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1 **Legacy effects of drought on plant-soil feedbacks and plant-plant interactions**

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30

31

32 **Summary**

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

50

51 **Running head:** Drought changes plant-soil feedbacks

52

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

55

56 **Introduction**

57

58 Ecologists have long sought to understanding how plant communities assemble and respond
59 to environmental change. The importance of plant-plant interactions for community dynamics
60 is well documented (Connell, 1983; Schoener, 1983; Hunter & Aarssen, 1988; Callaway,
61 1995), but evidence is growing that plant-soil feedbacks also influence various plant
62 community attributes, including plant species coexistence, invasion, and rarity (van der
63 Putten *et al.*, 2013). Plant-soil feedback describes the relative growth of a plant in its own
64 conspecific soil, compared to heterospecific soil conditioned by other plant species (Bever *et al.*,
65 1997; Ehrenfeld *et al.*, 2005), and is thought to arise through biotic changes in specific
66 plant associated microbial communities, but also through abiotic changes such as soil
67 chemical modification (e.g. nutrient depletion). As such, plant responses to plant-soil
68 feedback can be negative, mostly via the promotion of pathogens or reductions in nutrient
69 availability, or positive through promoting symbionts and/or soil nutrient availability (Bever
70 *et al.*, 1997; Klironomos 2002; Bever, 2003; van der Putten *et al.*, 2013). There is also
71 evidence that plant-soil feedbacks can mediate plant-plant interactions (van der Putten *et al.*,
72 2013; Baxendale *et al.*, 2014); for instance when two species compete in soil conditioned by
73 one species, the feedback effect of that one plant species can influence the performance of
74 itself (intraspecific feedback) or the competing species (interspecific feedback) (Jing *et al.*,
75 2015). By influencing plant-plant interactions in such as way, plant-soil feedbacks can have
76 consequences for the outcome of plant competition (van der Putten & Peters, 1997).

77

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

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

109

110 The aim of this study was to investigate how drought modifies plant-soil feedback, plant-
111 plant interactions, and their responses to a subsequent drought. Specifically, we tested three
112 hypotheses: first, we hypothesized that drought influences the strength and direction of plant-
113 soil feedback due to its impact on the composition of the soil microbial community; second,
114 we hypothesized that drought-driven changes in plant-soil feedback have consequences for
115 plant competitive interactions (through intraspecific and interspecific feedbacks); and third,
116 we hypothesized that the response of plants to subsequent drought events depends on the
117 legacy effect of previous drought on plant-soil interactions. We tested these hypotheses using
118 a two-phase, pairwise plant-soil feedback experiment with two co-existing, widely distributed
119 temperate grassland plant species: *Dactylis glomerata* and *Leontodon hispidus*. The first
120 phase of the experiment was designed as a classic plant-soil feedback experiment, which
121 involved conditioning of soil by plant communities dominated by either *D. glomerata* or *L.*
122 *hispidus* with or without drought, and then a second generation of each plant species was
123 grown in monoculture (hypothesis 1) or in competition (hypothesis 2) in conditioned soils.

124 During the second phase of experiment, the second plant generation was exposed to a new
125 drought. The resistance and recovery of plant and microbial communities to this drought
126 were measured to assess whether a soil biotic legacy of a previous drought influences plant-
127 soil feedback and plant competition during a subsequent drought.

128

129 **Materials and methods**

130

131 EXPERIMENTAL SETUP

132

133 **Soil and plants**

134 Two common grassland plant species were used in this experiment, namely *Dactylis*
135 *glomerata* L. and *Leontodon hispidus* L. These two species were selected because they
136 naturally co-exist and are widely distributed across European grasslands, but have contrasting
137 life history characteristics: *L. hispidus* is a slow-growing forb with a tap root system that
138 helps to sustain water supply in dry habitats, and which performs well in nutrient poor
139 situations; whereas *D. glomerata* is an exploitative, fast-growing grass with a high maximal
140 relative growth rate due to its ability to efficiently capture resource (Poorter & Remkes, 1990;
141 Ryser & Lambers, 1995). Seeds of *D. glomerata* and *L. hispidus* were obtained from a seed
142 company (Emorsgate Seeds, Norfolk, UK) and the 20 first cm of a local soil for the
143 experiment was collected from a permanent grassland at Hazelrigg Field Station, Lancaster
144 University, UK (54°1'N, 2°46'W, 94 m a.s.l), where the conditioning phase of the
145 experiment was done in field-based mesocosms (Fig. 1). The soil was a silt loam (Brickfield
146 2 association; Avis & Harrop, 1983) of pH 6.2, and had a C and N content of 3.13 and 0.25 g
147 kg⁻¹ respectively. Soil was homogenised manually and large stones and roots were removed
148 prior to planting.

149

150 **PHASE 1: Plant-soil feedback phase**

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

155

156 **Conditioning stage.** The soil was conditioned in field mesocosms by mixed plant
157 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

192 between 14.8 and 22.8 °C with an average of 18.5 °C. Moisture contents were monitored
193 gravimetrically throughout the incubation and were maintained at 60% water holding
194 capacity (WHC) by adding tap water. Microcosms were destructively sampled nine weeks
195 after the beginning of feedback period (Sampling S1).

196

197 **PHASE 2: Effects of subsequent drought on plant-soil feedback and plant-plant**
198 **interaction**

199 The goal here was to assess how a biotic legacy of a previous drought influences the
200 ecosystem response to subsequent drought and rewetting event (hypothesis 3). For this
201 purpose, all microcosms of phase 1 of the plant-soil feedback experiment were duplicated.
202 From the seventh week, duplicated microcosms were subjected to a drought for 2 weeks by
203 stopping watering until the soil water content reached on average 0.09 g g⁻¹ DW and up to
204 85% of plant leaves were senescent. After two weeks of drought, microcosms were rewetted
205 by adding 85 g of water to bring soil moisture back to about 60% WHC while simulating a
206 rainfall event of identical intensity (equal to 14 mm), and the recovery was followed for 5
207 weeks (Fig. 1). Droughted microcosms were destructively sampled at the end of the drought
208 period (Sampling S1) and 5 weeks after rewetting (Sampling S2). Microcosms of phase 1
209 (kept at constant moisture) were sampled at the same days and were used as control for phase
210 2 of the experiment. In total, this resulted in 192 soil microcosms comprising twelve
211 treatments (cf. feedback stage above), each replicated in four blocks of the field experiment,
212 incubated with or without subsequent drought, and destructively sampled at two dates. At
213 each of the two sampling dates, plants were removed from soil and roots were washed prior
214 to subsequent biomass quantification.

215

216 PLANT AND SOIL ANALYSES

217

218 Total leaf and root biomass was measured across all treatments as the dry weight after oven-
219 drying for 48h at 70 °C. In addition, to estimate plant resistance to subsequent drought (phase
220 2), the biomass of detached leaves at the end of the drying period (Sampling S1) was weighed
221 in order to calculate leaf biomass before the drying period. For all sampling times (S0, S1,
222 S2) and treatments, total genomic DNA was extracted from 0.35 g equivalent dry soil using
223 PowerSoil kit (MoBio, Carlsbad, CA). The composition of bacterial and fungal communities
224 was assessed by T-RFLP analysis, as detailed by Griffiths *et al.* (2011) and Plassart *et al.*
225 (2012). For bacteria, 16S DNA were PCR-amplified using the couple of primers 63F/530R.

226 For fungi, the internal transcribe spacer (ITS) region of DNA was amplified using the primers
227 ITS1/ITS4. Relative abundances of the different microbial units were calculated as the ratio
228 between the fluorescence of each terminal restriction fragment (T-RF) and the total integrated
229 fluorescence of all T-RFs, and bacterial and fungal diversity was estimated using Shannon
230 and evenness indices (Hill *et al.*, 2003).

231 At the end of the conditioning stage (sampling S0) a suite of soil properties were
232 measured. Total C and N was measured using a CN analyser (Elementar Vario El Cube,
233 Germany) after grinding in a ball-mill and using acetanilide for internal calibration, pH was
234 measured using a 1:5 soil-water ratio, and maximum soil water holding capacity was
235 measured as detailed by Haney and Haney (2010). For the three sampling times, we
236 measured water extractable carbon and nitrogen in soil (10 g soil + 70 ml MilliQ water,
237 shaken for 20 min). In these extracts, total dissolved organic carbon (TOC) was measured
238 with a TOC analyser (Shimadzu, Japan) and dissolved inorganic N (NH_4^+ and NO_3^-) was
239 assessed with an Auto Analyser (Seal Analytical, Mequon, USA). Additionally, soil
240 respiration was assessed two hours after rewetting the microcosms: fluxes of CO_2 were
241 measured by placing the microcosms in a dark chamber and measuring the accumulation of
242 CO_2 for two minutes with an IRGA (EGM-4 PP-System).

243

244 STATISTICAL ANALYSES

245

246 **Phase 1: Plant-soil feedback**

247

248 All statistical analyses were performed with R software version 3.1.3 (R Core Team, 2015)
249 and all mixed effect linear models were performed using lme in the nlme package (Pinheiro
250 *et al.*, 2015) with block as a random effect. For phase 1 of the experiment, effects of
251 conditioning treatments on soil properties and microbial diversity (conditioning stage,
252 Sampling S0) were analysed using lme with plant species and drought and their interaction as
253 fixed effects. We assessed T-RFLP data using ordination by nonmetric multidimensional
254 scaling (NMDS) and Adonis tests to determine the dissimilarity of the bacterial and fungal
255 communities at sampling S0. For the feedback stage of phase 1, which was designed to test
256 whether previous drought influenced plant-soil feedback (Hypothesis 1), we calculated
257 feedback responses using total plant biomass (Sampling S1). For plants in monoculture, we
258 calculated the average weight of the two plants in a pot in order to use an equal number of
259 plants for the statistical analyses for monoculture and competition treatments. We calculated

260 the plant-soil feedback in pairwise comparisons for the two sub-groups non-drought and
261 drought conditioning as in Brinkman *et al.* (2010):

262

$$263 \quad PSF_k = (O_k - F_k) / F_k$$

264

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

271

$$272 \quad \text{Competitiveness}_k = (C_k - M_k) / M_k$$

273

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

278

279 To test whether the influence of previous drought on plant-soil feedback and plant
280 competitiveness was related to an altered soil microbial community composition or soil
281 nutrient availability (hypotheses 1 and 2), we assessed the influence of the 12 treatments on
282 concentrations of dissolved organic C and inorganic N during phase 1 (Sampling S1). We
283 constructed lme models with previous drought, previous plant and growing plant species (*D.*
284 *glomerata* in monoculture, *L. hispidus* in monoculture, the two plants in competition), and
285 their interactions as fixed factors. Next we examined the effects of treatments on the
286 microbial community composition with two successive tests. First, an Adonis test was
287 performed on T-RFLP data to evaluate if soil conditioning by plant and drought, and plant
288 species identity influenced soil bacterial and fungal community composition. Then, we
289 selected the T-RFLP fragments (T-RF) that significantly varied with these factors (ANOVA
290 $P < 0.05$). The relative abundance of each of these T-RFs within communities in different
291 treatments were used for generation of cluster plots created by the heatmap2 function of the
292 gplots package in R; the double dendrogram allows to cluster the microbial communities

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

295

296 **Phase 2: Response to subsequent drought**

297

298 We assessed if biotic legacy effects of previous drought modified plant responses to a
299 subsequent drought (hypotheses 3). First, we calculated plant-soil feedback and
300 competitiveness as above for control and droughted microcosms at the end of the experiment
301 (Sampling S2). Then, to test whether an adaptation of microbial community to previous
302 drought prevents changes in drivers of plant-soil feedbacks and plant-plant interaction, the
303 response to a subsequent drought of plant growth, microbial community composition, soil
304 respiration and soil nutrient availability were assessed. At sampling S1, the soil compaction
305 at the end of drying period restricted the harvest of the entire root system; therefore the plant
306 growth response was assessed with leaves biomass only. Plant resistance to drought was
307 assessed as the leaf biomass lost during the drought; plant recovery as the increase in leaf
308 biomass between samplings S1 and S2. Two microbial responses to the subsequent drought
309 were measured: soil respiration two hours after rewetting and the intensity of changes in
310 microbial community composition at the end of the drought (Sampling S1). For this, the
311 similarity of microbial community composition between control and droughted microcosms
312 (horn index in “vegan” R package; Oksanen et al., 2015) was calculated for bacterial and
313 fungal T-RFs (Sampling S1). The smaller the horn similarity index, the more drought
314 changed microbial community composition compared to control. Plant-soil feedback,
315 competitiveness, plant resistance and recovery, horn index, soil respiration, and the
316 concentration of DOC, ammonium and nitrate (Sampling 1) were all analysed with lme with
317 previous drought, previous plant, growing plant species (*D. glomerata* in monoculture, *L.*
318 *hispidus* in monoculture, the two plants in mixture) and ‘subsequent drought effect’ as fixed
319 factors.

320

321 **Results**

322

323 **PHASE 1: Plant-soil feedback phase**

324

325 ***Conditioning stage.***

326 Conditioning of soils with plant communities dominated by the two different plant species
327 had limited effects on soil microbial community composition and physico-chemical
328 properties (Supporting Information Table S1), apart from soil extractable nitrate, which was
329 greater when *D. glomerata* was the dominant plant species, irrespective of the drought
330 treatment. However, the drought treatment, which was imposed after two years of soil
331 conditioning (Sampling S0), significantly changed bacterial and fungal community
332 composition (Adonis tests $P=0.012$ and $P=0.016$, respectively), albeit in different ways:
333 drought increased fungal diversity (increased evenness; $P_{\text{anova}}=0.02$), but decreased bacterial
334 diversity (decreased evenness; $P_{\text{anova}}=0.01$). The drought treatment had no detectable impact
335 on soil physico-chemical properties, except soil water retention capacity, which was higher in
336 drought treatment (Supporting Information Fig. S1).

337

338 ***Feedback stage.***

339 When grown in monoculture and in non-droughted soils, the plant-soil feedback responses of
340 the two plant species differed: the growth of *D. glomerata* did not differ when it was grown
341 in conspecific (i.e. home) or heterospecific (i.e. away) soil, whereas *L. hispidus* grew better in
342 conspecific soil, indicating a positive plant-soil feedback for this species (Fig. 2a and Table
343 1a). However, when grown in soil that had been subjected to drought the direction of plant-
344 soil feedback changed (Table 1a, $P=0.04$): both plant species performed worse in conspecific
345 than heterospecific soil, indicating that a previous drought caused both species to display
346 negative feedback. When grown in competition, both species displayed negative plant-soil
347 feedback in both droughted and non-droughted soils (Table 1a, $P=0.47$).

348

349 Drought had a legacy effect on plant competitive interactions, although effects differed for
350 the two plant species and depended on soil conditioning (Fig. 2b and Table 1a). There was a
351 significant legacy effect of drought on *D. glomerata* and *L. hispidus* competitiveness when
352 soils were conditioned by *L. hispidus* (Soil L; Tukey tests $P=0.06$ and $P<0.001$,
353 respectively), while there was no effect when soils were conditioned by *D. glomerata* (Soil
354 D; Tukey tests $P=1.00$ and $P=0.35$). Competitiveness of *D. glomerata* was slightly negative
355 (-0.2 ± 0.1) when grown in non-droughted soil that had been conditioned by *L. hispidus*,
356 while competitiveness of *L. hispidus* was neutral in this soil (-0.04 ± 0.19). However,
357 competitiveness of *L. hispidus* was positive (0.64 ± 0.09) when grown in conspecific soil that
358 had been subjected to drought, meaning that this species grew better in competition than in
359 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

394 PHASE 2: Response to subsequent drought

395

396 The effectiveness of the second, glasshouse-based drought was similar across all treatments,
397 with soil moisture contents being similar across treatments at the end of drying period ($0.09 \pm$
398 $0.02 \text{ g g}^{-1} \text{ DW}$) and after the rewetting period ($0.39 \pm 0.03 \text{ g g}^{-1} \text{ DW}$) (Supporting
399 Information Fig. S3). This second drought decreased leaf biomass across all treatments
400 ($P < 0.001$), and the response was proportional to leaf biomass before the drying period
401 (Supporting Information Fig. S4). Detected increases in leaf biomass over the five-week
402 recovery period following drought were also proportional to leaf biomass at the end of drying
403 period. As a consequence, the competitiveness values after the drought recovery (Sampling
404 S2) were similar to those observed during the feedback experiment (Table 1a,b) as well as the
405 plant-soil feedbacks of *L. hispidus* (Table 1b; $P < 0.001$). Therefore, our results showed a
406 persistent legacy effect of previous drought on plant-soil feedback, especially for *L. hispidus*,
407 and plant competitive interactions during a subsequent drought.

408

409 At the end of the second drought (Phase 2, Sampling S1), bacterial and fungal community
410 composition differed significantly between control and droughted microcosms (Adonis
411 $P = 0.034$ and $P = 0.001$, respectively; Supporting Information Table S2). The intensity of
412 changes in bacterial and fungal communities was assessed by calculating the similarity of
413 their composition (with horn index) for each treatment between control and second-droughted
414 microcosms at sampling S1 (Fig. 4a,b). No significant previous drought effect was observed
415 on horn similarity index (Fig. 4 a,b), therefore the intensity of the change in bacterial and
416 fungal community composition in response to the second drought was similar in previously
417 droughted and non-droughted soils, i.e. irrespective to previous drought history. In contrast,
418 the previous drought did have a strong legacy effect on soil functioning: CO_2 respiration (Fig.
419 4c) and DOC concentrations (Fig. 4d) after rewetting, and ammonium concentrations at the
420 end of new drought (Fig. 4e) were significantly lower when soils had been subject to
421 previous drought (Fig. 4 and Supporting Information Table S4), except for CO_2 respiration
422 from soils conditioned with *L. hispidus* when plants grew in competition.

423

424 The plant species present previously or during the second drought influenced effects of the
425 second drought on soil properties, although effects varied for different soil properties (Fig. 4).
426 For instance, for plants in monoculture, bacterial community composition changed more
427 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 plant 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 (Tawarayama, 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;

496 Kardol *et al.*, 2007; Baxendale *et al.*, 2014; Jing *et al.*, 2015), but also demonstrates that
497 drought strongly modifies the outcome of plant-soil feedbacks for plant competitive
498 interactions, and responses are species specific.

499

500 We propose that the opposite response of the two plant species to drought is related to their
501 different resource acquisition strategies and nutrient supply to the plants. We found that
502 under non-droughted conditions, *L. hispidus* and *D. glomerata* grew equally well in
503 monoculture and mixture, suggesting that competition for nutrients was low and, potentially,
504 that both species could benefit from nutrients provided by their own microbial community. In
505 contrast, in droughted soil, improved growth of *L. hispidus* and reduced growth of *D.*
506 *glomerata* occurred in mixtures compared to monoculture, despite no detectable effect of
507 mixtures on soil microbial community composition. This suggests that drought changed the
508 outcome of plant-soil feedbacks for plant competitive interactions because of drought-
509 induced changes in nutrient competition and nutrient supply by microbial-mediated
510 mechanisms. Indeed, the two plant species differ in their nutrient use strategies: *D. glomerata*
511 increased soil nitrate concentrations (Supporting Information Fig. S2), which was likely due
512 to a positive influence of this species on rates of nitrification (Bremer *et al.* 2009; Legay *et*
513 *al.*, 2016), whereas *L. hispidus* is known to have a high demand in nitrate, as shown by
514 Onipchenko *et al.* (2001). As such, nitrate provided by the soil microbial community
515 associated with *D. glomerata* could provide a more accessible nitrogen source for *L. hispidus*,
516 but only when its own microbial community became less efficient in nitrate supply. This
517 could be the case when *L. hispidus* grew in conspecific droughted soil, as indicated by its low
518 growth in monoculture.

519

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

530 control of plant productivity (Hendriks *et al.*, 2013) could evolve with drought. In contrast,
531 the growth of *D. glomerata* in mixture decreased in heterospecific droughted soil, but not in
532 monoculture nor in mixture in its conspecific soil. Therefore, *D. glomerata* had a lower
533 growth only when *L. hispidus* was present with its conspecific droughted microbial
534 community: this indicates a negative interspecific feedback of *L. hispidus* on *D. glomerata*.
535 These results support the view that, interspecific plant-soil feedback can influence plant-plant
536 competition (van de Voorde *et al.*, 2011; Jing *et al.*, 2015), which can evolve with drought
537 due to a change in nutrient availability related to biotic change (Meisner *et al.*, 2013).
538 Further, these results support our second hypothesis that drought influences plant competitive
539 interactions depending on plant-soil feedbacks, likely because of a desynchronization of the
540 plant-microbial partnership related to nutrient acquisition. So species-specific responses
541 suggest that drought could be a particular threat to plant species with a high dependence of
542 mycorrhizal fungi.

543

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

559

560 We found that the commonly observed flush of carbon and nitrogen following the second
561 drought (Birch, 1958) was less in soils that had previously been subjected to drought than in
562 soils that hadn't. The hypothesized mechanisms explaining the Birch effect generally
563 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.

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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) and869 (b) Competitiveness of *D. glomerata* (*D.g*) and *L. hispidus* (*L.h*) growing in soil previously870 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 that880 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* in895 monoculture and the both in mixture. Data are means \pm sd (n=4).

896

897 **Table 1: Analysis of variance of mixed linear models for plant performance** (i.e. plant-
898 soil feedback and competitiveness) (a) during the feedback experiment (phase 1, sampling
899 S1), and (b) after the subsequent drought (phase 2, sampling S2). Asterisks indicate a
900 statistically significant effect tested with mixed linear model: *, $P < 0.05$; **, $P < 0.01$; ***, P
901 < 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: **New Phytologist**
PLANT-SOIL FEEDBACK

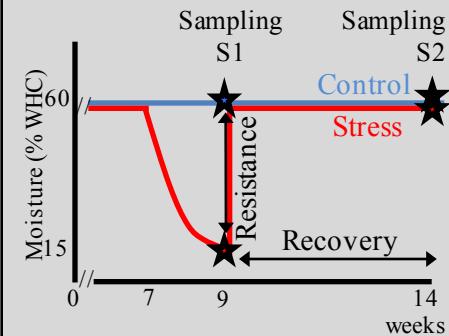
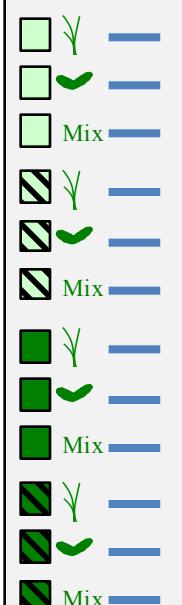
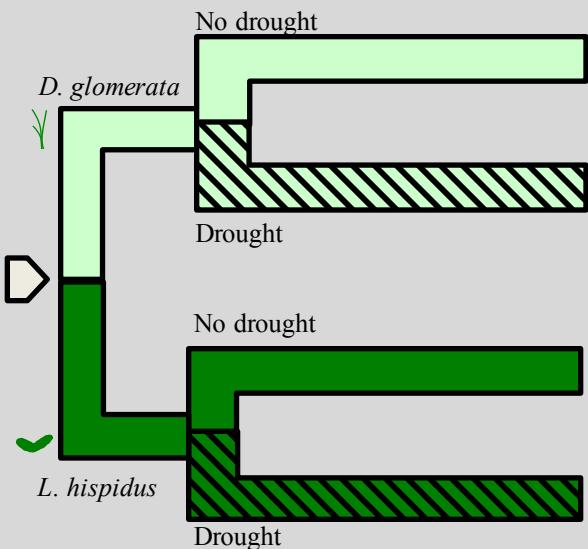
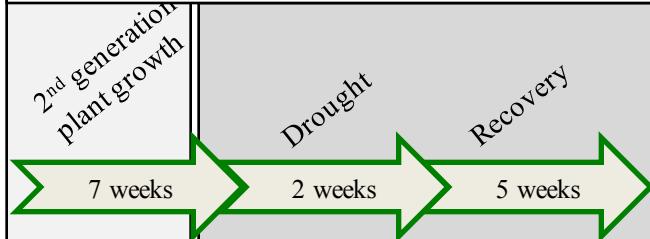
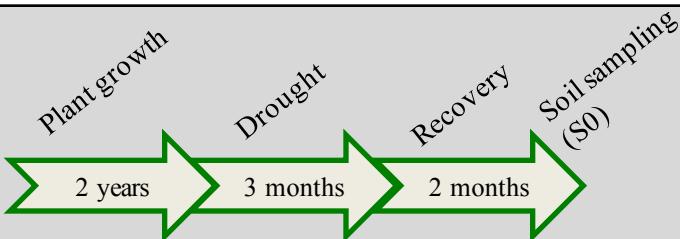
PHASE 2: **Page 30 of 34**
SUBSEQUENT DROUGHT

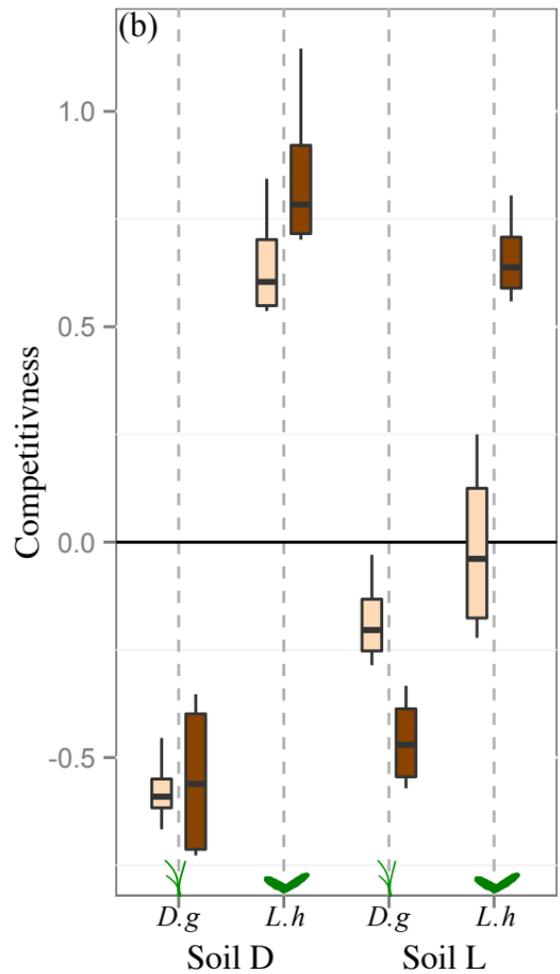
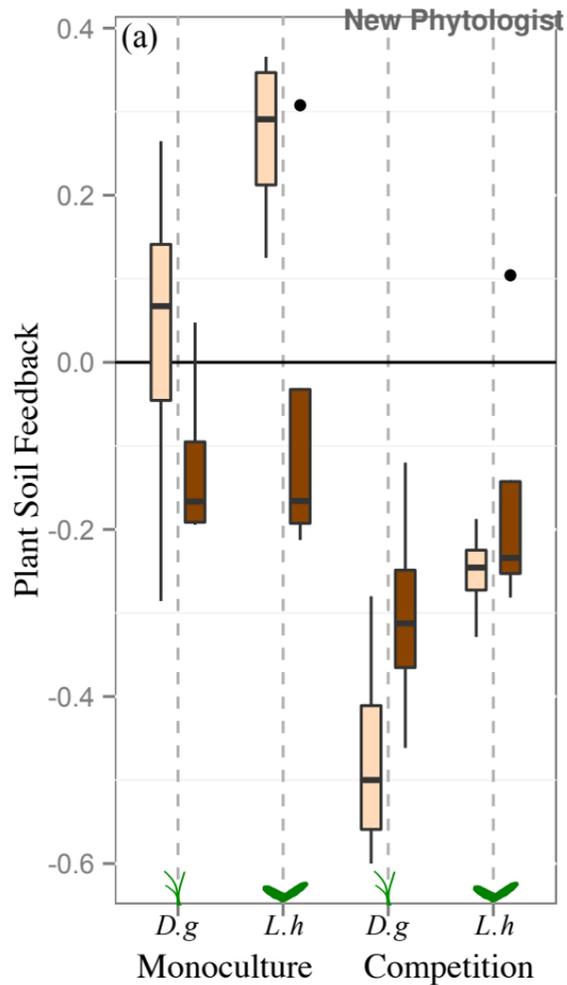
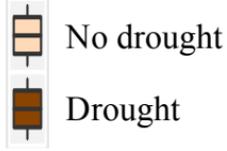
Soil conditioning stage

Feedback stage

FIELD EXPERIMENT

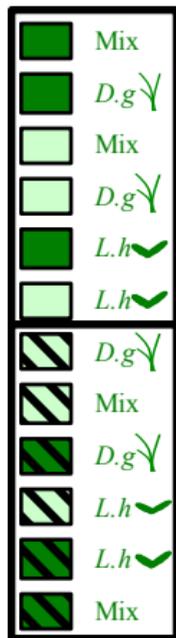
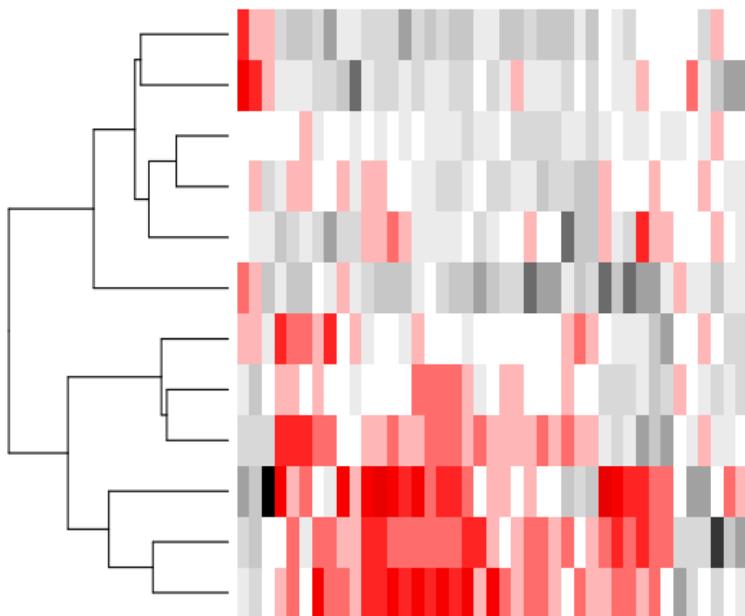
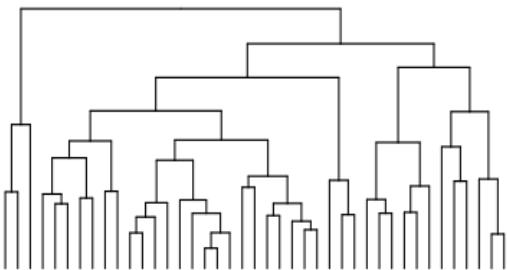
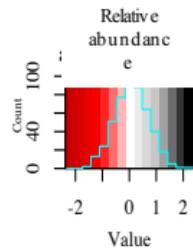
GREENHOUSE EXPERIMENT



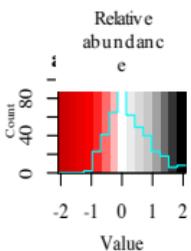


(a)

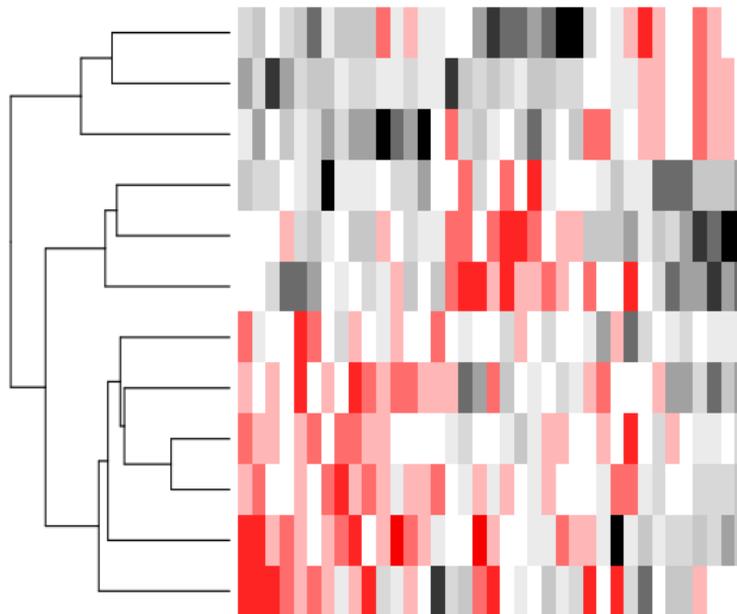
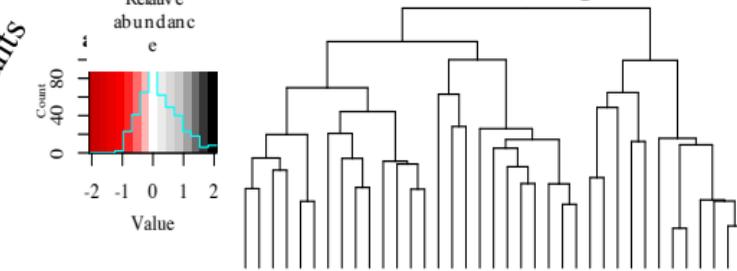
The 42 selected bacterial T-RFs



New Phytologist



The 41 selected fungal T-RFs



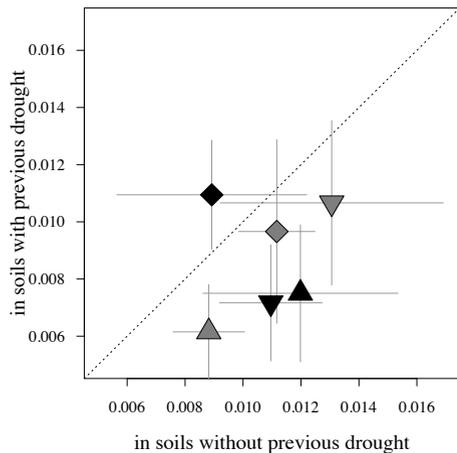
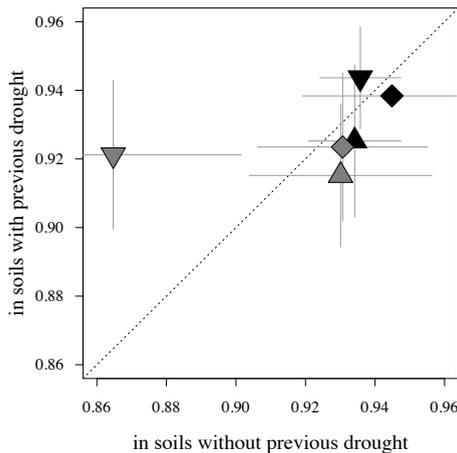
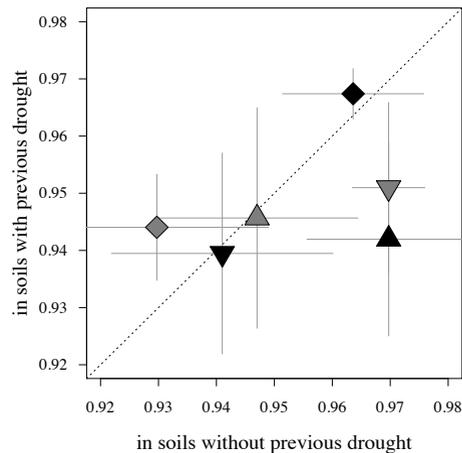
Soil conditioning
Growing plants

Soil conditioning
Growing plants

Bacterial horn similarity index

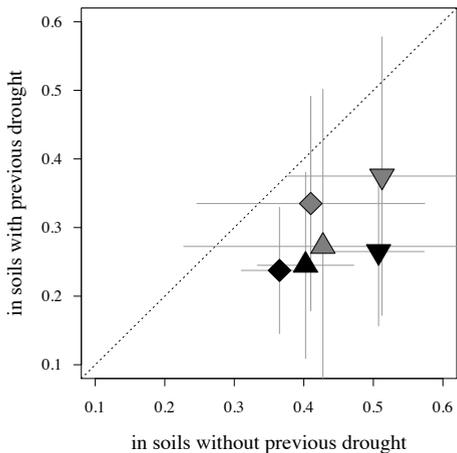
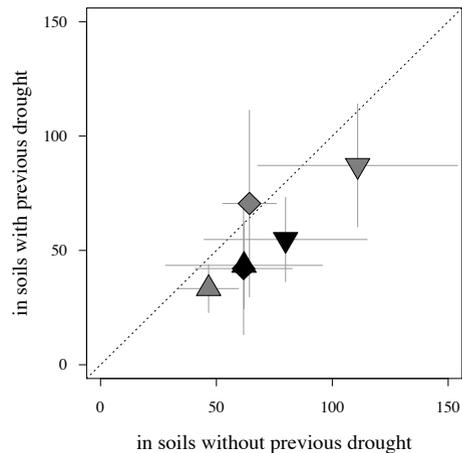
Fungal horn similarity index

Soil respiration (mg C - CO₂ . g dry soil⁻¹)



DOC (ug C . g dry soil⁻¹)

Ammonium (ug N . g dry soil⁻¹)



- ▲ D. glomerata in soil D
- ▲ D. glomerata in soil L
- ▼ L. hispidus in soil D
- ▼ L. hispidus in soil L
- ◆ Mixture in soil D
- ◆ Mixture in soil L

(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 monoculture, non drought vs. previous drought	-2.66	0.04 *	<i>D. glomerata</i> in soil D, non drought vs. previous drought	0.27 1.00
In competition, non drought vs. previous drought	1.45	0.47	<i>D. glomerata</i> in soil L, non drought vs. previous drought	-2.99 0.06
			<i>L. hipidus</i> in soil D, non drought vs. previous drought	2.20 0.35
			<i>L. hispidu s</i> 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
<i>D. glomerata</i> in monoculture non drought vs. previous drought	0.51	1.00	<i>D. glomerata</i> in soil D, non drought vs. previous drought	-0.73 1.00
<i>D. glomerata</i> in competition non drought vs. previous drought	0.99	0.98	<i>D. glomerata</i> in soil L, non drought vs. previous drought	-1.88 0.56
<i>L. hipidus</i> in monoculture non drought vs. previous drought	-4.74	< 0.001 ***	<i>L. hipidus</i> in soil D, non drought vs. previous drought	1.46 0.83
<i>L. hispidu s</i> in competition, non drought vs. previous drought	0.04	1.00	<i>L. hispidu s</i> in soil L, non drought vs. previous drought	5.48 <0.001 ***