Regulation of *Jacobaea vulgaris* by varied cutting and restoration measures

Regulation of *Jacobaea vulgaris*

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Abstract

13 The growth of the noxious grassland weed *Jacobaea vulgaris* Gaertn. in pastures is a threat to

14 grazing animals. This is especially true when it dominates vegetation cover, which often occurs

15 on non-intensively used pastures that are managed with the nature-conservation goal to

16 maintain and promote biodiversity. Thus, we wanted to find management techniques to reduce

17 *J. vulgaris* without harming the floral biodiversity on the pastures.

18 We tested six different mechanical and cultural methods to reduce the presence and spread of

19 *J. vulgaris*. Seven study sites in Northern Germany (Schleswig-Holstein) were treated with

20 various measurements, including: milling and seeding, hay transfer, mowing regimes at

21 different times and frequencies, and combinations of mowing and seeding.

22 Our results show that cutting within the bloom of the plant at the end of June and again four

23 weeks later, when the plant is in its second bloom was the most effective treatment in reducing

24 *J. vulgaris* abundance. This was the only treatment leading to a significant reduction in

25 population growth rate and density without reducing surrounding plant species richness.

26 The study reveals that management of *J. vulgaris* in non-intensively used pastures is possible,

27 while preserving species-rich grasslands.

Keywords

31

Introduction

33 *Jacobaea vulgaris* is a widespread noxious grassland weed native to Eurasia, and invasive in

34 North America, New Zealand, and Australia (1). As the plant's pyrrolizidine alkaloids pose a

35 health risk to cattle when consumed (2), the control of *J. vulgaris* is a primary management

36 goal of many farmers and in some countries even prescribed in their legislation (3). In intensive

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37 grasslands, high fertilizer input, high cutting frequency, and chemical weed management

38 precludes the occurrence of *J. vulgaris*. It's control is challenging, though, in low input

39 grasslands that are managed for high plant and animal diversity, yet prone to massive *J. vulgaris*

40 spread (4). Taking this into account, we tested management strategies that aim at controlling *J.*

41 *vulgaris* without jeopardizing wild plant diversity.

42 Several studies have aimed to find ways to manage *J. vulgaris* (3, 6–8). Some of these focused

43 on chemical control measures (3), intensified fertilization (4, 9) or the introduction of

44 antagonists to reduce *J. vulgaris* density (10). While these measures can be very effective, their

45 negative side-effects precludes their widespread application, especially in non-intensively

46 managed species-rich grasslands (4, 11) or are more promising in the weed's non-native range

47 (6). Studies on alternative *J. vulgaris* management measures that are compatible with nature

48 conservation goals, are scarce (12).

49 Therefore, we tested the effects of cutting regimes at different phenological stages of *J. vulgaris*

50 and restoration measures to increase plant species diversity as well as a combination of both on

51 the development of *J. vulgaris* populations.

52 Studies of the effectiveness of cutting on *J. vulgaris* populations in grasslands are scarce (12)

53 and so far ambiguous in their findings. While 13 (2008) showed that frequent cutting decreases

54 *J. vulgaris* abundance and fertility, other studies find that the species can re-grow within a few

55 weeks after being damaged or may switch to vegetative reproduction forming multiple rosettes

56 (2, 14). Thus, slashing or mowing per se may not be sufficient for weed control (15).

57 In order to increase the effectiveness of cutting in suppressing *J. vulgaris*, the frequency and

58 timing of cutting is essential because the proportion of nutrients and energy invested in different

59 plant parts varies among life-stages (16, 17). As *J. vulgaris* grows back quickly after cutting

60 (14), a second cut may be necessary before the second bloom. While this cutting regime may

61 prevent generative reproduction, it may induce vegetative reproduction, clonal growth or a

3

62   switch to a perennial life cycle (1). Since *J. vulgaris* usually dies off after seed production (18),

63   cutting before seed dispersal may be another option that may not only prevent vegetative re-

64   growth but also seed dispersal.

65   In addition to an adapted cutting regime, sowing other species can suppress weeds (19).

66   According to the biotic resistance hypotheses (20), increased plant diversity may also increase

67   plant community resistance against future invasion (21). Several studies showed that the

68   invasion of problematic plant species can be prevented through increasing plant species

69   diversity (e.g. 22, 23).

70   If species enrichment proves an effective measure in weed control, it could be combined with

71   the re-establishment of species-rich grassland communities, which have declined dramatically

72   (24). Various techniques of species enrichment are commonly applied in restoration (25). While

73   seeding seed mixtures is frequently applied for the re-creation of grasslands (25), transferring

74   freshly cut, seed-containing biomass from species-rich grasslands (green hay) is another

75   effective method to restore grasslands (26). When green hay is transferred, disturbance to an

76   existing sward has been shown to enhance seedling establishment (27). Under high seed

77   pressure from *J. vulgaris*, however, sward disturbance will also enhance the weed's

78   establishment (9); thus, slot drilling and broadcast sowing without sward disturbance may be

79   promising techniques (28) to combine with the adapted cutting regimes to reduce establishment

80   and growth of *J. vulgaris*. Therefore, we also applied a combination of cutting regimes and

81   species-enrichment measures, where the cutting regime is thought to weaken *J. vulgaris*

82   individuals and the increased plant diversity reduces new establishment.

83   Contrary to deliberate action against *J. vulgaris*, there is indication that waiting for its natural

84   disappearance might be another promising management approach (14, 29, 30). 1 (1957), have

85   observed a hump-shaped population development of *J. vulgaris*, with a population boom

86   followed by sudden decline. In addition, Bezemer et al. (2006a) reported a natural decrease in

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87   *J. vulgaris*. Nonetheless, management to reduce *J. vulgaris* is essential to avoid threat to

88   domestic animals.

89   The objective of our study was to find the optimal treatment, timing, and frequency for

90   grassland management on non-intensive pastures that leads to a maximal weed reduction and a

91   minimal loss of co-occurring vegetation. We studied six different management options for five

92   years.

93   We addressed the following research questions:

94   1. Which treatment is most effective in reducing the abundance and population growth rate of

95   *J. vulgaris*?

96   2. What are the effects of the different treatments on the co-occurring grassland vegetation?

Material and Methods

98

Study species

100 *Jacobaea vulgaris* (Asteraceae; syn. *Senecio jacobaea* L.) is native to Eurasia, but invasive in

101 many other countries (1). Despite being regarded as a character species of mesophilic pastures

102 (31) in its native range, its poisonousness leads to its rating as a noxious pasture weed

103 worldwide (3). Its occurrence is common in open disturbed sites, such as ruderal sites, fallow

104 land, and temperate non-intensively managed grasslands (1, 2).

105 *J. vulgaris* is a mostly monocarpic perennial herb (Fig. 2). After seed germination in late

106 summer or autumn, the rosette overwinters and flowers the next year, with a flowering peak at

107 the end of July. Flowering can be delayed if site conditions are not optimal or if first flowers

108 are damaged by herbivory or cutting (14).

109 *J. vulgaris* has several biological traits supporting its spread and hampering its regulation. It

110 produces thousands of wind-dispersed seeds and grows quickly at suitable sites, making it a

111 typical pioneer species of ruderal or disturbed sites (1). Seeds show a high germination rate

5

112   (about 80 %) and are long-term persistent (32). The plant tolerates disturbance during different

113   life stages, e.g. an early cut of the inflorescence does not preclude post-ripening of vital seeds

114   (33) and disturbance or injury to the vegetation bud promotes regrowth through roots and crown

115   buds (3).

116

117   Study sites

118   In 2015, we began management experiments on seven pastures with presence of *J. vulgaris*.

119   Sites were situated in two geographical regions (Moraine, Hill land) in Northern Germany (see

120   Fig. 1, Table S1, Supporting Information) on sandy soils (34).

121

122   **Fig. 1** – Study area in Schleswig-Holstein, Northern Germany.

123

124   At least 5 out of 7 sites were formerly used as arable land. The current nature-conservation

125   grazing scheme applied by the managing nature conservation organization aims at maintaining

126   and promoting biodiversity (35). At all sites, *J. vulgaris* abundance was very high with on

127   average 60 ± 5 *J. vulgaris* plants per square meter (excluding seedlings) at the experiments start.

128   Study design

129   Six different grassland management schemes, two measures of milling and species enrichment,

130   two cutting treatments, and two combinations of seeding (as a measure of species-enrichment)

131   and cutting, against a control with no measures applied (Table 1), were assessed in a randomized

132   block design. On each of the seven study sites, each of the six treatments was applied within an

133   area of 90 m² (block). All study sites were under non-intensive grazing during the study. To

134   minimize edge effects, *J. vulgaris* and the co-occurring vegetation were assessed within three

135   permanently marked nine m² plots in the central part of each block.

136   Table 1 Treatments

Name	Description
Biodiversity 1	Milling and broadcast-seeding in 2015, then left untouched in following years

6

Biodiversity 2	Milling and green hay (26) spread over the ground in 2015, then left untouched in following years
Flower cut	Flowers cut before each bloom (end of June and beginning of August) in every year (2015-2019)
Seed cut	Flowers cut before seeds disperse (mid/end of July) in every year (2015-2019)
Combination 1	Mowing and drill-seeding in 2015, then left untouched in following years
Combination 2	Mowing and broadcast-seeding in first two years (2015-2016), mowing for whole study period (2015-2019 mid/end of July)
Control	No treatment, still grazed as all other treatments

137

138 In both *biodiversity* treatments, the plots were milled to a depth of 30 cm prior to seed addition

139 in autumn 2015. For the *biodiversity 1* treatment, 5 g/m² of the "fertile meadow mixture"

140 (Rieger-Hoffmann seed supplier) containing 20 species (30 % forbs 70 % grasses) commonly

141 found and collected in northeast Germany (see Table S2, Supporting Information) were sown

142 and rolled. For the *biodiversity 2* treatment, freshly cut plant material was transferred from

143 species-rich donor-sites nearby. Thereafter, cattle were fenced out of the seed-enriched area

144 some weeks to allow seedling establishment. For the *combination* treatments, 1.5 g/m² of the

145 seed mixture mentioned above (see Table S2, Supporting Information) was sown by slit drill

146 (*combination 1*) or by hand in two subsequent years (2015, 2016; *combination 2*) after mowing

147 the standing vegetation. Sowing took place in September and October 2015 and in October

148 2016 for *combination 2*. At the second sowing date the *combination 2* received some additional

149 seeds of other characteristic grassland species. Cuttings remained on the plots. Mowing took

150 place between June and August every year from 2015 to 2019. The *flower cut* treatment was

151 cut in late June and before the second blooming peaked. The *seed cut* treatment was cut before

152 seed dispersal in mid to late July. Cutting of the *combination 2* treatment in late July was

153 continued annually until the end of the experiment in July 2019.

154 ### Data sampling

155 Within the central one square meter of the permanently marked plots, the position of each *J.*

156 *vulgaris* individual were recorded every year between 2015 and 2019. Recorded *J. vulgaris*

157 individuals were assigned to one of three life stages: (i) seedling (S), (ii) rosettes (with one

158 single rosette (SR) or multiple rosettes (MR)), and (iii) generative plants (G) with flower stalks.

159 Altogether, 40 466 individuals for one or more transitions were monitored, summing up to

160 100 199 observations.

161 The initial species composition of the vegetation was recorded from June to August 2015. In

162 subsequent years, vegetation assessment according to the Braun-Blanquet scale (36) took place

163 between May and August. Red List status of recorded species was noted (37). Target species

164 were defined as character species for species-rich grasslands in Northern Germany according

165 to 38 (2019).

166

167 ### Transition matrix model and population dynamics

168 Based on the frequency distribution of recorded life stages, a 4x4-transition matrix (based on

169 S, SR, MR, and G) was constructed for each study site, treatment, and year (Fig 2). Each matrix

170 element (a_{ij}) was calculated from the number of individuals in stage *j* in year *t* that passed to

171 stage *i* in year *t* + 1, divided by the column total of stage *j* (39). Fecundity was determined by

172 dividing the number of generative plants (G) in year *t* by the number of seedlings (S) in the

173 following year *t* + 1 (40).

174

		Stage at time *t*			
Stage		**S**	**SR**	**MR**	**G**
at time	**S**	0	0	0	F_{14}
t + 1	**SR**	G_{21}	S_{22}	R_{23}	R_{24}
	MR	G_{31}	G_{32}	S_{33}	R_{34}

8

	G	0	G_{42}	G_{43}	S_{44}

175 **Fig. 2** – Life cycle graph for *J. vulgaris* and the corresponding transition matrix. Boxes indicate

176 life stages, arrows represent the possible transitions between them, and letters show the

177 connection between each transition and its matrix entry (F, fecundity; G, growth; S, stasis).

178

179 The population growth rate was calculated by pooling all plants of the single life stages for all

180 study sites for each treatment and year. A 95 % confidence interval was established by

181 bootstrapping the data (5 000 iterations) for each treatment and year (39) in R (41). For fertility

182 values, we used mean fertilities per treatment and year.

183 Life-table response experiments (LTREs) were conducted using matrices based on vital rates

184 to analyze the contribution of different vital rates to the difference in the population growth rate

185 ($\Delta\lambda$) between each treatment and the control (39). Each matrix element is a product of the lower-

186 level vital rates: survival (σ_j), stasis ($\gamma_{i=j}$), growth ($\gamma_{i>j}$), retrogression ($\gamma_{i<j}$), and reproduction

187 (Φ_{ij}) (42). All analyses were performed with the program POPTOOLS version 3.2 (43).

188

189 ## Statistical Analysis

190 Population growth was analyzed using mixed effect models (44, 45). The first model included

191 a pseudo factor as a fixed factor. This pseudo factor consisted of the actual factors *treatment*

192 and *year*. This was necessary because the actual factors treatment and year are not orthogonal

193 for population growth. The second model included the fixed factors *treatment* and *year* without

194 an interaction effect. The two models were compared to test for significance of the interactive

195 effect. As the interactive effect was not significant, the second model with *study site* as an

196 additional random factor was used for further analyses. Residuals were visually checked for

197 normality and heteroscedasticity. Based on this model, a Pseudo R^2 was calculated (46) and an

198 analysis of variances (ANOVA) conducted, followed by multiple contrast tests (e.g., see 47) in

9

199   order to compare the influence of factors levels of *treatment* and *year*, respectively. To test if

200   treatments differed from the control, we used a Dunnett test.

201   *Numbers of plants and their life stages* in response to *year* were studied using analysis of

202   variances (ANOVA) for each treatment. For each study site, average numbers of plants per

203   square-meter from the three plots per block were used. To identify differences between the

204   treatments, years, and life stages, Tukey's HSD (honestly significant difference) post hoc test

205   was applied.

206   To examine species diversity, we calculated species richness and evenness (48). To investigate

207   whether species composition fluctuated more strongly in the treatments compared to the control

208   plots, the temporal species turnover rate for each population and treatment was calculated as

209    $(NR + D)/(n_t + n_{t+1})$ (49). NR denotes the number of species per plot that were newly recorded

210   in year $t + 1$ but did not occur on the plot in year t. D is the number of species that disappeared

211   during the transition from year t to year $t + 1$. n_t and n_{t+1} denote the species numbers in year t

212   and $t + 1$, respectively.

213   All statistical analyses were calculated using the statistical software R (41).

214

Results

Population dynamics of *J. vulgaris*

Abundance and life stages

218   The abundance of *J. vulgaris* individuals did not differ significantly between treatments;

219   however, a trend was identified (ANOVA $F_{6, 204} = 1.955$ P = 0.073). Year had a significant

220   effect on *J. vulgaris* abundance ($F_{4, 204} = 6.6478$, P < 0.0001).

221   The seven treatments led to three distinct response patterns (Fig. 3). Both *biodiversity*

222   treatments showed an initial decline in *J. vulgaris* abundance but a rebound after the fourth

223   year. The *combination* treatments, the *seed cut* treatment, and the control showed an oscillating

224   pattern with falling and rising *J. vulgaris* abundance every other year. Under all treatments,

225   *J. vulgaris* abundance was on average lower in the fourth year of the study compared to the first

10

226 year. Similarly, the abundance of rosettes and flowering plants was slightly lower at the end of

227 the study in 2019 compared to its start in 2015 under all treatments (52 ± 8 in 2019 vs. 60 ± 5

228 in 2015). Only the *flower cut* treatment led to a continuous decrease in *J. vulgaris* abundance,

229 while statistically this was only a trend ($F_{4,30}$= 2.558, P = 0.059).

230

231 **Fig. 3 Abundance of *J. vulgaris* plants per one squaremeter according to treatment and**

232 **year.** In the box plots, middle lines represent the median, boxes represent the first and third

233 quartiles, lower and upper bars represent the minimum and the maximum, and points represent

234 outliers (i.e. points above 1.5 SD). There were no significant differences between treatments

235 (TukeyHSD, P ≤ 0.05).

236

237 Fluctuations in *J. vulgaris* abundance in non-milled sites between years were mainly driven by

238 differences in the numbers of vegetative plants, while the number of flowering plants showed

239 an almost continuous decrease during the study period (see Fig. 4; ANOVA $F_{4,65}$= 10.46, P <

240 0.0001). This decrease was significant within *biodiversity* treatments, *seed cut*, and *combination*

241 *1* treatments.

242

243 **Fig. 4 Abundance of flowering *J. vulgaris* plants per one squaremeter according to**

244 **treatment and year.** In the box plots, middle lines represent the median, boxes represent the

245 first and third quartiles, lower and upper bars represent the minimum and the maximum, and

246 points represent outliers (i.e. points above 1.5 SD). Different letters indicate significant

247 differences between the years within the treatments (TukeyHSD, P ≤ 0.05).

248

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249   Population growth and Life-Table-Response-Experiments

250   Population growth rates did not differ significantly between treatments ($F_{6,153}$ = 1.541, P = 0.

251   168). For the *flower cut* treatment, the population growth rate was significantly below one

252   throughout the study period, indicating a constant decline in population size (Fig. 5). All other

253   treatments did not lead to a constant decline in population sizes but showed considerable

254   variation between years. Interestingly, years with relatively high population growth rates were

255   followed by years with lower population growth rates, which led to a significant year effect on

256   λ (ANOVA $F_{3, 158}$ = 15.35064, P < 0.0001). The substantial population growth setback in 2018

257   (year 17-18) was prominent.

258   ANOVA of our mixed model including *treatment* and *year* as main factors and *study site* as a

259   random factor revealed that *year* rather than *treatment*, mainly influenced the survival and

260   population growth rates of *J. vulgaris*. In the dry year of 2018, density and abundance decreased

261   but increased even more in the subsequent moist year 2019. Consequently, there was a positive

262   linear relationship between the amount of rainfall and the abundance of seedlings found in the

263   study plots (regression *y =- 55.5360 + 1.0612*N*, F_1 = 17.57, P < 0.0001).

264

265   **Fig. 5 Population growth rates for treatments according to years.** Shown are the means ±

266   95 % CI. Treatments did not differ significantly. Constant population is marked with the line

267   λ = 1.

268

269   The LTRE analysis showed that differences in the population growth rate (Δλ) between each

270   treatment and the control were mainly the result of differences in generative reproduction and

271   survival (Fig. 6). The analysis also demonstrates that the influence of vital rates on population

272   growth in the *flower cut* treatment is very different from all other treatments. Here stasis and

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273   retrogression have a considerable impact on positive population growth, as these vital rates are

274   much higher than in the control.

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276

277 **Fig. 6**. Contribution of vital rates to the difference in population growth rate (Δλ) between the control and other treatments (bio I, *biodiversity 1*; bio

278 II, *biodiversity 2*; cutflower, *flower cut* treatment; combi I, *combination* treatment 1; combi II, *combination* 2; cutseed, *seed cut* treatment) in the

279 pooled *J. vulgaris* population, as determined by LTRE (life-table response experiment) analysis. Bar sections above zero display the summed positive

280 contributions and bar sections below zero the summed negative contributions.

281   The elasticity analysis revealed that three main transitions are crucial for population growth: i)

282   the transition from seedling to single rosette (G_{21}), ii) from single rosette to flowering plant

283   (G_{24}), and iii) from flowering plant to seedling (F_{14}). The importance of these transitions did

284   not differ between treatment or year. All transitions contributed equally to population growth.

285

286   Changes in vegetation composition

287   The overall mean number of vascular plants per one square meter was 23 ± 1 in 2015 and 29 ±

288   1 in 2019. Species richness differed between treatments (ANOVA $F_{6,210}$ = 3.204, P < 0.005).

289   The seed addition in *biodiversity* and *combination* treatments led to species enrichment. About

290   five more plant species were found on plots with seed addition (*biodiversity* and *combination*)

291   compared to control plots. In the last year of the experiment only species richness in the

292   *combination* treatments was significantly higher than in the control treatment. All management

293   measures but the control enhanced species richness (Fig. 7).

294

295   **Fig. 7 Species richness according to year and treatment.** For explanation of the boxplot,

296   refer to Figure 3. Different letters indicate significant differences (P ≤ 0.05; Tukey HSD)

297   between years within one treatment. Dashed lines indicate mean species richness in 2015 (lower

298   line) and 2019 (upper line).

299

300   Richness patterns varied over the study period (Fig. 7) and were significantly different between

301   treatments and years (ANOVA treatment $F_{6,168}$ = 3.893, P < 0.005; year $F_{3,168}$ = 3.813, P <

302   0.05). While in the first and second years after seed addition, the *biodiversity* treatments resulted

303   in the highest species richness, in 2018 and 2019 the *combination* treatments were the most

304   species rich. During all study years, the control treatment resulted in the lowest species richness,

305   also only significantly different from the *biodiversity* treatments. Both cutting regimes had

306   slightly but not significantly higher species richness compared to the control. Throughout the

15

307   study period, species evenness was highest under treatments that included mowing (*flower* and

308   *seed cut*, *combination 2*).

309   The number of red-listed plant species differed significantly between treatments (ANOVA

310    $F_{6, 189} = 31.08$, $P < 0.0001$). Most endangered plants occurred in the *biodiversity 1* treatment

311   (mean: three endangered species per one square meter vs. zero or one species in the control).

312   Plants categorized as characteristic grassland species were most numerous in the *biodiversity 1*

313   treatment, followed by hay transfer (*biodiversity 2*) and *combination* treatments with mowing

314   and sowing (Fig. 8). *Biodiversity* treatments and the *combination 2* treatment led to significantly

315   higher numbers of endangered plants than in the control.

316

317   **Fig. 8 Percentage of value species per year and treatment.** For explanation of the boxplot,

318   refer to Figure 3. Different letters indicate significant differences ($P \leq 0.05$; Tukey HSD)

319   between years within one treatment.

320

321   Turnover rates of the vegetation differed significantly between treatments (ANOVA $F_{6, 140} =$

322   16.74, $P < 0.0001$) and were significantly highest under treatments with milling in combination

323   with seeding or plant material transfer, i.e. *biodiversity 1* and *2*, followed by *combination 2*,

324   which combined to sowing impulses and mowing (Fig. S3, Supporting Information). Turnover

325   rates of pure cutting treatments, *combination 1*, and the control were significantly lower and

326   did not differ from each other. The percentage of target species contributing to the turnover rate

327   was highest for *biodiversity 1* (37 ± 0.02 %) and *combination 2* (35 ± 0.03 %) and lower for

328   *combination 1* (29 ± 0.02 %) even though these differences were not significant.

329

16

Discussion

Effects of regulation measures on *J. vulgaris*

The *biodiversity* treatments led to a drastic decrease in *J. vulgaris* abundance in the first two to three years (Fig. 3). The decrease was especially pronounced, significant, and lasting in the *biodiversity 1* treatment for the flowering stage (Fig. 4). Although the general abundance decline in the *biodiversity* treatments after the first years was not significant, the results concur with previous findings that *J. vulgaris* densities decline as a result of sowing grassland species into the sward after soil disturbance (50, 51).

Declines in *J. vulgaris* abundance following milling, seem counterintuitive since establishment of *J. vulgaris* is favored by soil disturbance (9). Consequently, soil disturbance was combined with sowing grassland species. Lawson et al. (2004) revealed a reduction in *J. vulgaris* densities in the first year after ploughing and sowing grassland species. Similarly, 50 (2006) reported reduced *J. vulgaris* abundance on those plots that were sown with grassland species compared to control plots without seed addition over a period of eight years.

According to the biotic resistance theory (Levine et al. 2004), invasibility in species-rich systems should be lower than in species poor ones. While this might be true in the long run, at shorter temporal scales, species-rich plant communities may also be invaded if open soil patches remain that favor the establishment of light-demanding pioneers such as *J. vulgaris* (14). Thus, we cannot confirm the results of Bezemer et al. (2006) and Lawson et al. (2004) that *J. vulgaris* density declines after ploughing and sowing as we did not find lower *J. vulgaris* numbers in sown plots compared to untreated plots in the four years after milling and sowing took place. We assume that this is due to differences in the initial setting of the study by Bezemer et al. (2006) and our study. While in our study, grazed grassland was the control baseline, i.e. cattle caused continuous small-scale disturbances throughout the study, Bezemer et al. (2006) began with an un-vegetated control, which was mown once a year. Repopulation of the early successional *J. vulgaris* might not have been prevented as around 33% higher bare ground cover

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356   compared to control plots (Fig. S4, Supporting Information) offered enough establishment

357   opportunities for the weed.

358   The LTRE analysis showed that the population growth on milled sites was due to an increased

359   generative reproduction (Fig. 5). The increase in *J. vulgaris* might also have occurred due to

360   changes in soil biota after milling. While soil biota are thought to suppress *J. vulgaris* growth

361   over time (50, 52), soil disturbance might set soil biota back to an earlier developmental state,

362   where negative effects on *J. vulgaris* are less pronounced. 53 (2006) reported increased growth

363   of invaders in tilled soil due to changes in the soil community, which supports this assumption.

364   Besides milling, mowing is a commonly used tool to control noxious weeds (19). It can prevent

365   seed production, reduce carbohydrate reserves, and give advantages to desirable perennial

366   grasses. Our results show that the success of mowing depends on timing and frequency. While

367   mowing once before seed dispersal failed to reduce the population growth rate or abundance of

368   *J. vulgaris*, mowing twice shortly before the bloom resulted in a growth rate significantly and

369   constantly below one and constantly decreased *J. vulgaris* abundance. This concurs with the

370   finding that the optimal time for mowing noxious weeds is the flowering stage before seed

371   development (19). This too is true for *J. vulgaris*, as the highest proportion of nutrients and

372   energy is channeled into aboveground organs during bloom (Otzen 1977).

373   According to the pattern of population growth in the *flower cut* treatment, the LTRE analysis

374   showed that the lower population growth rate in *flower cut* compared to the *control* treatment

375   was mainly caused by the suppression of generative reproduction, the most important means of

376   mass colonization for pioneer plants (1).

377   Additionally, repeated mowing leads to a denser sward and thus less open soil patches, which

378   prevents emergence of *J. vulgaris* seedlings. Furthermore, we found that continuous mowing

379   twice a year for more than two years is needed to drive down *J. vulgaris* abundance. Otherwise,

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380 high numbers of plants either remaining in their life stage or becoming rosettes so that prolific

381 blooming will only be postponed.

382 In accordance with our results, 13 (2008) found that cutting *J. vulgaris* early and at least two

383 times significantly weakens the plant. The LTRE analysis showed that impeding the fertility

384 transition, i.e. the production of seeds, was the decisive property of the *flower cut* treatment

385 causing r *J. vulgaris* to decline, particularly in 2017, two years after the treatment began. The

386 need to mow repeatedly over several years for successful weed control is also mirrored in the

387 estimated survival rates in the *flower cut* treatment.

388 The LTRE-analysis showed that survival of *J. vulgaris* plants in the *flower cut* treatment in

389 2016/17 was higher than in the control. This is in accordance with findings of 54 (1989), who

390 found that plants defoliated by the butterfly *Tyria jacobaeae* die less than those setting seeds

391 probably due to higher resource investments involved in seed production than in regrowth.

392 Cumulative effects of constant mowing regimes were also found by 55 (2014). Increased

393 retrogression and stasis of plants that are ready to bloom in the next year reflects this mechanism

394 in our LTRE analysis.

395 Finding the optimal cutting date after which *J. vulgaris* will not bloom again and produce vital

396 seeds is difficult. Contrary to the effects of repeated cutting (*flower cut*), cutting once before

397 seeds disperse did not influence population dynamics or *J. vulgaris* abundance. Even though

398 more flowering plants completed their lifecycle and died, new seedlings from post-ripened

399 seeds compensated this mortality. In *J. vulgaris*, the first germinable seeds occur with the first

400 withered capitula (33). Eisele suggest cutting *J. vulgaris*, when 10 % of the capitula begin to

401 flower within half of the generative plants. However, under field conditions where the

402 phenology of *J. vulgaris* is not synchronous, this stage is rather difficult to assess and is close

403 to the emergence of the first germinable seeds. If cut too early however, enough resources

404 remain for vegetative reproduction (56).

19

405   Rosettes and seedlings constituted the largest proportion of *J. vulgaris* plants, which is in

406   accordance with 13 (2008), who also found that 50% of *J. vulgaris* populations are made up of

407   rosettes. Yet, *J. vulgaris* population structure and growth rates were highly variable across

408   years, such that year had a stronger effect on population growth than management. We observed

409   an oscillating pattern with falling and rising *J. vulgaris* abundance every other year in *seed cut*,

410   *combination*, and *control* treatments (Fig. 3). Extreme weather conditions, such as

411   exceptionally hot or dry summers, affect population growth through immediate effects on vital

412   rates (57, 58). The low rainfall in 2018 caused a decline in the population growth rate from

413   2017 to 2018 and a density decrease at all sites under all treatments. In accordance with our

414   general observation of increased seedling numbers when rainfall was higher, we found that the

415   population growth rate from 2018 to 2019 increased again after increased precipitation in 2019

416   (Fig. 5). *J. vulgaris* populations probably profited from more light on the ground from 2018 to

417   2019 because the sward was less dense after the drought of 2018. 54 (1989) found that plant

418   recruitment was microsite limited and depended very much on weather and activity of rabbits,

419   boars, and moles, i.e. enough light and water for germination (14). In our experiment, trampling

420   and grazing from cattle can create windows of opportunity for *J. vulgaris* establishment (59).

421   Our analysis showed that all transitions from seedling to rosette, from rosette to flowering plant,

422   and from flowering plant to seedling contribute equally to population growth rate. This is in

423   accordance with 60 (2012), who found that transitions from rosette to flowering plant and from

424   flowering plant to rosette were especially important for population growth.

425

426

427   ### Development of species richness and vegetation composition

428   Botanical richness in our study either remained constant or increased. Thus, none of the

429   measures applied to regulate *J. vulgaris* counteracted the goal of preserving species richness.

430   Highest species richness occurred in the combination treatment of seeding and cutting

431   (*combination 2*), where two sowing impulses with slightly different seed mixtures were set and

20

432   resident species and newly sown species coexisted, whereas species diversity in *control*

433   treatments stayed lowest. *Combination 2* also reached high evenness-indices, as did the *flower*

434   *cut* treatment. In general, evenness was significantly higher under mowing regimes (*flower cut*,

435   *seed cut*, and *combination 2*), in which more species were able to establish due to reduced

436   competition by dominant grasses (61). Diversity patterns of the treatments changed over time.

437   *Biodiversity* treatments were most species rich in the first two years after milling and seeding

438   or green hay transfer. However, species numbers and cover of target species in *biodiversity*

439   treatments declined after the third year (Fig. 7 - 8). Other studies also showed that initially high

440   species numbers in sown treatments decreased after some years (50, 62, 63). One reason for

441   this decrease is that ruderal species and former arable weeds occur initially after milling and

442   vanish later on (27, 64). Therefore, species turnover was highest in mill-sow treatments

443   (*biodiversity* treatments *1* and *2*) and seed-addition treatments (*combination* treatments *1* and

444   *2*), whereas turnover in the pure cutting treatments (*flower* and *seed cut*) did not differ from the

445   control.

446   Species turnover in *biodiversity* and *combination* treatments led to a higher share of

447   characteristic grassland species. Percentage of characteristic grassland species was highest in

448   *biodiversity* and *combination* treatments (Fig. 8). As numerous other studies showed, without

449   bridging dispersal limitation by actively introducing seeds, it is rather unlikely that species-rich

450   grasslands will develop on former arable land (65; 66). This is due to the disappearance of

451   characteristic grassland species in intensively used agricultural landscapes (67). This is

452   especially problematic within modern agricultural landscapes in Northern Germany, which are

453   characterized by severe habitat fragmentation and biodiversity losses in grasslands (68).

454   The percentage of characteristic grassland species in *biodiversity* treatments was approximately

455   40%, which was 10 % higher than in treatments without seed addition. While the number of

456   species in the *biodiversity* treatments decreased again, the number of species in the *combination*

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457 *2* treatment increased constantly over the study period and exceeded the number of species in

458 the *biodiversity* treatments. This increase in species richness may have been supported by

459 sowing different grassland species twice, which lowers the risk that establishment fails due to

460 unfavorable germination conditions when plants are sown in one year only. Besides the positive

461 effect of two sowing applications, the establishment of higher species numbers in the

462 *combination 2* treatment might have been supported by the yearly cut, which is known to favor

463 the occurrence of forbs over grasses (69). In the *control*, dominance of grasses and occurrence

464 of ruderal species was higher than in all other treatments. This suggests that residual soil fertility

465 on ex-arable land may reduce the survival of characteristic grassland species (70).

466 Most promising in promoting threatened plants is milling and sowing (*biodiversity 1*), probably

467 because germination conditions for seeds in milled plots are better than in non-milled plots. In

468 the latter, the accumulation of living and dead biomass and the encroachment of competitor

469 species can hinder germination and establishment (64, 71). However, there is also a noticeable

470 effect of seed addition by hand – probably because trampling of cattle can result in relatively

471 large bare patches, potentially increasing establishment opportunities for sown seeds (72).

472 Various studies (73, 74) showed that gap size in the sward is an important element of successive

473 seed introduction.

474

Conclusion

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477 Slight *J. vulgaris* decline occurred under all treatments and consequently we found no

478 significant differences in *J. vulgaris* abundance between the applied treatments and the control.

479 Even the most effective treatment, the *flower cut* treatment, which was significant by trend and

480 the only treatment resulting in a population growth rate below one during the complete study

481 period, did not lead to a complete disappearance of *J. vulgaris* but allowed on average five

22

482   *J. vulgaris* plants per square meter to occur. Therefore, *J. vulgaris* management in nature

483   conservation grasslands needs patience and will not eradicate the weed.

484   We probably did not find clear differences between the treatments as *J. vulgaris* is a

485   successional plant adapted to disturbance and quickly reacts to windows of opportunity being

486   continuously created by grazing animals or because of drought. Moreover, as our treatments

487   were designed to minimize unwanted harmful impacts on grassland ecosystems, we refrained

488   from drastic measures. Nonetheless, our results reveal some promising approaches for

489   *J. vulgaris* regulation that do not jeopardize nature conservation efforts.

490   The *flower cut* treatment, which led to a constant decline in *J. vulgaris* resembles a traditional

491   non-intensive meadow management technique, which supports high plant species and animal

492   species richness. Still, the two cuts that were employed for this treatment may harm

493   invertebrates. These drawbacks must be weighed against the treatment's regulating effect on

494   *J. vulgaris*. This might restrict the application of this measure to sites with high conflict

495   potential.

496

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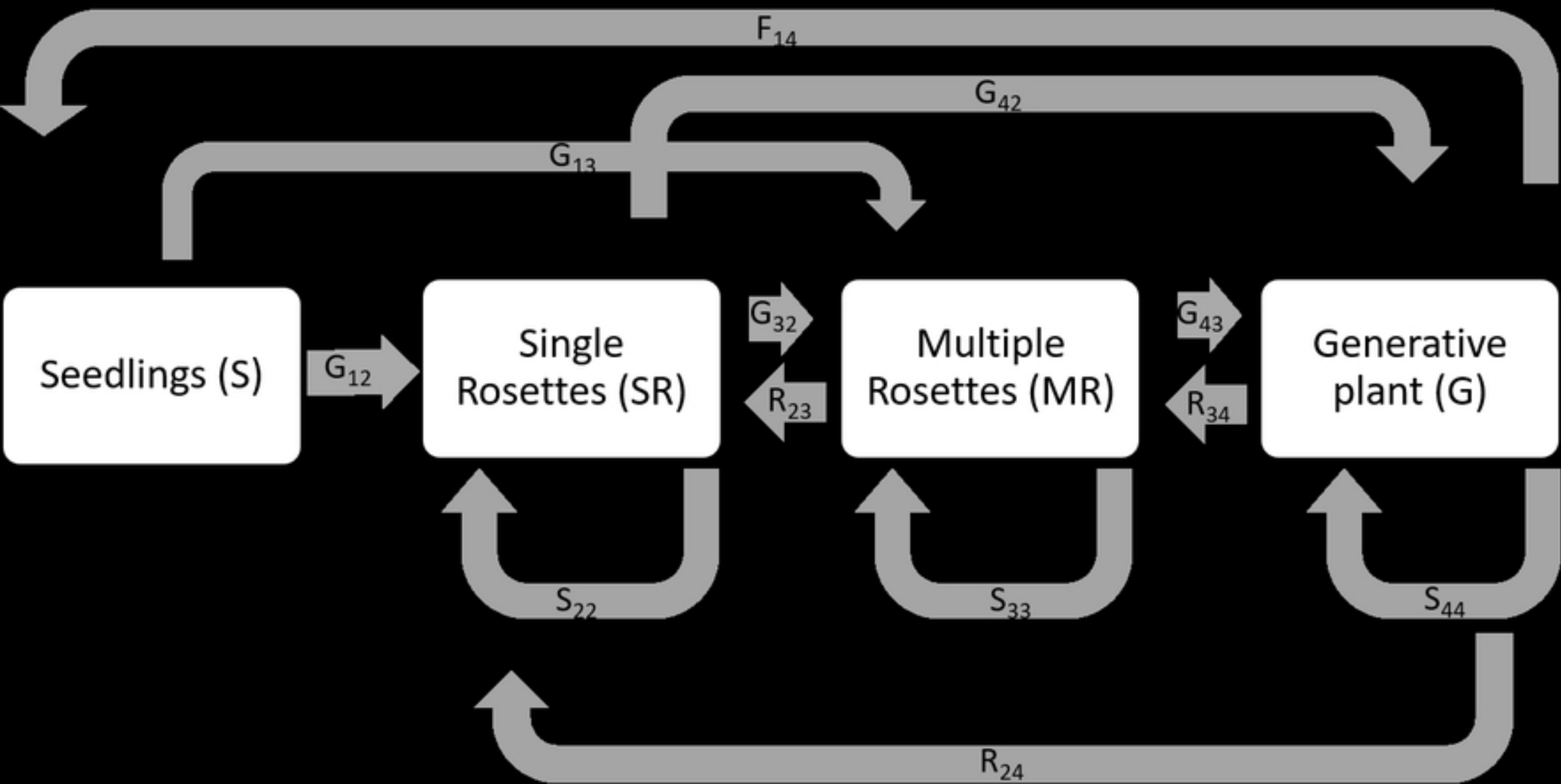550 TIKJTxYQZR9JIKXmrTLQt1b8iXDItQUnry81rHmEUF3l1h3bFy3gP9G53uATdw8.

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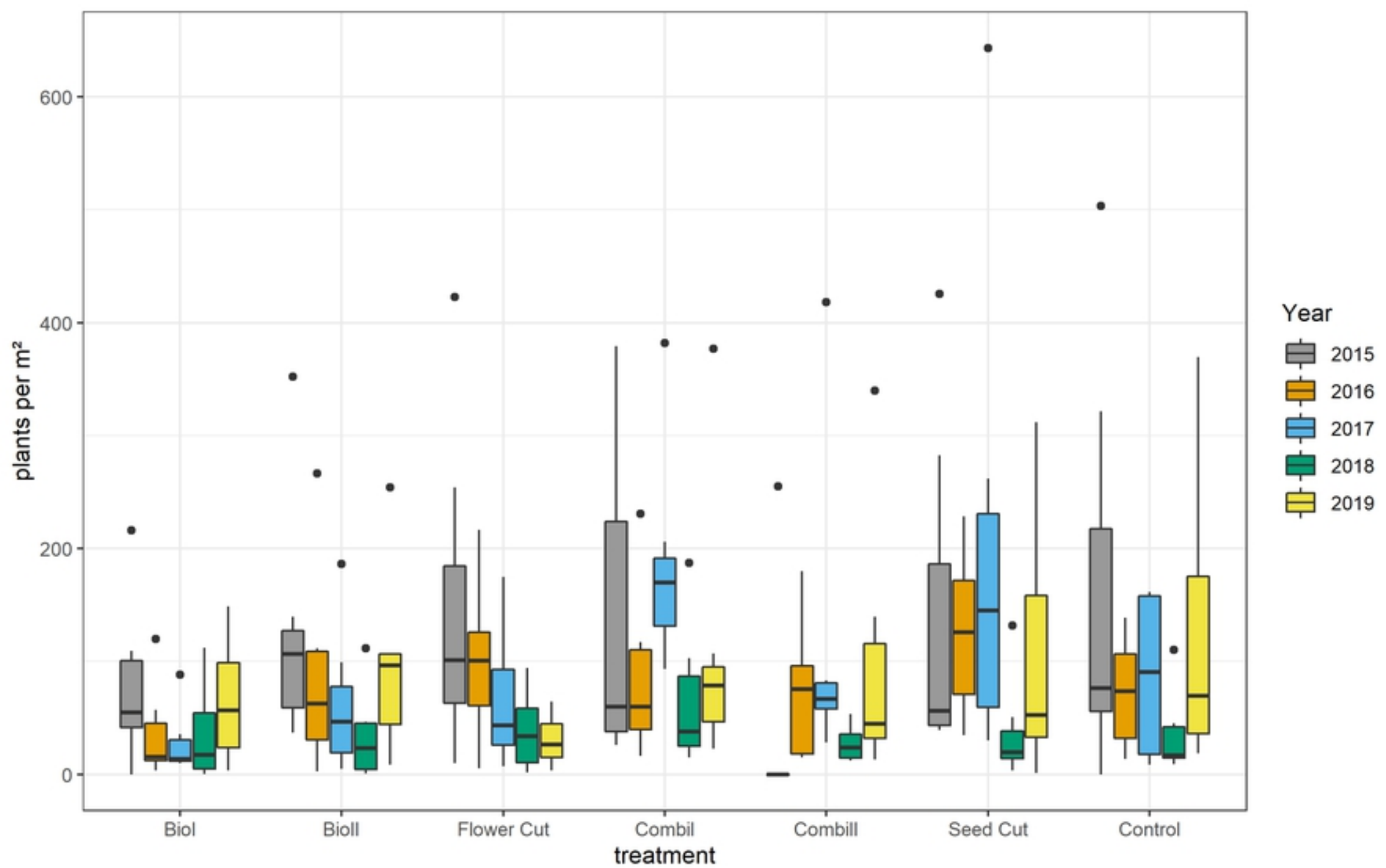631   49. Mühlenberg M. Freilandökologie. Heidelberg: Quelle & Meyer; 1989.

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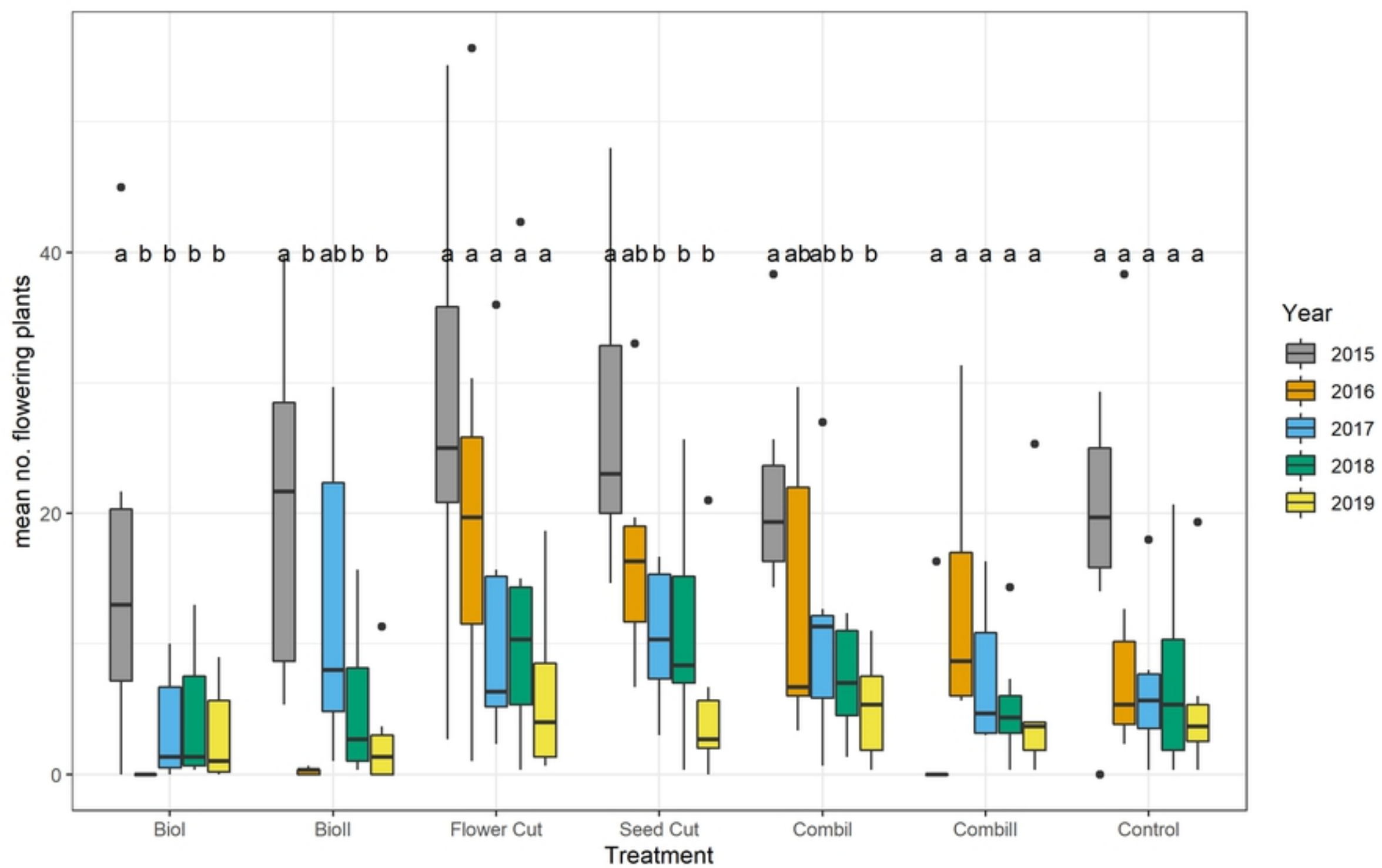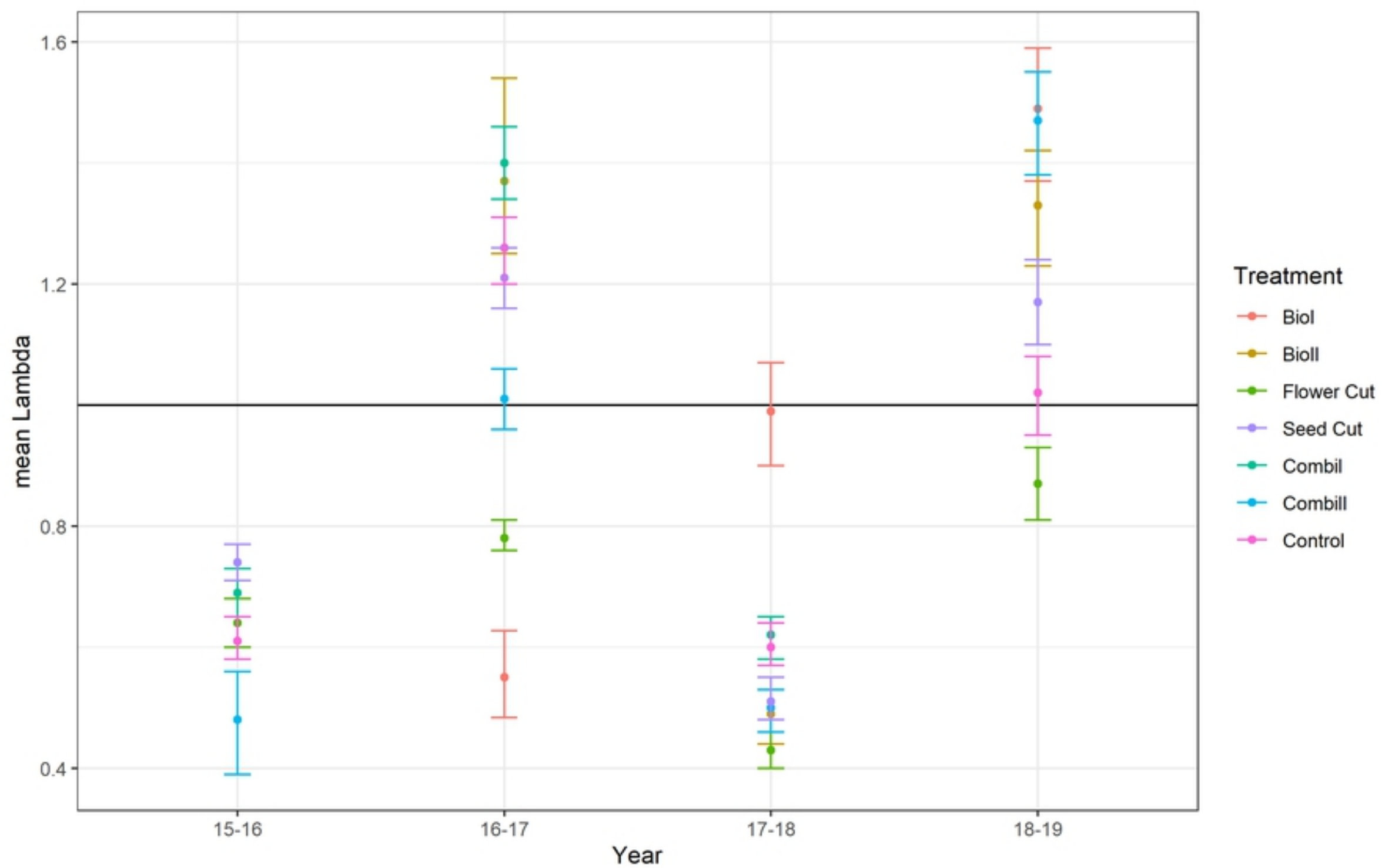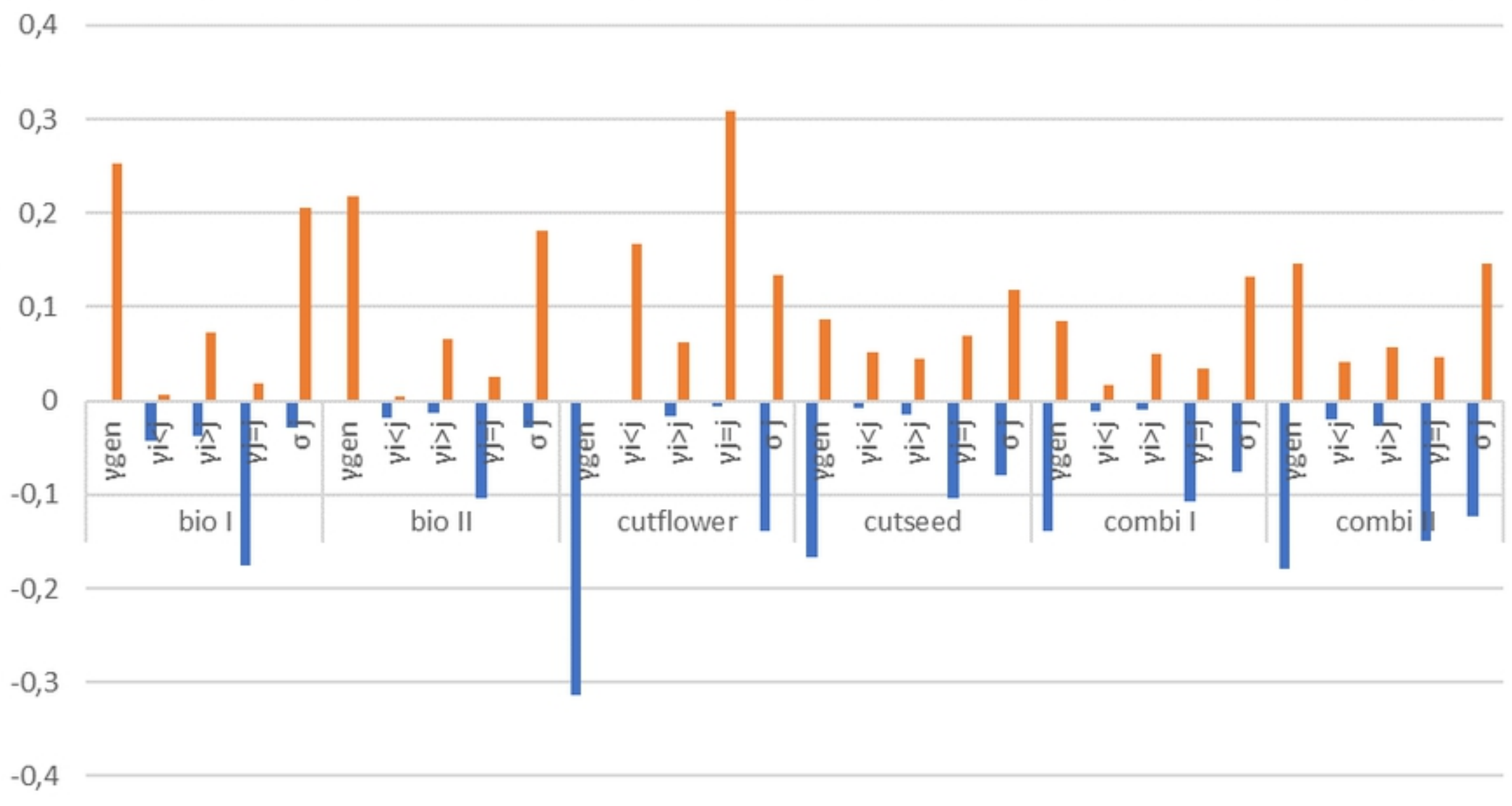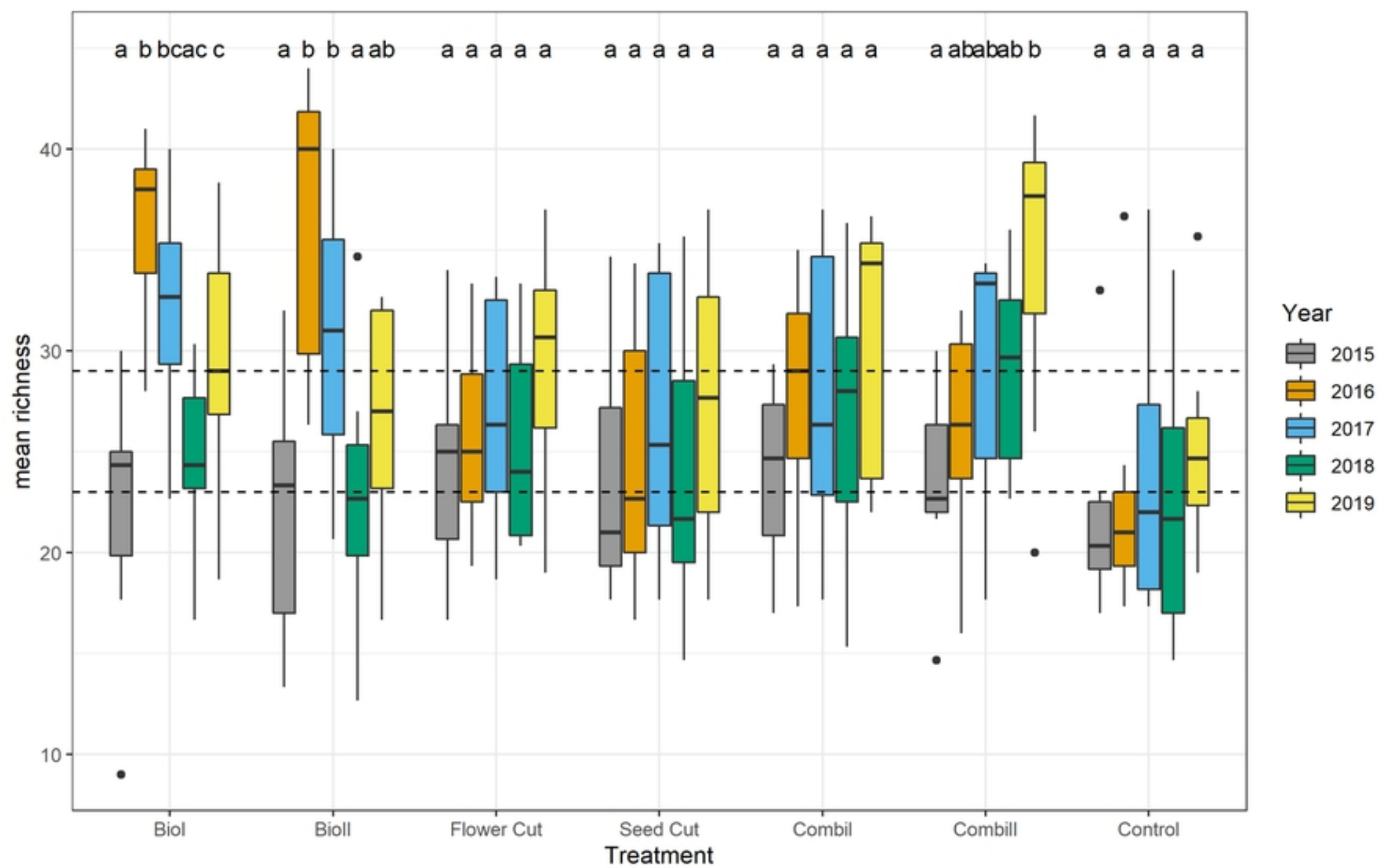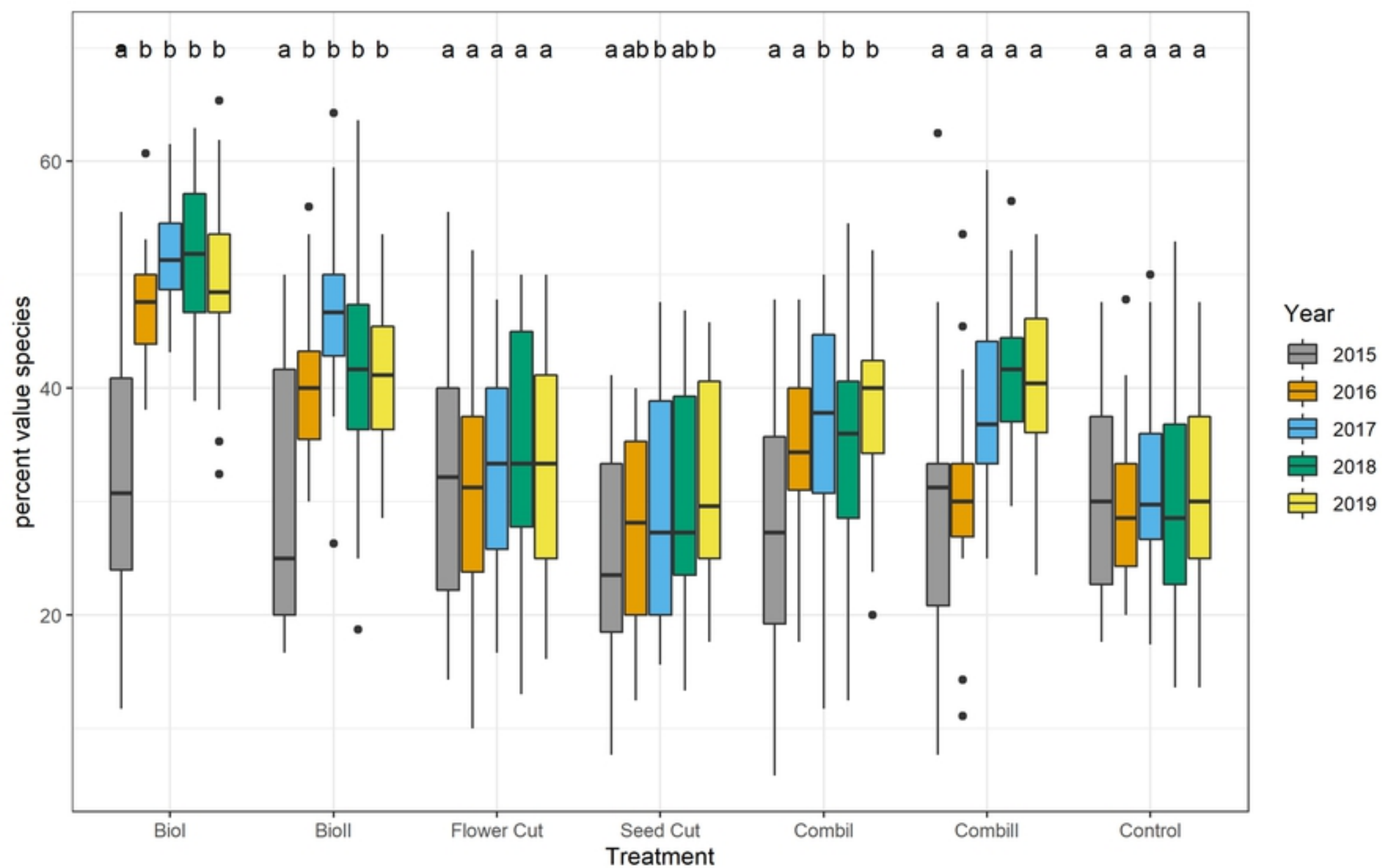
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