

# Grassland invasibility varies with drought effects on soil functioning

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## Grassland invasibility varies with drought effects on soil functioning

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1 Grassland invasibility varies with drought effects on soil functioning

2

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7

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9 disturbance, fluctuating resource hypothesis

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## 10 Summary

- 11 1. Although it is known that ecosystems are more susceptible to invasion when disturbed, our  
12 knowledge of the mechanisms involved remains limited. Recent studies indicate that  
13 disturbance-induced changes in soil nutrient availability could influence community  
14 invasibility, but the importance of this mechanism in the real world is not known.
- 15 2. We tested the hypotheses that: (a) exotic plant species profit from drought effects on soil  
16 functioning more than do natives; and (b) grassland invasibility depends on soil responses to  
17 drought disturbance, which are greater in soils that exhibit a larger nutrient pulse following  
18 drought.
- 19 3. This was tested in a series of grassland sites of contrasting management intensity which we  
20 subjected to an extreme (40-day) drought, after which seeds from four different plant families  
21 of native species and related exotics were added to soils originating from the drought and  
22 control treatments under glasshouse conditions. We also examined the performance of seeded  
23 native species in the field. We expected that intensively managed grasslands with bacterial  
24 dominated soils would exhibit a larger nutrient pulse following drought, and hence a greater  
25 window of opportunity for invasion, than in extensively managed grassland soils with fungal  
26 dominated microbial communities.
- 27 4. Results from the glasshouse experiment indicated that exotic species grew better in soil that  
28 had experienced drought, and had higher survival and growth rates than natives in both  
29 grassland types. Field results showed that drought increased invasibility in intensively  
30 managed grasslands, but had little impact on survival and growth of seeded species on  
31 extensively managed grassland soils. Increased invasibility of intensively managed grassland  
32 soils was associated with a significant soil nitrogen pulse following rewetting, which was not  
33 detected in the extensively managed grasslands.
- 34 5. *Synthesis.* Our results indicate that intensively managed grasslands are more prone to invasion  
35 following drought than are extensively managed grasslands, and that this response is in part  
36 related to differences in microbial community composition which regulate nutrient availability  
37 in soil following disturbance events. Given that extreme climate events are predicted to

38 increase, our findings suggest that invasion of exotic species will increase in ecosystems with  
39 soils that are less resilient to disturbance.

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42 **Introduction**

43

44 The invasion of habitats by non-native species is a global phenomenon with serious consequences for  
45 ecosystems worldwide (Dukes & Mooney 1999). However, we lack fundamental knowledge of the  
46 factors that render some ecosystems more vulnerable to invasion than others (Richardson & Pyšek  
47 2006). Determinants of invasiveness of exotic plant species have been overwhelmingly addressed  
48 from an above-ground perspective (Rejmánek & Richardson 1996; Lonsdale 1999; Kolar & Lodge  
49 2001; Lockwood, Cassey & Blackburn 2005; Stohlgren & Schnase 2006). Recently, there has been a  
50 considerable shift in focus to below-ground determinants of invasiveness (Reinhart *et al.* 2003;  
51 Agrawal *et al.* 2005), with a particular focus on the effect of pathogens (Van der Putten, Van Dijk &  
52 Peters 1993; Reinhart *et al.* 2003) and/or mutualists (Klironomos 2002). However, little is known  
53 about how below-ground determinants of invasion, including soil biota and availability of nutrients,  
54 are related to disturbances, such as those caused by extreme climatic events, which are predicted to  
55 increase in frequency in the future (Murphy *et al.* 2009).

56

57 Disturbance has long been considered a central prerequisite for enabling access to invasive species  
58 into established communities (Hobbs & Huenneke 1992; Parker, Mertens & Schemske 1993;  
59 Rejmánek & Richardson 1996; Davis, Grime & Thompson 2000). However, the mechanisms through  
60 which disturbance acts are not well known. It is well established that the disturbance from extreme  
61 climate events, such as drought, can strongly modify soil conditions, causing significant resource  
62 pulses on rewetting (Birch 1958; Davis, Grime & Thompson 2000; Borken & Matzner 2009). Recent  
63 evidence suggests that resource pulses, which are typically associated with drought disturbances, could  
64 play a key role in increasing the invasibility of ecosystems. For example, Parepa *et al.* (2013) showed  
65 that a highly invasive exotic species increased its cover most when nutrients were added in pulses as  
66 opposed to constant levels. This suggests that soil resource pulses, which commonly result from  
67 climatic disturbance events such as drought, could impact invisibility.

68

69 Evidence is also mounting that the composition of the soil microbial community plays a significant  
70 role in determining the ability of soils to buffer resource pulses following dry-rewetting events  
71 (Gordon, Haygarth & Bardgett 2008; de Vries *et al.* 2012b), suggesting a possible causal link between  
72 the soil microbial community and invasibility. Indeed, the short-lived flush of carbon and nitrogen  
73 following drought-rewetting cycles has been shown to be greater in soil with more bacterial dominated  
74 than fungal dominated communities (Gordon, Haygarth & Bardgett 2008; De Vries *et al.* 2012b), and  
75 it is well established that fungal dominated microbial communities are more effective at immobilising  
76 nitrogen than are bacterial dominated communities (Bardgett *et al.* 2003; de Vries *et al.* 2012a). Hence,  
77 pulse regimes associated with exposure of less resilient bacterial soil microbial communities to a  
78 drought disturbance, in terms of their ability to retain nutrients (Birch 1958; Gordon, Haygarth &  
79 Bardgett 2008; De Vries *et al.* 2012b), could potentially result in increased invasibility. Moreover, the  
80 size of the resulting ‘window of opportunity’ for species to enter a given community may depend on  
81 the composition of the microbial community and its reaction to a disturbance in terms of nutrient  
82 availability and retention in soil (De Vries *et al.* 2012b). Although not tested, differences in the  
83 resilience of soil microbial communities may therefore govern the susceptibility of different  
84 ecosystems to invasion.

85

86 The goal of this study was to test the hypothesis that grassland invasibility depends on the response of  
87 soil functioning to an extreme climatic disturbance, and that invasibility is greater in soils that are less  
88 resilient to drought in terms of their ability to buffer nutrient pulses. This was tested using a series of  
89 paired upland grasslands in the Yorkshire Dales, northern England, subject to long term differences in  
90 management intensity. Previously, it was shown that differences in management cause shifts in the  
91 composition of soil microbial communities that influence the capacity of soil to minimise nutrient loss  
92 following drought (Gordon, Haygarth & Bardgett 2008; de Vries *et al.* 2012a). Specifically, we  
93 expected that invasibility would be greater in intensively managed grasslands with bacterial dominated  
94 microbial communities that are less resilient to drought disturbance, than in extensively managed  
95 grasslands with fungal dominated microbial communities, which have been shown to be more resistant  
96 to drought. As a result, this would lead to a greater resource pulse in intensively managed grasslands

97 following drought than in extensively managed grasslands, and hence a window of opportunity for  
98 invasion. We compared the response of native species and exotic congeners to the drought disturbance  
99 in a glasshouse study, by seeding natives and related exotics in field soils that were taken from both  
100 grassland types. Because exotic species generally have higher growth rates (Dawson *et al.* 2012), we  
101 hypothesized they would exhibit a higher growth after drought disturbance than native congeners. To  
102 test whether results gained from this glasshouse experiment reflected natural invasibility, we  
103 introduced the native, but not the exotic species into the field plots in both grassland types, in plots  
104 that had experienced drought and no drought.

## 106 **Methods**

### 107 *Study site*

108 Twelve experimental sites were located in Ingleborough National Nature Reserve, which is part of the  
109 Yorkshire Dales National Park, northern England (54.18°N, 2.36°E, Figure 1). The climate is  
110 temperate maritime with a mean annual precipitation of approximately 1800 mm (Medina-Roldán,  
111 Paz-Ferreiro & Bardgett 2012) and all sites had a similar underlying geology (Yordale sandstone) and  
112 altitude (300-350m asl). At each of the 12 sites, we selected paired grasslands with similar soil type,  
113 topography, and edaphic characteristics. Each pair consisted of two treatments that had been subject to  
114 historic differences in land use intensity. Grasslands on one side of the wall had not received any  
115 fertilizer applications and were grazed at low stocking densities (1–2 ewes ha<sup>-1</sup>), and hereafter are  
116 referred to as ‘extensively managed grasslands’. Grasslands on the other side of the wall had received  
117 regular applications of fertilizer (NPK) (50-100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and farmyard manure, and were heavily  
118 grazed (10–15 ewes ha<sup>-1</sup>), and hereafter are referred to as ‘intensively managed grasslands’. These  
119 long-term differences in management have led to highly dissimilar vegetation composition (Figure  
120 S1), with intensively managed grasslands being dominated by fast-growing graminoids (*Agrostis*  
121 *cappilaris* – *Festuca ovina* – *Galium saxatile* grassland, National Vegetation Classification U4,  
122 Rodwell 1992), and extensively managed grassland being dominated by slow-growing graminoids and  
123 herbs (*Nardus stricta* – *Galium saxatile* grassland, National Vegetation Classification U5, Rodwell



124 1992). Soils were shallow brown earth (average depth 21 cm) and had a similar pH, and carbon (C)  
125 and nitrogen (N) content (Table 1).

### 126 *Experimental design of field experiment*

127 At each of the twelve sites, three treatments of 1.5m x 1m were established in the paired intensive and  
128 extensive grassland, between the 23<sup>rd</sup> of May and 2<sup>nd</sup> of July: (1) a full drought treatment, using  
129 drought shelters constructed of a 1.5 m corrugated roof placed 25 cm above the vegetation on a  
130 slightly tilted angle (hereafter “*drought*”); (2) a microclimatic control, which was similar to the full  
131 drought treatment, but had 64 2cm holes in the corrugated sheets to allow for rain water to come in,  
132 thus controlling for the microclimatic effect of the shelter (hereafter “*microclimate control*”); and (3) a  
133 control with no shelter (hereafter: “*control*”), amounting to a total of 72 plots. The 40-day drought  
134 period equals the probability of once in a 100-year drought period, according to IPCC projections for  
135 the north-west region of England (Murphy *et al.* 2009). Each set of treatments was protected against  
136 sheep using a 1.2 m high fence (Figure 1). During the drought treatments, soil moisture concentrations  
137 were tracked with a Theta Probe moisture sensor (Delta-T Devices, Cambridge, UK) on a weekly  
138 basis. At the onset of the drought period, soil moisture was similar in both grassland types, but rapidly  
139 decreased after setting up the shelters (Figure S2). At the end of the drought treatment, mean soil  
140 moisture in the drought treatment was below the wilting point, but not in the microclimatic control and  
141 control treatments (Figure 3). The rewetting event consisted of a rainfall peak following the removal  
142 of the shelters and amounted to 70 mm between the 5<sup>th</sup> and the 11<sup>th</sup> of July (Figure S2).

### 143 *Soil nutrient availability and microbial properties*

144 Soil samples were collected before shelters were erected, at the end of the 40 day drought period and  
145 two days after the rewetting event. This was done by collecting five randomly positioned soil cores  
146 (1.5 x 1.5 cm x 5cm depth) from the central 1x1m, which were subsequently pooled to create a  
147 composite sample for the plot. These samples were stored for 1 day at +4 degrees C until analysed for  
148 soil nutrient and microbial properties. We expected soils in the different grassland types to differ in  
149 soil organic matter quality (i.e. C:N ratio), nitrogen availability, and microbial community structure, in  
150 terms of the relative abundance of fungi and bacteria, but we expected no differences in pH and soil

151 moisture content. Soil carbon and nitrogen content was determined using an automated elemental  
152 analysis (vario EL Cube, Elementar Analysesysteme GmbH), whereas dissolved inorganic nitrogen  
153 (DIN: extractable soil ammonium ( $\text{NH}_4^+$ ) and nitrate/nitrite ( $\text{NO}_3^-/\text{NO}_2^-$ )) and dissolved organic  
154 nitrogen (DON) concentrations were determined using 1 M potassium chloride (25ml KCl: 5 g fresh  
155 soil) and analysed colourimetrically using an auto-analyser (Seal AA3, SEAL Analytical, UK).  
156 Microbial biomass C and nitrogen N were measured using the fumigation–extraction technique of  
157 Vance, Brookes & Jenkinson (1987), as described by Harrison, Bol & Bardgett (2007), and microbial  
158 community structure was assessed using phospholipid fatty acid analysis (PLFA), as described by  
159 Bardgett, Hobbs & Frostegård (1996). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0,  
160 cis18:1 $\omega$ 7 and cy19:0 were chosen to represent bacterial PLFAs (Federle *et al.* 1986; Frostegård,  
161 Tunlid & Bååth 1993) and 18:2 $\omega$ 6 and 18:1 $\omega$ 9 were used as an indicator of fungal biomass (De Deyn,  
162 Quirk & Bardgett 2010). The ratio of 18:2 $\omega$ 6 : bacterial PLFAs was taken to represent the ratio of  
163 fungal to-bacterial biomass in soil (Bardgett, Hobbs & Frostegård 1996; Frostegård & Bååth 1996)

#### 164 *Glasshouse experiment*

165 Because the study site was designated as a National Nature Reserve, and because the Wildlife and  
166 Countryside act ([www.legislation.gov.uk/ukpga/1981/69/schedule/9](http://www.legislation.gov.uk/ukpga/1981/69/schedule/9)) prohibits the introduction of the  
167 invasive species in this study, it was not possible to introduce exotic species in the field directly. To  
168 examine the effect of an extreme climate event and grassland type on invisibility, and compare the  
169 effect between natives and exotics, we carried out a glasshouse experiment. For this, we took field  
170 soils from drought and control treatments from both grassland types one day after the 40-day drought  
171 period. At this time, one kg of soil was harvested from the surface soil (0–7.5 cm) of each control and  
172 drought treatment plot in the field. Soils were transported to Manchester, where they were passed  
173 through a 5 mm sieve and stored at 4 °C for 48 hrs. Trays (18x23 cm) were filled with 0.25kg soil  
174 from each of the drought and control treatments and were brought to 60% WHC. Three of the four  
175 native species used in this study were congeners of exotic species (native mentioned first: *Epilobium*  
176 *montanum* and *E. ciliatum*; *Heracleum spondylium* and *H. mantezianum*; *Senecio jacobaea* (syn:  
177 *Jacobaea vulgaris*) and *S. inaequidens*), and one species pair consisted of two confamilial species

178 (*Rumex obtusifolius* and *Fallopia japonica*). All native species used in this study are part of the  
179 vegetation communities of mesotrophic montane grasslands (Rodwell 1992) and occur in the  
180 immediate vicinity of the experimental plots (<500 meters away from each of the plot locations), but  
181 were not present in the experimental plots themselves. All native species used have a fast-growing,  
182 ruderal life history strategy (Ellenberg *et al.* 1992), as do their exotic relatives (Pyšek *et al.* 2009).  
183 Moreover, exotic plant species with a ruderal, fast-growing life history strategy make up the majority  
184 of the exotic invaders in Europe (Pyšek *et al.* 2009), and all four exotic species used here are  
185 commonly found throughout Europe and were introduced in the UK in the last century (Williamson  
186 2002). Each species pair of native and exotic species was grown in a different tray containing five  
187 seeds per species, so the number of trays per site was 24, and the total number of trays was 196. Trays  
188 were randomized in the glasshouse within site. Survival (%) and biomass (g) of all established  
189 seedlings was determined at the end of the experiment (6 September 2014).

#### 190 *Introducing seeds in the field*

191 To examine the effect of drought on invasibility in a realistic field situation, and to compare with the  
192 results from the glasshouse experiment, we added seeds of the same four native plant species into each  
193 of the plots, three days after the rewetting event. In addition, *Urtica dioica* was sown in the field  
194 plots, as it is also an important weed species in the UK (Taylor 2009). To aid establishment, we  
195 created five artificial hoof prints per plot in the central 0.5m x 0.5m, thus mimicking a realistic soil  
196 disturbance by a grazing herbivore (Burke & Grime 1996). A single seed of each native species was  
197 seeded in each hoof print. Seedling survival and height were determined after two months, by  
198 measuring the proportion of surviving seeds and their height.

#### 199 *Data analysis*

200 Initial soil conditions were tested with paired Student's t-tests. Effects of the different drought  
201 treatments on soil moisture content at the end of the drought regime and soil nutrient availability after  
202 rewetting were tested with a general linear model with treatment (3 levels: control, microclimate,  
203 drought) and grassland type (two levels: intensive, extensive) and block effect (12 levels) as a random  
204 effect. Survival of seeded plants in the field experiment was tested with a generalized linear models

205 (GLZ) with a binomial-distributed dependent variable and treatment (two levels: drought and control),  
206 species identity (five levels: 5 species) as fixed effects and block (12 levels: 12 sites) as a random  
207 effect and all two-way interaction effects. Relative survival for field and lab seedlings was calculated  
208 for each species pair as follows: (proportion of seedlings survived in treatment with highest  
209 survival)/(proportion of seedlings survived in treatment with lowest survival) + 1. In case controls had  
210 a higher survival than in the drought treatment, this number was multiplied with + 1 and if the reverse  
211 were true, this number was multiplied with -1. The effect of drought on seedling height was tested  
212 using the same procedure but with a GLMM instead where the natural log of plant height was used as  
213 a normal-distributed dependent variable. Results from the glasshouse experiment were tested using a  
214 similar procedure but with species origin (2 levels: natives and exotics) as one extra level in both the  
215 GLZ (testing the effect on survival) and the GLMM (testing for the effect on biomass). To meet the  
216 assumption of normality of residuals, standardized residuals were tested on normality using a  
217 Kolmogorov–Smirnov test. Post hoc Tukey HSD tests were used to test for the pairwise difference  
218 between treatments. To inspect the relation between seedling growth in the field and nutrient  
219 availability after rewetting, we tested linear regressions between the natural log of plant height and the  
220 concentration of mineral nitrogen after rewetting for each species and each drought treatment  
221 separately. All statistics were carried out using Statistica 9.0 and R version 2.15.1.

222

## 223 **Results**

### 224 Vegetation and soil microbial community composition

225 Consistent with our expectations, across all sites, soils of more intensive grasslands had a significantly  
226 ( $t_{(11)} = 3.1, P = 0.01$ ) lower fungal:bacterial PLFA ratio than soils of extensive grasslands (Table 1;  
227 Figure 2), which was due to a significantly higher bacterial biomass in the intensive grassland plots  
228 ( $t_{(1,11)} = 3.7, P = 0.003$ , Table 1), whereas fungal biomass was not significantly different between  
229 grassland types. Soils in intensively managed grasslands had a higher concentration of mineral  
230 nitrogen (DIN: dissolved inorganic nitrogen =  $\text{NO}_3^- + \text{NH}_4^+$ ) before the drought shelters were erected

231 ( $t_{(11)} = 2.7$ ;  $P = 0.02$ , Table 1) and a higher mineral nitrogen to dissolved organic nitrogen (DIN/DON)  
232 ratio ( $t_{(11)} = 3.22$ ,  $P = 0.008$ ; Table 1).

### 233 Effects of drought in soil properties

234 Across all sites, simulated drought had significant measurable effects on soil moisture levels at the  
235 end of the drought period ( $F_{(2,22)} = 54.3$ ,  $P < 0.001$ ; Figure S2). Soil moisture in the drought treatments  
236 decreased stronger than in control and microclimate controls, both in the intensive (Tukey HSD,  
237  $P < 0.001$ ) and extensive grassland plots (Tukey HSD,  $P < 0.001$ , Figure S2). Soil temperature was not  
238 affected by the drought or microclimate treatment relative to the control (data not shown). Drought  
239 significantly increased DIN ( $\text{NO}_3^- + \text{NH}_4^+$ ) availability in the intensive, but not the extensive  
240 grasslands ( $F_{(2,55)} = 3.6$ ,  $P = 0.03$ ; Figure 2). Further, in the intensive grasslands, there was a significant  
241 correlation between the soil moisture at the end of the drought period and the resulting peak in mineral  
242 N availability ( $R^2 = 0.16$ ,  $P < 0.001$ , Figure 3).

### 243 Glasshouse experiment

244 Drought had a positive effect on seedling biomass in both intensively managed and extensively  
245 managed soils ( $F_{(1,225)} = 14.5$ ,  $P < 0.001$ ; Figure 4). Both exotic and native seedlings growing on soils  
246 from intensively managed grasslands had higher biomass than when grown on soil from extensively  
247 managed grasslands ( $F_{(1,11)} = 17.6$ ,  $P < 0.001$ , Figure 4). Overall, exotic species performed better than  
248 native congeners or confamilials ( $F_{(1,225)} = 52.5$ ;  $P < 0.001$ ; Figure 4). Survival of seedlings from exotic  
249 species was higher than for native seedlings, but only in soils of intensively managed grasslands (Wald  
250  $X^2_{(1)} = 12.27$ ,  $P < 0.001$ , Figure 5). Drought had a positive effect on survival of both native and exotic  
251 seedlings on intensively managed grassland soils (Wald  $X^2_{(1)} = 8.4$ ,  $P = 0.003$ ), but not on extensively  
252 managed grassland soils (Figure 5).

### 253 Field experiment

254 Two months after introduction, seeded species exhibited higher growth in intensively managed  
255 grassland plots than in extensively managed grassland plots ( $F_{(1, 184)} = 41.8$ ,  $P < 0.0001$ ; Figure 4).  
256 *Heracleum sphondylium* exhibited the greatest seedling growth, *Epilobium montanum* showed the

257 lowest growth. For the intensively managed grasslands, species growing in drought treatments  
258 exhibited 30% higher growth than in the control soils ( $F_{(2,83)} = 6.7$ ,  $P=0.002$ ; Figure 4). Survival for  
259 three out of five seeded species showed a similar pattern; survival was significantly greater in drought  
260 plots than in control plots, and one other species showed a similar trend, but this was not the case for  
261 the extensively managed grassland plots (Interaction effect grassland type x drought treatment  $Walt_{(22)}$   
262  $=16.5$ ,  $P < 0.001$ ; Figure 5). *Rumex obtusifolius* was the exception to this pattern, in that it did not  
263 perform better in plots that had experienced drought (Figure 4 and 5). Soil mineral N availability after  
264 rewetting was positively and significantly correlated with seedling growth across both treatments and  
265 for both soil types (Figure 6), with the exception of *R. obtusifolius*.

266

## 267 Discussion

268 Our study reveals a novel mechanism by which extreme climate events influence the invasibility of a  
269 range of exotic and native species in grassland plant communities. The findings of our glasshouse  
270 experiment indicate that, compared to natives, exotic plant species exhibit more vigorous growth,  
271 especially when grown in soils of high nutrient availability from intensively managed grasslands.  
272 Results from our field manipulation experiment, which was carried out across a broad range of  
273 grasslands sites, shows that drought had a strong, positive effect on species invasibility, but that this  
274 effect only occurred when species were seeded into productive, intensively managed grasslands, in  
275 which we observed a significant pulse in soil inorganic nitrogen following rewetting after a period of  
276 drought. In contrast, in extensively managed grasslands, which have fungal dominated soils of lower  
277 nitrogen availability, drought did not cause a significant nitrogen pulse or increase species invasibility,  
278 suggesting that these low productivity grasslands are more resistant to invasion following extreme  
279 climate events. Overall, our results suggest that extreme climate events can increase species grassland  
280 invisibility through creating pulses in soil nutrient availability, but that the strength of this effect varies  
281 across grasslands depending on their management intensity and the resilience of soil microbial  
282 processes of nutrient cycling to disturbance.

283 Results from our glasshouse experiment indicated that, compared to natives, exotic plant species  
284 exhibit more vigorous growth, especially in intensively managed grassland soils of greater nutrient  
285 availability, which is in line with other studies showing that exotics often outperform native species  
286 (Byers 2002; Klironomos 2002; Engelkes *et al.* 2008; Meisner *et al.* 2013). There are several  
287 mechanisms through which exotics may be promoted over native plant species in soils of high nutrient  
288 availability. First, exotics may face a weaker impact from soil pathogens and symbionts than natives  
289 as they lack the co-evolutionary history (Klironomos 2002; Engelkes *et al.* 2008; Meisner *et al.* 2013).  
290 Lower vulnerability to soil pathogens might explain the observed higher performance of exotic plant  
291 species compared to natives. Second, a high growth rate and the ability to rapidly exploit high resource  
292 conditions are widely recognized as fundamental plant strategies and a potential determinant of  
293 invasion success (Richards *et al.* 2006; Dawson *et al.* 2012). If land use intensification continues, as  
294 predicted by Lambin & Meyfroidt (2011), our results suggest that this may disproportionately favour  
295 invasions of exotics over natives.

296 Our data from the field manipulation experiment provide further evidence that invasibility of a broad  
297 range of plant species, covering four different families, differed between grassland types, being greater  
298 in intensively managed than in extensively managed grasslands, especially when a drought disturbance  
299 was applied. We propose that this response is due in part to management-induced differences in soil  
300 microbial community composition between the grassland types, which influence the availability of soil  
301 nitrogen, and hence plant nitrogen availability, following dry-rewetting events. Supporting this idea  
302 we found that soil inorganic nitrogen availability increased markedly after rewetting in soils of  
303 intensively managed grasslands with bacterial dominated communities, but not in soils of extensively  
304 managed grasslands, which have more fungal dominated microbial communities. Also, we found that  
305 seedling growth after rewetting was positively correlated with inorganic nitrogen availability in soil,  
306 suggesting that the pulse in nitrogen availability in soils of intensively managed grasslands following  
307 rewetting was associated with increase in acquisition of nitrogen by seeded plants. These findings are  
308 consistent with previous work done in grasslands showing that fungal dominated microbial  
309 communities are more effective in sequestering nutrients in soil than are bacterial dominated microbial

310 communities (Bardgett, Streeter & Bol 2003; de Vries *et al.* 2012a), and that nutrient pulses and losses  
311 following dry-rewetting cycles are greater in soils with bacterial dominated than fungal dominated  
312 microbial communities (Gordon, Haygarth & Bardgett 2008). Moreover, they are consistent with the  
313 finding that soils with bacterial dominated food webs are less resilient to climate extremes than are  
314 those with fungal dominated foods webs, and hence suffer greater carbon and nitrogen losses in  
315 response (De Vries *et al.* 2012b). Collectively, these findings, along with those from our glasshouse  
316 study, suggest that bacterial dominated soils exhibit a greater window of opportunity to species to  
317 invade, particularly when exposed to extreme drought conditions.

318 Although the majority of invasive species in Europe has been found to share the life-history  
319 characteristics with the species used in the current study (Pyšek *et al.* 2009), it remains unclear  
320 whether the observed patterns shown here also apply to exotic species with different life history  
321 characteristics. Given this, future experiments should consider the response to climate related  
322 disturbances of a broader range of exotic species, with different life history characteristics to those  
323 studied here, including species that are more dependent on mutualisms (e.g. Leguminosae or  
324 Ericaceae). Nevertheless, our study did show that, on the whole, plant species show a consistent  
325 positive response to nutrient pulses resulting climate extremes in soils of intensively managed  
326 grasslands. What is more, as exotics performed far better in the glasshouse than their native congeners,  
327 field results may be conservative estimates of responses of exotic species under the same field  
328 conditions.

329 In conclusion, our study reveals that grasslands differ in their vulnerability to invasion by exotic and  
330 native species following extreme climate events, and that this difference is related to differences in soil  
331 microbial community composition that influence nutrient availability following disturbance events.  
332 Given that the number of extreme climatic events is projected to increase, our data suggest that the  
333 number of successful plant invasions might increase in ecosystems with soils of high nutrient  
334 availability that are also less resilient to disturbance. Moreover, our data suggest that extensively  
335 managed grasslands may have a greater capacity to buffer invasions following extreme climate events,  
336 most likely due to the ability of their fungal dominated microbial communities to effectively retain



337 nutrients following drought (De Vries *et al.* 2015). In order to understand the consequences of changes  
338 in invasibility for the local dominance of these species, future studies need to be conducted over a  
339 much longer timescales in order to assess the long term success of exotic and native species following  
340 disturbance events. Nevertheless our study provides new evidence that invasion into grassland  
341 communities, which is a necessary first step to establishment, differs markedly in grasslands that vary  
342 in their resilience to extreme climate events, and that the response of microbial communities to  
343 drought could play an important role in species invasion.

344

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353 for assisting during the harvest of the glasshouse experiment. The authors declare they have no  
354 conflict of interest regarding this manuscript.

355

## Literature cited

356

- 357 Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. (2005)  
 358 Enemy release? An experiment with congeneric plant pairs and diverse above-and  
 359 belowground enemies. *Ecology*, **86**, 2979-2989.
- 360 Bardgett, R.D., Hobbs, P.J. & Frostegård, Å. (1996) Changes in soil fungal: bacterial biomass ratios  
 361 following reductions in the intensity of management of an upland grassland. *Biology and  
 362 Fertility of Soils*, **22**, 261-264.
- 363 Bardgett, R.D., Streeter, T.C. & Bol, R. (2003) Soil Microbes Compete Effectively With Plants For  
 364 Organic-Nitrogen Inputs To Temperate Grasslands. *Ecology*, **84**, 1277-1287.
- 365 Birch, H. (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Plant  
 366 and Soil*, **10**, 9-31.
- 367 Borken, W. & Matzner, E. (2009) Reappraisal of drying and wetting effects on C and N mineralization  
 368 and fluxes in soils. *Global Change Biology*, **15**, 808-824.
- 369 Burke, M.J. & Grime, J. (1996) An experimental study of plant community invasibility. *Ecology*, **77**,  
 370 776-790.
- 371 Byers, J.E. (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration  
 372 of selection regimes. *Oikos*, **97**, 449-458.
- 373 Davis, M.A., Grime, J.P. & Thompson, K. (2000) Fluctuating resources in plant communities: a  
 374 general theory of invasibility. *Journal of Ecology*, **88**, 528-534.
- 375 Dawson, W., Rohr, R.P., van Kleunen, M. & Fischer, M. (2012) Alien plant species with a wider  
 376 global distribution are better able to capitalize on increased resource availability. *New  
 377 Phytologist*, **194**, 859-867.
- 378 De Deyn, G.B., Quirk, H. & Bardgett, R.D. (2010) Plant species richness, identity and productivity  
 379 differentially influence key groups of microbes in grassland soils of contrasting fertility.  
 380 *Biology Letters*, rsbl20100575.
- 381 De Vries, F.T., Bloem, J., Quirk, H., Stevens, C.J., Bol, R. & Bardgett, R.D. (2012a) Extensive  
 382 management promotes plant and microbial nitrogen retention in temperate grassland. *PloS  
 383 one*, **7**, e51201.
- 384 De Vries, F.T., Bracht Jørgensen, H., Hedlund, K. & Bardgett, R.D. (2015) Disentangling plant and  
 385 soil microbial controls on carbon and nitrogen loss in grassland mesocosms. *Journal of  
 386 Ecology*, **103**, 629-640.
- 387 De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M. & Bardgett,  
 388 R.D. (2012b) Land use alters the resistance and resilience of soil food webs to drought. *Nature  
 389 Climate Change*, **2**, 276-280.
- 390 Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W. & Paulißen, D. (1992) Zeigerwerte von  
 391 pflanzen in Mitteleuropa.
- 392 Engelkes, T., Morriën, E., Verhoeven, K.J., Bezemer, T.M., Biere, A., Harvey, J.A., McIntyre, L.M.,  
 393 Tamis, W.L. & van der Putten, W.H. (2008) Successful range-expanding plants experience  
 394 less above-ground and below-ground enemy impact. *Nature*, **456**, 946-948.
- 395 Federle, T.W., Dobbins, D.C., Thornton-Manning, J.R. & Jones, D.D. (1986) Microbial biomass,  
 396 activity, and community structure in subsurface soils. *Ground Water*, **24**, 365-374.
- 397 Frostegård, Å. & Bååth, E. (1996) The use of phospholipid fatty acid analysis to estimate bacterial and  
 398 fungal biomass in soil. *Biology and Fertility of Soils*, **22**, 59-65.
- 399 Frostegård, Å., Tunlid, A. & Bååth, E. (1993) Phospholipid fatty acid composition, biomass, and  
 400 activity of microbial communities from two soil types experimentally exposed to different  
 401 heavy metals. *Applied and Environmental Microbiology*, **59**, 3605-3617.
- 402 Gordon, H., Haygarth, P.M. & Bardgett, R.D. (2008) Drying and rewetting effects on soil microbial  
 403 community composition and nutrient leaching. *Soil Biology and Biochemistry*, **40**, 302-311.
- 404 Harrison, K.A., Bol, R. & Bardgett, R.D. (2007) Preferences for different nitrogen forms by coexisting  
 405 plant species and soil microbes. *Ecology*, **88**, 989-999.
- 406 Hobbs, R.J. & Huenneke, L.F. (1992) Disturbance, diversity, and invasion: implications for  
 407 conservation. *Conservation biology*, **6**, 324-337.
- 408 Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in  
 409 communities. *Nature*, **417**, 67-70.

- 410 Kolar, C.S. & Lodge, D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in*  
411 *Ecology & Evolution*, **16**, 199-204.
- 412 Lambin, E.F. & Meyfroidt, P. (2011) Global land use change, economic globalization, and the  
413 looming land scarcity. *Proceedings of the National Academy of Sciences*, **108**, 3465-3472.
- 414 Lockwood, J.L., Cassey, P. & Blackburn, T. (2005) The role of propagule pressure in explaining  
415 species invasions. *Trends in Ecology & Evolution*, **20**, 223-228.
- 416 Lonsdale, W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**,  
417 1522-1536.
- 418 Medina-Roldán, E., Paz-Ferreiro, J. & Bardgett, R.D. (2012) Grazing exclusion affects soil and plant  
419 communities, but has no impact on soil carbon storage in an upland grassland. *Agriculture,*  
420 *Ecosystems & Environment*, **149**, 118-123.
- 421 Meisner, A., De Deyn, G.B., de Boer, W. & van der Putten, W.H. (2013) Soil biotic legacy effects of  
422 extreme weather events influence plant invasiveness. *Proceedings of the National Academy of*  
423 *Sciences*, **110**, 9835-9838.
- 424 Murphy, J.M., Sexton, D., Jenkins, G., Booth, B., Brown, C., Clark, R., Collins, M., Harris, G.,  
425 Kendon, E. & Betts, R. (2009) UK climate projections science report: climate change  
426 projections.
- 427 Parepa, M., Fischer, M. & Bossdorf, O. (2013) Environmental variability promotes plant invasion.  
428 *Nature communications*, **4**, 1604.
- 429 Parker, I.M., Mertens, S.K. & Schemske, D.W. (1993) Distribution of seven native and two exotic  
430 plants in a tallgrass prairie in southeastern Wisconsin: the importance of human disturbance.  
431 *American Midland Naturalist*, 43-55.
- 432 Pyšek, P., Lambdon, P.W., Arianoutsou, M., Kühn, I., Pino, J. & Winter, M. (2009) Alien vascular  
433 plants of Europe. *Handbook of alien species in Europe*, pp. 43-61. Springer.
- 434 Reinhart, K.O., Packer, A., Van der Putten, W.H. & Clay, K. (2003) Plant–soil biota interactions and  
435 spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046-  
436 1050.
- 437 Rejmánek, M. & Richardson, D.M. (1996) What attributes make some plant species more invasive?  
438 *Ecology*, **77**, 1655-1661.
- 439 Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006) Jack of all trades,  
440 master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters*, **9**,  
441 981-993.
- 442 Richardson, D.M. & Pyšek, P. (2006) Plant invasions: merging the concepts of species invasiveness  
443 and community invasibility. *Progress in Physical Geography*, **30**, 409-431.
- 444 Rodwell, J. (1992) *British Plant Communities Volume 3. Grasslands and montane communities.*  
445 *Cambridge, UK.* Cambridge University Press.
- 446 Stohlgren, T.J. & Schnase, J.L. (2006) Risk analysis for biological hazards: what we need to know  
447 about invasive species. *Risk analysis*, **26**, 163-173.
- 448 Taylor, K. (2009) Biological Flora of the British Isles: *Urtica dioica* L. *Journal of Ecology*, **97**, 1436-  
449 1458.
- 450 Van der Putten, W., Van Dijk, C. & Peters, B. (1993) Plant-specific soil-borne diseases contribute to  
451 succession in foredune vegetation.
- 452 Vance, E., Brookes, P. & Jenkinson, D. (1987) An extraction method for measuring soil microbial  
453 biomass C. *Soil Biology and Biochemistry*, **19**, 703-707.
- 454 Williamson, M. (2002) Alien plants in the British Isles. *Biological invasions: economic and*  
455 *environmental costs of alien plant, animal, and microbe species*, 91-112.
- 456
- 457
- 458

459 Tables and Figures

460 Captions

461 Table 1. Initial soil abiotic conditions ( $\pm$ standard error) in intensive and extensive grassland plots at  
462 the onset of the drought shelter treatments. %C: percentage total carbon in microbial biomass; %N:  
463 percentage total nitrogen in dry soil; C:N ratio: carbon:nitrogen ratio in soil organic matter; F:B ratio:  
464 ratio of fungal:bacterial biomass; DIN: Dissolved inorganic nitrogen; DON: Dissolved organic  
465 nitrogen; TN: total nitrogen; %C, %N, Bacterial and fungal fatty acid concentrations as well as the  
466 different nitrogen concentrations were determined per gram of dry soil. Significances were calculated  
467 using paired student t tests.

468

469 Table 1.

	% moisture (vol)	%C	%N	OM CN ratio	F:B ratio	Bacterial PLFA (ug g <sup>-1</sup> )	Fungal PLFA(ug g <sup>-1</sup> )	pH	DIN (ug g <sup>-1</sup> )	DON (ug g <sup>-1</sup> )	TN	DON/DIN
Intensive	51.1 ± 1.5	7.9 ± 0.7	0.6 ± 0.1	13.1 ± 0.4	0.34 ± 0.02	2.29 ± 0.2	0.51 ± 0.04	6.1 ± 0.1	5.18 ± 1.26	1.55 ± 0.28	3.25 ± 0.47	0.66 ± 0.06
Extensive	46.8 ± 1.5	8.2 ± 0.8	0.6 ± 0.1	13.9 ± 0.4	0.24 ± 0.02	1.38 ± 0.1	0.47 ± 0.03	6.0 ± 0.1	1.57 ± 0.12	0.61 ± 0.18	5.00 ± 0.90	1.06 ± 0.09
Sign.	P = 0.1	P = 0.5	P = 0.8	<b>P=0.04</b>	<b>P=0.01</b>	<b>P=0.003</b>	P=0.4	P=0.98	<b>P=0.01</b>	<b>P=0.02</b>	P=0.06	<b>P=0.005</b>

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472 Figure captions

473 Figure 1. Experimental setup at each of the 12 study sites at Ingleborough National Nature  
474 Reserve, Yorkshire Dales (54.18°N, 2.36°E), UK. Yellow stars indicate the different sites  
475 (A). Each site had three different drought treatments (A: Full drought, Microclimate control  
476 and Control treatment) in two grassland types (B; left: intensively managed grassland, right:  
477 extensively managed grassland). After the drought period, seeds of native species were sown  
478 in each of the drought treatments (C). Seedlings were protected against grazing using chicken  
479 wire mesh cages (D).

480 Figure 2. Effects of drought treatments in intensive and extensive grassland plots on dissolved  
481 inorganic nitrogen (DIN:  $\text{NO}_3^- + \text{NH}_4^+$ ) concentrations (in  $\mu\text{g g}^{-1}$  dry soil), 1 week after  
482 rewetting. Different letters indicate significant differences at  $\alpha = 0.05$ . Stars indicate  
483 significant differences \*  $0.01 < P < 0.05$ ; \*\*  $0.001 < P < 0.01$ ; \*\*\*  $P < 0.001$ ; NS = not  
484 significant.

485 Figure 3. Soil moisture (% w/w) at the last day of the drought treatment versus DIN  
486 (dissolved inorganic N). A) intensively managed grassland plots B) extensively managed  
487 grassland plots. The relation between drought effects and total available N is significant for  
488 the intensive soil, but not for the extensive soil.

489 Figure 4. Effect of drought and control treatments on growth of exotic and native seedlings in  
490 intensively managed grassland plots (A) and extensively managed grassland plots (B); results  
491 from glasshouse experiment. White bars represent control treatments, black bars represent  
492 drought treatments. Grey shades indicate native species.

493 Figure 5. Effect of drought on survival of exotic and native seedlings in intensively managed  
494 grassland plots (A) and extensively managed grassland plots (B). Results from glasshouse  
495 experiment. Grey shades indicate native species.

496 Figure 6. Growth (A,B) and survival (C, D) of seeded native species the field experiment in  
497 intensive (A, C) and extensive (B,D) grassland plots, 2 months after sowing. Black bars  
498 represent drought treatments, white bars represent control treatments. Survival values below  
499 zero (light grey bars) indicate relatively higher survival in the control treatments; values  
500 above zero (dark grey bars) indicate higher survival in the drought treatments. Statistics in  
501 panel C and D were carried out on the proportion of seedlings that survived, relative seedling  
502 survival is shown for illustration purposes.

503 Figure 7. Relationship between DIN (mineral N availability) after rewetting and seedling  
504 growth of each of the five native species that were seeded in the plots in the field. Fits include  
505 points from both intensive (closed symbols) and extensive (open symbols). Solid lines  
506 indicate significant fits, dotted line for *R. obtusifolius* indicates absence of significant  
507 correlation.

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509



510 Figure 1.

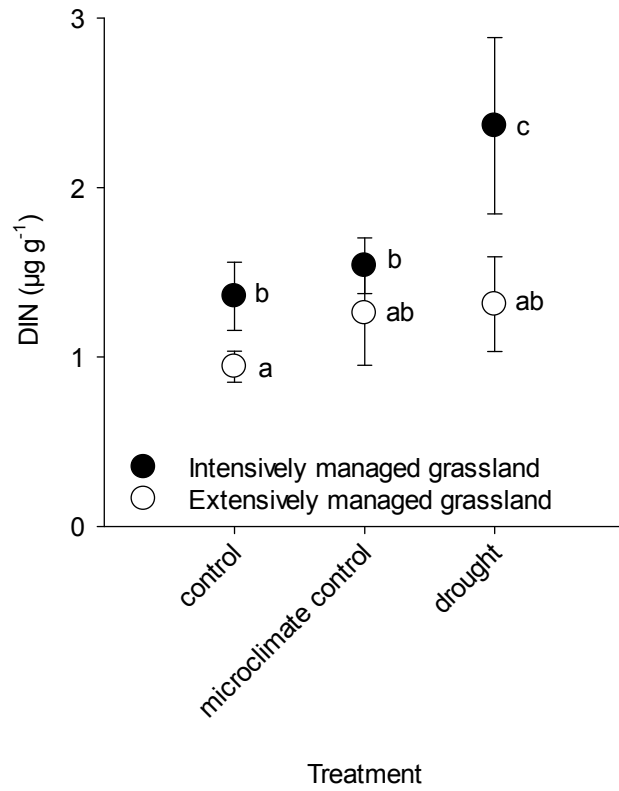
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512 Figure 2.

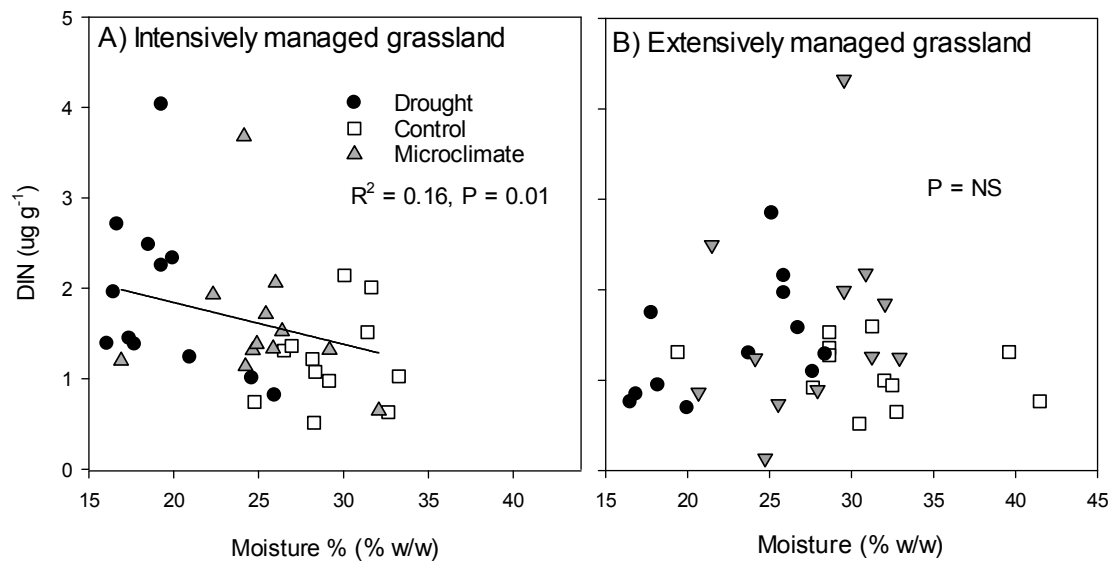


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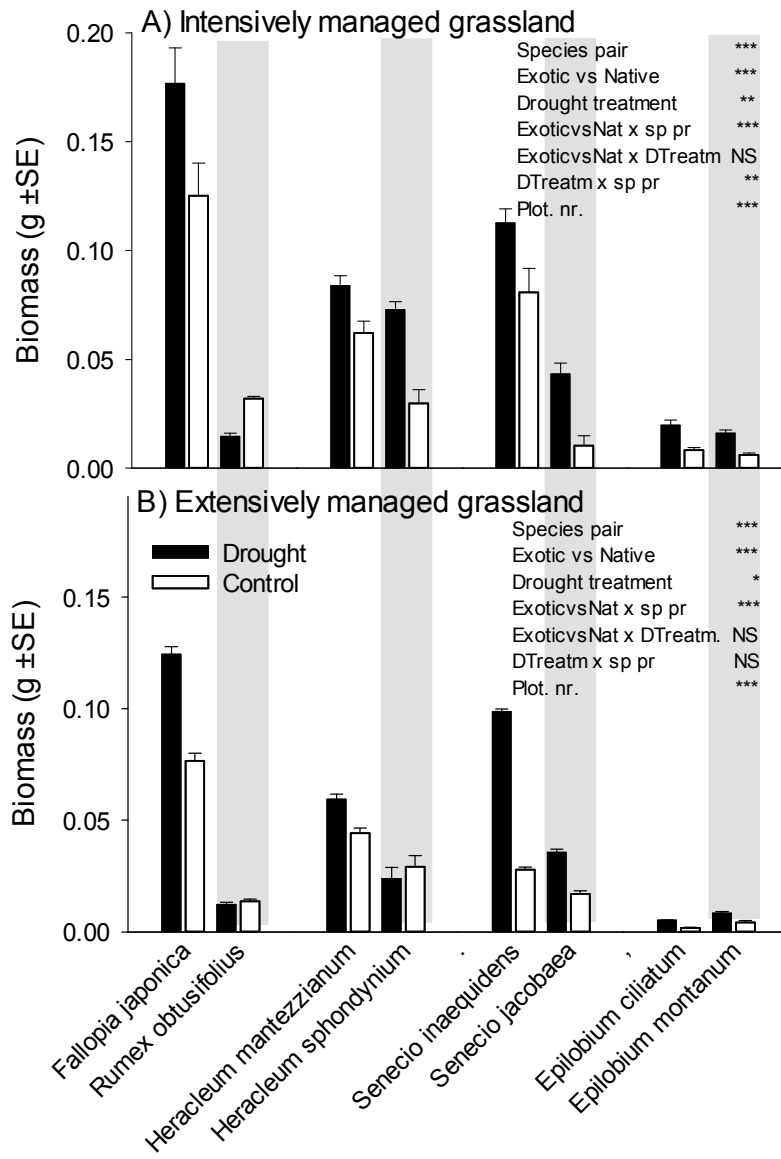
514 Figure 3.  
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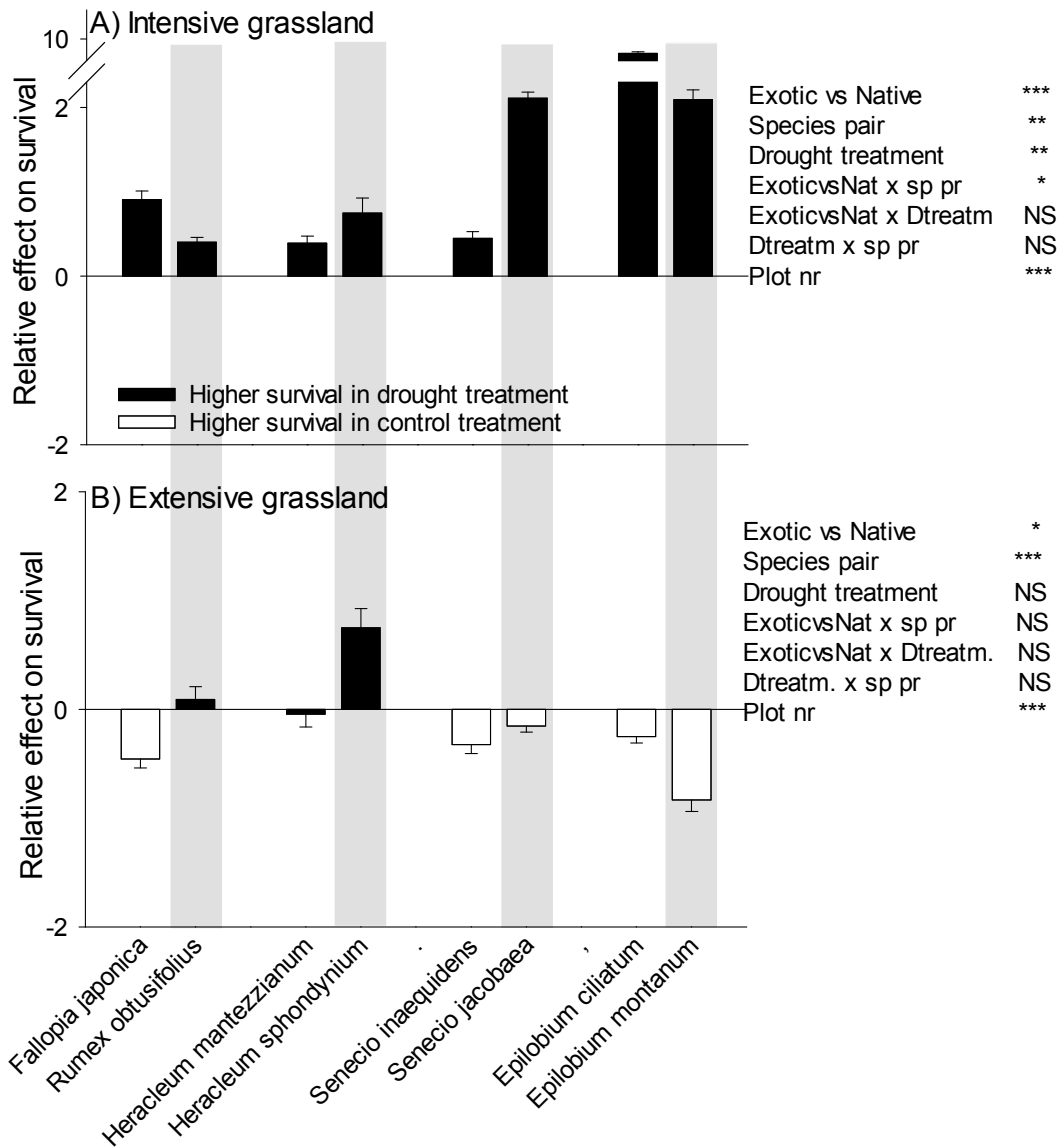
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518 Figure 4



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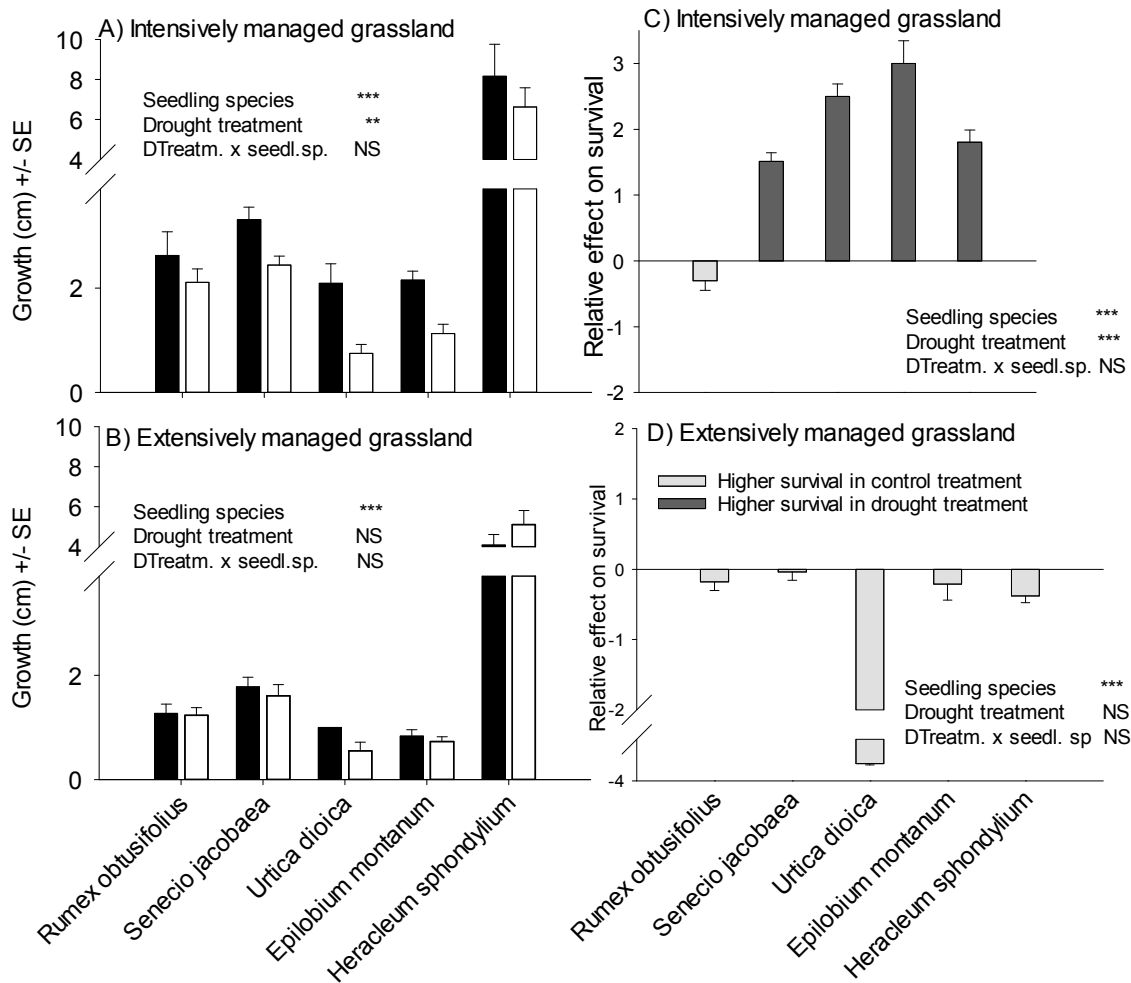
520 Figure 5.



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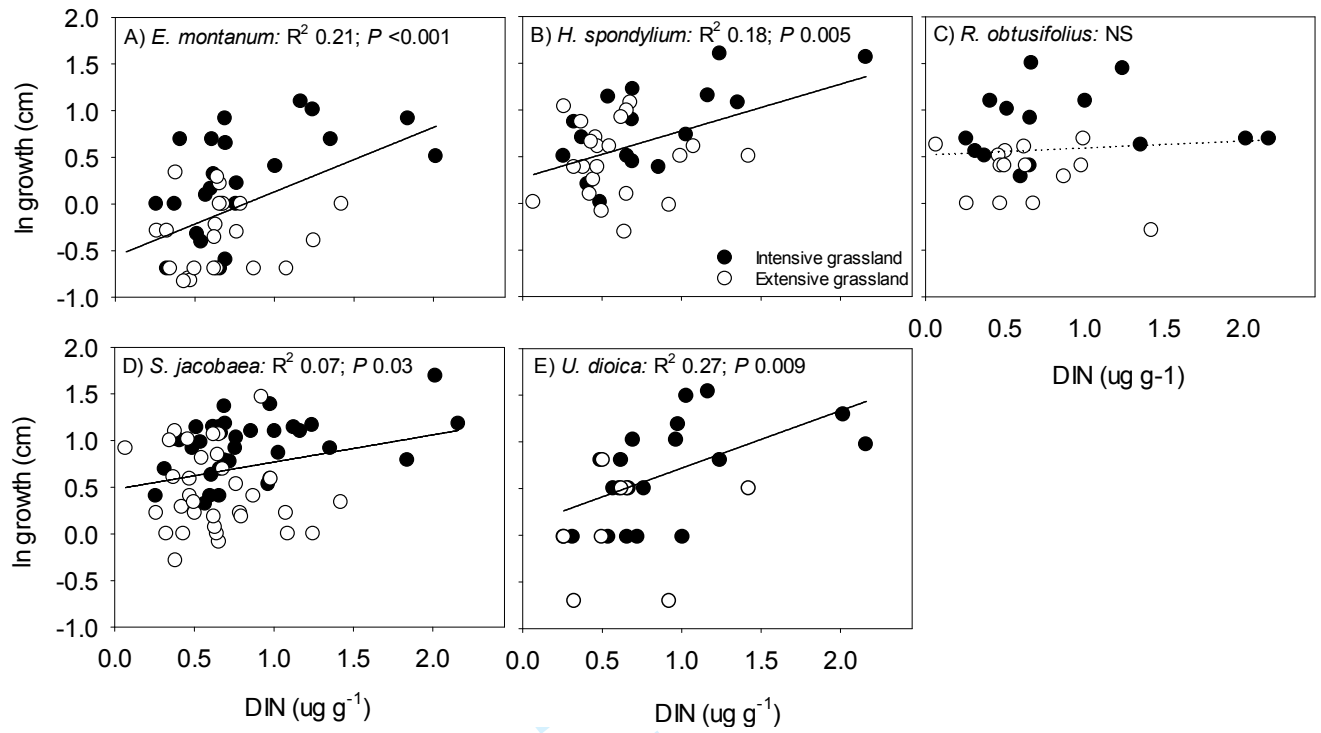
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523 Figure 6.



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526 Figure 7.



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Electronic appendix with M. Schrama & R. D. Bardgett “*Grassland invasibility varies with drought effects on soil functioning*”

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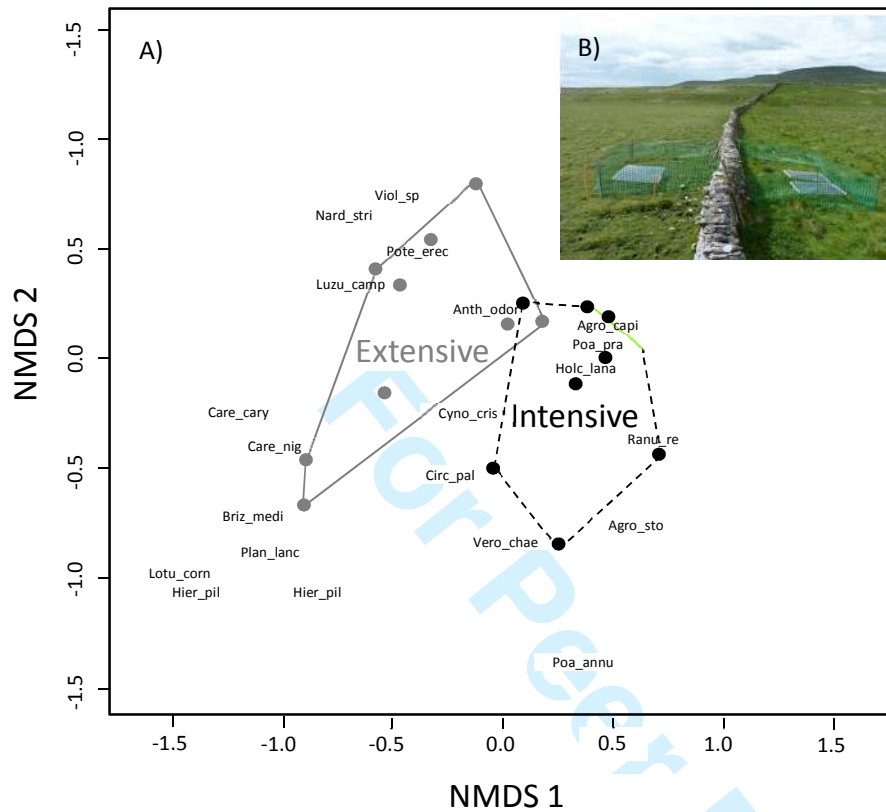


Figure S1. NMDS plot showing dissimilarity in species composition between plant communities in intensive and extensive grasslands. In general, intensive grassland communities were characterized by fast growing graminoids (e.g. *Lolium perenne*, *Agrostis stolonifera*, *A. capillaris*, *Festuca rubra*, *Holcus lanatus*) whereas extensive grasslands were associated with slow-growing grass species (e.g. *Nardus stricta*, *Sesleria caerulea*, *Briza media*), sedges (*Carex* spp.) and different herbs (*Potentilla erecta*, *Viola* spp.) B) Picture showing experimental setup with intensive grassland to the left of the wall and extensive grassland to the right of the wall.

**Methods.** Vegetation composition of individual plots was measured by visual assessments of percentage cover of each species. In June 2014, which were taken from the central 1x1 m square in each of the control plots. To inspect whether the composition of the plant species communities differed between grassland types, nonmetric multidimensional scaling (NMDS) analyses were conducted on plant species cover data (Oksanen *et al.* 2013). To link community invasibility of the different grassland types to community plant traits, we calculated community-weighted means (CWM)



of trait values for SLA and LDMC, which were calculated for each vegetation recording, based on

species biomass proportions according to the equation 
$$CWM = \sum_{i=1}^S p_i \times t_i$$
, where S is the number

of species in the community,  $p_i$  is the species biomass proportion and  $t_i$  are the given trait values for

each species from the TRY database. Vegetation composition was highly dissimilar between

intensively and extensively managed grassland plots. Results from the plant traits analysis shows that

intensively managed grassland communities were characterized by species with higher specific leaf

area (SLA) ( $F_{(1,18)}71.4$ ,  $P < 0.0001$ ) and lower LDMC ( $F_{(1,18)}7.0$ ,  $P = 0.01$ ).

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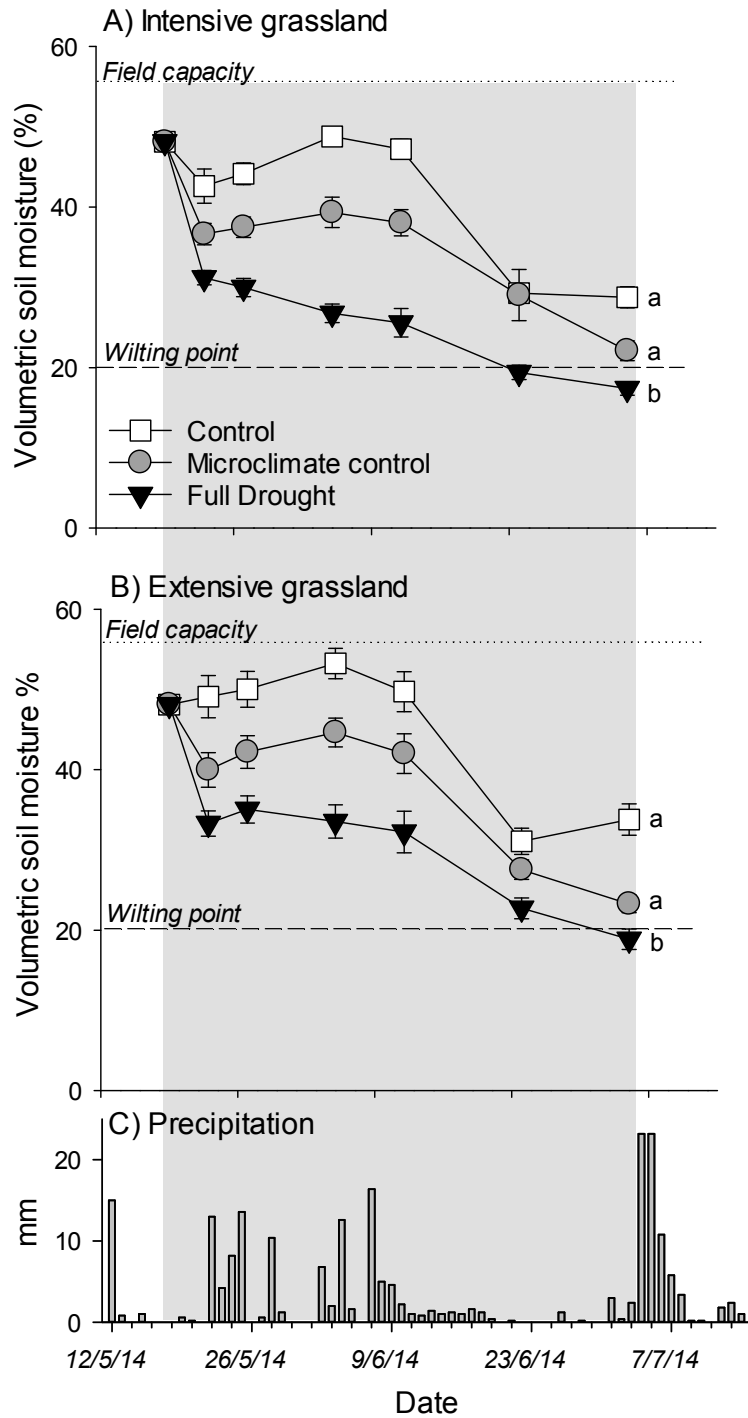


Figure S2. Effects of different drought shelter treatments in intensively (A) and extensively managed (B) grassland plots. Panel C shows the precipitation during the experiment. Grey area indicates 40-day period that drought shelters were set up in the field.