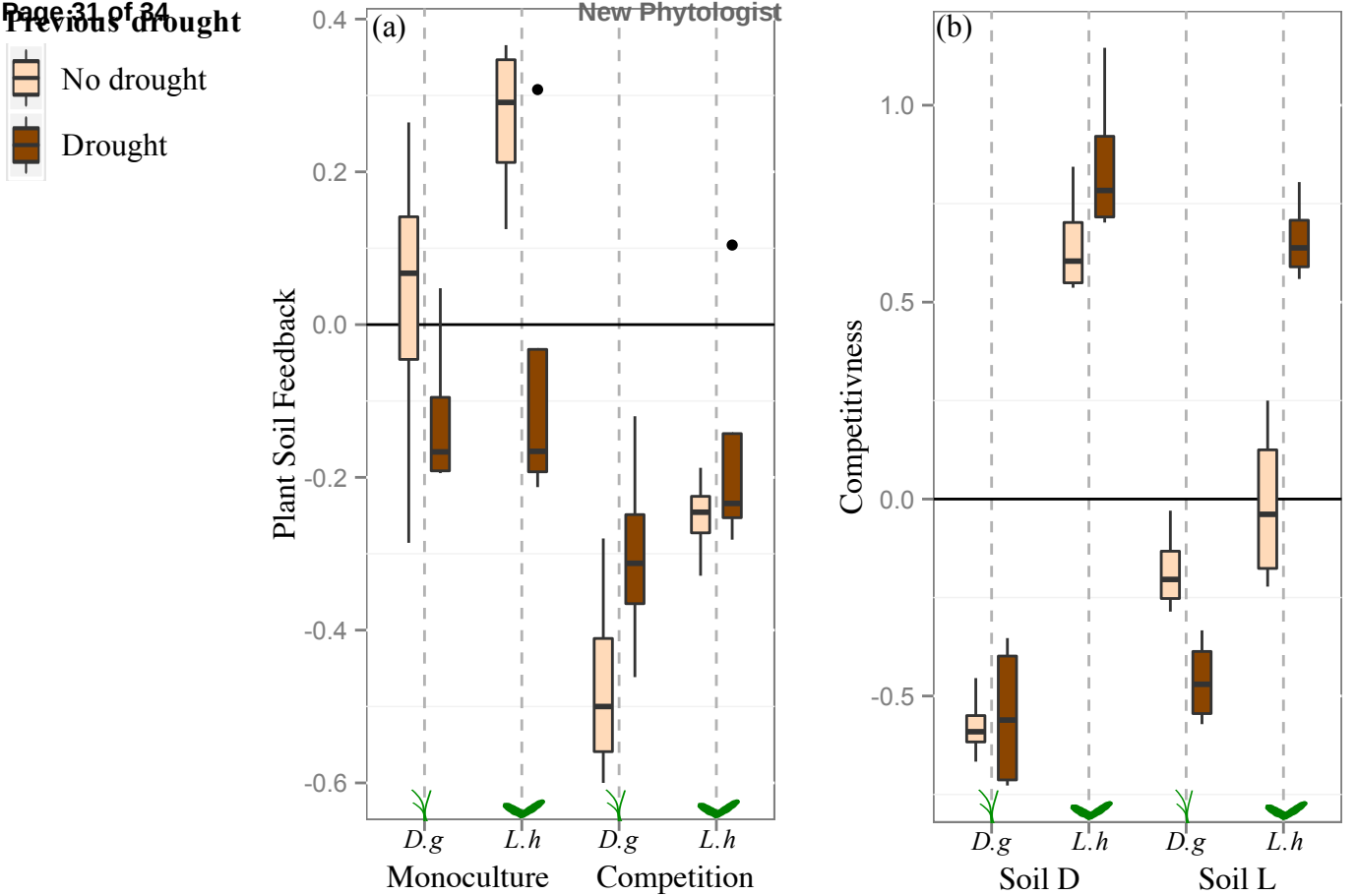
FIELD EXPERIMENT

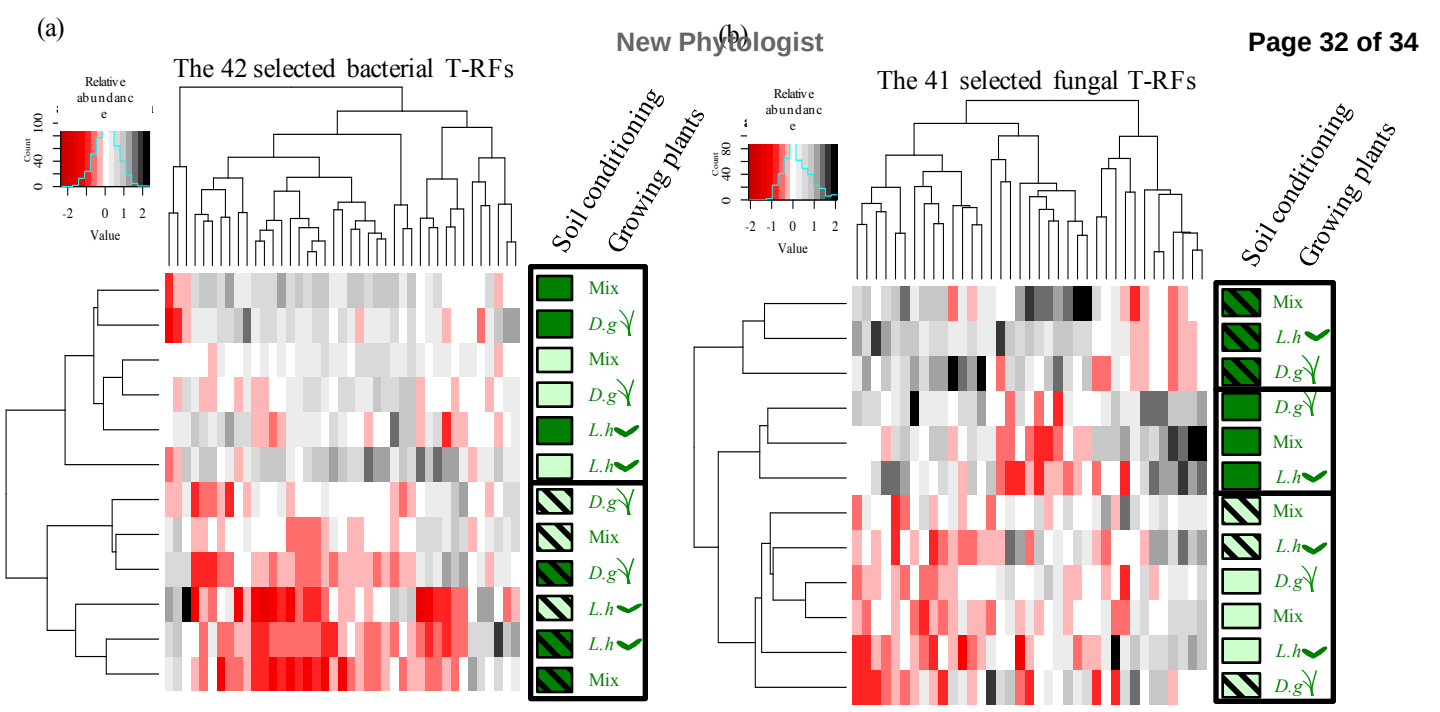
GREENHOUSE EXPERIMENT

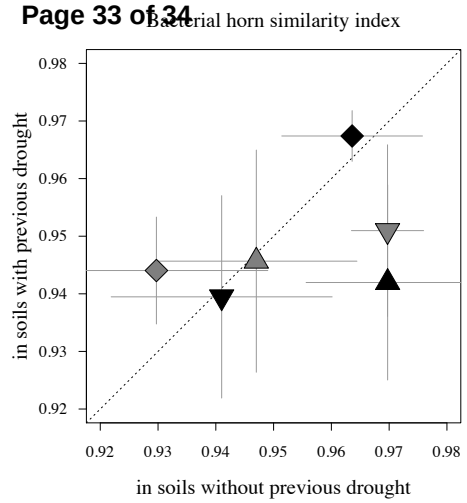


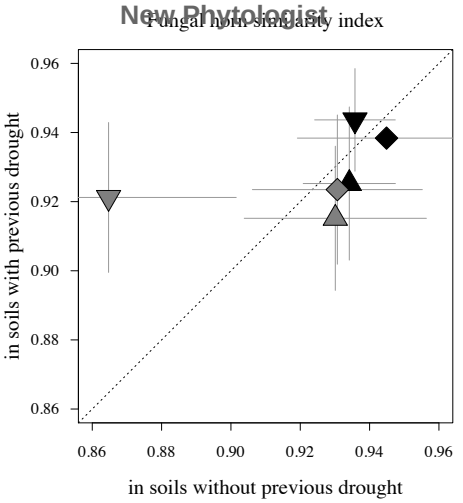


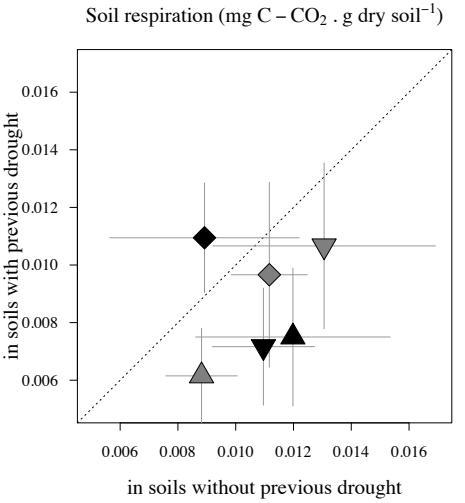


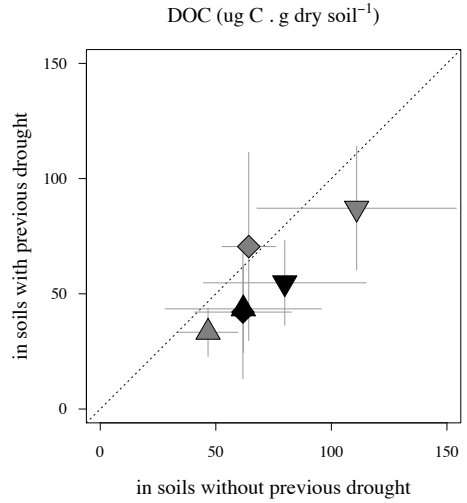

Previous drought
No drought
Drought
(a)
Plant Soil Feedback
0.4
0.2
0.0
-0.2
-0.4
-0.6
D.g
L.h
D.g
L.h
Monoculture
Competition
(b)
Competitivness
1.0
0.5
0.0
-0.5
D.g
L.h
D.g
L.h
Soil D
Soil L

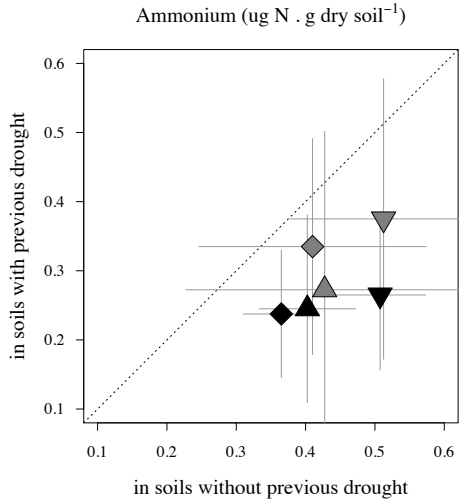

(a)
The 42 selected bacterial T-RFs
Relative abundance
Count
Value
Soil conditioning
Growing plants
Mix
D.g
Mix
D.g
L.h
L.h
D.g
Mix
D.g
L.h
L.h
Mix
(b)
The 41 selected fungal T-RFs
Relative abundance
Count
Value
Soil conditioning
Growing plants
Mix
L.h
D.g
D.g
Mix
L.h
Mix
L.h
D.g
Mix
L.h
D.g

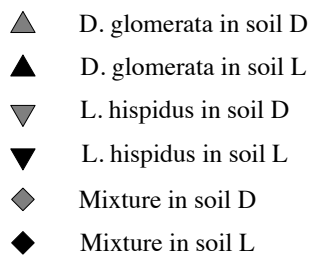












(a)	Plant-soil Feedback				Competitiveness		
	F-value	p-value			F-value	p-value	
Previous drought (A)	0.91	0.35		Previous drought (A)	11.06	0.003	**
Growing species (B)	8.43	0.01	***	Growing species (B)	436.60	<.0001	**
Community (C)	32.93	<.0001	***	Previous plant (C)	3.88	0.06	
A:B	1.28	0.27		A:B	36.62	<.0001	***
A:C	10.48	0.00	***	A:C	0.73	0.40	
B:C	0.06	0.80		B:C	50.92	<.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 monoculure, non drought *vs.* previous drought	-2.66	0.04	*	*D. glomerata* in soil D, non drought *vs.* previous drought	0.27	1.00	
In competititon, non drought *vs.* previous drought	1.45	0.47		*D. glomerata* in soil L, non drought *vs.* previous drought	-2.99	0.06	.
				L. hipidus in soil D, non drought *vs.* previous drought	2.20	0.35	
				L. hispidu s in soil L, non drought *vs.* previous drought	7.17	< 0.001	***

(b)	Plant-soil Feedback				Competitiveness		
	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	<.0001	***	Growing species (B)	260.76	<.0001	**
Community (C)	79.25	<.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	<.0001	***
A:C	6.90	0.01	*	A:C	1.96	0.17	
B:C	0.12	0.73		B:C	31.55	<.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 non drought *vs.* previous drought	0.51	1.00		*D. glomerata* in soil D, non drought *vs.* previous drought	-0.73	1.00	
D. glomerata in competition non drought *vs.* previous drought	0.99	0.98		*D. glomerata* in soil L, non drought *vs.* previous drought	-1.88	0.56	
L. hipidus in monoculture non drought *vs.* previous drought	-4.74	< 0.001	***	*L. hipidus* in soil D, non drought *vs.* previous drought	1.46	0.83	
L. hispidu s in competition, non drought *vs.* previous drought	0.04	1.00		*L. hispidu* s in soil L, non drought *vs.* previous drought	5.48	<0.001	***