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Direct and higher-order interactions in plant communities under increasing weather persistence

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1 Direct and higher-order interactions in plant communities under increasing 2 weather persistence

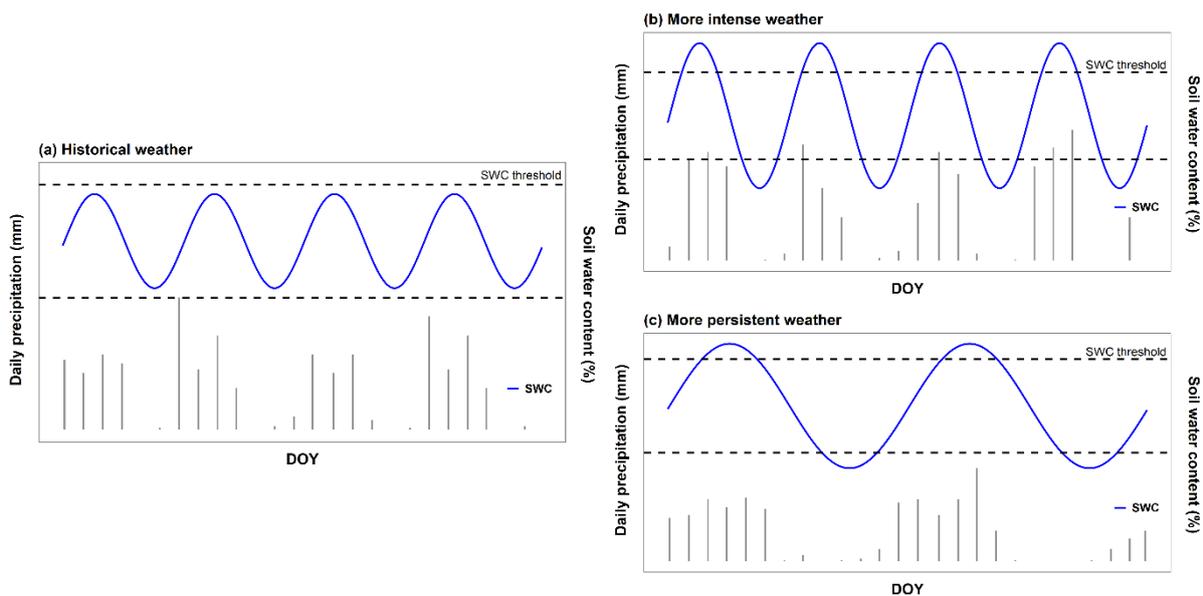
3 1. Abstract

4 Climate change is increasing the weather persistence in the mid-latitudes, prolonging both
5 dry and wet spells compared to historic averages. These newly emerging environmental
6 conditions destabilize plant communities, but the role of species interactions in this process
7 is unknown. Here, we tested how direct and higher-order interactions (HOIs) between species
8 may change in synthesized grassland communities along an experimental gradient of
9 increasing persistence in precipitation regimes. Our results indicate that species interactions
10 (including HOIs) are an important determinant of plant performance under increasing
11 weather persistence. Out of the 12 most parsimonious models predicting species
12 productivity, 75 % contained significant direct interactions and 92 % significant HOIs. Inclusion
13 of direct interactions or HOIs respectively tripled or quadrupled the explained variance of
14 target species biomass compared to null models only including the precipitation treatment.
15 Drought was the main driver of plant responses, with longer droughts increasing direct
16 competition but also HOI-driven facilitation. Despite these counteracting changes, drought
17 intensified net competition. Grasses were generally more involved in competitive interactions
18 whereas legumes were more involved in facilitative interactions. Under longer drought,
19 species affinity for nutrient rich or wet environments resulted in more negative direct
20 interactions or HOIs, respectively. We conclude that higher-order interactions, crucially
21 depending on species identity, only partially stabilize community dynamics under increasing
22 weather persistence.

23 **Keywords:** higher-order interactions, grasslands, facilitation and competition, species
24 interactions, increasing weather persistence, drought

25 **2. Introduction**

26 Climate warming changes the inter- and intra-annual variability of precipitation regimes in
27 the mid-latitudes (Pendergrass *et al.*, 2017), including shifts towards more persistent summer
28 weather with longer dry and wet spells compared to historic averages (Pfleiderer *et al.*, 2019).
29 With more extreme precipitation regimes (Fig. 1), we expect that ecosystems will be
30 subjected more often to prolonged plant stress-inducing extreme conditions, due to the
31 accompanying changes in soil water and temperature (Zhao & Khalil, 1993; Knapp *et al.*, 2008;
32 Felton *et al.*, 2021; Reynaert *et al.*, 2021), and potential changes in nutrient cycling (Borken &
33 Matzner, 2009). For example, when dry periods become longer, or occur at higher
34 temperatures (Collins *et al.*, 2013), soil water declines more, increasing the chance of crossing
35 mortality thresholds (Knapp *et al.*, 2008; De Boeck *et al.*, 2018). Moreover, longer droughts
36 can increase the risk of nutrient leaching upon rewetting, potentially leading to nutrient
37 shortages in the longer term (Borken & Matzner, 2009; Klaus *et al.*, 2020).



38

39 **Figure 1.** Theoretical framework indicating how increasing precipitation variability can
40 influence soil moisture regimes as an extension of Knapp *et al.* (2008), who primarily

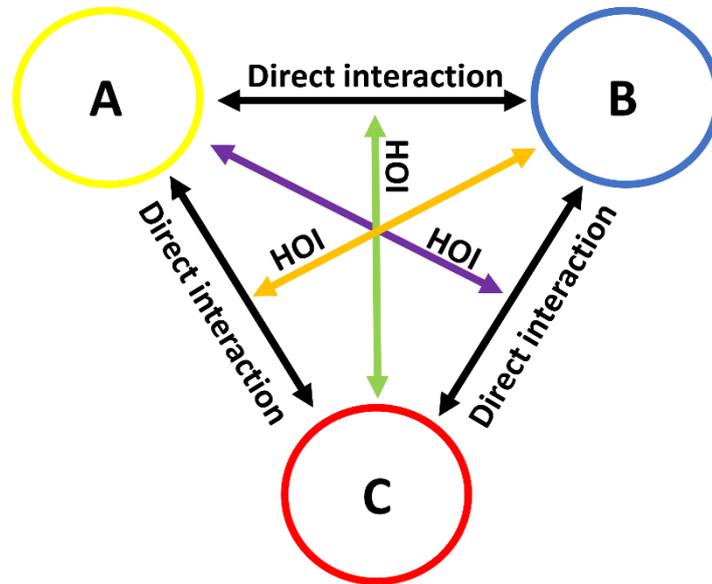
41 discussed the effects of increased weather intensity (i.e. larger, more irregular event sizes).
42 Grey bars represent daily precipitation. Day of the year and soil water content are abbreviated
43 by DOY and SWC, respectively. Under climate change, we expect an increase in both intensity
44 and persistence of summer weather in the mid-latitudes (Spinoni *et al.*, 2018; Pfliegerer *et*
45 *al.*, 2019). In all panels, the precipitation totals are equal to historical weather (a). More
46 intense weather (b) results in less frequent but larger rainfall events intermitted with longer
47 dry spells under higher temperatures, which could exacerbate drought/flooding effects.
48 More persistent weather (c) results in prolonged dry and wet periods, which could also lead
49 to more extreme variation in soil water content. In both cases, the chance increases that
50 critical soil moisture thresholds are exceeded, negatively affecting ecosystem functioning
51 (Ciais *et al.*, 2005).

52 While research has explored how climate shifts may alter selection of species with specific
53 traits (Siepielski *et al.*, 2017), whether interspecific interactions will change, or how species
54 interactions shape community responses to these regime changes remains unclear (Grant *et*
55 *al.*, 2014). Direct species interactions, i.e. the direct effect of plant A on plant B and vice versa
56 (Fig. 2), are important determinants of ecosystem functioning (Schmitz *et al.*, 2008). They can
57 be facilitative (positive) or competitive (negative). In grasslands, legumes often facilitate
58 grasses through N-fixation, which increases ecosystem productivity (Ball *et al.*, 2020), in
59 return for improved microclimatic conditions such as wind protection (Walker *et al.*, 2003).
60 Conversely, fast-growing dominant grasses often compete with one another for limited
61 resources, which can result in productivity loss (Fay *et al.*, 2003; Corbin & D'Antonio, 2004).
62 During episodes of extreme abiotic conditions, when water (drought) or oxygen (flooding)
63 become limited, direct interactions between species may become more positive, negative, or
64 not change (Grant *et al.*, 2014; Van den Berge *et al.*, 2014). The net effect of direct interaction
65 changes on ecosystem functioning (e.g., productivity) under such conditions is ecosystem
66 specific and depends on their strength and direction, but also on the affected species. For

67 example, under drought, increased competition between dominant species can exacerbate
68 the productivity decline (Fay *et al.*, 2003; Suttle *et al.*, 2007; Grant *et al.*, 2014).

69 In diverse communities, indirect interactions can also emerge, through interaction chains of
70 direct interactions (Wootton, 1993) or through higher-order interactions (HOIs) (Billick &
71 Case, 1994). With interaction chains, apparent indirect interaction effects may appear
72 between two distant plants, due to a shared relationship with a third (e.g., species A modifies
73 performance of species B, in turn affecting performance of species C). Unlike interaction
74 chains, HOIs remain poorly understood, although they play an important role in determining
75 net community dynamics and the stability of complex ecosystems by modifying direct
76 interactions (Billick & Case, 1994; Brooker *et al.*, 2008; Bairey *et al.*, 2016; Grilli *et al.*, 2017;
77 Mayfield & Stouffer, 2017; Xiao *et al.*, 2020; Li *et al.*, 2021; Singh & Baruah, 2021). HOIs can
78 either be defined as the combined effect of two or more neighboring plants on a third target
79 plant (Fig. 2), which is determined by the nature of these neighbors' own pairwise
80 interaction(s) (Levine *et al.*, 2017; Mayfield & Stouffer, 2017), or, as the effect of one species
81 on the interaction between two others (Billick & Case, 1994). Such interaction effects could
82 arise when a Nitrogen (N)-fixing legume C is introduced into a plant community with scarce
83 resources, reducing N competition between two neighboring grasses (Fig. 2). Recent studies,
84 both theoretical and observational, have indicated that HOIs may mitigate intense direct
85 competitive effects between neighbors, facilitating long-term species coexistence in highly
86 diverse plant communities (Grilli *et al.*, 2017; Xiao *et al.*, 2020; Li *et al.*, 2021; Singh & Baruah,
87 2021). Thus, Grilli *et al.* (2017) suggest that HOIs could make communities more robust
88 against ecological perturbations. However, their impact on ecosystem functioning and how
89 they are influenced by shifting climate regimes remains unclear (Brooker *et al.*, 2008; Bimler

90 *et al.*, 2018; Xiao *et al.*, 2020). Elucidating their role is important to better understand climate-
91 driven changes in diverse plant communities.



92

93 **Figure 2.** Schematic representation of differences between direct interactions and higher-
94 order interactions (HOIs). Direct interactions involve two plants, a neighbor and a target,
95 while HOIs (in their simplest form), include a neighbor pair and a target plant. For example,
96 although species A (neighbor) may outcompete species B (target) in the absence of species C
97 (negative direct interaction), a mixture also including species C (neighbor), reducing the
98 intensity of competition between A and B (less negative interaction) by supplying a scarce
99 resource to both, may actually benefit (facilitate) the growth of species B (positive HOI).

100 Exposure to more extreme dry and wet spells may lead to fundamental changes in species
101 interaction networks (Grant *et al.*, 2014; Olsen *et al.*, 2016). The stress-dominance hypothesis
102 predicts that the relative importance of environmental filtering increases while competition
103 decreases along a gradient of environmental stress (Weiher & Keddy, 1995; Coyle *et al.*,
104 2014). By extension, the stress-gradient hypothesis presumes an increase in the relative
105 importance of facilitation compared to competition along such gradients (Bertness &
106 Callaway, 1994). Despite recent evidence questioning the generality of these hypotheses
107 (Maestre & Cortina, 2004; Grant *et al.*, 2014; Metz & Tielborger, 2016; De Boeck *et al.*, 2018;

108 Singh & Baruah, 2021), directional shifts in mean interactions along environmental gradients
109 have been observed in many ecosystems, indicating important changes in ecosystem
110 functioning (Coyle *et al.*, 2014; Olsen *et al.*, 2016; De Boeck *et al.*, 2018).

111 We explored if and how increasing persistence in summer precipitation regimes (i.e.
112 precipitation variability) changes the number, strength and directionality of species
113 interactions that influence plant biomass production, including both direct interactions and
114 HOIs. We subjected experimental grassland mesocosms with 12 common C3 species (six
115 grasses, three non-N-fixing forbs, three legumes) to an 8-level gradient of precipitation
116 regimes. These regimes varied the duration of consecutive dry and wet periods from 1 to 60
117 days while keeping total precipitation constant (Reynaert *et al.*, 2021). Our research questions
118 were whether species interactions, either direct ones or HOIs, determine species biomass
119 under altered precipitation persistence, and whether these interactions change as drought
120 stress intensifies (i.e., the main driver of plant responses to increasing summer precipitation
121 persistence; Reynaert *et al.* (2021); Reynaert *et al.* (2022)).

122 We hypothesized that (1) HOIs would generally counteract direct competitive interactions,
123 weakening net species interactions and stabilizing community dynamics; (2) species
124 interactions would overall become more positive (or less negative) across the community
125 with increasing drought severity, indicating either a decline in competitive interactions and
126 potentially increased facilitation, or a more prominent role of environmental filtering, leading
127 to increased competitive release; (3) species identity would determine the nature of
128 interactions, with more facilitation in interactions with N-fixing legumes and more
129 competition when considering dominant grasses.

130 **3. Methods & materials**

131 **3.1. Data collection**

132 From July to November 2019, a 120-day open air precipitation manipulation experiment was
133 conducted in Flanders, Belgium (51°09'41"N, 04°24'9"E). In total, 256 mesocosms (29.5 cm
134 inner diameter and 50 cm depth), each containing the same synthesized grassland plant
135 community with three individuals of 12 common C3 species (36 plants per community; Fig.
136 S1), were evenly distributed across eight identical plots of 3 m diameter and subjected to a
137 gradient of increasingly longer dry-wet period alternations (Reynaert *et al.*, 2021). The
138 precipitation persistence gradient contained eight different regimes of dry-wet period
139 alternations, ranging from 1 to 60 consecutive days with or without precipitation and starting
140 with either a dry or a wet period. This yielded 16 regimes in total, including 1, 3, 6, 10, 15, 20,
141 30 and 60 consecutive days dry/wet. All regimes had a total of 60 days with and 60 days
142 without precipitation. Historically, Belgian weather patterns following 1-day dry-wet
143 alternations are most common (KMI, 2019; Reynaert *et al.*, 2021). The precipitation regimes
144 were created by blocking out ambient precipitation with rainout screens which were
145 automatically deployed only during rain, and applying dripper irrigation (Reynaert *et al.*,
146 2021). Precipitation was equal in all regimes on each irrigation day (6.87 L/m², being 1.5 times
147 the Belgian daily average of a rainy day to compensate for excess evapotranspiration in
148 mesocosms) as well as in total across the experimental period. Mesocosms had holes in the
149 bottom, allowing free drainage and preventing flooding (Reynaert *et al.*, 2021). Per regime, a
150 total of 16 replicate mesocosms was evenly distributed across 4 plots. The position of these
151 replicates was different in every plot to account for potential edge effects.

152 The 12 grassland species represented a broad gradient in plant functional traits (Table S1) and
153 included six grasses (*Agrostis capillaris* L. (AC), *Anthoxanthum odoratum* L.

154 (AO), *Deschampsia cespitosa* (L.) P. Beauv. (DC), *Phleum pratense* L. (PHP), *Poa pratensis* L.
155 (POP), *Holcus lanatus* L. (HL)); three N-fixing forbs (*Lotus corniculatus* L. (LC), *Trifolium*
156 *pratense* L. (TP), *Trifolium medium* L. (TM)); and three non-N-fixing forbs (*Centaurea jacea* L.
157 (CJ), *Lychnis flos-cuculi* L. (SF), *Plantago lanceolata* L. (PL)). Seeds were obtained from a seed
158 company in the Netherlands (Cruydt-Hoeck) and sown in separate seedling containers at the
159 start of April 2019. By May, viable seedlings of similar size per species were transplanted
160 directly into the mesocosms (Reynaert *et al.*, 2021). We maximized interspecific interactions
161 by planting them in a hexagonal grid where each individual had different neighbors at 4.5 cm
162 interspace, and avoiding clumping (Fig. S1). The spatial configuration in our containers was
163 aimed at representing each species pair at the most comparable extent possible, with 92% of
164 the 66 possible pairs occurring either one or two times (Table S2). Species positions and north-
165 south orientation were equal in all mesocosms. Mesocosms were weeded regularly over the
166 course of the experiment to prevent (re)colonization.

167 Average volumetric soil water content (SWC) over 0-30 cm depth was recorded half-hourly in
168 two to four mesocosms per precipitation regime by a CS650-DS Reflectometer (Campbell®
169 Scientific INC., Logan, Utah, USA). Because the impact of increasing weather persistence was
170 shown to be mainly related to drought in the 2019 growing season (Reynaert *et al.*, 2021), the
171 daily averages of mean SWC values per regime were utilized to calculate different drought
172 indices which represented the persistency of the precipitation regime. These included
173 drought stress intensity (at relative extractable water (REW) of 0.4 which is the most common
174 but also at REW of 0.2 and 0.1 since sensitivity to soil moisture decline may differ among
175 vegetation types; Granier *et al.* (2007)), total time below permanent wilting point, and the
176 length of the longest consecutive period below permanent wilting point (Vicca *et al.*, 2012;
177 Reynaert *et al.*, 2021; Reynaert *et al.*, 2022). Following Vicca *et al.* (2012), drought stress

178 intensity was calculated by determining a soil moisture threshold, and integrating the
179 duration and extent to which soil moisture declined below it over the experimental period.

180 At the end of the experiment, all standing dead and live biomass above 4.5 cm height was
181 harvested per species per mesocosm, in 12 out of the 16 replicate mesocosms per regime (all
182 available individuals of the same species per mesocosm per paper bag). Dead biomass was
183 included since death at the time of measurement, does not indicate a lack of previous
184 interaction with its neighbors. In half of these 12 mesocosms, one third of the individuals per
185 species was randomly removed for another study. We corrected for this by multiplying the
186 plant mass in these bags by 1.5 (per regime, every species was discarded twice in every unique
187 position; Fig. S1). Combining the biomass of all harvested individuals per species in a
188 mesocosm accounted for variation caused by differences in local neighbor setting. Biomass
189 bags were oven-dried at 70 °C for >72 h and immediately weighed with 0.01 g accuracy. To
190 achieve dry plant mass per species per mesocosm, we subtracted the average mass of 58
191 oven-dried empty bags (SD = 0.08 g). Plants that had little or no biomass above 4.5 cm height
192 because they remained too small during the experiment (< 17 %, n = 2281) were given a mass
193 of 0.01 to account for being below our detection limit (Lubbe *et al.*, 2021).

194 **3.2. Statistical analysis**

195 All statistical analyses were performed in R version 4.0.2 (R Core Team, 2019). Significance
196 was assumed for p-values < 0.05. Graphs were constructed utilizing the packages dplyr
197 (Wickham *et al.*, 2015), igraph (Csardi & Nepusz, 2006) and ggplot2 (Wickham, 2016).

198 To model direct interactions and HOIs, we adapted the approach from Mayfield and Stouffer
199 (2017), which allows the use of biomass data. Their methodology suggests to construct
200 models with the characteristic of interest of a target species in the community (here: biomass)

201 as response variable and (the biomass of) all neighboring species and their statistical
202 interactions as explanatory variables, after which all significant terms from the most
203 parsimonious equivalent models (e.g., $\Delta AIC < 2$) per target species are of interest. The
204 estimates of significant model terms can then be utilized to obtain information about species
205 interactions, direct ones and HOIs, in the studied plant community. Because biomass data
206 was pooled per species per mesocosm in our experiment, we did not focus on the role of
207 individual plant position but rather on species identity. Our central question was if and how
208 the growth performance of a target species, expressed as accumulated dry biomass over the
209 growing season, was affected by the growth performance of neighboring species under
210 increasingly persistent precipitation regimes in diverse grassland communities.

211 As described earlier, we opted to represent our treatments in the models by a drought index.
212 Out of all calculated drought indices (section 3.1), the length of the longest consecutive period
213 below permanent wilting point captured species biomass effects caused by increasingly
214 persistent summer precipitation regimes the best (lowest AIC, adj. $R^2 = 0.56$; Table S3),
215 allowing us to more accurately linearize the biomass responses (Fig. S2 & S3) and model the
216 wet and dry start regimes along a continuous, common dimension (Reynaert *et al.*, 2021).

217 First, we normalized all biomass data by taking the natural logarithm of each biomass
218 observation divided by the mean biomass per species across all regimes. This approach is
219 similar to the log-response ratio, a technique often applied in meta-analysis, where values >0
220 indicate above average plant performance, and values <0 below average (Van Sundert *et al.*,
221 2021). By doing so, effect sizes become comparable among species with distinct intrinsic
222 characteristics, i.e. the coefficients of models with different target species are standardized
223 and directly comparable. Since all effects become relative, they can be interpreted as the

224 extent to which a change in mass of a neighbor species (relative to its mean size) affects the
 225 mass of a target species (relative to its mean size). We then created multiple linear models of
 226 all possible species combinations including every species as response variable (target) and all
 227 others (neighbors), the drought index and their two-way interactions as explanatory variables
 228 (Fig. 2; Eqn. 1).

$$229 \mathbb{E}(BM_{A,i}) = \text{Intercept} + \alpha_1 BM_{B,i} + \alpha_2 BM_{C,i} + \alpha_3 BM_{B,i} BM_{C,i} + \gamma \text{Index} + \beta_1 BM_{B,i} \text{Index} + \beta_2 BM_{C,i} \text{Index} + \beta_3 BM_{B,i} BM_{C,i} \text{Index} \\ 230 + \dots + \epsilon_i \quad (\text{Eqn 1})$$

231 With $\mathbb{E}(BM_{A,i})$ the expected standardized total dry biomass (BM) of target species A in a
 232 specified mesocosm i, $BM_{B,i}$ and $BM_{C,i}$ the standardized total dry biomass of neighbor species
 233 B and C, respectively, in that mesocosm, Index the length of the longest consecutive period
 234 below permanent wilting point, α and β the species interaction (model) coefficients and ϵ_i the
 235 errors. Model terms including only one species ($BM_{B,i}$ or $BM_{C,i}$) represent direct interactions
 236 as explanatory variable, since these test for the effect of a single neighbor species on the
 237 target. Model terms including both species ($BM_{B,i} BM_{C,i}$) indicate HOIs, since they test how the
 238 interaction between two neighboring species affects the target.

239 To avoid overfitting and other issues related to insufficient data (Martyn *et al.*, 2021), we only
 240 included the simplest, i.e. the three-way, HOIs (Fig. 2). This resulted in a total of 12 full models
 241 (one per target species as response variable). Intraspecies interactions were excluded from
 242 the models because we pooled the species biomass for the three individuals per mesocosm,
 243 which accounts for variation caused by individual plant position. Following Grant *et al.* (2014),
 244 we interpret the general variation in interspecies interactions knowing that distant (i.e. non-
 245 direct neighbor; Fig. S1) intraspecies effects may also contribute to the estimated interaction
 246 coefficients. Because plant density and positions are the same in all mesocosms we assume

247 that the found model coefficients primarily reflect variation in plant performance related to
248 changes in interspecies interactions and drought effects. Per response variable (i.e., each of
249 the 12 target species), we then compared the full model (including the drought index, all
250 direct interactions and all HOIs as explanatory variables) with direct-interaction (including the
251 droughty index and all direct interactions as explanatory variables) and null models (only
252 including the drought index as explanatory variable), retaining only the most parsimonious
253 model per target species ($\Delta AIC < 2$). In the remnant 12 models, the strength and direction of
254 the interaction(s) was determined by the model estimates of the explanatory variable(s).
255 Significant model coefficients remaining when filling in a drought index of zero in the model
256 equation (α coefficients; Eqn 1) represent interaction strengths at a drought length of zero.
257 All other significant model coefficients (β coefficients; Eqn 1) indicate a change of interaction
258 strength along the gradient of increasing drought length. To calculate interaction strengths at
259 maximum drought index, the maximum index value (39), was used to fill in Eqn 1. If the model
260 coefficient was not significant, the interaction strength was assumed to be zero. By doing so,
261 we were able to isolate the most important direct interactions and three-way HOIs per target
262 species in the plant community.

263 Although our models suggest the capacity to detect unidirectional effects of species on each
264 other and thus causation (influenced species as dependent variable or target, influencing
265 species as independent variables or neighbors in Eq. 1), they can actually only detect the
266 balance of these unidirectional effects, and thus correlation. We therefore considered every
267 found interaction coefficient as the bidirectional outcome of the (potential) unidirectional
268 effects, regardless of whether the species of interest was included as target or neighbor in
269 the model.

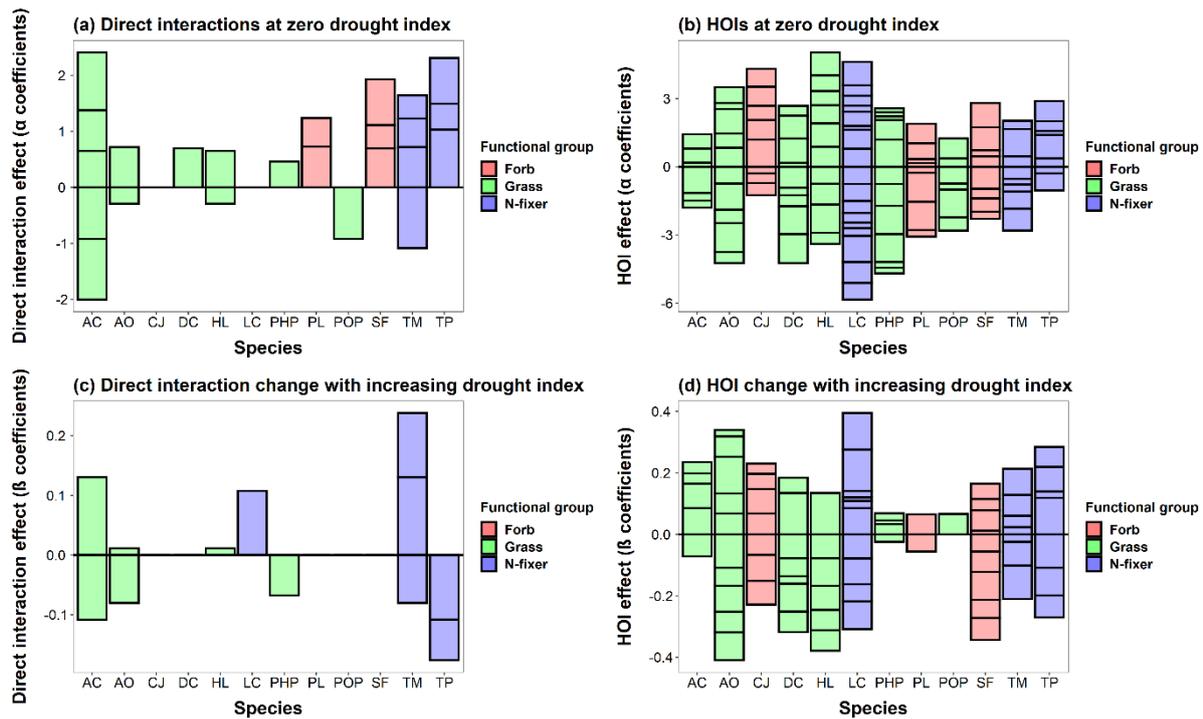
270 Utilizing the interaction coefficients obtained in this way, we then calculated the total number
271 of interactions that involved this species (either as target or neighbor in the models). This was
272 done separately for direct interactions and HOIs. Direct interaction coefficients between the
273 same species pair originating from different models were only counted once per species.
274 Other coefficients represented unique and equally relevant 3-way HOIs and were all counted
275 per species. Next, we calculated the mean direct interaction and HOI strengths by averaging
276 all positive and negative interactions per species, again utilizing the mean values of
277 coefficients between the same species pairs for direct interactions and all coefficients for
278 HOIs. We then visualized species interaction networks for direct interactions, HOIs, net
279 interactions (sum of direct interactions and HOIs per species pair) and their changes along the
280 drought gradient. Finally, to test if species characteristics determined any of the observed
281 patterns, we conducted various non-parametric Spearman correlation tests, utilizing mean
282 interaction strengths and total number as response variables and average species biomass
283 (at zero or maximum drought index) as well as Ellenberg nutrient and moisture indices as
284 explanatory variables.

285 **4. Results**

286 Species interactions (including HOIs) played an important role in determining species
287 performance, expressed as aboveground biomass production, under changing precipitation
288 regimes. Out of the 12 most parsimonious models ($\Delta AIC < 2$), 75% contained significant direct
289 interactions and 92% significant HOIs (Table S4). Null models, which only included the drought
290 index as explanatory variable, explained 10% of the variance in species biomass on average
291 (Table S4). Inclusion of direct interactions increased the variance explained to 28% on average

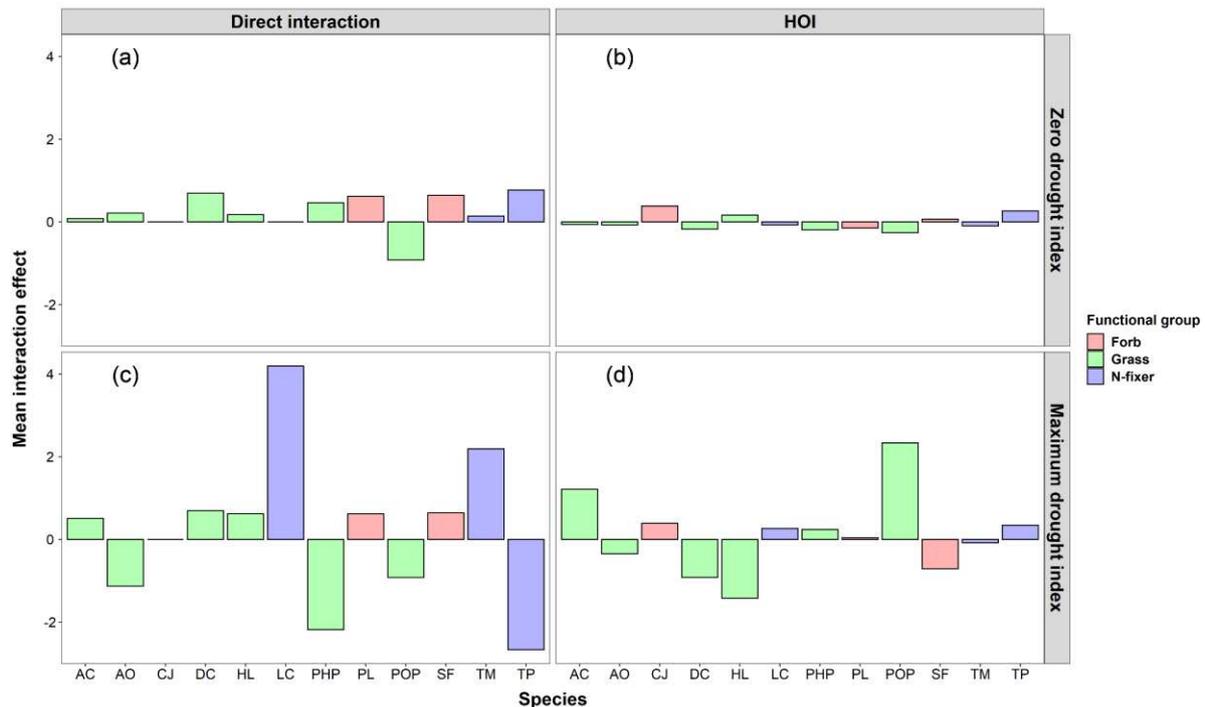
292 (Table S4). Full models, also including HOIs, explained 43% of variance in target species
293 biomass on average (Table S4).

294 At a drought index of zero, the majority of direct interactions (75%, n = 24) (stacked bars
295 above zero; Fig 3a) were positive (i.e., facilitative) compared to only half of the HOIs (50%,
296 n=108) (Fig. 3b). Although half of the significant direct interactions became more negative
297 (i.e., less facilitative or more competitive; 50%, n = 12) (stacked bars below zero; Fig. 3c) with
298 increasing drought length (i.e., index change), there were still more positive (i.e., facilitative)
299 mean direct interactions at maximum drought index overall (72%, n = 11; Fig. 4c). For HOIs,
300 the total number of positive (i.e., facilitative) and negative (i.e., competitive) interactions
301 stayed similar (52% positive, n = 75) (Fig. 3d), though on average, more species were involved
302 in facilitative HOIs at maximum drought index (58%, n = 12; Fig. 4d). Whereas opposite
303 interaction effects generally neutralized each other at zero drought index, overall,
304 competition and facilitation were more pronounced at maximum drought stress (Fig. 4). As
305 such, average direct competition was much stronger and more prevalent at maximum
306 drought stress while more HOIs became facilitative (Fig. 4). Despite these opposite changes,
307 mean direct interaction and HOI strength did not inversely correlate at either side of the
308 drought gradient (Fig. S4).



309

310 **Figure 3.** Direct interaction and higher-order interaction (HOI) effects grouped per species
 311 (as target or neighbor) at zero drought index (a, b; α coefficients in Eqn 1) and their change
 312 (c, d; β coefficients in Eqn 1) with increasing length of the longest consecutive period below
 313 permanent wilting point (PWP). Positive values indicate facilitative effects, while negative
 314 values indicate competitive effects. The number of interactions (number of blocks inside each
 315 bar) indicates how often a species is involved in a direct interaction or HOI with a different
 316 species or species pair. Direct interactions between the same species pairs from different
 317 models were averaged. The size and orientation of individual boxes indicates the size and
 318 direction (+/-) of effects (standardized effect sizes). Colors refer to functional group of the
 319 species (red: non-N-fixing Forb, green: Grass, blue: N-fixer). Non-significant interactions were
 320 excluded. For full species names, see methods section 3.1.



321

322 **Figure 4.** Mean direct (a, c) and higher-order interaction (b, d) effects grouped per species in
 323 the most parsimonious models at zero and maximum drought index. To calculate mean direct
 324 interactions, the average of bidirectional direct interactions is taken. Positive values indicate
 325 facilitative effects, while negative values indicate competitive effects. Colors refer to
 326 functional group of the species (red: non-N-fixing Forb, green: Grass, blue: N-fixer). Higher-
 327 order interaction is abbreviated by HOI. Non-significant interactions were excluded. For full
 328 species names, see methods section 3.1.

329 Species identity determined the nature of interactions. Out of the four species involved in
 330 competitive interactions on average at maximum drought index, three were grasses in both
 331 direct interactions and HOIs (Fig. 4c,d). Forbs (incl. N-fixers) were on average more involved
 332 in facilitation relative to their species number, especially at maximum drought index (Fig. 4).
 333 Furthermore, Ellenberg's moisture and nutrient indices correlated with average interaction
 334 effects. At maximum drought index, direct interactions became more negative with increasing
 335 affinity to nutrient rich environments ($p = 0.02$; Fig. S5b), while HOIs became more negative

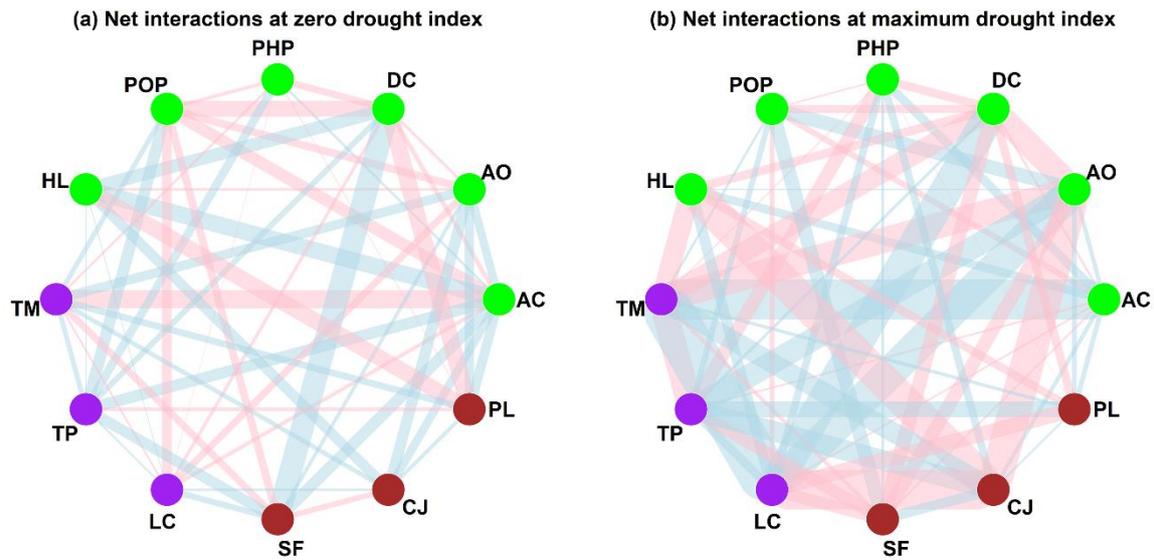
336 with increasing hydrophilicity ($p = 0.04$; Fig. S5a). No correlations were found between
 337 average interaction strength or number and average species biomass (Fig. S6, Table S5).

338 Interaction networks became stronger and more negative (i.e., less facilitative or more
 339 competitive) at maximum drought index (Fig. 5). Net interactions (sum of direct interactions
 340 and HOIs) were generally most positive (i.e., more facilitative or less competitive) on average
 341 for N-fixers, more negative (i.e., less facilitative or more competitive) for non-N-fixing forbs
 342 and most negative (i.e., less facilitative or more competitive) for grasses (Table 1). Moreover,
 343 the majority of net interactions became negative (i.e., competitive) for grasses and non-N-
 344 fixing forbs at maximum drought index, while the majority of net N-fixer interactions
 345 remained facilitative. Both at zero and maximum drought index, N-fixers were proportionally
 346 most involved in net interactions, followed by forbs and grasses (Table 1). Average net
 347 interaction strengths did not change noticeably with increasing drought index within
 348 functional groups (Table 1).

349 **Table 1.** Number and average strength of all significant positive (facilitative) and negative
 350 (competitive) net interactions (sum of direct interactions and HOIs) involving grasses, (non-
 351 N-fixing) forbs or N-fixers at zero and maximum drought index, respectively. Non-significant
 352 interactions were excluded. See Figure 5 for the complete network overview.

	At zero drought index			At maximum drought index		
	Grass	Forb	N-fixer	Grass	Forb	N-fixer
# Facilitative (+) interactions	20	14	15	21	12	16
# Competitive (-) interactions	19	7	10	23	13	14
Mean interaction effect	-0.23	-0.08	0.11	-0.23	-0.03	0.2

353



354
 355 **Figure 5.** Species networks indicating the net strength of interactions (sum of direct
 356 interactions and higher-order interactions per species pair) in the most parsimonious models
 357 ($\Delta AIC < 2$) at zero (a) and at maximum (b) drought index. The color of the network links
 358 indicates whether the net interaction is positive (blue) or negative (red). The color of the
 359 species relates to its functional group including grasses (green), non-N-fixing-forbs (brown)
 360 and N-fixers (purple). When multiple interactions were found between the same species pair
 361 in different models, their strengths were averaged. The thickness of the connections indicates
 362 net interaction strength. Non-significant interactions were excluded. For full species names,
 363 see methods section 3.1.

364 5. Discussion

365 Inclusion of species interactions (including HOIs) increased the accuracy of plant performance
 366 models along the drought gradient compared to models only including the drought effect.
 367 Direct interactions improved the explanatory power of models more on average compared to
 368 HOIs, though the latter were present in a larger number of the most parsimonious models
 369 (Table S4). This finding affirms the necessity to include at least some HOIs to improve net
 370 species interaction estimates in complex and diverse ecological networks (Mayfield &
 371 Stouffer, 2017; Li *et al.*, 2020; Li *et al.*, 2021; Martyn *et al.*, 2021). Particularly in plant

372 communities subjected to drought, where species interactions can change drastically (Grant
373 *et al.*, 2014; Van den Berge *et al.*, 2014), facilitation and/or competition may be overestimated
374 without inclusion of HOIs, limiting our ability to predict ecosystem trajectories (Brooker *et al.*,
375 2008). Incorporating HOI approaches in more studies could thus improve the predictability of
376 species performance under climate change.

377 Contrasting with the stress gradient hypothesis (Bertness & Callaway, 1994), more net
378 interactions became negative on average along the drought gradient. This occurred because
379 direct interactions predominantly shifted to more negative with longer drought, while HOIs
380 were doing the opposite, albeit without fully neutralizing intensified competition (Fig. 4). In
381 line with Xiao *et al.* (2020) and Li *et al.* (2021), these findings indicate that HOIs may influence
382 community stability by partially counteracting increased direct competitive effects under
383 increasing precipitation variability. While Xiao *et al.* (2020) argue that especially intraspecific
384 HOIs play a stabilizing role, our experimental findings suggest that interspecific HOIs may also
385 contribute to stabilizing community dynamics (Bairey *et al.*, 2016; Grilli *et al.*, 2017; Singh &
386 Baruah, 2021). Hence, persistence of highly diverse communities under increasing drought
387 stress could be promoted by interspecies HOIs counteracting stronger direct competitive
388 effects, thus stabilizing community dynamics, and preventing dominance of one single species
389 group (Brooker *et al.*, 2008). Since such stabilizing HOIs are more likely to be generated in
390 multi-species assemblages (Grilli *et al.*, 2017), this observation may also point at an additional
391 mechanism through which more diverse communities could be better equipped to withstand
392 climate extremes (Brooker *et al.*, 2008; De Boeck *et al.*, 2018).

393 In terms of directionality, the majority of HOIs showed a shift towards stronger facilitation (or
394 weaker competition) along the drought gradient on average (positive values in Fig. 3d), in line

395 with the stress gradient and dominance hypotheses (Bertness & Callaway, 1994; Coyle *et al.*,
396 2014). However, three additional species exerted direct competitive pressures at maximum
397 drought index on average (Fig. 4c), and more net interactions became competitive (Fig. 5;
398 Table 1). Hence, while the majority of direct competitive interactions was weakened by
399 positive HOIs, the strengthening of some competitors may have led to a destabilized system
400 where more species were being outcompeted under longer drought (Table 1; Fig. 5). This is
401 in agreement with some studies (Maestre *et al.*, 2005; Saccone *et al.*, 2009; Olsen *et al.*, 2016),
402 but contradicted by others (Brooker *et al.*, 2008; He *et al.*, 2013). Having more species in
403 negative direct interactions (and more net competitive interactions) under longer drought
404 indeed suggests that some species (e.g. *Phleum pratense*) could become more competitive
405 under water shortage because of, for example, favorable resource acquisition traits (Kraft *et*
406 *al.*, 2015; Olsen *et al.*, 2016).

407 In that regard, we observed that direct interactions became more negative with increasing
408 Ellenberg's nutrient indices under longer drought (Fig. S5b), indicating that species with an
409 affinity for resource rich environments became involved in more direct competitive
410 interactions. Hence, increasing weather persistence seemed to not only affect species
411 interaction networks directly through intrinsic negative responses to changes in soil water
412 availability (i.e. Ellenberg's moisture indices; Fig. S5a), but also indirectly through effects of
413 drought on nutrient availability. These intensified direct competitive effects may thus also
414 reflect differences in plant resilience during post-drought rewetting and recovery phases,
415 when the flush of nutrients related to Birch effects induces transient periods of high nutrient
416 availability (Birch, 1958; Borken & Matzner, 2009), allowing rapid recovery of more resilient
417 species.

418 However, positive interactions still made up half of all interactions under extreme drought
419 (Fig. 5b), and the average strength of net interactions per functional group did not change
420 noticeably with drought (Table 1). Together with the striking productivity declines (Fig. S2 &
421 S3) and diversity losses related to the intrinsic drought sensitivity of individual species
422 (Reynaert *et al.*, 2021), these observations may indicate a primary role of environmental
423 filtering (i.e. drought), and only to a lesser extent shifts in species interactions as the primary
424 cause for aboveground productivity declines under drought (Coyle *et al.*, 2014; Elst *et al.*,
425 2017). Following Maestre *et al.* (2009), we pose that under extreme environmental stress
426 (e.g., prolonged drought), species could potentially also stop growing due to exceedance of
427 intrinsic ecological thresholds, mostly independently of the community dynamics (i.e.
428 facilitative or competitive interactions) in their growing environment (Soliveres *et al.*, 2015;
429 De Boeck *et al.*, 2018; Reynaert *et al.*, 2021). The non-linearity in survival responses (Fig. S5;
430 Reynaert *et al.* (2021)), could indeed suggest such a mechanism. Similar negative threshold
431 responses of aboveground plant productivity have been observed in many terrestrial
432 ecosystems, particularly in relation to the duration of dry spell anomalies (Felton *et al.*, 2021).
433 Taken together, these results indicate that both intrinsic responses to increasing drought and
434 intensifying interspecific competitive effects (Olsen *et al.*, 2016) lead to the observed changes
435 in community dynamics by the end of the first 120 days of the experiment.

436 In line with our third hypothesis, forbs (particularly N-fixers) tended to be more involved in
437 facilitative interactions on average, while grasses seemed the strongest competitors (Table 1;
438 Fig. 5). This role of species identity (Weigelt *et al.*, 2002; Martyn *et al.*, 2021) was confirmed
439 by the relationships with Ellenberg's moisture and nutrient indices at maximum drought
440 index, with species with an affinity for wetter and more resource rich environments
441 experiencing or exerting more negative HOIs or direct interactions on average, respectively

442 (Fig. S5a,b). Additionally, the disproportionate contribution of N-fixers to positive interactions
443 and grasses to negative interactions suggests that some species (or species groups) are crucial
444 in defining the dynamics of complex species networks (Singh & Baruah, 2021). With the
445 available information, we cannot fully determine whether N-fixer importance is because of
446 active facilitative traits (e.g., improving water or nutrient supply), or due to passive
447 competitive release as these smaller species rapidly disappeared with increasing drought
448 stress (i.e. were more susceptible to environmental filtering), or because they prevent other
449 more competitive neighbors from colonizing (e.g., via vegetative propagation) (Maestre *et al.*,
450 2003).

451 Some aspects of this study's methodology should be considered. First, the presented direct
452 vs three-way higher-order counteracting interaction system may not fully reveal the nature
453 of net community dynamics. Inclusion of HOIs beyond simple three-way terms may shed more
454 light on the overall strength of facilitation and competition, but was not feasible in this study
455 because of insufficient data (Martyn *et al.*, 2021). Nonetheless, previous studies have
456 indicated that inclusion of more complex (i.e., 4-way, 5-way, etc.) HOIs leads to progressively
457 diminished returns in explaining community dynamics (Li *et al.*, 2021), highlighting the
458 importance of the modelled interactions. Second, the exclusion of intraspecific and density
459 effects likely perturbed the estimation of interaction coefficients, since intraspecific
460 interactions (both direct and HOIs) and competitor density influence net community
461 dynamics in diverse ecosystems (Chesson, 2000; Xiao *et al.*, 2020; Li *et al.*, 2021). However,
462 because individuals of the same species were never planted directly next to one-another and
463 the initial planting density was identical in every mesocosm, we believe that the observed
464 changes primarily reflect plant responses in function of neighbor performance and drought
465 (Grant *et al.*, 2014). Third, the species interactions investigated here represent short-term

466 responses to altered persistence in summer precipitation (Reynaert *et al.*, 2021). Since we
467 already observed strong differences in species performance related to the extremity of the
468 precipitation regime, later assessment of interactions would likely disproportionately reflect
469 the effects of only a few single species since many (e.g., *Silene flos-cuculi*) had died already
470 under extreme summer drought after 120 days (Reynaert *et al.*, 2021). We still decided to
471 include these sensitive species in our models since they still produced some biomass during
472 their initial growth and only disappeared after some time, thus interacting with others at least
473 across part of the experimental duration. Finally, as is true for most (if not all) other
474 experimental or observational studies on species interactions, including those on HOIs (e.g.,
475 Mayfield and Stouffer (2017)), emergence of synergistic or antagonistic relationships in
476 species performance, presence/absence or fecundity is not necessarily proof of interactions
477 amongst the investigated entities (Barner *et al.*, 2018). In fact, it is possible that such effects
478 are an emergent property related to similar or contrasting species-specific responses to an
479 unknown underlying driver (Barner *et al.*, 2018). However, since it is impossible to measure
480 and control everything in real-world experiments, we believe that our model and
481 methodology, despite its shortcomings, has advantages compared to other studies regarding
482 the estimation of true species interactions since it (i) investigates plant communities which
483 deviated from an identical starting point (i.e., identical mesocosm) across the same time
484 period and (ii) normalizes for variance related to the most important driver of all measured
485 responses (see Reynaert *et al.* (2021)). Consequently, the identified interactions likely
486 represent the most important ones that contributed to the observed declines of species
487 under the imposed climate trend.

488 **6. Conclusion**

489 Modelling and disentangling the role of HOIs in community dynamics remains challenging
490 because of their complexity, extensive dataset requirements and a lack of widely applicable
491 methodologies. More targeted manipulation experiments filtering out the environmental
492 noise that perturbs the estimation of interaction coefficients could aid in bridging the gap
493 between theoretical modelling and meaningful ecological understanding of diverse
494 communities. We took a first step in that direction, by experimentally demonstrating that
495 species interactions, including HOIs, can significantly change along a gradient of increasingly
496 persistent precipitation regimes. Inclusion of HOIs did not only improve model explanatory
497 power, but also shed more light on potential stabilizing mechanisms for increasing
498 competition under drought. Hence, HOI approaches could further improve the accuracy of
499 species performance models under rapidly changing environmental conditions, an imperative
500 prerequisite for targeted and efficient ecosystem management.

501 **7. Conflict of interest**

502 The authors declare that they have no known competing financial interests or personal
503 relationships that could have appeared to influence the work reported in this paper.

504 **8. Data availability**

505 The corresponding data of this study will be made publicly available online in a Zenodo
506 repository at [INSERT DOI] and in the previously published Dryad repository at
507 <https://doi.org/10.5061/dryad.k98sf7m52>.

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