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1 **Altering native community assembly history influences the performance of**  
2 **an annual invader**

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12 **Abstract**

13           Understanding the determinants of early invasion resistance is a major challenge for designing plant  
14 communities that efficiently repel invaders. Recent evidence highlighted the significant role of priority effects in  
15 early community assembly as they affect species composition, structure and functional properties, but the  
16 consequences of native community assembly history on the success of subsequent invasions has not been  
17 elucidated yet. In a greenhouse experiment, we investigated how (1) the identity of the first native colonizing  
18 species (one of two grasses: *Dactylis glomerata* and *Lolium perenne*, or two legumes: *Onobrychis viciifolia* and  
19 *Trifolium repens*), each introduced four weeks before the rest of the native community, and (2) timing of species  
20 establishment (synchronous vs. sequential sowing), influenced early establishment success of *Ambrosia*  
21 *artemisiifolia*, an annual noxious weed in Europe. First colonizer identity and establishment timing both affected  
22 early biomass production and composition of the community, and had implications for *A. artemisiifolia* early  
23 invasion success. Invasion success decreased when all native individuals were sown simultaneously, quickly  
24 generating a high biomass production, while it increased when the productive N-fixing legume *T. repens* was sown  
25 first. These findings support that native species assembly history matters to invasion resistance in the early growth  
26 stages, thus opening the way to more effective invasive species management strategies in restoration.

27

28 **Keywords:** priority effects, historical contingencies, revegetation, restoration, competition, *Ambrosia*  
29 *artemisiifolia*

30

## 31 **Introduction**

32           An increasing number of invasive species is causing major detrimental effects on the environment  
33 (Brondizio et al., 2019), human well-being and economy (Charles & Dukes, 2008), and this increase is expected  
34 to intensify (Sala et al., 2000). Invasions are also often a serious impediment to the successful restoration of  
35 damaged environments (Norton, 2009; Rowe, 2010; Stromberg et al., 2007). Restorative activities, including soil  
36 disturbance and vegetation clearing, increase resource availability and decrease competition from neighbors, and  
37 can promote plant invasion (Cherwin et al., 2009; Davis et al., 2000; Jauni et al., 2015; McIntyre & Lavorel, 1994).  
38 Susceptibility to invasion is expected to decrease over time as the restored community establishes and displays a  
39 more complete use of available space and resources (Vitousek & Walker, 1987). Reducing invasive species  
40 establishment success in the early, vulnerable stages of community growth is therefore critical. Restoring a rapidly  
41 competitive native plant cover has been advocated as a method to reduce invasions (Byun & Lee, 2017; Larson et  
42 al., 2013; Middleton et al., 2010; Yannelli, 2021). However, the mechanisms generating rapid invasion resistance  
43 are still poorly known.

44           Recent advances in community ecology have highlighted the defining role of historical contingencies  
45 (i.e., the effect of the order and timing of past events, being either abiotic or biotic) in community assembly and  
46 invasibility (Fukami, 2015; Švamberková et al., 2019; Werner et al., 2016; Young et al., 2016). Priority effects,  
47 the ability of an early-arriving species to either inhibit or facilitate the establishment, growth or reproductive  
48 success of species arriving later (Drake, 1991; Helsen et al., 2016), have recently received particular attention.  
49 Although priority effects often do not systematically generate changes persisting in the long term (Collinge & Ray,  
50 2009; Young et al., 2016), numerous studies showed that even small differences in species arrival can induce  
51 dramatic changes in composition, structure and functional properties (e.g., productivity) at least for one growing  
52 season (e.g., Delory et al., 2019a; Grman & Suding, 2010; Martin & Wilsey, 2012; Plückers et al., 2013; Sarneel  
53 et al., 2016; Stevens & Fehmi, 2011; Stuble & Young, 2020; Vaughn & Young, 2015; Weidlich et al., 2018;  
54 Werner et al., 2016). Priority effects could therefore affect early invasion resistance (Lang et al., 2017; Stevens &  
55 Fehmi, 2011; Vaughn & Young, 2015) and could be manipulated to design invasion resistant restored communities  
56 (Hess et al., 2019). In the early stages of community development, we expect priority effects to be particularly  
57 impactful by influencing (1) biomass production, since higher biomass production reflects higher competitive  
58 ability (Gaudet & Keddy, 1988) and is associated with lower invasibility (e.g., Byun & Lee, 2017; Hess et al.,  
59 2020; Lulow, 2006; Weigelt et al., 2002) and/or (2) species composition (the identity of the dominant specie or  
60 functional group; e.g., Byun et al., 2013; Fargione & Tilman, 2005; Mason et al., 2017; Symstad, 2000).

61 In the context of invasions, priority effects have usually been examined in terms of the consequences for  
62 invasive species to arrive before or after natives (Delory et al., 2019b; Grman & Suding, 2010; Lang et al., 2017;  
63 Stevens & Fehmi, 2011; Stuble & Souza, 2016; Vaughn & Young, 2015). Studies investigating how differences  
64 in native assembly history affect subsequent invasion events are scarcer (Mason et al., 2013). In a greenhouse  
65 experiment, we established native perennial communities differing by (1) the identity of the first colonizer (either  
66 of two grasses: *Dactylis glomerata* and *Lolium perenne*, or two legumes: *Onobrychis viciifolia* and *Trifolium*  
67 *repens*), and (2) timing of species establishment (synchronous vs. sequential sowing), in which we subsequently  
68 simulated invasion by introducing seeds of *Ambrosia artemisiifolia*, a noxious weed in Europe (Ozaslan et al.,  
69 2016). We sought to examine whether these differences in community assembly influence the success of  
70 subsequent invasion, and if so, assess whether invasion success is related to variations in biomass production  
71 and/or community composition.

## 72 **Materials and methods**

### 73 *Native species selection*

74 Six perennial plant species widely used for revegetation in France were selected to compose the recipient  
75 native communities: *Dactylis glomerata* (Poaceae), *Lolium perenne* (Poaceae), *Onobrychis viciifolia* (Fabaceae),  
76 *Trifolium repens* (Fabaceae), *Plantago lanceolata* (Plantaginaceae), and *Poterium sanguisorba* (Rosaceae).  
77 Commercial seeds were purchased from seed suppliers ZYGENE and SCHEIER France.

### 78 *Invasive species seed collection and stratification*

79 The common ragweed (*Ambrosia artemisiifolia* L., Asteraceae) is an annual plant native from North  
80 America (Heckel, 1906) successfully invading disturbed areas such as roadsides, riverbanks, abandoned and  
81 cultivated fields in numerous European countries (Smith et al., 2013). The species can produce up to 14,000  
82 achenes per plant, which are mainly dispersed by human activities (Bassett & Crompton, 1975). Achenes from *A.*  
83 *artemisiifolia* were collected from at least ten individuals from each of three mature populations in South-eastern  
84 France in autumn 2018 and pooled (population 1: 43°33'4.5"N, 4°7'40.8"E; population 2: 43°31'2.2"N,  
85 5°19'56.2"E; population 3: 43°34'17.8"N, 4°17'8.8"E). Before starting the experiment, achenes were put between  
86 two layers of cotton soaked with distilled water and cold-stratified for 20 weeks (wet, dark stratification at 4 °C)  
87 in order to break primary dormancy. After stratification, we assessed the germination capacity of 50 seeds placed  
88 in five Petri dishes on cotton soaked in distilled water. Petri dishes were placed in optimum germination conditions

89 (25 °C/12 h day and 15 °C/12 h night; Fumanal, 2007), and germination was recorded every two days until no  
90 additional germination was recorded. After ten days, the final germination rate was of 98.0%±2.0 (mean±SE).

### 91 *Study site and infrastructure*

92 The experiment was conducted over six months (early March 2019 until early September 2019) in a  
93 greenhouse at the Research Institute of Tour du Valat, France (43°30'N, 4°40'E, 1 m a.s.l.). Walls and roof of the  
94 greenhouse are made of Diatex mesh (500-600 µm) so that similar ambient climate conditions occurred inside but  
95 seed dispersal was prevented. The site is subjected to a Mediterranean climate, characterized by warm and dry  
96 summers and mild, wet winters, with high variability between years (Lionello et al., 2006). Precipitation and air  
97 temperature data were recorded using a meteorological station located close to the experimental site (Appendix A:  
98 Fig. 1). We established the artificial plant communities in square plastic pots with an upper width of 30.5 cm and  
99 a bottom width 25 cm, 27 cm deep, which were filled with, from bottom to top (1) a 27 cm diameter polyester  
100 tissue (© Diatex, 50-70 µm mesh size) to prevent loss of substrate, (2) a 20 cm width × 10 cm deep polystyrene  
101 bloc allowing water to flow at the sides, and (3) a substrate mixture of vermiculite (30 vol%; © Projar, 2.6 kg/m<sup>3</sup>)  
102 and commercial organic fertile topsoil (70 vol%; © Géolia). Pots were watered with equal amounts of water  
103 through sprinklers. The amount of supplied water was regularly adjusted to ensure moisture conditions suitable to  
104 germination and plant development. We ended the experiment in early September to avoid confounding effects of  
105 *A. artemisiifolia* natural senescence (Li et al., 2015).

### 106 *Experimental design*

107 We designed six types of recipient plant communities differing by the identity of the first species  
108 established, but all getting all six species eventually (Fig. 1). We ensured an identical final spatial arrangement of  
109 the communities by using cardboard patterns with holes, each hole corresponding to a seeding location. On 6  
110 March, we created four priority treatments by introducing 18 seeds of either (1) *D. glomerata* (treatment name  
111 *Dactylis<sub>f</sub>*, with “f” standing for “first”), (2) *L. perenne* (*Lolium<sub>f</sub>*), (3) *O. viciifolia* (*Onobrychis<sub>f</sub>*), or (4) *T. repens*  
112 (*Trifolium<sub>f</sub>*) per pot. We also introduced (5) three seeds of each of the six species per pot, for the same number of  
113 initial seeds as the priority treatments (*Synchronous<sub>seq</sub>*) or (6) 18 seeds of each of the six species per pot, which  
114 corresponds to the simultaneous sowing of all individuals at full density (*Synchronous*). Ungerminated seeds were  
115 regularly replaced by individuals sown in separate pots on 6 March to ensure similar age and density of individuals.  
116 After four weeks (i.e., on 3 April), we carried out a second sowing (except for *Synchronous*) by adding the rest of  
117 the species so that each pot contained 18 individuals of each of the six species, amounting a total of 108 seeds

118 (corresponding to 1200 seeds/m<sup>2</sup>). *Plantago lanceolata* and *P. sanguisorba* were added to the four priority-tested  
119 species in order to create communities that were more diverse and to reach the desired density of individuals while  
120 keeping an identical spatial pattern. Therefore, Synchronous<sub>seq</sub> had the same density and sowing timing as  
121 *Dactylis*<sub>f</sub>, *Lolium*<sub>f</sub>, *Onobrychis*<sub>f</sub> and *Trifolium*<sub>f</sub>, but did not give priority to any particular species. This assembly  
122 type tested the role of sowing density in itself on invasion resistance. Ungerminated seeds were regularly replaced  
123 by individuals sown in separate pots on 3 April.

124 On 18 April, six weeks after the first sowing event and two weeks after the second sowing event, we  
125 introduced in each pot nine seeds of *A. artemisiifolia* at fixed positions, 8 cm apart from each other (Fig. 1). There  
126 were 12 replicates of each assembly type (*Dactylis*<sub>f</sub>, *Lolium*<sub>f</sub>, *Onobrychis*<sub>f</sub>, *Trifolium*<sub>f</sub>, Synchronous<sub>seq</sub> and  
127 Synchronous), totaling 72 pots. Pot distribution in the greenhouse followed a randomized design and pots were  
128 randomized every two weeks.

#### 129 *Data collection*

##### 130 Invasive species early establishment success

131 We assessed early establishment success of *A. artemisiifolia* by monitoring seedling emergence and  
132 survival visually each week from 30 April to 2 September and final aboveground biomass (2 September).  
133 Aboveground biomass of each individual was collected 0.5 cm above ground level, dried at 60 °C for 72 h and  
134 weighed.

##### 135 Recipient communities

136 We measured aboveground and belowground biomass of the communities three times during the  
137 experiment. For this purpose we harvested (1) three pots per assembly type when *A. artemisiifolia*'s seeds were  
138 introduced (hereafter 'initial' – on 18 April), (2) three pots per assembly type midway through the experiment, on  
139 13 June (hereafter 'intermediate'), and (3) six pots per assembly type at the end of the experiment (hereafter 'final'  
140 – 2 September). For each pot, aboveground biomass was harvested, sorted by species and dried at 60° C for 72 h  
141 until weighed. Belowground biomass was measured by collecting one soil core (12 cm diameter × 16 cm deep) at  
142 the center of each pot. Since we were not able to identify and sort the roots of the different species, we measured  
143 total belowground dry biomass including native species and *A. artemisiifolia* roots. Roots were isolated from soil,  
144 washed and sieved with a 250 μm mesh, dried at 60 °C for 72 h, and weighed.

145 Soil analyses

146           Established community composition and biomass production can influence subsequent colonizing species  
147 success through modification of soil resource availability (Fargione & Tilman, 2005; MacLaren et al., 2019;  
148 Scherer-Lorenzen et al., 2003; Temperton et al., 2007). We therefore analyzed organic carbon, nitrogen, nitrates,  
149 ammonium and available phosphorus on soil samples collected from each pot at the end of the experiment. For  
150 each assembly type, we collected 24 soil cores of 10 mm diameter × 100 mm deep (four per pot), which were  
151 pooled and dried for 48 h at 40 °C and sieved (<2 mm) to remove roots and rocks. Control soil (i.e., soil before  
152 recipient community establishment) was also added to the analyses. Samples were analyzed for organic carbon by  
153 sulfochromic oxidation (NF ISO 14234, 1998), total nitrogen by the modified Kjeldhal method (NF ISO 11261,  
154 1995), nitrate NO<sub>3</sub><sup>-</sup> and ammonium NH<sub>4</sub><sup>+</sup> (NF ISO 14256-2, 2007), and available phosphorus P<sub>2</sub>O<sub>5</sub> by the Dyer  
155 method (NF X31-160, 1999). Standard French method AFNOR or standard international method ISO were used  
156 to determine soil parameters. Analyses were performed by the accredited soil analysis laboratory Teyssier  
157 (Bordeaux, France), following standard protocols.

158 *Statistical analyses*

159 Invasive species early establishment success

160           We used generalized linear mixed models (GLMMs) with Laplace approximation ('glmer' function in  
161 the 'lme4' package; Bates et al., 2013) for maximum likelihood estimation of the parameters (Bolker et al., 2009)  
162 to analyze variations in cumulative final seedling emergence (until 4 June; no new emergence was recorded after  
163 this date) and survival (2 September) of *A. artemisiifolia* seedlings, with a binomial error distribution and a logit-  
164 link function. We analyzed variations in final aboveground biomass (i.e., 2 September) of *A. artemisiifolia* using  
165 a linear mixed model with Gaussian error distribution (data was log transformed to fit into a Gaussian distribution;  
166 'lmer' function of the 'lme4' package; Bates et al., 2013). Analyses of seedling emergence, survival and final  
167 aboveground biomass included assembly type (Dactylis<sub>f</sub>, Lolium<sub>f</sub>, Onobrychis<sub>f</sub>, Trifolium<sub>f</sub>, SynchronouS<sub>seq</sub>,  
168 SynchronouS) as fixed predictor variable and pot as random factor. Upon finding a significant effect of the fixed  
169 effect assembly type (significance tested using type II sums of squares using the 'Anova' function in 'car' package;  
170 Fox et al., 2020), we conducted *post-hoc* pairwise contrasts comparisons with a Tukey adjustment ('emmeans'  
171 package; Lenth et al., 2019).

172 Analyses of invasive species seedling emergence was based on the 81 seeds sown per assembly type.  
173 Analyses of survival included emerged individuals in six pots per assembly type (from 50 to 54 individuals;  
174 Appendix A: Table 1). Final aboveground biomass considered all individuals surviving until the end of the  
175 experiment in six pots per assembly type (from 40 to 53 individuals; Appendix A: Table 1).

176 Recipient communities

177 We analyzed the effect of assembly type on (1) total recipient species aboveground biomass and (2) total  
178 belowground (native and invasive) biomass for the three harvests using a one-way ANOVA. When a significant  
179 effect was found, we performed pairwise comparisons on the least-squares means (LSM) with a Tukey adjustment  
180 ('emmeans' package; Lenth et al., 2019). When residuals did not satisfy normality and/or homoscedasticity  
181 assumptions, we performed Welch's heteroscedastic  $F$  tests with trimmed means and Winsorized variances  
182 ('welch.test' function in 'onewaytests' package; Dag et al., 2018; Welch, 1951), which are relatively insensitive  
183 to the combined effects of non-normality and heteroscedasticity (Keselman et al., 2008). When a significant effect  
184 was found, *post-hoc* multiple pairwise comparison tests were performed with a BH adjustment ('paircomp'  
185 function in 'onewaytests' package; Dag et al., 2018). All analyses of biomass were conducted on three pots per  
186 assembly type, except final aboveground biomass for which six pots were available per assembly type. We also  
187 calculated the contribution of legumes and grasses by summing aboveground biomass of *O. viciifolia* and *T.*  
188 *repens*, and *D. glomerata* and *L. perenne*, respectively.

189 All analyses were performed using R software (ver. 3.6.2; R Development Core Team, 2020). The  $p$ -  
190 values lower than 0.05 were considered as statistically significant.

## 191 **Results**

### 192 *Invasive species early establishment success*

193 Seedling emergence and survival of *A. artemisiifolia* were high across all assembly types (i.e., 97.3%±1.0  
194 and 93.3%±3.1, mean±SE, respectively; Appendix A: Table 1). Assembly type did not significantly affect *A.*  
195 *artemisiifolia* seedling emergence (Wald  $\chi^2=3.70$ ,  $df=5$ ,  $p=0.59$ ) or survival (Wald  $\chi^2=4.48$ ,  $df=5$ ,  $p=0.48$ ).  
196 Assembly type significantly affected final aboveground biomass (Anova Type II: Wald  $\chi^2 = 59.056$ ,  $df=5$ ,  
197  $p<0.001$ ), which was significantly lower in Synchronous than in all other assembly types except Lolium<sub>f</sub> (Fig. 2),  
198 and (2) significantly higher in Trifolium<sub>f</sub> than in Lolium<sub>f</sub>, Synchronous<sub>seq</sub> or Synchronous.

199 *Recipient community biomass*

200 Assembly type significantly affected recipient communities above- and belowground biomass, at all  
201 harvest times (Fig. 3; Appendix A: Table 2). For the initial harvest, aboveground biomass was significantly higher  
202 in Synchronous than in all other assembly types and significantly lower in Trifolium<sub>f</sub> than in all other assembly  
203 types except Synchronous<sub>seq</sub> (Fig. 3A), and belowground biomass was significantly higher in Synchronous than in  
204 all other assembly types (Fig. 3D). Legumes contributed to aboveground biomass more than grasses in  
205 Onobrychis<sub>f</sub>, Trifolium<sub>f</sub> and Synchronous<sub>seq</sub>, and less than grasses in Dactylis<sub>f</sub>, Lolium<sub>f</sub> and Synchronous (Table  
206 1). For the intermediate harvest, aboveground biomass was significantly higher in Trifolium<sub>f</sub> than in all other  
207 assembly types (Fig. 3B), and belowground biomass was not significantly different between assembly types (Fig.  
208 3E). Legumes contributed to aboveground biomass more than grasses in Trifolium<sub>f</sub>, and less than grasses in all  
209 other assembly types (Table 1). For the final harvest, aboveground biomass was significantly lower in Dactylis<sub>f</sub>  
210 and Lolium<sub>f</sub> than in Synchronous (Fig. 3C), and belowground biomass was significantly higher in Lolium<sub>f</sub> than in  
211 Onobrychis<sub>f</sub> and Trifolium<sub>f</sub> (Fig. 3F). Legumes contributed to aboveground biomass less than grasses in Lolium<sub>f</sub>,  
212 and more than grasses in all other assembly types (Table 1).

213 *Soil analyses*

214 Compared to control soil (i.e., soil before recipient community establishment), all planted treatments  
215 depleted the soil of available phosphorus and nitrates (Table 2). Trifolium<sub>f</sub> communities produced soils with  
216 greater concentrations of nitrates and ammonium than any other treatments, including the other legume species.  
217 Soil supporting Lolium<sub>f</sub> communities showed the lowest nitrate content.

218 **Discussion**

219 This study provides evidence for a role of assembly history and priority effects in invasion resistance  
220 during the early stages of community development and thus corroborates several recent studies (e.g., Delory et al.,  
221 2019b; Grman & Suding, 2010; Lang et al., 2017; Stevens & Fehmi, 2011; Stuble & Young, 2020; Vaughn &  
222 Young, 2015). The novelty of our results lies in the fact that the history of native species establishment (i.e., timing  
223 and order of species arrival) affects the success of the subsequent invasion event (i.e., not only the timing and order  
224 of invasive species arrival, as classically addressed). Altering (1) the identity of the first native colonizer, the  
225 establishment of which preceded other species arrival by four weeks, and (2) species establishment timing (i.e.,  
226 synchronous vs. sequential sowing) both significantly affected the biomass production of the invasive plant species

227 *A. artemisiifolia* (Figs. 2 and 3). Seedling emergence and survival did not significantly differ and were high across  
228 all communities ( $97.3\% \pm 1.0$  and  $93.3\% \pm 3.1$ , mean  $\pm$  SE, respectively; Appendix A: Table 1). A high germination  
229 success is crucial for annual species, such as *A. artemisiifolia* because they reproduce sexually. The reduction of  
230 its biomass goes along with a decrease in the number of produced seeds (Chauvel & Fumanal, 2009), therefore  
231 potentially resulting in a lower invasion success the following season (Gentili et al., 2015). This way, revegetation  
232 strategies may help to reduce invasion by annual species. These findings are in accordance with the results of the  
233 meta-analysis of Levine et al. (2004) showing that competitive interactions with native species are more likely to  
234 reduce the performance of invaders than totally repel invasions.

235 Differences in assembly history influenced early community composition (i.e., species contribution to  
236 biomass), although it tended to converge after six months (Fig. 3). The invasive species exhibited the lowest overall  
237 performance in Synchronous, where all recipient individuals were sown at the same time (Fig. 2). Receiving more  
238 seeds in the first two weeks allowed Synchronous to reach high biomass more quickly (Fig. 3A,D), which is likely  
239 to be responsible for lower invasive species success at the end of the experiment. This assumption is supported by  
240 the lower resistance of Synchronous<sub>seq</sub> (sequential sowing of all six species), since both assembly types differed in  
241 initial biomass production while sharing close intermediate and final biomass (Fig. 3B,C), species composition  
242 (Fig. 3B,C; Table 1) and soil nutrient contents (Table 2). These results emphasize the importance of an initial quick  
243 and high biomass production to counter invasion (Lulow, 2006; Mason et al., 2013, 2017; Rinella et al., 2007;  
244 Symstad, 2000). These results also indicate that sequential sowing may increase community vulnerability to  
245 invasion (Martin & Wilsey, 2012; Stuble & Young, 2020), challenging the implementation of this sowing  
246 technique in invaded restoration sites (e.g., sequential sowing may sometimes be beneficial to promote positive  
247 interactions between early and target late-successional species; Weidlich et al., 2021). Additional native species  
248 sowings might still improve invasion resistance when environmental conditions at the time of initial sowing are  
249 uncertain or not ideal for establishment.

250 A prior establishment of the productive N-fixing legume *T. repens* boosted *A. artemisiifolia* biomass  
251 (Figs. 2 and 3C,D), which is consistent with previous studies (Mwangi et al., 2007; Scherber et al., 2006). The  
252 facilitative effect of legumes has been attributed to their ability to fix atmospheric nitrogen, directly transferring it  
253 to neighbors via root exudation and mycorrhizal links (Govindarajulu et al., 2005; Paynel et al., 2001), or releasing  
254 it into the soil by decomposition (Tomm et al., 1995). In addition, nitrogen-fixing legumes display a small root  
255 system and preempt little soil nitrogen, leaving more opportunities for root and nutrient foraging by later arriving  
256 species ('N sparing'; Temperton et al., 2007; von Felten et al., 2009). In our study, the reduced invasion resistance

257 of *Trifolium*<sub>f</sub> may result from a lower competition for root space and belowground resources arising from (1) a  
258 low initial biomass production of the community (Fig. 3A,D), (2) a greater response of *A. artemisiifolia* to elevated  
259 soil N level, which is more likely to result from N sparing (Table 2; Govindarajulu et al., 2005; Paynel et al., 2001;  
260 Temperton et al., 2007; von Felten et al., 2009), and/or (3) a low grass content (Table 1), since perennial grass  
261 dominance has been associated with higher invasion resistance *via* a strong competition for belowground  
262 resources, as seems to be the case here for *L. perenne* (Fargione et al., 2003; Frankow-Lindberg, 2012; Scherber  
263 et al., 2010; Scherer-Lorenzen et al., 2003).

264 Our study focused on exploring the influence of immigration history in a perennial native community on  
265 *A. artemisiifolia* establishment success during early community assembly. The few existing long term experiments  
266 highlight long-lasting consequences of early differences in species arrival on invasibility and community structure  
267 (i.e., several years; Švamberková et al., 2019; Vaughn & Young, 2015; Werner et al., 2016), acknowledging the  
268 importance of studying mechanisms driving early community assembly. For instance, Vaughn and Young (2015)  
269 showed that giving a two-week advance to perennial native species favored those species over annual invasives  
270 for four years. In ecosystems characterized by seasonal rainfall and a high proportion of annual species,  
271 phenological gaps between native and invasive species might particularly matter to invasion success (Wolkovich  
272 & Cleland, 2011). In these environments, repeated invasive species management actions might be required  
273 (Wainwright et al., 2012).

274 Although the direction, strength and persistence of priority effects appear dependent on biotic and abiotic  
275 site conditions (Young et al., 2015) such as soil biota (Reinhart & Callaway, 2006), the identity (or traits) of  
276 interacting species (Cleland et al., 2015; Delory et al., 2019b; Hess et al., 2020; Stuble & Souza, 2016) and resource  
277 availability (Kardol et al., 2013), there is growing evidence that priority effect-based management strategies help  
278 decreasing plant invasions across many systems (e.g. Grman & Suding, 2010; Vaughn & Young, 2015; Yannelli  
279 et al., 2020; Young et al., 2016). A successful application of priority effect-based strategies requires the  
280 identification of the main drivers underlying site effects. Notably, understanding how phenological, morphological  
281 and physiological traits of early and later colonizers interact and how both affect priority effects would help  
282 building strategies adapted to specific species.

## 283 **Conclusions**

284 Overall, our study supports that native species colonization history matters to invasion success of the  
285 annual invader *A. artemisiifolia* when invasion occurs in the early stages of community assembly. Altering the

286 timing of species establishment (all together or sequential sowing) and the identity of the first native colonizer  
287 affected early biomass production and composition of the community, which influenced invader performance.  
288 Notably, communities reached a higher invasion resistance when quickly displaying a high native biomass. Thus,  
289 when immediate invasion risk is high in a restored site, establishing productive, densely sown native perennial  
290 communities and avoiding an early planting of highly productive N-fixing legumes may help decrease invasion  
291 success of *A. artemisiifolia* in the first stages of community assembly. Our results are in line with Gentili et al.  
292 (2015) who showed that establishing a dense, competitive vegetation cover in autumn helps reducing *A.*  
293 *artemisiifolia*'s success the following spring. Whether variations in community assembly might influence longer-  
294 term invasion success of *A. artemisiifolia* remains however uncertain. In the study of Gentili et al. (2017), passive  
295 and active (seeding of hayseed or a commercial seed mixture) revegetation resulted in *A. artemisiifolia* suppression  
296 over three years, whatever initial species compositions and vegetation percent covers. According to these results  
297 and to ours, showing biomass convergence for the various community types within six months (Fig. 3), we expect  
298 the decline of *A. artemisiifolia* in perennial communities over time, resulting in few or no long-lasting  
299 consequences of initial conditions on invader success. Further real-world studies are needed to evaluate whether  
300 such priority effect-based management strategies (i.e. altering native species colonization history) are efficient to  
301 limit various invasive species across systems.

## 302 **Supplementary data**

### 303 **Appendix A.** Supplementary data

304 Supplementary data associated with this article can be found, in the online version, at XXXXX.

## 305 **References**

- 306 Bassett, I. J., & Crompton, C. W. (1975). The Biology of Canadian Weeds. 11. *Ambrosia artemisiifolia* L. and *A.*  
307 *psilostachya* DC. *Canadian Journal of Plant Science*, 55(2), 463–476.
- 308 Bates, D., Maechler, M., & Bolker, B. (2013). Package 'lme4'. Linear mixed-effects models using S4 classes.
- 309 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S.  
310 (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in*  
311 *Ecology & Evolution*, 24(3), 127–135.

312 Brondizio, E. S., Settele, J., & Díaz, S. (2019). IPBES. 2019 Global assessment report on biodiversity and  
313 ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem  
314 Services. *Bonn, Germany*.

315 Byun, C., Blois, S., & Brisson, J. (2013). Plant functional group identity and diversity determine biotic resistance  
316 to invasion by an exotic grass. *Journal of Ecology*, *101*(1), 128–139.

317 Byun, C., & Lee, E. J. (2017). Ecological application of biotic resistance to control the invasion of an invasive  
318 plant, *Ageratina altissima*. *Ecology and Evolution*, *7*(7), 2181–2192.

319 Charles, H., & Dukes, J. S. (2008). Impacts of invasive species on ecosystem services. In *Biological invasions*  
320 (pp. 217–237). Springer.

321 Chauvel, B., & Fumanal, B. (2009). Seed production of *Ambrosia artemisiifolia* under stress conditions. In  
322 XIIIème Colloque International sur la Biologie des Mauvaises Herbes, Dijon, France, 8-10 September  
323 2009 (pp. 465–472). *Association Française de Protection des Plantes (AFPP)*,

324 Cherwin, K. L., Seastedt, T. R., & Suding, K. N. (2009). Effects of nutrient manipulations and grass removal on  
325 cover, species composition, and invasibility of a novel grassland in Colorado. *Restoration Ecology*,  
326 *17*(6), 818–826.

327 Cleland, E. E., Esch, E., & McKinney, J. (2015). Priority effects vary with species identity and origin in an  
328 experiment varying the timing of seed arrival. *Oikos*, *124*(1), 33–40.

329 Collinge, S. K., & Ray, C. (2009). Transient patterns in the assembly of vernal pool plant communities. *Ecology*,  
330 *90*(12), 3313–3323.

331 Dag, O., Dolgun, A., & Konar, N. M. (2018). Onewaytests: An R Package for One-Way Tests in Independent  
332 Groups Designs. *R Journal*, *10*(1).

333 Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: A general  
334 theory of invasibility. *Journal of Ecology*, *88*(3), 528–534.

335 Delory, B. M., Weidlich, E. W., von Gillhaussen, P., & Temperton, V. M. (2019a). When history matters: The  
336 overlooked role of priority effects in grassland overyielding. *Functional Ecology*, *33*(12), 2369–2380.

337 Delory, B., Weidlich, E. W., Kunz, M., Neitzel, J., & Temperton, V. M. (2019b). The exotic species *Senecio*  
338 *inaequidens* pays the price for arriving late in temperate European grasslands. *BioRxiv*, 542563.

339 Drake, J. A. (1991). Community-assembly mechanics and the structure of an experimental species ensemble. *The*  
340 *American Naturalist*, *137*(1), 1–26.

341 Fargione, J., Brown, C. S., & Tilman, D. (2003). Community assembly and invasion: An experimental test of  
342 neutral versus niche processes. *PNAS*, *100*(15).

343 Fargione, J. E., & Tilman, D. (2005). Diversity decreases invasion via both sampling and complementarity  
344 effects. *Ecology Letters*, *8*(6), 604–611.

345 Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Heilberger, R. (2020). Package “car”:  
346 Companion to applied regression. <https://cran.r-project.org/web/packages/car/car.pdf>. Accessed 22 May 2020

347 Frankow-Lindberg, B. E. (2012). Grassland plant species diversity decreases invasion by increasing resource  
348 use. *Oecologia*, *169*(3), 793–802.

349 Fukami, T. (2015). Historical contingency in community assembly: Integrating niches, species pools, and  
350 priority effects. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 1–23.

351 Fumanal, B. (2007). *Caractérisation des traits biologiques et des processus évolutifs d'une espèce envahissante*  
352 *en France: Ambrosia artemisiifolia L.* [Montpellier SupAgro].

353 Gaudet, C. L., & Keddy, P. A. (1988). A comparative approach to predicting competitive ability from plant  
354 traits. *Nature*, *334*(6179), 242–243.

355 Gentili, R., Gilardelli, F., Ciappetta, S., Ghiani, A., & Citterio, S. (2015). Inducing competition: intensive  
356 grassland seeding to control *Ambrosia artemisiifolia*. *Weed Research*, *55*(3), 278-288.

357 Gentili, R., Montagnani, C., Gilardelli, F., Guarino, M. F., & Citterio, S. (2017). Let native species take their  
358 course: *Ambrosia artemisiifolia* replacement during natural or “artificial” succession. *Acta Oecologica*,  
359 *82*, 32-40.

360 Govindarajulu, M., Pfeffer, P. E., Jin, H., Abubaker, J., Douds, D. D., Allen, J. W., Bücking, H., Lammers, P. J.,  
361 & Shachar-Hill, Y. (2005). Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*,  
362 *435*(7043), 819–823.

363 Grman, E., & Suding, K. N. (2010). Within-year soil legacies contribute to strong priority effects of exotics on  
364 native California grassland communities. *Restoration Ecology*, *18*(5), 664–670.

365 Heckel, M. E. (1906). Sur l’*Ambrosia artemisiaefolia* L. et sa naturalisation en France. *Bulletin de La Société*  
366 *Botanique de France*, *53*(8), 600–620.

367 Helsen, K., Hermy, M., & Honnay, O. (2016). A test of priority effect persistence in semi-natural grasslands  
368 through the removal of plant functional groups during community assembly. *BMC Ecology*, *16*(1), 22.

369 Hess, M. C. M., Buisson, E., Fontes, H., Bacon, L., Sabatier, F., & Mesléard, F. (2020). Giving recipient  
370 communities a greater head start and including productive species boosts early resistance to invasion.  
371 *Applied Vegetation Science*, 23(3), 340-352.

372 Hess, M. C.M., Mesléard, F., & Buisson, E. (2019). Priority effects: Emerging principles for invasive plant  
373 species management. *Ecological Engineering*, 127, 48–57.

374 Jauni, M., Gripenberg, S., & Ramula, S. (2015). Non-native plant species benefit from disturbance: A meta-  
375 analysis. *Oikos*, 124(2), 122–129.

376 Kardol, P., Souza, L., & Classen, A. T. (2013). Resource availability mediates the importance of priority effects  
377 in plant community assembly and ecosystem function. *Oikos*, 122(1), 84-94.

378 Keselman, H. J., Algina, J., Lix, L. M., Wilcox, R. R., & Deering, K. N. (2008). A generally robust approach for  
379 testing hypotheses and setting confidence intervals for effect sizes. *Psychological Methods*, 13(2), 110.

380 Lang, M., Hanslin, H. M., Kollmann, J., & Wagner, T. (2017). Suppression of an invasive legume by a native  
381 grass—High impact of priority effects. *Basic and Applied Ecology*, 22, 20–27.

382 Larson, D. L., Bright, J. B., Drobney, P., Larson, J. L., Palaia, N., Rabie, P. A., Vacek, S., & Wells, D. (2013).  
383 Using prairie restoration to curtail invasion of Canada thistle: The importance of limiting similarity and  
384 seed mix richness. *Biological Invasions*, 15(9), 2049–2063.

385 Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2019). *emmeans: Estimated Marginal Means, aka*  
386 *Least-Squares Means (Version 1.3. 4)*.

387 Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant  
388 invasions. *Ecology Letters*, 7(10), 975–989.

389 Li, X.-M., She, D.-Y., Zhang, D.-Y., & Liao, W.-J. (2015). Life history trait differentiation and local adaptation  
390 in invasive populations of *Ambrosia artemisiifolia* in China. *Oecologia*, 177(3), 669–677.

391 Lionello, P., Malanotte-Rizzoli, P., Boscolo, R., Alpert, P., Artale, V., Li, L., Luterbacher, J., May, W., Trigo,  
392 R., & Tsimplis, M. (2006). *The Mediterranean climate: An overview of the main characteristics and*  
393 *issues*. Elsevier.

394 Lulow, M. E. (2006). Invasion by non-native annual grasses: The importance of species biomass, composition,  
395 and time among California native grasses of the Central Valley. *Restoration Ecology*, 14(4), 616–626.

396 MacLaren, C., Swanepoel, P., Bennett, J., Wright, J., & Dehnen-Schmutz, K. (2019). Cover Crop Biomass  
397 Production Is More Important than Diversity for Weed Suppression. *Crop Science*, 59(2), 733-748.

398 Martin, L. M., & Wilsey, B. J. (2012). Assembly history alters alpha and beta diversity, exotic-native proportions  
399 and functioning of restored prairie plant communities. *Journal of Applied Ecology*, 49(6), 1436–1445.

400 Mason, T. J., French, K., & Jolley, D. (2013). Arrival order among native plant functional groups does not affect  
401 invasibility of constructed dune communities. *Oecologia*, 173(2), 557–568.

402 Mason, Tanya J., French, K., & Jolley, D. F. (2017). Functional Richness and Identity Do Not Strongly Affect  
403 Invasibility of Constructed Dune Communities. *PloS One*, 12(1), e0169243.

404 McIntyre, S., & Lavorel, S. (1994). Predicting richness of native, rare, and exotic plants in response to habitat  
405 and disturbance variables across a variegated landscape. *Conservation Biology*, 8(2), 521–531.

406 Middleton, E. L., Bever, J. D., & Schultz, P. A. (2010). The effect of restoration methods on the quality of the  
407 restoration and resistance to invasion by exotics. *Restoration Ecology*, 18(2), 181–187.

408 Mwangi, P. N., Schmitz, M., Scherber, C., Roscher, C., Schumacher, J., SCHERER-LORENZEN, M., Weisser,  
409 W. W., & Schmid, B. (2007). Niche pre-emption increases with species richness in experimental plant  
410 communities. *Journal of Ecology*, 95(1), 65–78.

411 NF ISO 112361 (1995) Soil Quality – Determination of total nitrogen – modified Kjeldahl method. International  
412 Organization for Standardization, Geneva, Switzerland

413 NF ISO 14235 (1998) Soil Quality – Determination of organic carbon by sulfochromic oxidation. International  
414 Organization for Standardization, Geneva, Switzerland

415 NF ISO 14259-2 (2007) Soil Quality – Determination of nitrate, nitrite and ammonium content in crude soils by  
416 extraction with potassium and chloride solution – Part 2: automated method with segmented flow  
417 analysis. International Organization for Standardization, Geneva, Switzerland

418 NF X31-160 (1999) Soil Quality – Determination of soluble phosphorus in a 20g/l solution of citric acid  
419 monohydrate – Dyer Method. Association Française de Normalisation, Paris, France

420 Norton, D. A. (2009). Species invasions and the limits to restoration: Learning from the New Zealand  
421 experience. *Science*, 325(5940), 569–571.

422 Ozaslan, C., Onen, H., Farooq, S., Gunal, H., & Akyol, N. (2016). Common ragweed: An emerging threat for  
423 sunflower production and human health in Turkey. *Weed Biology and Management*, 16(1), 42-55.

424 Paynel, F., Murray, P. J., & Cliquet, J. B. (2001). Root exudates: A pathway for short-term N transfer from  
425 clover and ryegrass. *Plant and Soil*, 229(2), 235–243.

426 Plückers, C., Rascher, U., Scharr, H., von Gillhaussen, P., Beierkuhnlein, C., & Temperton, V. M. (2013).  
427 Sowing different mixtures in dry acidic grassland produced priority effects of varying strength. *Acta*  
428 *Oecologica*, 53, 110–116.

429 Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New phytologist*, 170(3), 445-  
430 457. Rinella, M. J., Pokorny, M. L., & Rekaya, R. (2007). Grassland invader responses to realistic  
431 changes in native species richness. *Ecological Applications*, 17(6), 1824–1831.

432 Rowe, H. I. (2010). Tricks of the trade: Techniques and opinions from 38 experts in tallgrass prairie restoration.  
433 *Restoration Ecology*, 18, 253–262.

434 Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.  
435 F., Jackson, R. B., & Kinzig, A. (2000). Global biodiversity scenarios for the year 2100. *Science*,  
436 287(5459), 1770–1774.

437 Sarneel, J. M., Kardol, P., & Nilsson, C. (2016). The importance of priority effects for riparian plant community  
438 dynamics. *Journal of Vegetation Science*, 27(4), 658–667.

439 Scherber, C., Milcu, A., Partsch, S., Scheu, S., & Weisser, W. W. (2006). The effects of plant diversity and  
440 insect herbivory on performance of individual plant species in experimental grassland. *Journal of*  
441 *Ecology*, 94(5), 922–931.

442 Scherber, C., Mwangi, P. N., Schmitz, M., Scherer-Lorenzen, M., Beßler, H., Engels, C., Eisenhauer, N.,  
443 Migunova, V. D., Scheu, S., & Weisser, W. W. (2010). Biodiversity and belowground interactions  
444 mediate community invasion resistance against a tall herb invader. *Journal of Plant Ecology*, 3(2), 99–  
445 108.

446 Scherer-Lorenzen, M., Palmberg, C., Prinz, A., & Schulze, E.-D. (2003). The role of plant diversity and  
447 composition for nitrate leaching in grasslands. *Ecology*, 84(6), 1539–1552.

448 Smith, M., Cecchi, L., Skjøth, C. A., Karrer, G., & Šikoparija, B. (2013). Common ragweed: A threat to  
449 environmental health in Europe. *Environment International*, 61, 115–126.  
450 <http://www.sciencedirect.com/science/article/pii/S0160412013001682>

451 Stevens, J. M., & Fehmi, J. S. (2011). Early Establishment of a Native Grass Reduces the Competitive Effect of  
452 a Non-Native Grass. *Restoration Ecology*, 19(3), 399–406.

453 Stromberg, M. R., D'Antonio, C. M., Young, T. P., Wirka, J., & Kephart, P. R. (2007). California grassland  
454 restoration. *California Grasslands: Ecology and Management*. University of California Press, Berkeley,  
455 California, USA, 254–280.

456 Stuble, K. L., & Souza, L. (2016). Priority effects: Natives, but not exotics, pay to arrive late. *Journal of*  
457 *Ecology*, 104(4), 987–993.

458 Stuble, K. L., & Young, T. P. (2020). Priority Treatment Leaves Grassland Restoration Vulnerable to Invasion.  
459 *Diversity*, 12(2), 71.

460 Švamberková, E., Doležal, J., & Lepš, J. (2019). The legacy of initial sowing after 20 years of ex-arable land  
461 colonisation. *Oecologia*, 1–11.

462 Symstad, A. J. (2000). A test of the effects of functional group richness and composition on grassland  
463 invasibility. *Ecology*, 81(1), 99–109.

464 Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., & Buchmann, N. (2007). Positive  
465 interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity  
466 experiment. *Oecologia*, 151(2), 190–205.

467 Tomm, G. O., Walley, F. L., van Kessel, C., & Slinkard, A. E. (1995). Nitrogen cycling in an alfalfa and  
468 brome grass sward via litterfall and harvest losses. *Agronomy Journal*, 87(6), 1078–1085.

469 Vaughn, K. J., & Young, T. P. (2015). Short-term priority over exotic annuals increases the initial density and  
470 longer-term cover of native perennial grasses. *Ecological Applications*, 25(3), 791–799.

471 Vitousek, P. M., & Walker, L. R. (1987). Colonization, succession and resource availability: ecosystem-level  
472 interactions. In *Symposium of the British Ecological Society*.

473 von Felten, S., Hector, A., Buchmann, N., Niklaus, P. A., Schmid, B., & Scherer-Lorenzen, M. (2009).  
474 Belowground nitrogen partitioning in experimental grassland plant communities of varying species  
475 richness. *Ecology*, 90(5), 1389–1399.

476 Wainwright, C. E., Wolkovich, E. M., & Cleland, E. E. (2012). Seasonal priority effects: implications for  
477 invasion and restoration in a semi-arid system. *Journal of Applied Ecology*, 49(1), 234–241.

478 Weidlich, E. W., Nelson, C. R., Maron, J. L., Callaway, R. M., Delory, B. M., & Temperton, V. M. (2021).  
479 Priority effects and ecological restoration. *Restoration Ecology*, 29(1), e13317.

480 Weidlich, E. W., von Gillhausen, P., Max, J. F., Delory, B. M., Jablonowski, N. D., Rascher, U., & Temperton,  
481 V. M. (2018). Priority effects caused by plant order of arrival affect below-ground productivity. *Journal*  
482 *of Ecology*, 106(2), 774–780.

483 Weigelt, A., Steinlein, T., & Beyschlag, W. (2002). Does plant competition intensity rather depend on biomass  
484 or on species identity? *Basic and Applied Ecology*, 3(1), 85–94.

485 Welch, B. L. (1951). On the comparison of several mean values: An alternative approach. *Biometrika*, 38(3/4),  
486 330–336.

487 Werner, C. M., Vaughn, K. J., Stuble, K. L., Wolf, K., & Young, T. P. (2016). Persistent asymmetrical priority  
488 effects in a California grassland restoration experiment. *Ecological Applications*, 26(6), 1624–1632.

489 Wolkovich, E. M., & Cleland, E. E. (2011). The phenology of plant invasions: a community ecology perspective.  
490 *Frontiers in Ecology and the Environment*, 9(5), 287-294.

491 Yannelli, F. (2021). Applying competition theory to ensure ecological restoration and prevent plant invasions.  
492 *Biodiversity*, 1–5.

493 Yannelli, F. A., MacLaren, C., & Kollmann, J. (2020). Moving away from limiting similarity during restoration:  
494 Timing of arrival and native biomass are better proxies of invasion suppression in grassland  
495 communities. *Frontiers in Ecology and Evolution*, 8, 238.

496 Young, T. P., Stuble, K. L., Balachowski, J. A., & Werner, C. M. (2016). Using priority effects to manipulate  
497 competitive relationships in restoration. *Restoration Ecology*, 25, S114-S123.

498 Young, T. P., Zefferman, E. P., Vaughn, K. J., & Fick, S. (2015). Initial success of native grasses is contingent  
499 on multiple interactions among exotic grass competition, temporal priority, rainfall and site effects. *AoB*  
500 *Plants*, 7.

501

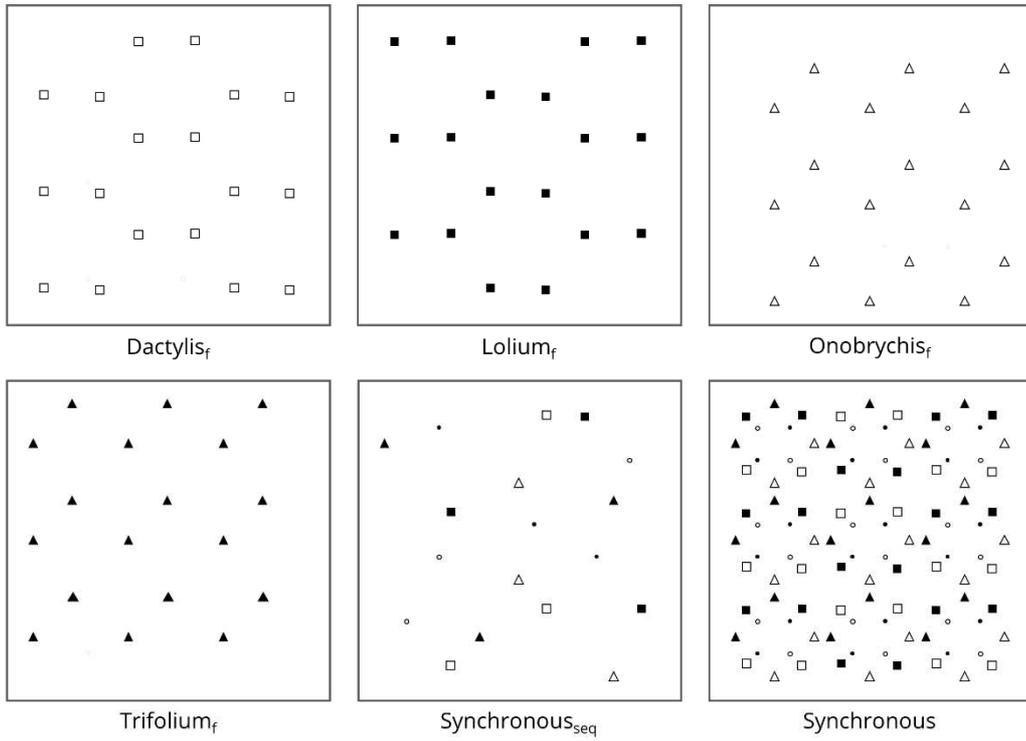
502 **Figure captions**

503 **Fig. 1.** Experimental design. Spatial arrangement of individuals in pots (represented by squares) is depicted  
504 depending on assembly type. In the second sowing, all communities were completed to achieve same species  
505 abundance and spatial pattern (no seed was added for Synchronous)

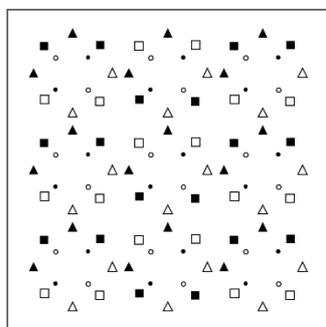
506 **Fig. 2.** *Ambrosia artemisiifolia* final aboveground biomass depending on assembly type (mean per individual  $\pm$ SE,  
507  $n_{\text{tot}}=324$ ). Assembly types with no letter in common are significantly different (pairwise contrasts comparisons  
508 with Tukey adjustment;  $p<0.05$ )

509 **Fig. 3.** Above- and belowground biomass of the recipient communities depending on assembly type for (A,D)  
510 initial (18 April), (B,E) intermediate (13 June), and (C,F) final (2 September) harvests. Aboveground biomass  
511 (A,B,C) represents total biomass per pot (mean $\pm$ SE) and includes native species only, while belowground biomass  
512 (D,E,F) represents sample biomass per pot (mean $\pm$ SE) and includes both native and invasive species. Treatments  
513 with no letter in common are significantly different (pairwise comparisons with Tukey adjustment;  $p<0.05$ ). 'NS'  
514 indicates to non-significant *post-hoc* differences between assembly types

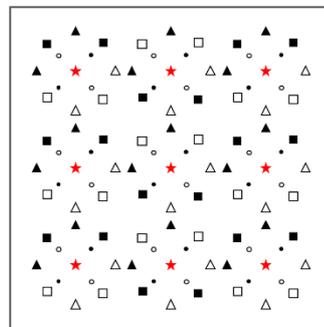
(A) First sowing (6 March)



(B) Second sowing (3 April)

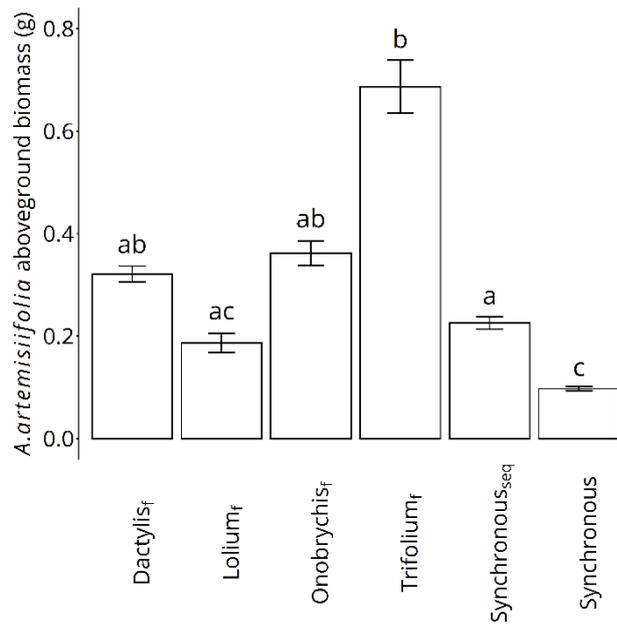


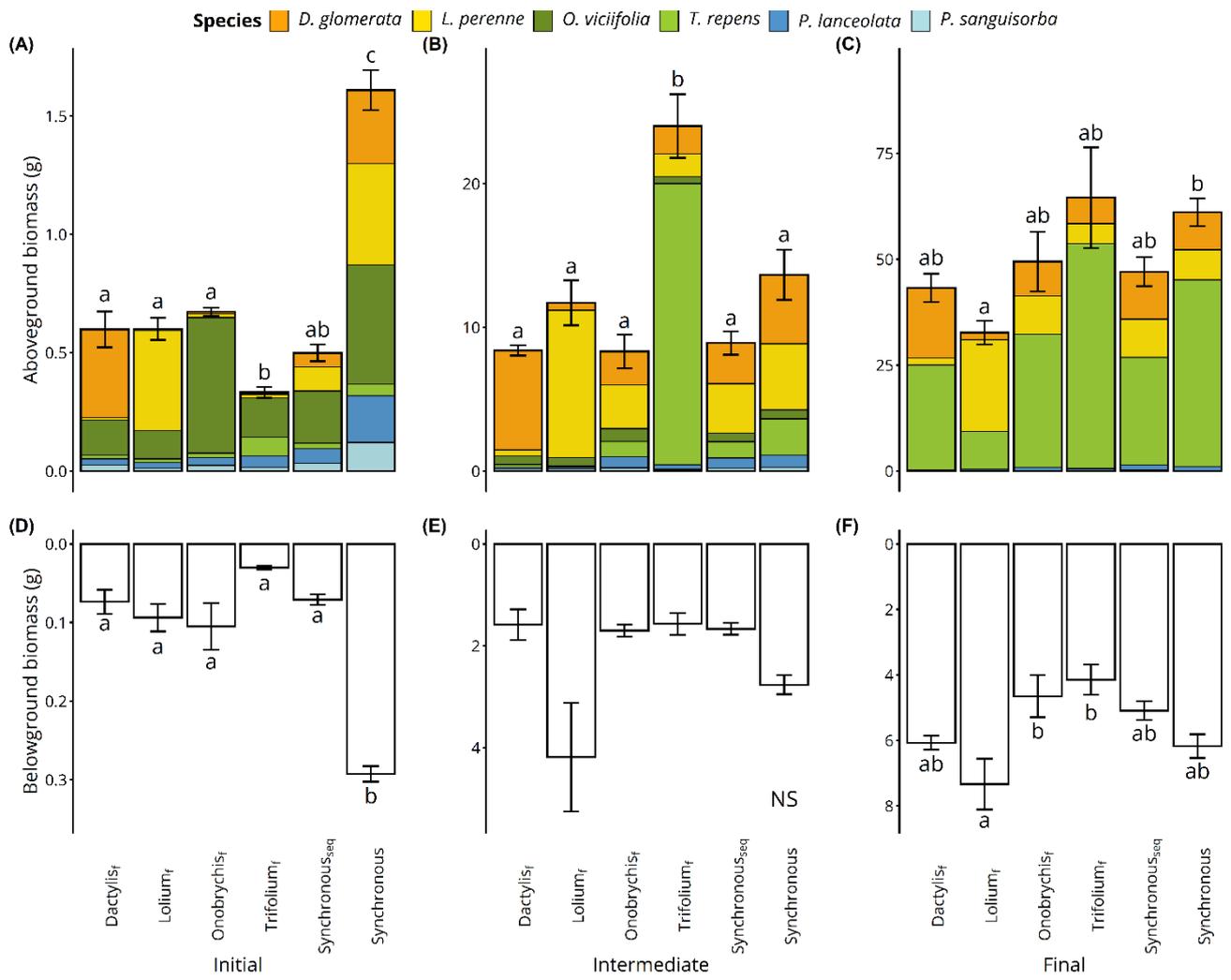
(C) Invasion by *A. artemisiifolia* (\* , 18 April)



□ *D. glomerata*    ■ *L. perenne*    △ *O. viciifolia*    ▲ *T. repens*    ○ *P. lanceolata*    ● *P. sanguisorba*

516 Fig. 2.





519 **Table 1.** Aboveground biomass of legumes (sum of *O. viciifolia* and *T. repens*, mean±SE) and grasses (sum of *D.*  
520 *glomerata* and *L. perenne*, mean±SE) for each assembly type for initial (18 April, n=3), intermediate (13 June,  
521 n=3), and final (2 September, n=6) harvests. Contribution to total biomass (mean%±SE), and ratio between mean  
522 biomass of legumes and grasses ('Ratio L:G'; mean±SE) are also indicated, with ratios > 1 in bold (i.e. mean  
523 biomass of legumes exceeds mean biomass of grasses)

	Legumes		Grasses		Ratio L:G
	Biomass (g)	Contribution to biomass (%)	Biomass (g)	Contribution to biomass (%)	
Initial					
Dactylis <sub>f</sub>	0.16±0.00	28.2±3.9	0.38±0.09	62.1±8.4	0.42
Lolium <sub>f</sub>	0.13±0.01	22.3±1.1	0.43±0.04	71.6±1.7	0.30
<b>Onobrychis<sub>f</sub></b>	<b>0.59±0.02</b>	<b>87.9±0.2</b>	<b>0.02±0.00</b>	<b>3.6±0.1</b>	<b>29.50</b>
<b>Trifolium<sub>f</sub></b>	<b>0.25±0.03</b>	<b>73.1±5.6</b>	<b>0.02±0.00</b>	<b>7.1±0.6</b>	<b>12.50</b>
<b>Synchronous<sub>seq</sub></b>	<b>0.24±0.02</b>	<b>48.8±2.7</b>	<b>0.16±0.03</b>	<b>32.0±3.3</b>	<b>1.50</b>
Synchronous	0.55±0.03	34.5±1.4	0.74±0.03	46.0±1.5	0.74
Intermediate					
Dactylis <sub>f</sub>	0.81±0.15	9.6±1.6	7.34±0.3	87.5±1.5	0.11
Lolium <sub>f</sub>	0.64±0.04	5.6±0.5	10.79±1.56	91.5±1.1	0.06
Onobrychis <sub>f</sub>	1.98±0.64	22.6±4.0	5.36±0.52	65.2±2.6	0.37
<b>Trifolium<sub>f</sub></b>	<b>20.05±2.29</b>	<b>83.2±2.1</b>	<b>3.5±0.19</b>	<b>14.9±1.9</b>	<b>5.72</b>
Synchronous <sub>seq</sub>	1.72±0.24	19.3±2.3	6.28±0.62	70.5±2.1	0.27
Synchronous	3.16±0.77	22.7±3.0	9.40±0.95	69.4±3.0	0.37
Final					
<b>Dactylis<sub>f</sub></b>	<b>24.75±3.18</b>	<b>47.5±3.2</b>	<b>18.23±1.44</b>	<b>35.7±2.6</b>	<b>1.35</b>
Lolium <sub>f</sub>	8.90±3.09	20.3±6.0	23.35±1.65	61.5±6.5	0.38
<b>Onobrychis<sub>f</sub></b>	<b>31.38±7.59</b>	<b>49.1±7.0</b>	<b>17.15±1.11</b>	<b>32.8±5.5</b>	<b>1.79</b>
<b>Trifolium<sub>f</sub></b>	<b>53.15±11.73</b>	<b>64.1±4.3</b>	<b>10.91±1.81</b>	<b>14.2±2.5</b>	<b>4.87</b>
<b>Synchronous<sub>seq</sub></b>	<b>25.40±4.17</b>	<b>41.8±4.5</b>	<b>20.27±1.40</b>	<b>35.1±3.4</b>	<b>1.25</b>
<b>Synchronous</b>	<b>44.08±3.58</b>	<b>55.3±2.2</b>	<b>15.98±0.98</b>	<b>20.5±1.9</b>	<b>2.75</b>

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525 **Table 2.** Results of soil analyses performed at the end of the experiment. Control soil refers to soil before recipient  
 526 community establishment. Since one soil sample was analyzed per treatment, no statistical test was performed

	Total organic matter (%)	C (g/kg)	N (g/kg)	NO <sub>3</sub> <sup>-</sup> (g/kg)	NH <sub>4</sub> <sup>+</sup> (g/kg)	P <sub>2</sub> O <sub>5</sub> (g/kg)
Dactylis <sub>f</sub>	11.0	63.6	4.1	0.007	0.012	0.078
Lolium <sub>f</sub>	11.1	64.6	4.4	0.003	0.010	0.074
Onobrychis <sub>f</sub>	11.3	65.7	5.0	0.005	0.012	0.076
Trifolium <sub>f</sub>	11.1	64.3	5.2	0.010	0.015	0.070
Synchronous <sub>seq</sub>	11.3	65.7	4.6	0.007	0.012	0.070
Synchronous	11.4	66.1	4.4	0.008	0.010	0.083
Control soil	11.2	65.0	4.5	0.011	0.005	0.136

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