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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/m³)
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_f*, with “f” standing for “first”), (2) *L. perenne* (*Lolium_f*), (3) *O. viciifolia* (*Onobrychis_f*), or (4) *T. repens*
112 (*Trifolium_f*) 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_{seq}*) 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/m²). *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_{seq} had the same density and sowing timing as
121 *Dactylis*_f, *Lolium*_f, *Onobrychis*_f and *Trifolium*_f, 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*_f, *Lolium*_f, *Onobrychis*_f, *Trifolium*_f, Synchronous_{seq} 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_3^- and ammonium NH_4^+ (NF ISO 14256-2, 2007), and available phosphorus P_2O_5 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_f, Lolium_f, Onobrychis_f, Trifolium_f, SynchronouS_{seq},
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_f (Fig. 2),
198 and (2) significantly higher in Trifolium_f than in Lolium_f, Synchronous_{seq} 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_f* than in all other assembly
203 types except *Synchronous_{seq}* (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_f*, *Trifolium_f* and *Synchronous_{seq}*, and less than grasses in *Dactylis_f*, *Lolium_f* and Synchronous (Table
206 1). For the intermediate harvest, aboveground biomass was significantly higher in *Trifolium_f* 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_f*, and less than grasses in all
209 other assembly types (Table 1). For the final harvest, aboveground biomass was significantly lower in *Dactylis_f*
210 and *Lolium_f* than in Synchronous (Fig. 3C), and belowground biomass was significantly higher in *Lolium_f* than in
211 *Onobrychis_f* and *Trifolium_f* (Fig. 3F). Legumes contributed to aboveground biomass less than grasses in *Lolium_f*,
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_f* communities produced soils with
216 greater concentrations of nitrates and ammonium than any other treatments, including the other legume species.
217 Soil supporting *Lolium_f* 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_{seq} (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*_f 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.

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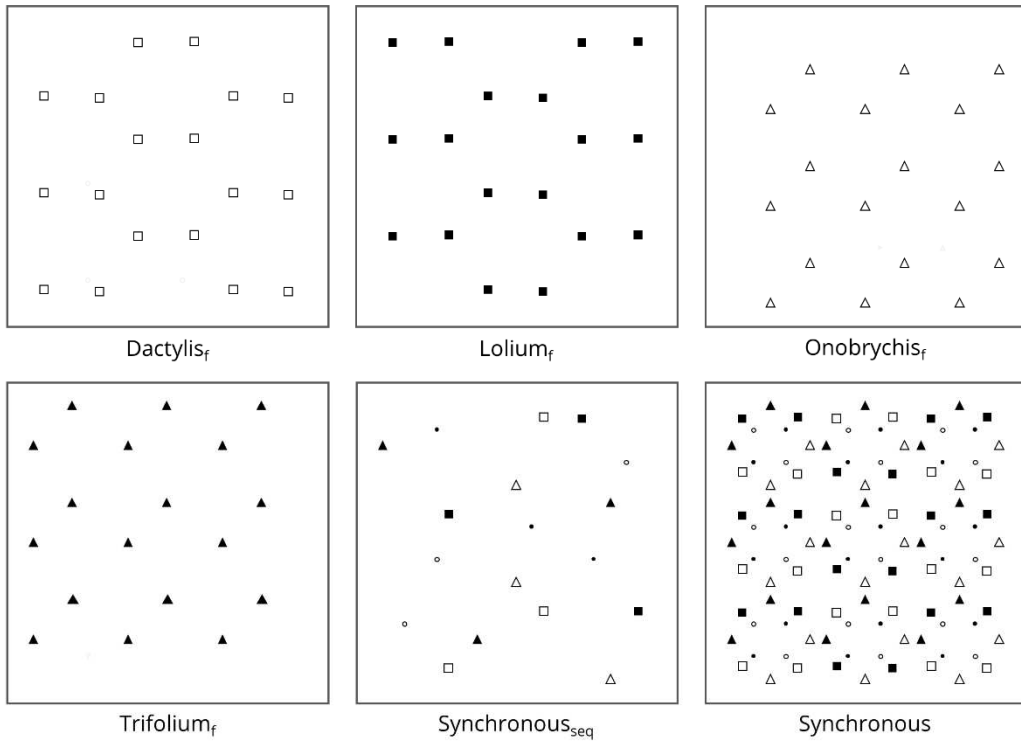
502 **Figure captions**

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)

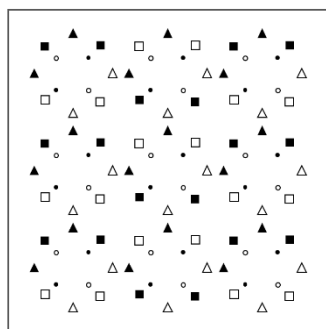
506 **Fig. 2.** *Ambrosia artemisiifolia* final aboveground biomass depending on assembly type (mean per individual \pm SE,
507 $n_{\text{tot}}=324$). Assembly types with no letter in common are significantly different (pairwise contrasts comparisons
508 with Tukey adjustment; $p<0.05$)

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

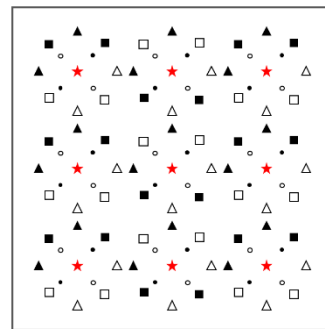
(A) First sowing (6 March)



(B) Second sowing (3 April)

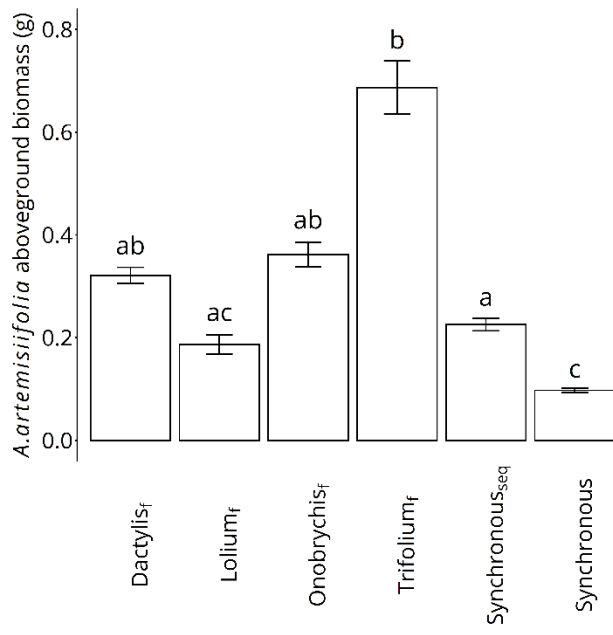


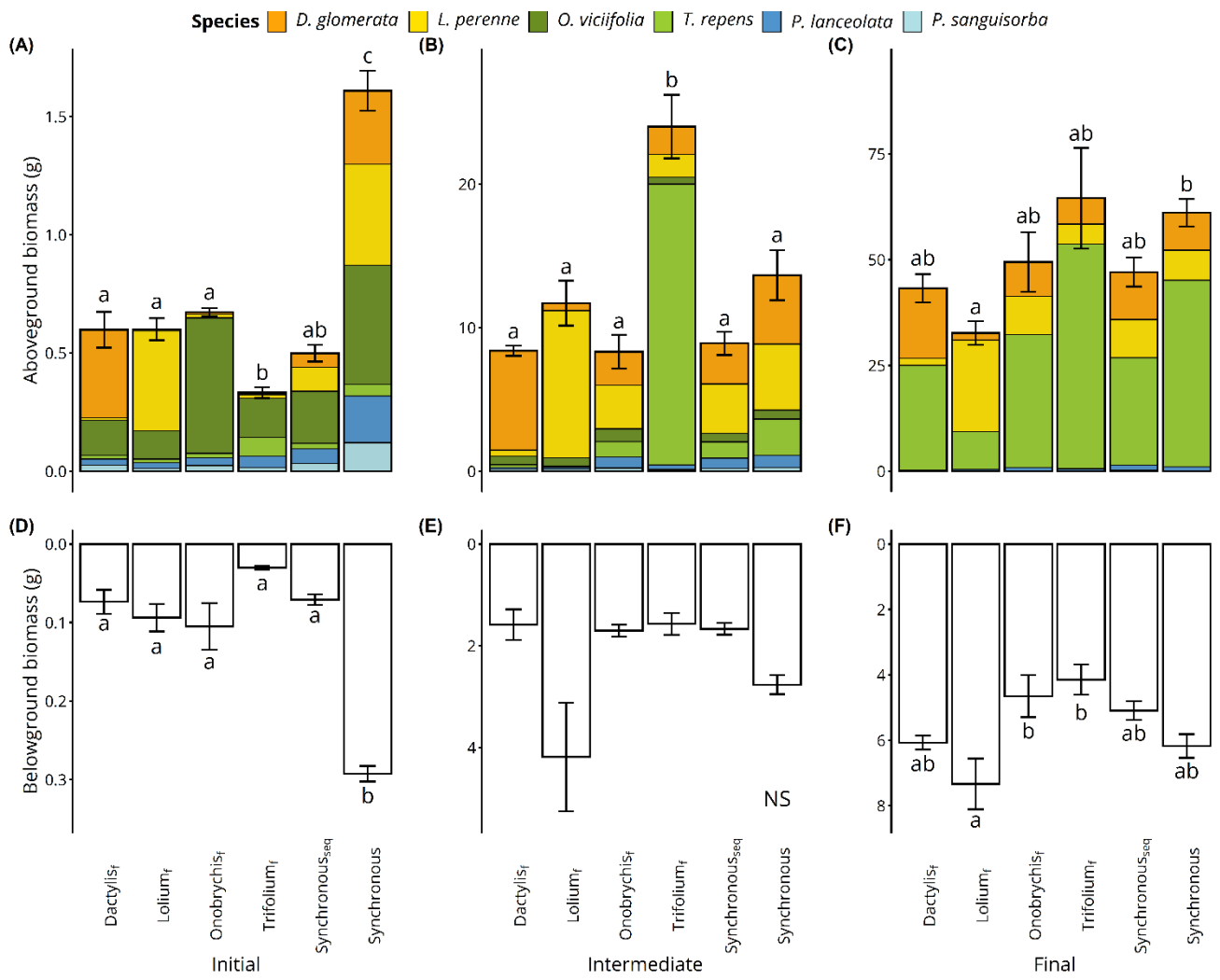
(C) Invasion by *A. artemisiifolia* (* , 18 April)



□ *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 _f	0.16±0.00	28.2±3.9	0.38±0.09	62.1±8.4	0.42
Lolium _f	0.13±0.01	22.3±1.1	0.43±0.04	71.6±1.7	0.30
Onobrychis_f	0.59±0.02	87.9±0.2	0.02±0.00	3.6±0.1	29.50
Trifolium_f	0.25±0.03	73.1±5.6	0.02±0.00	7.1±0.6	12.50
Synchronous_{seq}	0.24±0.02	48.8±2.7	0.16±0.03	32.0±3.3	1.50
Synchronous	0.55±0.03	34.5±1.4	0.74±0.03	46.0±1.5	0.74
Intermediate					
Dactylis _f	0.81±0.15	9.6±1.6	7.34±0.3	87.5±1.5	0.11
Lolium _f	0.64±0.04	5.6±0.5	10.79±1.56	91.5±1.1	0.06
Onobrychis _f	1.98±0.64	22.6±4.0	5.36±0.52	65.2±2.6	0.37
Trifolium_f	20.05±2.29	83.2±2.1	3.5±0.19	14.9±1.9	5.72
Synchronous _{seq}	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					
Dactylis_f	24.75±3.18	47.5±3.2	18.23±1.44	35.7±2.6	1.35
Lolium _f	8.90±3.09	20.3±6.0	23.35±1.65	61.5±6.5	0.38
Onobrychis_f	31.38±7.59	49.1±7.0	17.15±1.11	32.8±5.5	1.79
Trifolium_f	53.15±11.73	64.1±4.3	10.91±1.81	14.2±2.5	4.87
Synchronous_{seq}	25.40±4.17	41.8±4.5	20.27±1.40	35.1±3.4	1.25
Synchronous	44.08±3.58	55.3±2.2	15.98±0.98	20.5±1.9	2.75

524

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 ₃ ⁻ (g/kg)	NH ₄ ⁺ (g/kg)	P ₂ O ₅ (g/kg)
Dactylis _f	11.0	63.6	4.1	0.007	0.012	0.078
Lolium _f	11.1	64.6	4.4	0.003	0.010	0.074
Onobrychis _f	11.3	65.7	5.0	0.005	0.012	0.076
Trifolium _f	11.1	64.3	5.2	0.010	0.015	0.070
Synchronous _{seq}	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

527