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Alicia Rouge, Guillaume Adeux, Hugues Busset, Rodolphe Hugard, Juliette Martin, Annick Matejcek, Delphine Moreau, Jean-Philippe Guillemin, Stéphane Cordeau

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1 *Original article*

2 **Weed suppression in cover crop mixtures under contrasted levels of resource**
3 **availability**

4 Alicia Rouge ^{a, b}, Guillaume Adeux ^a, Hugues Busset ^a, Rodolphe Hugard ^c, Juliette
5 Martin ^c, Annick Matejcek ^a, Delphine Moreau ^a, Jean-Philippe Guillemain ^a, Stéphane
6 Cordeau ^{a, *}

7 ^a Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne-Franche-
8 Comté, F-21000 Dijon, France

9 ^b AgroParisTech, 75005 Paris, France

10 ^c INRAE, U2E, Unité Expérimentale du domaine d'Epoisses, F-21110 Bretenière,
11 France

12

13 *Corresponding author: stephane.cordeau@inrae.fr; Tel. +33 3 80 69 32 67

14

15 **Abstract**

16 Cover crop (CC) mixtures appear as a promising agroecological tool for weed
17 management. Although CC suppress weed growth by competing for resources, their
18 suppressive effect under contrasting levels of soil resource availability remains poorly
19 documented. A two field:year experiment was conducted to investigate the weed
20 suppressive effect of four CC mixtures. They were composed of 2 or 8 species
21 including or not legume species and compared to a bare soil control. The experiment
22 included two levels of irrigation and nitrogen fertilisation at CC sowing. The objectives
23 were to assess (i) weed and CC aboveground biomass response to CC mixtures and
24 resource availability, (ii) the weed suppressive effect of CC mixtures across a gradient
25 of CC biomass and (iii) weed community response to CC mixtures and resource
26 availability. CC and weed biomass were mainly influenced by interactions between CC
27 mixtures and fertilisation and between CC mixtures and irrigation, with contrasted
28 effects between field:years. Nitrogen fertilisation increased biomass of non-legume
29 based CC mixtures but this only resulted into a further reduction of weed biomass of
30 little biological relevance. Legume-based CC mixtures suppressed weeds to a greater
31 extent without nitrogen fertilisation in year 2 but not in year 1, possibly due to
32 contrasted initial soil nitrogen availability (lower in year 2). Weed biomass generally
33 benefited more from irrigation than CC mixtures. Among the 33 weed species
34 recorded, weed communities in each plot were dominated by wheat volunteers,
35 *Geranium dissectum*, *Veronica persica* and *Echinochloa crus-galli*, whose biomass
36 varied depending on the interaction between year, CC mixture and resource
37 availability. Our results revealed that competitive outcomes between CC mixtures and
38 weed species were driven by a complex interaction between resource availability and
39 species traits. Further experiments focusing on plant traits should improve our
40 understanding of weed:CC competitive outcomes under various levels of resource
41 availability.

42 **Keywords:** cover crop mixtures, weed community, biomass, competition, nitrogen,
43 water

44 1. Introduction

45 Weeds are a major concern in agriculture due to important potential crop yield losses
46 (Oerke, 2006) and their management currently often relies on herbicide use. However,
47 the over-reliance on chemical weed control is now questioned for its impact on the
48 environment (Kamrin, 1997), biodiversity (McLaughlin and Mineau, 1995) and human
49 health (Alavanja et al., 2004). The number of active herbicide substances on the
50 market is decreasing (Chauvel et al., 2012), thereby increasing the probability of weed
51 resistance through recurrent use of a reduced number of herbicides at the crop rotation
52 scale (Powles and Yu, 2010). Biodiversity-based options are sought in order to
53 diversify the selection pressures at the crop rotation scale (Petit et al., 2018). Cover
54 crops (CC) are plant species cultivated between two main cash crops which can
55 provide multiple ecosystem services (*e.g.* reducing soil erosion, improving soil
56 properties or crop yields), including weed suppression (Teasdale et al., 2007; Mirsky
57 et al., 2011; Cordeau et al., 2015). The weed suppressive effect of CC can be defined
58 as the capacity of CC to reduce weed biomass (Christensen, 1995). However,
59 mechanisms underlying the weed suppressive effect of CC remains unclear, especially
60 in CC mixtures (Nichols et al., 2020).

61 Farmers and extension services are promoting and implementing CC as species-
62 diverse mixtures (CTIC, 2017). However, the systematic review by Florence and
63 MacGuire (2020) reported that weed suppression was not greater in CC mixtures than
64 the most suppressive species of the mixture (grown in monoculture) in 98% of cases.
65 The relationship between CC mixtures, CC biomass production and weed suppression
66 remains unclear. On the one hand, numerous studies have reported enhanced weed
67 suppression through the use of key CC functional groups producing high biomass,
68 such as grasses and *Brassicaceae* (Bybee-Finley et al., 2017; Smith et al., 2020). On
69 the other hand, other studies have reported higher CC biomass production when
70 combining species with complementary traits (*e.g.* legumes and non-legumes) (Suter
71 et al., 2017), with no direct consequence on weed suppression (Ranaldo et al., 2020).
72 Biomass is commonly used indicator to quantify competition between CC and weeds
73 because it results from resource availability and competitive outcome with
74 neighbouring plants (ability to tolerate or suppress) (Trinder et al., 2021). Thus, most
75 authors reporting a weed suppressive effect of CC showed a negative relationship
76 between CC and weed biomass (Finney et al., 2017; Wittwer et al., 2017; Osipitan et
77 al., 2019), probably because of a greater ability of CC to uptake soil resources, produce
78 biomass and shade competing weeds. Nevertheless, a recent study from Adeux et al.
79 (2021) showed that the relationship between weed and CC biomass was dependent
80 on CC species, with *Brassica juncea* being more weed suppressive than *Vicia villosa*
81 at low levels of CC biomass. Such results suggest that for a given level of CC biomass,
82 certain CC species traits (*e.g.* nitrogen uptake ability or early ground cover) could
83 provide a competitive advantage.

84 CC and weed biomass depends on soil nutrient availability (Ryan et al., 2011), water
85 availability (Ruis et al., 2019), light availability (MacLaren et al., 2019), plant density
86 (Bybee-Finley et al., 2022) and plant traits such as the ability to acquire and transform
87 these resources into biomass (Tribouillois et al., 2015; Tribouillois et al., 2016; Blesh,
88 2018). Thus, competition is expected to be more intense when resource availability is

89 low and when CC and weeds share the same pool of resources in the same time and
90 space (Garnier and Navas, 2012). Light is often considered as the main driver of
91 competition between crops and weeds on agricultural lands (Perry et al., 2003). But in
92 summer-sown CC, competition for soil resources can be as important as competition
93 for light because soil water and nitrogen can be scarce (Kiær et al., 2013). Therefore,
94 competition between CC and weeds under contrasted levels of water and nitrogen
95 availability needs to be explored to improve the weed suppressive effect of CC
96 mixtures. However, we found no study which explored nitrogen competition between
97 weeds and CC mixtures by quantifying plant nitrogen uptake.

98 Focusing on weed community composition could shed light on specific interactions
99 between weed species, CC mixtures and/or resource availability (Smith and Gross,
100 2007; Navas, 2012). Weed community composition, diversity and abundance are
101 mainly driven by the expression of the weed seed bank (Smith et al., 2015), but could
102 be influenced directly by soil nitrogen availability or indirectly by variations in CC
103 nitrogen acquisition (Pyšek and Lepš, 1991). Smith et al. (2015) reported a general
104 negative relationship between CC biomass and weed species richness across all CC
105 species, but lacked to identify a filtering effect of certain CC mixtures on specific weed
106 species. However, this study only tested CC monocultures and one CC mixture
107 composed of five species. Testing a greater diversity of CC mixtures under contrasted
108 levels of water and nitrogen availability could help to further investigate the filtering
109 effect of CC mixtures on weed communities.

110 The objective of this study was to investigate the weed suppressive effect of summer-
111 sown CC mixtures during the fallow period, under contrasted levels of soil water and
112 nitrogen availability. To our knowledge, our study is the first to manipulate contrasted
113 levels of soil resource availability (nitrogen, water) and CC mixtures and investigate
114 their effect on (i) weed and CC aboveground biomass, (ii) the relationship between the
115 latter and (iii) weed community composition. We hypothesised that (1) weed and CC
116 biomass depend on interactions between CC mixture and resource availability, (2)
117 higher CC biomass generally ensures higher weed suppression but that some CC
118 mixtures are more weed suppressive than others at low levels of CC biomass, and (3)
119 weeds show species-specific response to contrasted levels of CC mixtures and
120 resource availability. The present study is based on a factorial experiment repeated on
121 two field:years, which compared four CC mixtures to a bare soil control, under different
122 levels of nitrogen and water availability. A total of 360 weed and CC biomass samples
123 and their nitrogen quantities were analysed.

124 **2. Material and methods**

125

126 *2.1. Site characteristics*

127 The experiment was replicated on two field:years (one field in 2016 and another in
128 2017, further denoted year 1 and year 2, respectively) at the INRAE experimental site
129 in Bretenièrre (47°14'11.2" N, 5°05' 56.1" E), 15 km south-east of Dijon, France. Both
130 fields were characterised by a clay (40%) - silt (50%) soil texture and a calcareous
131 bedrock. The site is subject to a semi-continental climate, characterised by mean
132 annual precipitation of 765 mm and an average daily temperature of 10.5°C, with

133 strong variations between winter and summer. Average daily temperature and daily
134 rainfall over the course of the two years can be found in Supp. Fig. 1. The
135 environmental conditions were quite similar in both years with an average of 25 mm of
136 rainfall around CC sowing and moderate temperatures in summer. A winter wheat crop
137 preceded CC sowing in both field:years. Straw were exported and stubble was
138 superficially tilled after winter wheat harvest. Unfortunately, post-harvest soil inorganic
139 nitrogen was not measured at the field but rather estimated for each year (Supp. Table
140 1) using the nitrogen balance method (Sainju, 2017). Residual nitrogen was higher in
141 year 1 (~74 kg N ha⁻¹) than in year 2 (~0 kg N ha⁻¹), mostly due to a combination of
142 high mineral nitrogen fertilisation and low winter wheat uptake in year 1.

143 2.2. Experimental set-up

144 Four CC mixtures (2leg-: 2 CC species without legumes, 2leg+: 2 CC species with
145 legume, 8leg-: 8 CC species without legumes and 8leg+: 8 CC species with four
146 legumes) including a wide range of species commonly used by French farmers (Table
147 1, black oat, winter rye, sorghum, foxtail millet, common vetch, berseem clover, faba
148 bean, brown hemp, brown mustard, field mustard, lacy phacelia, niger seed,
149 buckwheat, linseed) were compared to a bare soil control. CC were sown in summer
150 (August 10th and July 28th year 1 and 2, respectively) at their recommended pure
151 stand seeding rate divided by the number of species in the mixture and majored by
152 15% (Table 1). CC mixtures were created to occupy distinct spaces along a species
153 richness and biomass productivity gradient, determined by their ability to respond to
154 resource availability. *Poaceae* and *Brassicaceae* species were expected to quickly pre-
155 empt nitrogen and produce high biomass while *Fabaceae* species could avoid nitrogen
156 competition through dinitrogen fixation. Moreover, CC species were likely to differ in
157 their root and aerial architecture, thereby modifying competition for light and/or soil
158 resources. CC mixtures were composed of two or 8 species, as two is the minimum to
159 obtain a mixture and because 8 represents the end of the species richness gradient
160 commonly used by farmers in CC mixtures. Further increasing CC species richness
161 would have either induced functional redundancy or the inclusion of less relevant
162 species. Two levels of irrigation (0 mm or 40 mm) and nitrogen fertilisation (0N: 0 kg N
163 ha⁻¹ or 30N: 30 kg N ha⁻¹ of ammonium nitrate 33.5%) were applied around CC sowing
164 to mimic contrasted levels of soil resources. The experiment was set-up as a double
165 entry split-plot in a randomised complete block design (Supp. Fig. 2, n blocks = 3).
166 Main plots were characterised by the intersection of the two irrigation and nitrogen
167 fertilisation strips for practical reasons. CC mixtures and bare soil control were
168 replicated 3 times within each main plot due to a forthcoming CC termination factor
169 (not studied here). As a result, each combination of CC mixture (N=5), nitrogen level
170 (N=2) and irrigation level (N=2) was replicated 9 times, resulting in a total of 180 plots
171 (10 m² each). Since weeds are known to be patchily distributed within a field (Hughes,
172 1990), four weed species (*Echinochloa crus-galli*, *Veronica persica*, *Geranium*
173 *dissectum*, *Chenopodium album*) with contrasted functional attributes (e.g. height, leaf
174 area) were sown at 60 seeds m⁻² per species across all plots at CC sowing to
175 homogenise weed pressure within each experiment.

176 *2.3. Biomass sampling and nitrogen quantity*

177 CC (all species combined) and weed (per species) aboveground biomass were
178 sampled 90 days after CC sowing in each experiment, in all 180 plots, in one 1 m² and
179 two 0.25 m² quadrats per plot in year 1 and 2, respectively. This type of sampling was
180 chosen because competition occurs in a close environment and two quadrats were
181 used year 2 to improve the technical feasibility during biomass sampling. All biomass
182 samples were oven dried at 80°C for 72 hours and weighed.

183 In order to partition nitrogen uptake between CC and weeds, plant nitrogen was
184 quantified. The nitrogen concentration (g N / g aboveground biomass) in CC (all
185 species summed) and weed (per species) was measured using the Dumas method
186 (ThermoScientific™ FLASH 2000 CHNS/O Analyzer). The two quadrats were summed
187 in year 2 so as to quantify one value of nitrogen concentration per plot for both CC (all
188 species summed) and weeds (per species). The quantity of nitrogen in plants (g N m⁻¹)
189 was calculated by multiplying the nitrogen concentration (g N / g biomass) by
190 biomass (g m⁻¹) at the plot:year scale.

191 The two CC and weed biomass samples per plot in year 2 were averaged at the plot
192 level to avoid an additional hierarchical level (pseudo-replication) and obtain a
193 balanced design between years. Weed biomass and nitrogen quantity per species
194 were summed at the plot level to obtain total weed biomass and total weed nitrogen
195 quantity.

196 *2.4. Nitrogen nutrition index*

197 The nitrogen nutrition index (NNI) of CC mixtures was calculated at the plot:year scale
198 to quantify their nitrogen status, following Eq. 1 provided by Lemaire and Gastal (1997):

199
$$NNI = \frac{\%N_{\text{measured}}}{\%N_c} \quad (\text{Eq. 1})$$

200 where, %N_{measured} (g N / g biomass) is the nitrogen concentration measured at the
201 plot:year scale in CC mixtures (all species summed) and %N_c (g N / g biomass) is
202 computed as Eq. 2:

203
$$\%N_c = 5.1 * (DM)^{-0.34} \quad (\text{Eq. 2})$$

204 where, DM is the total (CC mixture+weeds) biomass (t ha⁻¹).

205 This equation shows the critical nitrogen dilution curve (*i.e.* relationship between
206 nitrogen and biomass accumulation at optimal canopy nutrition). Initially developed for
207 monospecies canopies (Lemaire and Gastal, 1997), it was adapted to multispecies
208 canopies (Louarn et al., 2021). The parameters of Eq. 2 are valid for legume as well
209 as non-legume C3 plants (Munier-Jolain et al., 2010), *i.e.* the vast majority of CC and
210 weed species in the experiment.

211 When NNI = 1, nitrogen nutrition is considered optimal, while NNI > 1 and < 1 indicate
212 excess and deficient nitrogen nutrition, respectively.

213 In our study, a NNI < 1 would indicate nitrogen deficiency in CC mixtures, either as a
214 result of below optimal initial soil nitrogen availability or as a result of nitrogen uptake

215 from weeds. To investigate whether fertilisation enhanced the weed suppressive effect
216 of CC mixtures through enhanced nitrogen nutrition, NNI of CC mixtures were
217 compared between fertilised and non-fertilised plots.

218 *2.5. Statistical analysis*

219 Statistical analysis were performed with the R software version 4.0.2 (R Core Team,
220 2021). CC and weed biomass, nitrogen quantities and CC NNI were analysed at the
221 plot scale (*i.e.* average of the two pseudo replicates for year 2) with tweedie
222 generalised mixed effect models using a log link function, available in the R package
223 glmmTMB (Brooks et al., 2017). All response variables were modelled as a function of
224 year (as factor, confounded with field), CC mixture, fertilisation (as factor), irrigation
225 (as factor) and all possible interactions between these four factors. Bare soil controls
226 were removed from the analysis when focusing only on CC mixtures (*i.e.* CC biomass
227 and nitrogen quantity, and CC NNI). To identify whether the relationship between CC
228 and weed biomass was dependent on CC mixtures, weed biomass was also modelled
229 as a function of year, CC mixture, CC biomass and all possible interactions between
230 the three latter.

231 For all six models an *a priori* set of five plausible random effect structures were defined
232 and compared using Akaike's Information Criterion (AIC), in order to best capture the
233 structure of the experimental design (Supp. Table 2). The model with the lowest AIC
234 was retained for analysis. The absence of confounding factors with the non-
235 randomised nitrogen strip was confirmed through an investigation of within-field yield
236 variability of the preceding winter wheat crop and soil resistivity (Supp. Fig. 3). The
237 split-split-plot model with irrigation as main plot factor, fertilisation as subplot factor and
238 CC mixture completely randomised within was retained for the analysis of CC NNI,
239 weed biomass, weed nitrogen quantity and the relationship between weed and CC
240 biomass, while a spatial exponential model was chosen to analyse CC biomass and
241 CC nitrogen quantity. Significance of effects was determined through Type III Wald
242 Chi-Square tests using the function Anova of the R package car (Fox and Weisberg,
243 2020). Contrasts were adjusted using the R package emmeans (Lenth, 2021).

244 Partial Redundancy Analysis (*i.e.* pRDA) was performed at the plot scale (*i.e.* average
245 of the two pseudo replicates for year 2) for each experiment so as to investigate
246 individual weed species response to experimental factors, after the removal of block
247 effect, using the rda function of the R package vegan (Oksanen et al., 2020). The
248 choice of a linear method was confirmed by assessing the length of the first axis of a
249 detrended correspondence analysis (<3 SD unit). The response matrix consisted of 144
250 row (one for each plot sown with a CC mixture) and 19 (one for each weed species
251 observed in year 1) or 20 columns (year 2). Weed species biomass were ln-
252 transformed to reduce the influence of dominants. Significance of effects were
253 determined through restricted permutation tests (n=999) taking into account blocks and
254 main plots (combinations of irrigation and fertilisation), using the R function permute of
255 R package permute (Simpson, 2019). Significant effects were graphically represented
256 using the CANOCO Software.

257

258 3. Results

259

260 3.1. Cover crop biomass, nitrogen quantity and nitrogen nutrition index

261 CC biomass was driven by the interactions between year, fertilisation and CC mixture
262 and between year, irrigation and CC mixture (Table 2). Across all other factors, CC
263 biomass was greatest in 2leg-, intermediate in 8leg- and 8leg+ and lowest in 2leg+
264 (Fig. 1a). All CC mixtures (except 2leg+ in year 2) responded significantly and
265 positively to nitrogen fertilisation (+80 to 121 g DM m⁻²), but to a lower extent for 2leg+
266 in year 1 (+42 g DM m⁻²) and 8leg+ in year 2 (+32 g DM m⁻²) (Fig. 2a). Only 8leg+ in
267 year 2 responded significantly and positively (+61 g DM m⁻²) to irrigation while biomass
268 of other CC mixtures remained unchanged (Supp. Fig. 4a).

269 CC nitrogen quantity was driven by the interaction between year, fertilisation and CC
270 mixture (Table 2). All CC mixtures (except legume-based CC in year 2) showed a
271 significant increase in nitrogen quantity (+0.95 to 1.93 g N m⁻²) with nitrogen fertilisation
272 (Supp. Fig. 5a), potentially corresponding to one to two-thirds of the 30 kg N ha⁻¹ (= 3
273 g N m⁻²) supplied by fertilisation at CC sowing. Nitrogen nutrition was suboptimal for
274 all CC mixtures (NNI<1) each year of the experiment (Fig. 3). NNI of CC mixtures was
275 driven by the interaction between year, fertilisation and CC mixture (Table 2). All NNI
276 values of CC were higher with nitrogen fertilisation in year 1, except 8leg+ (Fig. 3). In
277 year 2, nitrogen fertilisation increased NNI of non-legume-based CC mixtures (not
278 significantly for 8leg-) whereas NNI of legume-based CC mixtures decreased (Fig. 3).

279 3.2. Weed biomass, nitrogen quantity and community composition

280 Weed biomass was driven by the interactions between year, fertilisation and CC
281 mixture and between year, irrigation and CC mixture (Table 2). Across all other factors,
282 2leg+ reduced weed biomass by 68% (-130 g DM m⁻²) whereas all other CC mixtures
283 reduced weed biomass by 90 to 94% (-170 to -180 g DM m⁻²), with respect to the bare
284 soil control (Fig. 1b). Weed biomass was generally unaffected or slightly reduced (-5
285 and -7 g DM m⁻² for 8leg- and 8leg+, respectively, in year 1) with nitrogen fertilisation
286 for all CC mixtures (Fig. 2b). The only exception was for legume-based CC mixtures in
287 year 2 where weed biomass increased with nitrogen fertilisation (+12 and 34 g DM m⁻²
288 for 8leg+ and 2leg+, respectively). Irrigation increased weed biomass in 6 out of 8 CC
289 mixtures:year combinations (+7 to 30 g DM m⁻²) (Supp. Fig. 4b).

290 Weed nitrogen quantity was driven by the interaction between year, fertilisation and
291 CC mixture (Table 2). Nitrogen fertilisation increased weed biomass in two
292 combinations of CC mixture:year out of eight (+0.17 and 0.75 g N m⁻² for 2leg+ in year
293 1 and 2, respectively), decreased weed biomass in one (-0.03 g N m⁻² for 8leg- in year
294 1) and had no effect for the five others (Supp. Fig. 5b).

295 Among the 33 weed species recorded, total weed biomass was dominated by wheat
296 volunteers (61 and 12% in year 1 and 2, respectively) and two of the sown weed
297 species each year, *i.e.* *Geranium dissectum* and *Veronica persica* in year 1 (21 and
298 9%, respectively) and *Geranium dissectum* and *Echinochloa crus-galli* in year 2 (64
299 and 10%, respectively). Wheat volunteers were homogeneous among plots whereas
300 the two sown weed species varied across CC mixtures and resource availability each
301 year. Multivariate analysis showed that irrigation increased biomass of *Geranium*

302 *dissectum* and *Veronica persica* in year 1, especially in 2leg- and 2leg+ (Supp. Fig.
303 6a). Irrigation and nitrogen fertilisation increased biomass of *Geranium dissectum* and
304 *Echinochloa crus-galli* in year 2, especially in 2leg+ and 8leg+ (Supp. Fig. 6b).

305 3.3. Weed - cover crop biomass relationship

306 Weed biomass was driven by year, CC biomass, CC mixture and all possible
307 interactions between these three latter (Table 3). The relationship between weed and
308 CC biomass was negative in all CC mixtures in year 1 but only in 8leg+ in year 2 (Fig.
309 4). Increasing CC biomass from 100 to 400 g DM m⁻² had little effect on weed biomass
310 in all CC mixtures (-10 to -15 g DM m⁻²), except for 2leg+ in year 1 (-45 g DM m⁻²) and
311 8leg+ in year 2 (-33 g DM m⁻²).

312

313 4. Discussion

314

315 4.1. Increasing resource availability does not ensure a higher weed suppressive 316 effect of cover crop mixtures

317 Our study confirmed that CC could suppress weed biomass, with respect to the bare
318 soil control, as previously reported in numerous studies (Teasdale et al., 2007;
319 Baraibar et al., 2018; Florence and McGuire, 2020; Adeux et al., 2021). However, CC
320 biomass was highly variable depending on CC mixture and resource availability, and
321 weed biomass mainly varied depending on CC mixture, which partly validates our first
322 hypothesis of an interaction effect between CC mixture and resource availability on CC
323 and weed biomass. Weed suppression was higher in the 8-species non-legume based
324 CC mixtures although the 2-species non-legume based CC mixtures produced the
325 highest CC biomass, suggesting that CC biomass alone does not fully explain weed
326 suppression. In both years, when nitrogen fertilisation increased biomass of CC
327 mixtures, no repercussions on weed biomass of biological relevance were observed.
328 In contrast, irrigation increased weed biomass whereas biomass of CC mixtures
329 remained stable. Besides, in 5 out of 8 CC mixture:year combinations, the relationship
330 between weed and CC biomass was negative as previously observed many times
331 (Finney et al., 2017; Wittwer et al., 2017; Osipitan et al., 2019), but the major part of
332 weed suppression was done at low levels of CC biomass (< 200 g DM m⁻²). In the other
333 3 out of 8 CC mixture:year combinations, no relationship between weed and CC
334 biomass was found, suggesting that nitrogen fertilisation did not hastened CC canopy
335 close, which did not limited weed growth. The interaction between CC mixture and CC
336 biomass partly confirmed our second hypothesis that a higher CC biomass generally
337 ensure higher weed suppression but that some CC mixtures are more weed
338 suppressive than others at low CC biomass levels. All these observations suggest that
339 CC biomass is not the only factor of weed suppression contrary to conclusions of many
340 studies (Mirsky et al., 2013; Bybee-Finley et al., 2017; MacLaren et al., 2019; Florence
341 and McGuire, 2020).

342 4.2. Trait complementarity within cover crop mixtures

343 Our results showed that weed suppression was mainly driven by CC mixtures and to
344 a less extent by its interaction with nitrogen fertilisation. In our study, non-legume
345 based CC mixtures were mainly composed of *Poaceae*, *Brassicaceae* and/or other
346 species (e.g. lacy phacelia) that generally produce high biomass through high nitrogen
347 uptake (Tribouillois et al., 2015). However, these CC mixtures were nitrogen deficient
348 in both years of the experiment ($NNI < 1$), despite the high level of soil mineral nitrogen
349 estimated after wheat harvest in year 1. Such discrepancies could arise from either the
350 loss of a part of soil mineral nitrogen between wheat harvest and CC sowing (e.g.
351 through nitrate leaching), or a limitation in nitrogen absorption by plants due to water
352 stress. Nitrogen fertilisation increased biomass, nitrogen quantity and nitrogen nutrition
353 level in non-legume based CC mixtures but did not lead to reduced weed biomass or
354 weed nitrogen uptake. It suggests that competition for nitrogen was not the major driver
355 of weed biomass. Rather, we hypothesise that light was the limiting factor for weed
356 growth in non-legume based CC mixtures. Higher weed suppression in 8-species non-
357 legume based CC mixtures than in 2-species non-legume based CC mixtures (in both
358 years) could be related to trait complementarity between CC species (e.g. early growth
359 rate, leaf area establishment, root architecture), leading to a more efficient use of light
360 (Ranaldo et al., 2020), and a suppressive effect of weeds of short stature (Cressman
361 et al., 2011). Tribouillois et al. (2015) showed that the species composing the 8-species
362 CC mixtures, such as buckwheat, niger or brassica species, had a higher potential
363 growth rate than phacelia or the *Poaceae* species (either winter or summer species)
364 composing the 2-species CC mixtures, thereby supporting our hypothesis of greater
365 weed suppression through a more efficient use of light in highly diversified CC mixtures
366 (Suter et al., 2017).

367 The effect of nitrogen fertilisation on weed suppression by legume-based CC mixtures
368 (either 2- or 8-species) was inconsistent across the two field:years (i.e. experiments).
369 Nitrogen fertilisation had no (or slightly positive) effect on the weed suppressive effect
370 of legume-based CC mixtures in year 1 (i.e. when soil mineral nitrogen at crop harvest
371 was estimated high), whereas it reduced their weed suppressive effect in year 2 (i.e.
372 when soil mineral nitrogen at crop harvest was estimated low). Legume species are
373 favoured in nitrogen-poor environment due to their ability to symbiotically fix
374 atmospheric dinitrogen (Clark et al., 2007), and disfavoured in nitrogen-rich
375 environment because of their low initial growth (Voisin et al., 2002; Möller et al., 2008).
376 Hence, the differences in weed suppression between fertilised and non-fertilised plots
377 of legume-based CC mixture could be related to a modification of the relative
378 contribution of each CC species present in the mixture. We hypothesise that the
379 estimated high level of available soil nitrogen in year 1 at crop harvest did not promote
380 the competitive ability of legumes (Andersen et al., 2005), while non-legumes were
381 favoured in the mixtures (albeit at a reduced density than if legumes were not present),
382 resulting in a similar weed suppressive effect of legume-based CC than of non-legume
383 based CC mixtures during that year. Under limited soil nitrogen availability (i.e. soil
384 mineral nitrogen was estimated low in year 2), fertilisation probably favoured the growth
385 of non-legume species while limited the growth of the legumes in the legume-based
386 CC mixture, resulting in stable CC biomass across both fertilised and non-fertilised
387 plots (i.e. only a change in the relative composition). In addition, non-legume species
388 used to exhibit a lower nitrogen rate (%) in their biomass than legume species (Guinet

389 et al., 2020). Thus, the reduction of legume biomass relatively to the increase of non-
390 legume biomass (*i.e.* change in the relative composition of the biomass), combined
391 with the lower nitrogen rate of non-legume species compared to legume species,
392 decreased the N percent in the total biomass, decreasing NNI. Weed suppression was
393 then enhanced in non-fertilised plots through a direct effect of lower soil nitrogen
394 availability and a better complementarity between legume and non-legume CC species
395 to outcompete weeds for light and nitrogen (Suter et al., 2017; Ranaldo et al., 2020).

396 Finally, weed biomass depended also on water availability because weed biomass
397 increased with irrigation whereas CC biomass did not (except in one combination of
398 year:CC mixture out of 8). We hypothesise that weed germination/emergence and
399 early growth was enhanced by irrigation (Evans and Etherington, 1990), resulting in
400 higher weed biomass at sampling. Indeed, weed seed imbibition is related to seed
401 traits (Gardarin et al., 2011) such as seed weight, with smaller seeds at a competitive
402 advantage on the soil surface because of improved seed:soil contact. Hence, the
403 legume species in CC mixtures could have showed greater sensitivity to water
404 availability than the *Poaceae* and *Brassicaceae* species we selected because of their
405 large seeds (Tribouillois et al., 2016). In addition, weed seeds were placed in ideal
406 position to germinate, *i.e.* superficially incorporated in the soil (Cordeau et al., 2015),
407 since they were sown with CC seeds to ensure within plot homogeneity of their
408 distribution. Differences in base water potential for germination between weeds
409 (Guillemin et al., 2013) and CC species (Tribouillois et al., 2016) could explain the
410 positive effect of irrigation on weeds and the absence on CC species. Even if weed:CC
411 competition is intense after canopy closure, it could be determined at early stage by
412 the relative growth differences (Cordeau et al., 2015), particularly when soil water
413 availability varies (Cordeau et al., 2018).

414 *4.3. Influence of cover crop mixtures and soil resources on individual weed species* 415 *biomass*

416 Weed biomass was mostly represented by two annual and sown species each year,
417 (*i.e.* *Geranium dissectum* and *Veronica persica* in year 1 and *Geranium dissectum* and
418 *Echinochloa crus-galli* in year 2), although homogeneous wheat volunteers across
419 plot:years. Individual weed species did not respond to the same manner across year,
420 reflected by the interaction between CC mixture and resource availability, confirming
421 our third hypothesis of a CC mixture and resource availability effect on the biomass of
422 some weed species (Bàrberi and Mazzoncini, 2001; Smith et al., 2015). Irrigation
423 increased biomass of *Geranium dissectum* and *Veronica persica* in year 1 probably
424 because of increased weed seed germination (Cordeau et al., 2018) and absence of
425 CC response. Nitrogen fertilisation increased biomass of *Geranium dissectum* and
426 *Echinochloa crus-galli* in year 2, especially in legume-based CC mixtures because of
427 a lower competitive ability of legume-based CC mixtures at higher levels of soil nitrogen
428 (Ellenberg, 1979; Moreau et al., 2014). Such results revealed that competitive
429 outcomes between CC mixtures and weed species were driven by a complex
430 interaction between resource availability and species traits. Thus, we assume that the
431 selection of CC mixtures to control specific weed species seems difficultly practicable
432 due to the interaction between resource availability and species traits, which govern
433 competitive outcomes (Goldberg, 1990; Funk and Wolf, 2016).

434 4.4. Practical avenues for improving weed suppression by cover crops

435 In our experiment, all CC mixtures significantly reduced weed biomass, with respect to
436 the bare soil control, and most of this reduction was done at low levels of CC biomass
437 ($< 200 \text{ g DM m}^{-2}$). From a practical point of view, it is difficult to forecast CC biomass
438 in a given year and pedo-climatic context because it will depend on the choice of CC
439 species, sowing date/rate (Mirsky et al., 2017), resource availability, and in the case of
440 CC mixtures, on the relative seeding rate between species (Bybee-Finley et al., 2022),
441 which will affect intra- and interspecific competition. Moreover, we argue that
442 identifying a CC biomass threshold, above which the weed suppression objective is
443 reached, is of little relevance. Firstly, weed suppression is never complete and thus
444 the relative risk of residual community will be a matter of farmer perception (Wilson et
445 al., 2009) and relative to the forthcoming management practices such as CC
446 termination or tillage practices associated to the subsequent cash crop (Adeux et al.,
447 2021). Unfortunately, little is known about weed phenology in CC mixtures and further
448 investigation on weed communities in subsequent crops remains primordial. Secondly
449 and most importantly, biomass production (whether weed or CC) is a matter of
450 resource availability and relative uptake ability, illustrated by the fact that our threshold
451 of 2 t ha^{-1} ($= 200 \text{ g DM m}^{-2}$) is lower than the 3 t ha^{-1} threshold often relayed by studies
452 under temperate European climates (*e.g.* Gfeller et al. (2018)) and clearly lower than
453 the 8 t ha^{-1} threshold identified as consistent across Northeastern US (Mirsky et al.,
454 2013). Along these lines, Björkman et al. (2015) identified that weed suppression was
455 irrespective of pure-stand mustard biomass.

456 High weed suppression observed at low CC biomass in our experiment could be partly
457 attributed to high seeding rates (+15% with respect to a pure stand and the number of
458 species in the mixture). Previous studies on pure stands of legumes (Mirsky et al.,
459 2017) or grasses (Boyd et al., 2009) or on grass-legume mixtures (Uchino et al., 2011;
460 Bybee-Finley et al., 2022) showed that increasing seeding rates increased CC biomass
461 and thus, their weed suppressive effect (at a given level of resource availability). This
462 high suppressive effect could also be attributed to certain species traits such as leaf
463 area, growth rate or nitrogen acquisition rate, with CC mixtures being more competitive
464 at low levels of biomass due to a higher capacity to pre-empt resources through diverse
465 strategies (Tribouillois et al., 2015). These findings suggest that farmers require
466 knowledge on CC species traits (Tribouillois et al., 2015; Tribouillois et al., 2016) to
467 guide their selection for composing CC mixtures. The development of a decision tool
468 to compose CC mixtures, based on trait hierarchies between CC and weed species
469 (*e.g.* base temperature, water base potential for germination, relative growth rate, etc.)
470 should improve the predictability of CC:weed competitive outcomes between weeds
471 and CC mixtures.

472 Temperature and water availability are major drivers of seed emergence (Baskin and
473 Baskin, 1988), which influence CC establishment (Tribouillois et al., 2016), especially
474 when CC are sown in summer when temperatures are high and water availability low.
475 We showed that irrigation increased weed biomass but not CC biomass. In the context
476 of restricted use of irrigation, as is often the case in the summer, we argue that irrigation
477 may not be a suitable lever to increase weed suppression. However in some cases,
478 irrigation could ensure the minimal CC establishment and development required to

479 deliver ecosystem services. Selecting CC species tolerant to dry conditions could thus
480 be a lever to ensure ecosystem services associated to CC, with water limited
481 conditions expected to become more and more frequent in the coming years (Allen et
482 al., 2018). In addition, broadcasting CC seeds earlier, *e.g.* before the preceding crop
483 harvest, could enhance their establishment, and procure them a competitive
484 advantage over weeds germinating later in the season (Dorn et al., 2015; Baraibar et
485 al., 2018; Osipitan et al., 2018).

486 We showed that increased soil nitrogen availability increased biomass, nitrogen
487 quantity and nitrogen nutrition level in CC mixtures but this did not lead to a biologically
488 meaningful decrease in weed biomass or weed nitrogen uptake. It suggests that CC
489 fertilisation may not be a suitable mean to increase weed suppression. In certain cases
490 (*e.g.* legume-based CC mixtures in year 2), nitrogen fertilisation even increased weed
491 biomass, potentially resulting in higher weed seed set, albeit weeds completed their
492 life cycle. Unfortunately, weed phenology was not recorded, and this information is
493 currently lacking in the scientific literature.

494 In our study, CC biomass was not the only driver of weed suppression contrary to what
495 is commonly affirmed (Bybee-Finley et al., 2017; MacLaren et al., 2019; Florence and
496 McGuire, 2020). However, we did not relate greater weed suppression observed in 8-
497 species non-legume CC mixtures to a better use of water, as observed by (Nielsen et
498 al., 2015), nor nitrogen, but to species characteristics such as rapid growth rate
499 (Tribouillois et al., 2015) and early soil coverage (Brennan and Smith, 2005). When
500 composing CC mixtures, attention should be paid to species characteristics and their
501 response to contrasted levels of water and nitrogen availability.

502

503 **5. Conclusion**

504 The weed suppressive effect of CC was analysed during a 2-year experiment
505 combining four CC mixtures and a bare soil under various manipulated levels of soil
506 resources (nitrogen and water). Our study showed that CC biomass was not the only
507 factor responsible for weed suppression and that CC and weed biomass were mainly
508 influenced by interactions between CC mixtures and fertilisation, and between CC
509 mixtures and irrigation. Nitrogen fertilisation increased biomass of non-legume based
510 CC mixtures but this only resulted into a further reduction of weed biomass of little
511 biological relevance. Legume-based CC mixture showed differences in weed
512 suppression between fertilised and non-fertilised plots when nitrogen availability before
513 CC sowing was estimated low, which was probably related to a modification of the
514 relative contribution of each CC species present in the mixture. Nitrogen fertilisation
515 may have favoured non-legume CC species, which in turn reduced legume growth and
516 weed suppression, whereas non-fertilisation of legume-based CC mixtures enhanced
517 weed suppression due to complementarity between non-legume and legume species.
518 Weed biomass generally benefited more from irrigation than CC mixtures, which could
519 be attributed to a higher probability of weed seed germination. Among the 33 weed
520 species recorded, weed communities in each plot were dominated by homogeneous
521 densities of wheat volunteers and *Geranium dissectum*, *Veronica persica* and

522 *Echinochloa crus-galli*, whose species biomass varied depending on the interaction
523 between year, CC mixture and resource availability. Results revealed that competitive
524 outcomes between CC mixtures and weed species were driven by a complex
525 interaction between resource availability and species traits. Thus, we assume that
526 selecting CC mixtures to reduce one problematic weed species would be difficultly
527 practicable, considering the interaction between the environment and species traits,
528 which govern competitive outcomes. The development of a CC mixture composition
529 decision tool based on trait hierarchies between CC and weed species (*e.g.* base
530 temperature, water base potential for germination, relative growth rate, etc.) could
531 improve the predictability of CC mixture:weed competitive outcomes for given levels of
532 resource availability. Experiments focusing on plant traits could further improve our
533 understanding of weed:CC competitive outcomes under various levels of resource
534 availability.

535

536 **Author's contribution**

537 SC, HB and JM designed the experiment. SC, DM and JPG funded the research. RH
538 and JM conducted the experiment, and collected and formatted soil data and preceding
539 winter wheat yield. SC, GA, HB and AM sampled biomass and quantified nitrogen
540 concentration. AR and GA analysed the data. AR, DM, GA, JPG and SC were involved
541 in the interpretation of the results and contributed to writing the original version of the
542 manuscript and improving the subsequent ones. All authors agreed to the final version
543 of the manuscript.

544

545 **Declaration of Competing Interest**

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

548

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802 **Figures and tables**

803 **Table 1.** Cover crop (CC) species and seeding rates, used in 2 or 8 CC species
 804 mixtures including (leg+) or not (leg-) legumes in year 1 (2016) and 2 (2017).
 805 Seeding rates were computed as number of seeds m⁻² = [seeding rate in pure stand /
 806 number of species in the mixture * (1000/Thousand-seed weight)] majored by 15%.
 807 Winter grasses in year 1 were replaced by summer grasses in year 2 to avoid grass
 808 volunteers in the subsequent cereal crop.

809

Botanical family	CC species (latin name)	Seeding rate (number of seeds m ⁻²)								Recommended pure stand (g m ⁻²)‡	Thousand-seed weight in seed weight (g 1000 seeds ⁻¹)
		Year 1				Year 2					
		2leg-	2leg+	8leg-	8leg+	2leg-	2leg+	8leg-	8leg+		
<i>Poaceae</i>	Black oat (<i>Avena strigosa</i>)		68	17						2	16.9
	Winter rye cv. Multicaule (<i>Secale cereale</i>)	286		71	71					8	16.1
	Sorghum (<i>Sorghum bicolor</i>)					33	8			1.5	26.3
	Foxtail millet cv. Early bird (<i>Setaria italica</i>)						351	88	88	2.5	4.1
<i>Fabaceae</i>	Common vetch cv. Nacre (<i>Vicia sativa</i>)		43		11		43		11	4	54
	Berseem clover cv. Tabor (<i>Trifolium alexandrinum</i>)				90				90	2.5	4
	Faba bean cv. Irena (<i>Vicia faba</i>)				3				3	10	490
	Brown hemp (<i>Crotalaria juncea</i>)				14				14	3.5	36.8
<i>Brassicaceae</i>	Brown mustard cv. Vitamine (<i>Brassica juncea</i>)			25	25			25	25	0.5	2.9
	Field mustard cv. Chicon (<i>Brassica rapa</i>)			45	45			45	45	1	3.2
	Others										
Others	Lacy phacelia (<i>Phacelia tanacetifolia</i>)	288		72		288		72		1	2
	Niger seed cv. Regyne (<i>Guizotia abyssinica</i>)			42	42			42	42	1	3.4
	Buckwheat cv. Harpe (<i>Fagopyrum esculentum</i>)			22				22		3	20
	Linseed cv. Omegalin (<i>Linum usitatissimum</i>)			38				38		2	7.5

810 ‡ (Thomas and Archambeaud, 2016)

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814 **Table 2.** ANOVA table (type III Wald Chi-Square tests) highlighting the effect of year (as factor), fertilisation (N), irrigation (irr), cover
815 crop mixture (CCm), and all possible interactions between these four factors on cover crop biomass, cover crop nitrogen uptake,
816 cover crop nitrogen nutrition index (NNI), weed biomass and weed nitrogen uptake. Results were obtained with generalised mixed
817 effect models using a tweedie distribution and a log link. Significant p-values at $p < 0.05$ are highlighted in bold.

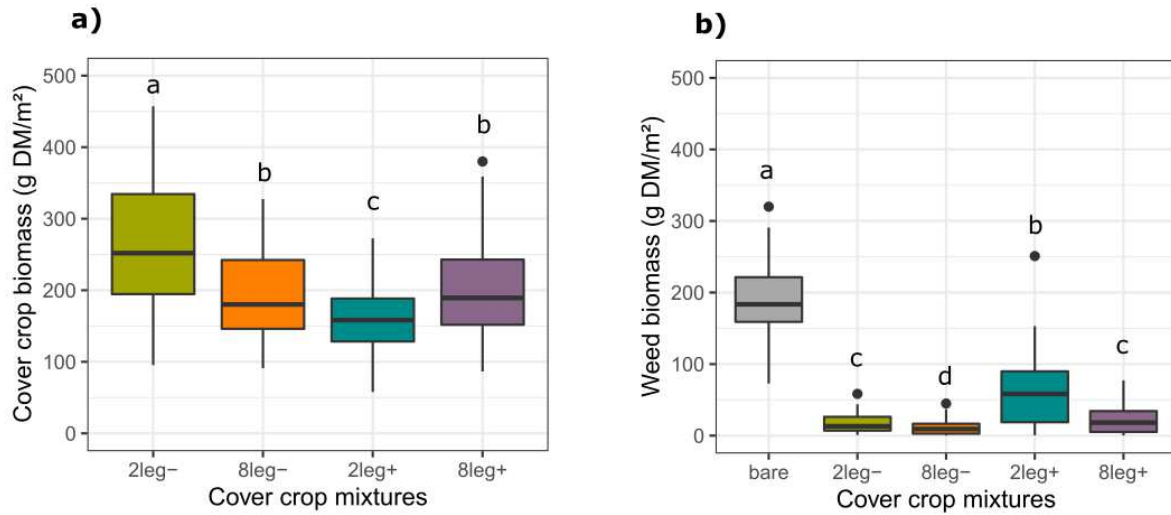
Factors	Cover crop									Weeds					
	Biomass			N quantity			NNI			Biomass			N quantity		
	χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value
year	0.12	1	0.72	7.74	1	5e-03	8.09	1	4e-03	105	1	< 2e-16	230	1	< 2e-16
N	121	1	< 2e-16	64.4	1	1e-15	11.1	1	9e-04	1.72	1	0.19	1.37	1	0.24
irr	3.70	1	0.05	0.00	1	0.95	0.60	1	0.44	74.9	1	< 2e-16	55.4	1	1e-13
CCm	265	3	< 2e-16	145	3	< 2e-16	389	3	< 2e-16	2281	4	< 2e-16	2412	4	< 2e-16
year:N	1.69	1	0.19	10.6	1	1e-03	16.1	1	6e-05	23.7	1	1e-06	6.79	1	0.01
year:irr	0.23	1	0.63	0.21	1	0.65	0.12	1	0.73	3.97	1	0.05	0.86	1	0.35
N:irr	0.31	1	0.58	2.59	1	0.11	6.69	1	0.01	0.64	1	0.42	1.29	1	0.26
year:CCm	29.3	3	2e-06	106	3	< 2e-16	122	3	< 2e-16	180	4	< 2e-16	297	4	< 2e-16
N:CCm	31.6	3	6e-07	36.8	3	5e-08	19.6	3	2e-04	31.1	4	3e-06	27.2	4	2e-05
irr:CCm	15.7	3	1e-03	9.31	3	0.03	3.07	3	0.38	29.3	4	7e-06	40.4	4	4e-08
year:N:irr	3.72	1	0.05	0.06	1	0.81	2.37	1	0.12	0.11	1	0.74	0.24	1	0.62
year:N:CCm	11.4	3	0.01	18.1	3	4e-04	15.9	3	1e-03	19.7	4	6e-04	10.1	4	0.04
year:irr:CCm	10.6	3	0.01	2.92	3	0.40	0.45	3	0.93	16.6	4	2e-03	17.4	4	1e-03
N:irr:CCm	6.34	3	0.10	3.66	3	0.30	4.61	3	0.20	5.92	4	0.21	1.38	4	0.85
year:N:irr:CCm	3.47	3	0.32	2.38	3	0.50	3.50	3	0.32	3.01	4	0.56	1.07	4	0.90

819 **Table 3.** ANOVA table (type III Wald Chi-Square tests) highlighting the effect of year
 820 (as factor), cover crop mixture (CCm), cover crop biomass (CC_biomass) and all
 821 possible interactions between these three factors on weed biomass. Results were
 822 obtained with generalised mixed effect models using a tweedie distribution and a log
 823 link. Significant p-values at $p < 0.05$ are highlighted in bold.

Variables	Weed biomass		
	χ^2	df	p-value
year	38.9	1	5e-10
CCm	135	3	< 2e-16
CC_biomass	50.1	1	1e-12
year:CCm	37.0	3	5e-08
year:CC_biomass	27.4	1	2e-07
CCm:CC_biomass	15.9	3	1e-03
year:CCm:CC_biomass	13.5	3	4e-03

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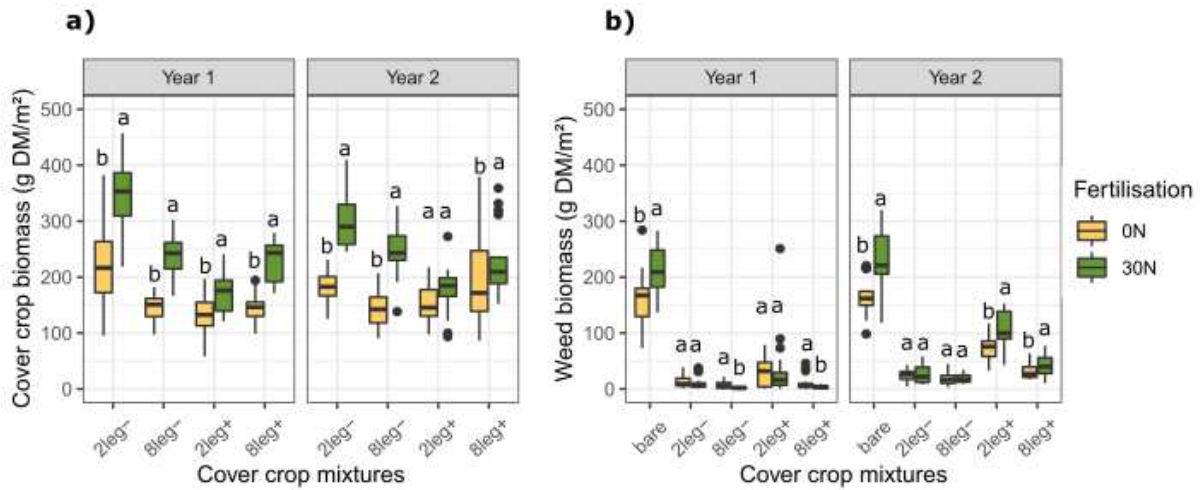
825 **Fig. 1.** Effect of cover crop mixtures on a) cover crop (CC) biomass (g of dry matter
 826 (DM)/m², N=288) and b) weed biomass (N=360). Data from year 1 and 2 were
 827 pooled. Boxplots sharing the same letter are not significantly different at p < 0.05.
 828 bare: bare soil control; 2leg-: 2 CC species without legumes; 8leg-: 8 CC species
 829 without legumes; 2leg+: 2 CC species with legumes; 8leg+: 8 CC species with
 830 legumes.



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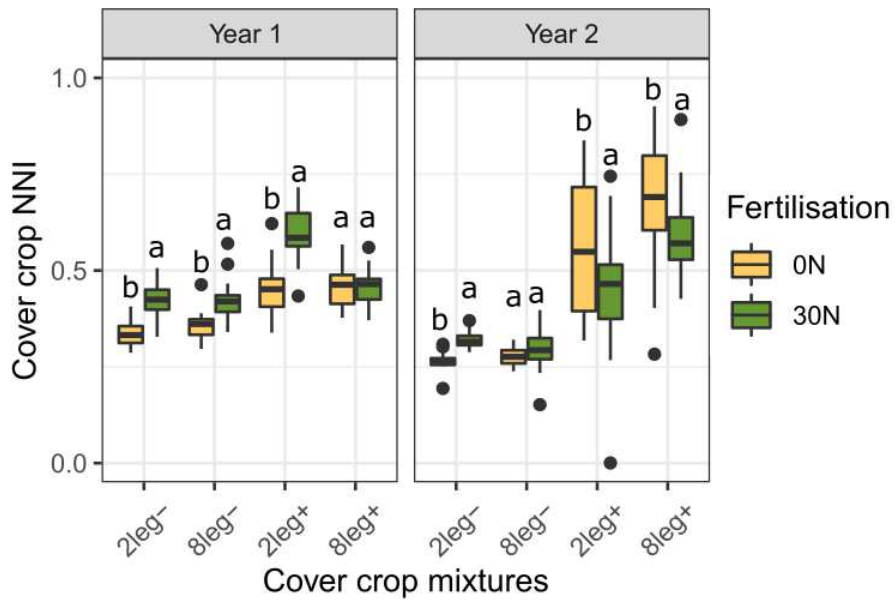
833 **Fig. 2.** Effect of nitrogen fertilisation rate on a) cover crop (CC) biomass (g of dry
 834 matter (DM)/m², N=288) and b) weed biomass (N=360) for each combination of CC
 835 mixture and year. Comparisons are made between 0N to 30N for each combination
 836 of CC mixture and year, boxplots sharing the same letter are not significantly different
 837 at $p < 0.05$. 0N: no nitrogen fertilisation, 30N: 30 kg N ha⁻¹ of ammonium nitrate at
 838 CC/weeds sowing; bare: bare soil control; 2leg-: 2 CC species without legumes; 8leg-
 839 : 8 CC species without legumes; 2leg+: 2 CC species with legumes; 8leg+ : 8 CC
 840 species with legumes.



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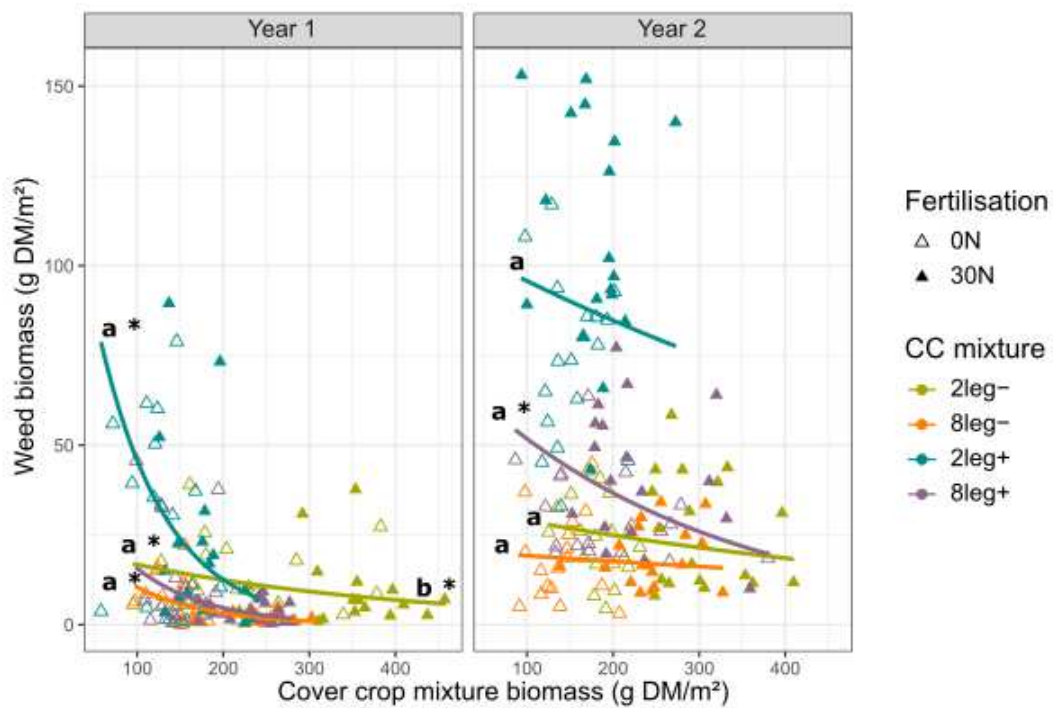
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843 **Fig. 3.** Effect of nitrogen fertilisation rate on cover crop (CC) nitrogen nutrition index
 844 (NNI, unitless) (N=288) for each combination of CC mixture and year. Comparisons
 845 are made from 0N to 30N for each combination of CC mixture and year, boxplots
 846 sharing the same letter are not significantly different at $p < 0.05$. 0N: no nitrogen
 847 fertilisation, 30N: 30kg N ha⁻¹ of ammonium nitrate at CC/weeds sowing; 2leg⁻: 2 CC
 848 species without legumes; 8leg⁻: 8 CC species without legumes; 2leg⁺: 2 CC species
 849 with legumes; 8leg⁺: 8 CC species with legumes.



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851 **Fig. 4.** Interaction between cover crop (CC) mixture biomass (g of dry matter
 852 (DM)/m²) and CC mixture identity on weed biomass in year 1 and 2. Effect of nitrogen
 853 fertilisation was partially redundant with cover crop mixture biomass so nitrogen
 854 fertilisation levels were simply distinguished graphically. Predictions were based on
 855 generalised linear mixed model taking into account random effect (Supp. Table 2).
 856 The regression lines show an average value (*i.e.* population level slope). Slopes
 857 sharing the same letter are not significantly different at $p < 0.05$. Slopes significantly
 858 different from zero are indicated with an asterisk. 0N: no fertilisation; 30N: 30kg N ha⁻¹
 859 of ammonium nitrate at CC/weeds sowing; 2leg-: 2 CC species without legumes;
 860 8leg-: 8 CC species without legumes; 2leg+: 2 CC species with legumes; 8leg+: 8 CC
 861 species with legumes.



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