

1 **Cover crops promote crop productivity but do not enhance weed management in tillage-**
2 **based cropping systems**

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15 **Abstract**

16 Cover crops (CC) have been proposed as a promising ecological tool to manage weeds and increase
17 crop productivity. We hypothesized that the repeated use of CC could increase crop yield directly
18 through nitrogen release or indirectly through a modification of weed communities. Data were
19 collected on CC biomass, weed biomass, weed community composition, and crop yield during one
20 complete rotation cycle (CC-sunflower-durum wheat-CC-maize-durum wheat) from 2011 to 2015, 18
21 years after the beginning of a long-term, single-site, split-split plot experiment focusing on tillage
22 systems (conventional (CT) vs. reduced (RT)), nitrogen rates and CC species (*Brassica juncea* (L.)
23 Czern. (Bj), *Vicia villosa* Roth (Vv), *Trifolium squarrosum* L. (Ts) and a winter baresoil control).
24 Univariate response variables were analyzed with generalized mixed effect models and community
25 data was analyzed with multivariate linear models. During the fallow period, Bj suppressed weed
26 biomass by 79, 75, 34, and 28% in CT:2012, RT:2012, CT:2014 and RT: 2014, respectively, whereas Vv
27 only suppressed weed biomass by 69 and 37% in CT and RT in 2012, respectively. Greater weed
28 suppression for Bj than Vv or Ts at lower levels of CC productivity (200 g dry biomass/m²) was
29 attributed to the importance of CC traits such as nitrophily, allelopathy and/or quick soil coverage.
30 The weed suppressive effect of CC during the fallow period was greater in CT ($\beta_{\text{slope}}=-0.28$) than in RT
31 ($\beta_{\text{slope}}=-0.16$), possibly due to contrasted weed flora and/or CC growth dynamics. Tillage and
32 herbicides overrode the potential effect of CC on weed communities in the subsequent crops. The
33 integration of a highly productive legume CC, such as Vv, allowed to increase maize productivity by
34 65 and 23% at N0 and N1, respectively. CC effects on sunflower and durum wheat yield were limited
35 due to dry weather conditions and quick nitrogen release in time, respectively. These results
36 highlight the importance of legume CC for sustaining crop productivity while reducing nitrogen
37 fertilisation. Further studies need to identify less intensive weed management practices that can
38 complement potential CC effects rather than override them.

39 **Keywords:** weed:crop interference, weed composition, *Vicia villosa* Roth, *Brassica juncea* (L.) Czern.,
40 reduced tillage, ploughing

41 **Highlights:**

- 42 • The weed suppressive potential of cover crops is not limited to biomass production
- 43 • Cover crops had no clear effect on weed communities in the subsequent weeded crops
- 44 • Management intensity overrode potential cover crop effects on weed communities
- 45 • *Vicia villosa* allowed to reduce N fertilisation and reach maximum maize yield

46

47 **1. Introduction**

48 Heavy reliance on agricultural inputs (*e.g.* herbicides, nitrogen (N) fertilisers) and intensive tillage to
49 increase crop productivity has generated a wide array of environmental impacts (*e.g.* water and air
50 pollution, soil erosion, decline in weed diversity and soil fertility) (Stoate et al., 2009). Hence,
51 reconciling crop productivity and environmental sustainability represents one of the main challenges
52 of agriculture worldwide (Stoate et al., 2009). Cover crops (CC) appear as a promising tool to improve
53 weed management and crop productivity (Blanco-Canqui et al., 2015). However, farmers currently
54 lack information on how to maximize the long-term contribution of contrasted CC species to weed
55 management and crop productivity (Blanco-Canqui et al., 2015).

56 CC effects on crop productivity have been related to the amount of inorganic N released by CC
57 through residue mineralisation and its dynamics with respect to the subsequent crop N requirements
58 in time (Magdoff, 1991; Salmerón et al., 2011). Beside soil resource availability, CC traits (*e.g.* relative
59 growth rate, N acquisition rate) and management (*e.g.* termination date and strategy) dictate the
60 total amount of N accumulated in CC biomass and its C:N ratio (Wayman et al., 2015), which
61 determines the proportion of N released from CC residues through N mineralisation (*e.g.* *Fabaceae*
62 with low C:N mineralize quicker than *Brassicaceae* or *Poaceae* with high C:N) (Justes et al., 2009). CC
63 effects on the productivity of subsequent crops have been shown to decrease with increasing levels
64 of N fertilisation of the subsequent crops (Marcillo and Miguez, 2017). However, little is known on
65 the long-term cumulative effect of contrasted cover crop types on crop productivity and their
66 potential to reduce N fertilisation (Constantin et al., 2011). Similarly, little is known on how tillage
67 intensity may influence CC biomass production (Salmerón et al., 2011; Büchi et al., 2018) or
68 mineralisation rate of CC residues over the long-term (Varco et al., 1989; Drinkwater et al., 2000). CC
69 residues are expected to mineralize faster under conventional than reduced tillage because of
70 greater soil temperatures, pore space, and contact between CC residues and soil bacteria (Lupwayi et
71 al., 2004). Nevertheless, reduced tillage has been shown to improve soil aggregate stability (Sapkota
72 et al., 2012), soil biological activity, and soil water content (Blevins et al., 1983), conditions which are
73 also favourable to CC residue mineralization. Furthermore, increased N availability after CC
74 termination could stimulate weed germination (Wayman et al., 2015), alleviate weed:crop
75 competition for N, or increase weed:crop competition for a new limiting resource (Casper and
76 Jackson, 1997).

77 CC can offset weed:crop competitive relationships through a modification of weed community
78 abundance and/or structure in the subsequent crops (Buchanan et al., 2016; Baraibar et al., 2018).
79 The weed suppressive effect of CC has been repeatedly affirmed based on the negative relationship

80 between CC and weed biomass during the fallow period (Wittwer et al., 2017). However, authors
81 have also highlighted the importance of CC traits (*e.g.* rapid emergence, early soil cover, N uptake,
82 allelopathy) in suppressing weeds during the fallow period (Dorn et al., 2015). Hence, uncertainty
83 remains as to whether or not the weed suppressive effect of CC during the fallow period can be
84 simply attributed to biomass productivity (Baraibar et al., 2018; Smith et al., 2020). Moreover, little is
85 known on how CC management practices can be adapted to maximize weed suppression during the
86 fallow period. Excessive N fertilisation of previous cash crops could give N-demanding CC species,
87 such as *Brassica juncea* (L.) Czern., a competitive advantage over weeds during the subsequent fallow
88 period (Sturm et al., 2017). Certain CC species could be better suited to grow in specific tillage
89 systems and compete with the target weed community (*e.g.* grasses under reduced tillage).
90 Furthermore, little research has focused on how weed suppression during the fallow period
91 transcribed into weed:crop interference in the subsequent crops (Brennan and Smith, 2005;
92 Buchanan et al., 2016).

93 The weed suppressive effect of CC in the subsequent crops has mainly been affirmed in no-till
94 systems (*i.e.* cropping systems in which CC residues are left on the soil surface and act as a
95 mechanical barrier against weed germination) (Teasdale, 1996), based on the negative relationship
96 between biomass of CC mulch and weed abundance measured early in the next crop growing season
97 (Buchanan et al., 2016). Little is known about the long-term contribution of CC to weed management
98 in tillage-based cropping systems (*i.e.* cropping systems in which termination of CC is ensured via
99 ploughing (CT) or superficial disking (RT) and in which in-crop weed control is ensured by herbicides)
100 (Brennan and Smith, 2005; Baraibar et al., 2018).

101 The objective of this study was to investigate the long-term contribution of contrasted CC species to
102 weed management and crop productivity in tillage-based systems. We hypothesized that (i)
103 contrasted CC species generate contrasted weed communities and levels of N availability and that (ii)
104 these changes affect crop productivity and reliance on N fertilisers. In order to benefit from potential
105 long-term effects, the present study focused on four years of data (CC biomass, crop yield, weed
106 biomass and weed community composition in both CC and subsequent crops), *i.e.* one rotation
107 cycle, collected 18 years after the beginning of a long-term factorial experiment on tillage systems, N
108 rates and CC types.

109 **2. Material and Methods**

110 **2.1. Site characteristics**

111 The long-term experiment was located at the Center for Agri-environmental Research 'E. Avanzi' of
112 the University of Pisa, Pisa, Central Italy (43°40'N, 10°19'E). The soil was a Typic Xerofluvent (further

113 information concerning soil characteristics can be found in Supp. Tab. 1). The site was subject to a
114 Mediterranean climate, with mild winters, very warm summers and rainy autumns. Temperatures
115 (maximum, average, minimum) and monthly precipitations over the four-year period in which data
116 were collected can be found in Supp. Fig. 1. Further information concerning cropping sequence and
117 weed communities prior to the beginning of the long-term experiment can be found in Bàrberi &
118 Mazzoncini (2001), Moonen & Bàrberi (2004), and Mazzoncini et al. (2011).

119 **2.2. Experimental set-up**

120 Over the 1993-2015 period of the long-term experiment, three crop sequences followed one
121 another: a maize (*Zea mays* L.) monoculture (1993-1998), a two-year durum wheat (*Triticum*
122 *turgidum* L. subsp. *durum* (Desf.) Husn.) – maize rotation (1999-2006) and a four-year durum wheat –
123 sunflower (*Helianthus annuus* L.) – durum wheat – maize rotation (2007-2015). The experiment was
124 set up as a split-split plot in a randomized complete block design (PennState, 2019). The three
125 experimental factors were tillage system (main plots), N fertilisation (sub-plots), and CC type (sub-
126 sub plots). The two tillage systems tested, *i.e.* conventional tillage (CT) and reduced tillage (RT),
127 differed in terms of tillage disturbance (intensity and frequency) and herbicide use (type and
128 quantity). CT averaged 1.0 primary tillage operations per year (*i.e.* disk or mouldboard ploughing, 30
129 cm depth, performed in autumn every year), 3.4 secondary tillage operations per year (*i.e.* disk or
130 rotary harrow and field cultivator, 10-20 cm in depth) and 0.8 superficial tillage operations per year
131 before sowing (*i.e.* tine harrow, <10 cm in depth). RT averaged 2.0 secondary tillage operations per
132 year and 0.5 superficial tillage operations per year carried out before sowing. Chiselling (30 cm
133 depth) was introduced in RT prior to cover crop sowing in 2011, 2013 and 2015 and represented the
134 main tillage tool for the subsequent summer crops. Mechanical weeding frequency was slightly
135 higher in CT than in RT (0.6 vs. 0.3 operations per year). CT averaged 1.3 equivalent full dose
136 herbicide applications per year (9% glyphosate, 24% pre-emergence, and 67% post-emergence)
137 whereas RT averaged 2.0 applications (41% glyphosate, 7% pre-emergence, and 52% post-
138 emergence). The four mineral N rates tested were N0 (0 kg N ha⁻¹ for all crops), N1 (50 kg N ha⁻¹ for
139 sunflower, 60 kg N ha⁻¹ for winter wheat, 100 kg N ha⁻¹ for maize), N2 (two times N1), and N3 (three
140 times N1). Only half of the theoretical dose for winter wheat was applied in 2013 because heavy
141 rainfall and waterlogging limited crop establishment. The four CC types tested were a non-legume
142 (*Secale cereale* L. from 1993 to 2001 and *Brassica juncea* (L.) Czern. (Bj) onwards), a low nitrogen
143 supply legume (*Trifolium incarnatum* L. from 1993 to 2003 and *Trifolium squarrosum* L. (Ts)
144 onwards), a high nitrogen supply legume (*Trifolium subterraneum* L. from 1993 to 2001, 50% *Vicia*
145 *villosa* Roth (Vv) plus 50% *Secale cereale* L. in 2003 and *Vicia villosa* Roth onwards) and a control
146 (baresoil, C). Cover crops were sown every autumn during the maize monoculture phase and

147 between every winter (*i.e.* durum wheat) and summer crop in the subsequent phases (*i.e.* every two
148 years). In both tillage systems, CC species were broadcast seeded manually in autumn (on September
149 22nd and 19th in 2011 and 2013, respectively, see Supp. Tab. 2 for CC sowing rates) and terminated at
150 the early flowering stage (BBCH 60) in the following spring (April 10th and March 20th in 2012 and
151 2014, respectively). In both tillage systems, CC sowing was systematically preceded by tillage (disk
152 harrow, rotary harrow or chisel). In CT, CC were terminated mechanically (*i.e.* CC residues were
153 incorporated in the soil with a disk harrow). In RT, CC were terminated chemically (*i.e.* CC residues
154 were left on the soil surface after an application of glyphosate at 0.9 kg a.i. ha⁻¹) from 1994 to 2002
155 and mechanically with a disk harrow afterwards. Control plots (*i.e.* baresoil fallow) were managed
156 identically to CC plots, with the exception of CC sowing. Each of the 32 treatments (2 tillage systems x
157 4 N rates x 4 CC types) was replicated four times in 21 m wide and 11 m long plots, resulting in a total
158 of 128 elementary plots of 231 m² (*i.e.* statistical unit). More detailed information on agricultural
159 practices over the four-year period on which data were collected can be found in Supp. Tab. 3.

160 **2.3. Weed, cover crop and crop sampling**

161 Weed and crop data were collected over one complete rotation cycle, *i.e.* CC 2011-2012, sunflower
162 2012, winter wheat 2012-2013, CC 2013-2014, maize 2014 and winter wheat 2014-2015. Cover crop
163 and weed biomass (in CC) were collected prior to CC termination in two 0.5 m² quadrats per
164 elementary plot. Crop biomass at maturity was collected in two 1 m² (durum wheat) or two 2 m²
165 (sunflower and maize, *i.e.* row crops) quadrats per elementary plot. Weed biomass at crop maturity
166 was collected in the same quadrats as crop biomass but was limited to half of the quadrat surface for
167 sunflower (1 m²) and to a fourth for maize (0.5 m²). All samples were oven dried for 48 h at 60°C.
168 Crop samples were then passed through a threshing machine to assess grain yield at 0% grain
169 moisture content.

170 Weed community composition was assessed in all phases of the crop sequence (*i.e.* CC and cash
171 crops) by visually estimating the percent cover of each weed species on a positively unbounded
172 scale, in order to account for different strata of vegetation (each species cover was however
173 bounded to a maximum of 100%) (Galland et al., 2019). This visual assessment took place at grain
174 filling, *i.e.* after weeding operations, within two 1 m² (cover crop 2012, sunflower 2012 and durum
175 wheat 2013), two 0.5 m² (cover crop 2014), four 0.5 m² (maize 2014) or four 1 m² (durum wheat
176 2015) quadrats per elementary plot.

177 **2.4. Numerical and statistical analysis**

178 **2.4.1. Weed diversity assessment**

179 Weed diversity was assessed as the effective number of species at the quadrat level, *i.e.* the
180 exponential of the Shannon diversity index: $\exp(-\sum_{i=1}^{SR} p_i \log(p_i))$ where SR: species richness, *i*: one
181 of the SR species of the community and p_i : relative cover of species *i*. This index has the advantage of
182 being expressed on a linear scale, in terms of number of equally-common species, *i.e.* a true measure
183 of diversity (Chao and Jost, 2015).

184 **2.4.2. Univariate analysis**

185 All univariate response variables were analyzed at the quadrat level with linear or generalized mixed
186 effect models, using the lme4 package of the R software version 3.5.1 (R Development Core Team,
187 2019), in order to account for the nature of the different response variables and the hierarchical
188 structure of the experiment.

189 Cover crop biomass, crop yield, weed biomass and weed diversity were regressed against block,
190 tillage system, N, CC species and all possible interactions between the latter three factors. To identify
191 if the experimental factors could offset the negative effect of weed biomass on crop yield while
192 avoiding model overfitting, we compared, based on Akaike's Information Criterion with small-sample
193 correction (AICc), a full model which integrated all possible interactions between weed biomass,
194 tillage system, N, and CC to all possible nested models (while respecting marginality constraints)
195 (Barton, 2019). The model with the lowest AICc was selected. The same strategy was used to identify
196 if the experimental factors could modify the relationship between CC and weed biomass, except that
197 the response variable was weed biomass and the covariate was CC biomass.

198 Block was always considered as a fixed factor. Crop density (as continuous variable) was added as a
199 covariate for the analysis of sunflower and maize yield (in both approaches, *i.e.* considering weed
200 biomass or not). Year (and its interaction with experimental factors) was added as a fixed factor
201 when multiple years of data were available for the same response variables (*i.e.* response variables
202 related to cover crops or durum wheat). Control variables (block, year, crop density) were never
203 subject to model selection. Block:tillage, block:tillage:N and block:tillage:N:CC were always
204 considered as random effects whereas block:year, block:year:tillage, block:year:tillage:N and
205 block:year:tillage:N:CC were considered as additional random effects when multiple years of data
206 were available for the same response variables.

207 **2.4.3. Multivariate analysis**

208 Weed community composition was averaged as to obtain one value per elementary plot:year and,
209 hence, avoid an additional level of nesting (pseudoreplication level). Similarly, all phases of the crop
210 sequence were analyzed independently to account for differences in sampling effort and quadrat size

211 between years, resulting in six sub-datasets (one for each CC:year and crop:year) of 128 rows (*i.e.*
212 elementary plots). For each sub-dataset, a Bray-Curtis dissimilarity matrix was computed between all
213 possible pairs of rows. The effect of experimental factors was assessed by fitting a full multivariate
214 linear model on each of the six Bray-Curtis dissimilarity matrices using the RRPP R package (Collyer
215 and Adams, 2018). To respect the hierarchy of the design (PennState, 2019), random effects
216 (block:tillage, block:N, block:tillage:N) were explicitly modelled and effects were tested against the
217 appropriate error term (block and tillage were tested against the block:tillage error term, N and
218 tillage:N against the pooled block:N and block:tillage:N error term, and the remaining effects against
219 the residuals).

220 **3. Results**

221 **3.1. Determinants of cover crop biomass**

222 Cover crop biomass was mainly driven by year x CC species, tillage x CC species and N x CC
223 interactions (Fig. 1, Supp. Tab. 4). Bj, Vv and Ts produced 176, 186 and 71% more biomass in 2012
224 than 2014, respectively (Fig. 1a). Bj outperformed Vv and Ts by 15 and 111% in 2012, respectively,
225 and by 17 and 66% in 2014, respectively. Bj and Ts were 38 and 76% more productive in CT than in
226 RT, respectively, whereas Vv showed similar productivity in both tillage systems (Fig. 1b). Bj
227 outperformed Vv and Ts by 53 and 230% respectively in CT whereas Bj and Vv showed similar
228 productivity in RT and outperformed Ts by 4-fold (Fig. 1b). Bj was the only CC species which
229 responded positively to N levels, *i.e.* 103% increase from N0 to N2 (Fig. 1c). Vv outperformed Bj by
230 41% at N0 whereas Bj outperformed Vv by 27, 81, and 86% at N1, N2, and N3, respectively. Ts was
231 the least productive CC at all N levels.

232 [Figure 1]

233 **3.2. Determinants of crop yield**

234 Sunflower yield was driven by N x CC (Fig. 2a, Supp. Tab. 4). Significance of the interaction was mainly
235 justified by the fact that Vv outperformed Bj by 64% at N0 (Fig. 2a). The most parsimonious model of
236 sunflower yield did not support the inclusion of weed biomass on top of N, CC and N x CC.

237 [Figure 2]

238 Maize yield was driven by tillage system x N and N x CC interactions (Fig. 2b and Fig. 2c, Supp. Tab. 4).
239 Maize yield did not significantly differ between tillage types from N0 to N2 (Fig. 2b). However, CT
240 outyielded RT by 17% at N3 (Fig. 2b). This was congruent with the fact that maize yield increased by
241 12% from N1 to N2/N3 in CT whereas it remained stable from N1 to N3 in RT (Fig. 2b). At N0, Vv and
242 Ts outyielded C by 65 and 26%, respectively, whereas no significant differences were observed

243 between Bj and C. At N1, Vv outyielded C by 23% while no differences were observed between C and
244 Bj (Fig. 2c). No differences between CC species were observed at N2 or N3 (Fig. 2c). Across all
245 combinations of CC species and N, only N1:Vv and N1:Ts were able to reach the level of productivity
246 observed at N2 and N3 (*i.e.* maximum productivity). The most parsimonious model of maize yield did
247 not support the inclusion of weed biomass (either as a main effect or in interaction with the
248 experimental factors) on top of N, CC species, tillage system, N x CC species and N x tillage system.

249 Durum wheat yield was driven by tillage x N x CC interactions and year x tillage x N (Fig. 2d, Supp. Fig.
250 2, Supp. Tab. 4). In 2013, RT outyielded CT by 25% at N1 whereas CT outyielded RT by 40% at N3. In
251 2015, CT outyielded RT by 23 and 13 % at N1 and N3, respectively. Vv outyielded C by 33 and 35% at
252 CT:N0 and RT:N1,, respectively (Fig. 2d). No CC species were able to maintain maximum durum
253 wheat yield while reducing N fertilisation. In CT, durum wheat yield gradually increased from N0 to
254 N3 in both 2013 and 2015 (Supp. Fig. 2). In RT, maximum durum wheat was observed at N1 and N2 in
255 2013 and 2015, respectively. The most parsimonious model of durum wheat included N x tillage x
256 weed biomass (on top of CC, N, tillage, weed biomass, N x weed biomass, N x tillage, year x N, tillage
257 x weed biomass, year x tillage, and year x tillage x N). However, the interaction was only justified by
258 that fact that the slope between weed biomass and durum wheat yield was steeper at N3 than at N0
259 in CT (Supp. Fig. 3).

260 **3.3. Factors shaping weed communities**

261 **3.3.1. Weed biomass**

262 Weed biomass in CC was driven by year x tillage x CC species and year x tillage x N (Fig. 3a, Supp. Fig.
263 4, Supp. Tab. 4). In 2012, Bj, Vv, and Ts reduced weed biomass by 79, 69, and 24% in CT, respectively
264 (Fig. 3a). Only Bj (-75%) and Vv (-37%) were capable of significantly reducing weed biomass in RT in
265 2012. In 2014, Bj was the only CC capable of significantly reducing weed biomass (-34% in CT and -
266 28% in RT). Weed biomass was greater at N3 than at N0 across all combinations of tillage system and
267 years, except in CT, in 2012 (Supp. Fig. 4). The most parsimonious model of weed biomass in cover
268 crop included CC x CC biomass and tillage x CC biomass (on top of tillage, N, CC, CC biomass, CC x N,
269 CC x tillage, CC x year, N x CC biomass, tillage x year, and CC x tillage x year). The slope (on square
270 root scale) between CC biomass and weed biomass was steeper in CT than in RT and steeper for Vv
271 and Ts than for Bj (Fig. 4 for 2012 and Supp. Fig. 5 for 2014). All slopes were significantly different
272 from zero except Bj in CT.

273 [Figure 3]

274 [Figure 4]

275 Weed biomass in sunflower was not influenced by any of the experimental factors (Supp. Tab. 4).

276 Weed biomass in maize was driven by N and by tillage system x CC species (Fig. 3b, Supp. Tab. 4).
277 Weed biomass at N3 was 68, 42 and 41% greater than at N0, N1 and N2 respectively. On average,
278 weed biomass was 3 times greater in RT than in CT. The first order interaction was justified by the
279 fact that C, Bj and Ts showed 2.6 – 4.4 times more weed biomass in RT than CT whereas Vv only
280 showed 1.7 times more weed biomass in RT than CT (Fig. 3b).

281 Weed biomass in durum wheat was driven by year x tillage x N, year x N x CC, and tillage x N x CC
282 interactions (Fig. 3c, Supp. Tab. 4). Across all N rates and CC species, weed biomass was 2 and 39
283 times greater in RT than in CT in 2013 and 2015, respectively (Fig. 3c). Within all combinations of year
284 and tillage system, no differences between combinations of N rates and CC species were found
285 (except within CT, in 2015, but weed biomass was extremely low, *i.e.* 1 to 5 g of dry matter m⁻²).

286 3.3.2. Weed diversity

287 Weed diversity in CC was driven by a CC species x year interaction (Supp. Tab 4). In 2012, weed
288 diversity was greatest in C, intermediate in Ts (-16% compared to C), and lowest in Bj (-38%) and Vv (-
289 46%). In 2014, all CC reduced weed diversity to a similar extent (-13 to -15%). Weed diversity in
290 sunflower was driven by tillage system (Supp. Tab. 4) and was slightly higher in CT than in RT. Weed
291 diversity in maize was driven by N rate (Supp. Tab. 4) and was slightly higher at N3 than at N0. Weed
292 diversity in durum wheat was mainly driven by tillage system x N interactions (Supp. Tab. 4, Fig. 5): it
293 gradually decreased from N0 to N3 in CT whereas it remained stable across all N levels in RT.

294 [Figure 5]

295 3.3.3. Weed community composition

296 In both cover crop seasons, *Poa annua* L., *Capsella bursa-pastoris* (L.) Medik. and *Veronica* spp. were
297 associated to RT whereas *Lolium* spp. was associated to CT (Supp. Fig. 6a-b, Supp. Tab. 5a). Bj was the
298 only CC capable of completely outcompeting *C. bursa-pastoris* (L.) Medik. in both tillage systems of
299 the 2012 CC season (*C. bursa-pastoris* (L.) Medik. was not a dominant species in the 2014 CC season).
300 However, tillage system significantly interacted with CC in both CC seasons (Supp. Tab. 5a). The
301 interaction was justified by that fact that, unlike other CC species, Vv consistently reduced the
302 percent cover of *Poa annua* L., thereby homogenizing weed communities between tillage systems
303 (Supp. Fig. 6a-b).

304 In all subsequent crops, *Cynodon dactylon* (L.) Pers. and *Equisetum arvense* L. were consistently
305 associated with RT whereas *Solanum nigrum* L., *Datura stramonium* L., *Xanthium orientale* subsp.
306 *italicum* (Moretti) Greuter, and *Polygonum* spp. were associated with CT (Supp. Fig. 6c-f, Supp. Tab.
307 5b-c). Tillage system significantly interacted with N in sunflower (Supp. Tab. 5b). Increasing levels of

308 N generated a reduction of *Cynodon dactylon* (L.) Pers. and an increase of *Datura stramonium* L. in CT
309 whereas weed communities remained homogeneous across the different levels of N in RT (Supp. Fig.
310 6c). In maize, N fertilisation significantly interacted with CC species (Supp. Tab. 5b). Vv was the only
311 CC capable of reducing the percent cover of *Equisetum arvense* L. at N0 (Supp. Fig. 6d).

312 **4. Discussion**

313 **4.1. Weed suppression by cover crops during the fallow period**

314 In accordance with previous studies (Buchanan et al., 2016; Alonso-Ayuso et al., 2018), highly
315 productive CC allowed to suppress weed growth during the fallow period, with respect to the
316 baresoil fallow (*e.g.* -79 and -69% for Bj and Vv in CT in 2012, respectively)). In 2014, intense
317 precipitations (*i.e.* 1010 mm from the beginning of September to the end of April, vs. 332 mm in
318 2012) most likely limited CC development and hence, weed suppression. Nevertheless, the weed
319 suppressive potential of CC could not be simply attributed to biomass productivity (Campiglia et al.,
320 2010; Radicetti et al., 2013). The relationship between CC and weed biomass (*i.e.* slope and intercept
321 of the regressions in Fig. 4) also depended on CC species. In 2012, Bj suppressed weeds to a greater
322 extent than Vv or Ts at low levels of CC productivity (200 g DM m⁻²) whereas Bj and Vv suppressed
323 weeds to a similar extent at higher levels of CC productivity (400 g DM m⁻²). Within all combinations
324 of tillage systems and years, the lowest weed biomass was observed for Bj in N0 even though Vv was
325 4 to 100% more productive at that N rate. Such results challenge the beliefs that higher CC biomass is
326 necessarily required to reduce weed growth during the fallow period (MacLaren et al., 2019) or than
327 N fertilisation enhances the weed suppressive potential of N-demanding CC species, such as Bj
328 (Sturm et al., 2017). Bj's greater potential to reduce weed growth at lower levels of CC productivity
329 (such as in 2014) could be related to quick N uptake (Tribouillois et al., 2015), early soil cover
330 (Teasdale, 1996), and/or allelopathic effects (as suspected with *Capsella bursa-pastoris* (L.) Medik.
331 (Couëdel et al., 2017)). In contrast, Vv's potential to reduce weed growth at high levels of CC
332 productivity could be related to a smothering effect. Ts showed the same slope and intercept as Vv
333 but was never able to reach high biomass productivity (maximum observed biomass for Ts was 386
334 and 185 g DM/m² in 2012 and 2014, respectively) and hence smother weeds.

335 At the same biomass level, CC were more suppressive in CT than in RT. Two hypotheses can be
336 formulated to explain this undocumented phenomenon. First, coarser soil structure in RT than in CT
337 (Schlüter et al., 2018) could have delayed CC emergence and CC:weed interference through reduced
338 seed:soil contact (Teasdale, 1996; Munkholm et al., 2008; Büchi et al., 2018). This may also explain
339 why small seeded CC species (*i.e.* Bj and Ts; 1000 seed weight for Bj, Vv and Ts was 2.9, 34.8 and 3.3 g
340 respectively) were less productive in RT than CT (Uchino et al., 2011; Büchi et al., 2018). In addition,

341 weed communities selected by RT could have shown trait values that allowed them to overcome CC
342 competition (Adeux et al., 2019). For example, *Poa annua* L., one of the dominant weeds in RT, might
343 have avoided CC competitive effects thanks to its rapid growth rate, shallow root system, and high
344 tolerance to shading (Warwick, 1979).

345 Finally, the high level of weed biomass (*i.e.* 58 and 134 g DM/m² in 2012 and 2014, respectively)
346 observed in the most suppressive treatment (*i.e.* CT:N0:Bj) could question the importance of CC for
347 weed suppression in real farming conditions (Teasdale, 1996). Further studies aiming to characterize
348 weed seed rain (Doisy et al., 2014) or weed phenology would allow to identify which species (if any)
349 are capable of producing seeds during the fallow period (Brennan and Smith, 2005) and whether or
350 not they may jeopardize long-term weed management.

351 **4.2. Weed suppression by cover crops during the subsequent crops**

352 Contrary to previous studies (Campiglia et al., 2010; Wayman et al., 2015; Buchanan et al., 2016), CC
353 showed no clear long-term effect on weed communities (*i.e.* abundance and structure) in the
354 subsequent crops. Such discrepancies may be explained by differences in CC termination method and
355 timing of weed samplings. Studies reporting an effect of CC on weed communities in the subsequent
356 crops (Campiglia et al., 2010; Wayman et al., 2015; Buchanan et al., 2016) have mainly resorted to
357 non-chemical termination methods which maintained CC residues on the soil surface (*e.g.* flail
358 mowing, roller-crimping) and to weed samplings carried out before direct weed control. By contrast,
359 in the present study, CC residues were soil incorporated (*i.e.* CC were terminated by tillage in both
360 tillage systems) and weed samplings were carried out after herbicide applications. The incorporation
361 of CC residues did not allow the expression of a weed suppressive mulch (Teasdale, 1996) and the
362 weed sampling strategy adopted did not allow to identify whether (i) CC had simply no effect on
363 weed communities or whether (ii) CC effects on weed communities were overridden by tillage and/or
364 herbicides. Tillage could have overridden differences in weed seed production during the fallow
365 period (Brennan and Smith, 2005) and herbicides could have overridden differences in weed
366 communities before weeding. Patch spraying could have allowed to adjust herbicide use based on
367 emerged weed flora and therefore, reveal the potential of CC to reduce herbicide use in tillage-based
368 systems. Finally, the fact that no CC species were able to alleviate weed:crop interference suggests
369 that was not driven by N availability (Casper and Jackson, 1997) or that increasing N availability led to
370 competition for other resources, such as light and/or water (Hautier et al., 2009).

371 **4.3. Cover crop effects on crop yield**

372 Cover crop effects on crop yield were determined by CC type and biomass, adequate combination
373 between N fertilisation – CC N release – crop N requirements, weather conditions, and the position

374 of the crop in the rotation with respect to CC termination. In both tillage systems, growing Vv during
375 the fallow period resulted in the highest maize grain yield increase at N0 (+65%) and N1 (+23%) with
376 respect to the control. The positive effect of Vv on maize grain yield decreased with increasing levels
377 of N, highlighting the opportunity to reduce N fertilisation while maintaining maize grain yield
378 (Liebman et al., 2012; Marcillo and Miguez, 2017; Wittwer et al., 2017). The integration of Ts also
379 allowed a gain in maize grain productivity, but the gain was significant only at N0 (+26% with respect
380 to C), most likely because of lower cover crop biomass (76 g DM/m² across tillage systems, N rates
381 and years) and hence, lower potential N release (Mazzoncini et al., 2011). Such results are in line with
382 the results obtained by Mazzoncini *et al.* (2011) on the same experiment in 2008, which highlight
383 greater total soil nitrogen content for Vv (5.71 Mg/ha) than Bj (5.40 Mg/ha) and an intermediate
384 level for Ts (5.50 Mg/ha). Nevertheless, measures of soil available N could have provided a finer
385 understanding of CC effects on crop productivity as total soil nitrogen content does not reflect the
386 quantity of N available for crop growth after CC termination. The lack of a legume CC or N effect in
387 sunflower, even though CC biomass was 151% higher before sunflower (2012) than maize (2014),
388 suggests that sunflower yield was restricted by other factors than N, such as the extremely dry
389 weather conditions encountered in 2012 (176 mm of rainfall from sunflower sowing to harvest). The
390 only negative CC effect was observed for Bj at N0 in sunflower. The high quantity of residues
391 produced by Bj prior to sunflower planting in 2012 was possibly associated to a high C:N ratio, which
392 could have resulted in soil inorganic N immobilization by microbial biomass (Trinsoutrot et al., 2000).
393 Furthermore, Bj might also have depleted soil N resources during the fallow period. In contrast, Bj
394 residues possibly showed a more balanced C:N ratio in 2014 due to lower CC productivity. Therefore,
395 sufficient N could have been released by mineralization to meet microbial N requirements but in
396 insufficient amounts to generate net N mineralization and promote crop growth (Trinsoutrot et al.,
397 2000). A more systemic approach could have allowed to maximize CC benefits by adapting CC
398 termination date depending on CC species level of maturity (Mirsky et al., 2009). To maximize CC
399 benefits, we would encourage farmers to select CC species based on the subsequent crop N
400 requirements but to possibly reconsider the choice of the subsequent crop based on actual CC
401 performance. Finally, it is important to note that the intensity of legume CC effects on crop yield
402 decreased considerably in time after CC termination. The effect of Vv was still visible in durum wheat
403 at CT:N0 and RT:N1, which may justify the importance of CC in stockless cereal-based organic
404 systems. However, our results also suggest that legume CC released N quickly in time and that little
405 benefit can be expected from CC one year after CC termination in more productive systems. Finally,
406 the fact that Vv had a positive effect on durum wheat yield at RT:N1 but not at RT:N0 may highlight
407 that N fertilisation is required to stimulate long-term CC mineralization in RT systems (Drinkwater et
408 al., 2000).

409 **5. Conclusion**

410 The complexity of long-term CC effects on crop productivity and weed management were revealed
411 through an in-depth analysis of CC biomass, weed biomass, weed community composition, and crop
412 yield during one complete rotation cycle, sampled 18 years after the beginning of a factorial
413 experiment on tillage systems, N fertilisation and CC. Our findings should encourage the selection of
414 weed suppressive CC based on traits other than biomass productivity. Increased mineral N
415 availability did not appear as a sustainable solution to increase the weed suppressive potential of CC.
416 Further experiments encompassing a wider diversity of CC types may identify CC species or mixtures
417 best adapted to grow in reduced or conventional tillage systems and to suppress the associated
418 weed flora. Further research should also address weed seed production during the fallow period to
419 characterize potential long-term risks. In our study, potential CC effects on weed communities in the
420 subsequent crops were possibly overridden by tillage and herbicides, highlighting the necessity to
421 reduce management intensity to maximize potential cover crop benefits on weed management.
422 Mechanical methods of CC termination which maintain residue on the soil surface (e.g. roller-
423 crimping) appear as a promising solution to reduce herbicide reliance and increase CC benefits on
424 weed management. Patch spraying could also allow growers to benefit from reduced weed pressure.
425 Finally, we encourage to position highly productive legume CC prior to high N-demanding summer
426 crops with the aim of reducing standard nitrogen fertilisation levels.

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435 **Author's contribution**

436 MM designed the study. MM, PB and DA funded the experiment and planned data collection. SCO
437 and NMJ provided additional funding for GA's work. SCa and DA collected the data. GA analyzed the
438 data. All authors were involved in the interpretation of the results and contributed to writing the
439 original version of the manuscript and improving the subsequent ones.

440 **Conflict of interest**

441 The authors declare no conflict of interest.

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558 Figure 1: Boxplots highlighting the interaction effect between cover crop species and a) year, b)
559 tillage system, and c) nitrogen level on cover crop biomass in 2012 and 2014 (years were pooled for
560 graphs b) and c)). Boxplots sharing the same letter are not significantly different at $p < 0.05$ (p value
561 adjustment: multivariate t method). 384 observations (2 pseudoreplications x 96 elementary plots x
562 2 years) were available for all graphs. CT: Conventional tillage; RT: Reduced tillage; Bj: *Brassica*
563 *juncea*; Vv: *Vicia villosa*; Ts: *Trifolium squarrosum*.

564 Figure 2: Boxplots highlighting the interaction effect between a) cover crop species and nitrogen level
565 on sunflower yield in 2012, b) tillage system and nitrogen level on maize yield in 2014, c) nitrogen
566 level and cover crop species on maize yield in 2014, and d) tillage system, nitrogen level and cover
567 crop species on durum wheat yield in 2013 and 2015 (pooled data). Boxplots sharing the same
568 symbol (lower or uppercase letter, arabic numbers) are not significantly different at $p < 0.05$. Within a
569 graph or panel, different symbols refer to different families of contrasts (p value adjustment:
570 multivariate t method). 256 observations (2 pseudoreplications x 128 elementary plots) were
571 available for both graph a), b), and c) whereas 512 observations (2 pseudoreplications x 128
572 elementary plots x 2 years) were available for graph d). CT: Conventional tillage; RT: Reduced tillage;
573 C: Control (baresoil); Bj: *Brassica juncea*; Vv: *Vicia villosa*; Ts: *Trifolium squarrosum*.

574 Figure 3: Boxplots highlighting the interaction effect between a) cover crop species and year on weed
575 biomass in cover crops (2012 and 2014), b) cover crop species and tillage system on weed biomass in
576 maize in 2014 and c) tillage system, nitrogen level and cover crop species on weed biomass in durum
577 wheat in 2013 and 2015 (pooled data). Boxplots sharing the same letter are not significantly different
578 at $p < 0.05$ (p value adjustment: multivariate t method). 256 observations (2 pseudoreplications x
579 128 elementary plots) were available for both graph b) whereas 512 observations (2
580 pseudoreplications x 128 elementary plots x 2 years) were available for both graph a) and b). CT:
581 Conventional tillage; RT: Reduced tillage; Bj: *Brassica juncea*; Vv: *Vicia villosa*; Ts: *Trifolium*
582 *squarrosum*.

583 Figure 4: Fitted values of weed biomass in cover crops as a function of tillage system, nitrogen level,
584 cover crop species and cover crop biomass in 2012. Predictions were based on generalized linear
585 mixed model taking into account random effects. The regression line shows an average plot value
586 (*i.e.* prediction at the population level). Slopes sharing the same letter are not significantly different
587 at $p < 0.05$ (p value adjustment: multivariate t method). All slopes are significantly different from
588 zero, except Bj in CT. 8 observations (2 pseudoreplications x 4 blocks) were available for each slope
589 (384 observations total). CT: Conventional tillage; RT: Reduced tillage; Bj: *Brassica juncea*; Vv: *Vicia*
590 *villosa*; Ts: *Trifolium squarrosum*.

591 Figure 5: Boxplots highlighting the interaction effect between tillage system and nitrogen level on the
592 effective number of weed species in durum wheat in 2013 and 2015 (pooled data). Boxplots sharing
593 the same letter are not significantly different at $p < 0.05$ (p value adjustment: multivariate t method).
594 256 observations (2 pseudoreplications x 128 elementary plots) were available. CT: Conventional
595 tillage; RT: Reduced tillage.









