

Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate

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1 **Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the**
2 **development of the vine mealybug under a Mediterranean climate**

3

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13

14

15 **ABSTRACT**

16

17 The influence of complete cover cropping (inter- and intra-row) on grapevine growth, yield
18 and must quality was evaluated in a three-year field trial in a commercial vineyard in
19 northwestern Sardinia (Italy). Effects on developmental and reproductive parameters of the
20 vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), were also
21 investigated. The cover crop treatments were: natural covering, legume mixture, grass
22 mixture, and conventional soil tillage, which was included as the reference treatment. Relative
23 to soil tillage, cover crops reduced grape production by modifying yield components in
24 different ways: legume mixture reduced the cluster weight, whereas grass mixture led to a
25 lower number of clusters per vine coupled with a lower cluster weight. Cover crops also
26 altered the must qualities relative to soil tillage. Grass mixture increased the content of sugar,
27 anthocyanins and polyphenols, whereas legume mixture and natural covering reduced total
28 polyphenols and anthocyanin content, respectively. All the *P. ficus* biological parameters
29 examined were affected by the floor management practices. Mealybugs reared on grapevines
30 subjected to soil tillage and legume covering showed a faster development time and higher
31 survival, fecundity and fertility than those developed on natural covering and grass plots. The
32 vine mealybug showed a higher performance on grapevines with a higher nitrogen content
33 and vigor. Effects of cover crop treatments appear to be mediated through nutrient availability
34 and content in grape plants. Consequently, utilizing competitive cover crops, while reducing
35 yields, would improve must quality and reduce pest development.

36

37 **Keywords:** *Vitis vinifera*; Cover crops; Grape quality; *Planococcus ficus*; Mealybug
38 development; Mealybug fecundity.

39

40 **1. Introduction**

41

42 Cover crops are important ecological vineyard management tools, which improve the soil
43 structure and soil erosion control, enrich nitrogen and organic matter content, and regulate
44 excessive grapevine vigor (Pardini et al., 2002). Many experiments have been carried out to
45 better identify the influence of different floor covers in grapevine vegetative growth, yield,
46 berry and wine quality (Monteiro and Lopes, 2007; Guerra and Steenwerth, 2012; Mercenaro
47 et al., 2014). Today, cover crops are widely used in vineyard inter-rows combined with
48 herbicide strips under the vines.

49 Cover cropping the entire vineyard floor (intra and inter-row) may increase the control of
50 excessive vine vigor, with consequent changes in grape quality, and reduce the herbicide use
51 and associated risks, such as plant injury by spray drift, evolution of weed resistance (Powles
52 et al., 1997), contamination of groundwater (Thurman et al., 1996), and reduction in agro-
53 ecosystem biodiversity (Danne et al., 2010; Sanguaneko and León, 2011). The reduction in
54 herbicide use would also facilitate compliance with EU directives and regulations that restrict
55 or ban the use of several pesticides and promote the development of integrated control
56 techniques and the use of environmentally friendly tools (European Union, 2009a, 2009b).

57 Few studies have investigated the influence of complete floor cover crops (inter- and intra-
58 row) on grapevine, especially when cultivated in semi-arid conditions. In a Chenin blanc
59 vineyard under dryland conditions in South Africa, weeds and cover crops competed with
60 grapevines during the growing season, thus decreasing vegetative growth and yield (Van
61 Huyssteen and Weber, 1980). Other studies found similar effects, but alterations in the canopy
62 architecture and reductions in grapevine vigor and crop yield were only observed after several
63 years (Tescic et al., 2007; Gontier et al., 2011).

64 In order to reduce the excessive grape vigor and crop yield and thus improve the grape
65 quality, several crop regulation techniques, such as shoot and cluster thinning (Naor et al.,
66 2002; Calderon-Orellana et al., 2014; Gamero et al., 2014) and early defoliation (Poni et al.,
67 2006; Silvestroni et al., 2016) have been evaluated. Inter-row cover crops have also been
68 tested in multi-year experiments for regulating grape production. The overall results showed
69 no influence on crop yield, while changes in the must composition were observed after 2-3
70 years (Lopes et al., 2008; Mercenaro et al., 2014). One of the aims of the present work was to
71 study various complete floor cover crops as a cultural practice to reduce excessive grape vigor
72 and productivity by evaluating grapevine growth, yield and fruit composition parameters.

73 Cover crops can also alter vineyard insect pest dynamics and may play a role in integrated
74 pest management programs. Cover crops can affect pest dynamics through altering plant and
75 natural enemy diversity (top-down effects) as well as modifying nutrient status and vigor of
76 vines (bottom-up effects) (Landis et al., 2000; Thomson and Hoffmann, 2013; Veres et al.,
77 2013). However, increasing plant diversity does not always increase pest control (Bone et al.,
78 2009; D'Alberto et al., 2012). Evidence suggests that when cover crops reduce the nitrogen
79 content in crops, the growth and development of plant-feeding insects are reduced as
80 individual and population growth of these insects is typically N-limited (Wilson et al., 1988;
81 Hunt et al., 1992; Cocco et al., 2015).

82 In vineyards, cover crops have had variable effects on pest densities. For example,
83 competition for water and nutrients caused lower plant vigor and reduced leafhopper density
84 due to a poorer host quality (Costello and Daane, 2003). On the other hand, a higher
85 abundance of the vine mealybug, *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae),
86 was observed as a consequence of the suppression of tillage which promoted the development
87 of ant populations and, therefore, the disruption of its natural enemies (Serra et al., 2006;
88 Mgocheki and Addison, 2010; Mansour et al., 2012). *P. ficus* is a key widespread pest in the

89 main grape growing areas which severely reduces the economic yield of table grape and the
90 quality of wine grape, in addition to being a vector of several viruses and diseases (Daane et
91 al., 2012).

92 From the perspective of a more sustainable viticulture oriented towards high-quality
93 production with a reduced use of insecticides and herbicides, we investigated the influence of
94 different complete floor covers on the grapevine yield and must quality, and the bottom-up
95 effects of cover crops on development and reproduction of the vine mealybug in a three-year
96 survey conducted in a commercial vineyard under Mediterranean climatic conditions.

97

98 **2. Materials and methods**

99

100 *2.1 Study site and experimental design*

101

102 The experiment was carried out between 2013 and 2015, in a 17-year-old vineyard, cv.
103 Carignano, located at 40 m a.s.l. in northwestern Sardinia (Italy, 40°33'28"44 N;
104 08°19'19"56 E). Prior to this study, the site was used for a separate cover crop trial
105 (Mercenaro et al., 2014). The cultivar Carignano is widely cultivated in Sardinia, Spain
106 (known as Cariñena and Mazuela) and southern France (Carignan noir), and it is a highly
107 productive and vigorous cultivar when cultivated in fertile soils (Christensen et al., 2003).
108 Vines were grafted onto 779 P rootstock, trained by a spur-pruned cordon (commonly with
109 five spurs with two buds each) and spaced 2.7 m between rows and 1.0 m within rows. The
110 site has a relatively uniform calcareous alluvial soil, with an average depth of 60-70 cm, and
111 the following physico-chemical characteristics: sand 51.0%, clay 24.9%, silt 24.1%; pH =
112 7.44; organic matter content = 16 g kg⁻¹. Vines were drip-irrigated three times per year from
113 late June to mid August (corresponding to about 700 m³ ha⁻¹ year⁻¹). The experimental

114 vineyard is characterized by a typical central Mediterranean climate, with mild winters and
115 hot dry summers, and precipitations concentrated between October and May (560 mm average
116 total annual rainfall). Daily temperature, relative humidity and rainfall during the survey were
117 recorded by a weather station positioned in the vineyard. In 2013, annual and spring rainfall
118 were higher compared with 2014 and 2015, while summer precipitations were generally
119 scarce, especially in 2014 when the dry season lasted from June to October. Temperatures
120 varied among years. 2015 had a relatively colder winter and hotter summer, resulting in
121 increased abiotic stress for plant growth.

122 The present study was conducted in a randomized complete block design with four
123 replications. Each plot was 32 m long and 5.4 m wide (width of two inter-rows) and consisted
124 of a central experimental row of 32 grapevines and two adjacent inter-rows on either side of
125 the study row. Plots were separated by a single border row. The following floor management
126 systems were compared: natural covering (NC) with a dominance of annual grasses (*Bromus*
127 *hordeaceus* L., *Avena sterilis* L. and *Vulpia myuros* L.); cover crop of an annual self-
128 reseeded legume mixture (LM): *Medicago polymorpha* L. cv Anglona (50%) and *Trifolium*
129 *yanninicum* Katzn. and Morley cv Gosse (50%); grass mixture (GM) cover consisting of a
130 summer semi-dormant perennial grass, *Dactylis glomerata* L. cv Currie (80%) and an annual
131 self-reseeded grass, *Lolium rigidum* Gaud. cv Nurra (20%); soil tillage (ST) as the reference
132 treatment. Grass and legume mixtures are expressed by the percentage of viable seed number
133 m⁻². LM was over-seeded by hand in the inter-rows, whereas a full covering of *D. glomerata*
134 was present in the GM inter-rows from the previous trial.

135 Cover crops were seeded along LM and GM rows in mid November 2012 at a rate of 30 kg
136 ha⁻¹, and plots were rolled immediately afterwards. Since the re-establishment of LM in
137 autumn 2013 was unsatisfactory due to adverse weather conditions, an over-sowing was
138 performed in mid February 2014 at the rate of 20 kg ha⁻¹. No herbicides or fertilizers were

139 used on cover crop plots during the trial. The only exception was on LM plots where the non-
140 residual herbicide glyphosate (Roundup Power 2.0, Monsanto, Milano, Italy) was sprayed
141 once in late October 2012 at the rate of 2.5 L ha⁻¹ before LM sowing in order to remove a
142 severe infestation of annual and perennial grasses. Glyphosate is most effective against
143 perennial weeds and less costly than pre-emergence herbicides or soil tillage (Monteiro and
144 Moreira, 2004; Tourte et al., 2008).

145

146 *2.2 Cover crop assessment*

147

148 In each cover crop plot, the following parameters were observed:

- 149 - establishment and re-establishment of autumn swards by counting in each plot the number of
150 seedlings (annuals) or plants (*D. glomerata*) in four sampling areas (25 × 50 cm) when
151 legumes reached the third trifoliate leaf stage;
- 152 - seasonal sward covering rate (%) and presence of unsown species by monthly visual
153 estimation of the whole plots;
- 154 - dry matter yield (DMY) and its botanical composition in four sampling areas of 100 × 50 cm
155 in each plot. Swards were mowed when their height reached 10-15 cm in order to control the
156 cover crop vegetative growth and ensure a proper establishment and self-reseeding of annuals.
157 Plant samples were oven-dried at 60 °C to constant weight and then weighed to determine the
158 above-ground dry matter yield.

159

160 *2.3 Grapevine leaf nitrogen content, vegetative growth and crop yield*

161

162 The content of nitrogen on leaves was estimated with the SPAD 502 Chlorophyll Meter
163 (Minolta, Osaka, Japan), which is a non-destructive portable tool to measure the chlorophyll

164 concentration in leaves (Shaahan et al., 1999; Porro et al., 2001). The nitrogen content in
165 grapevine leaves is closely related with SPAD readings ($r^2 = 0.989$) (Cocco et al., 2015). The
166 leaf nitrogen content was estimated on six dates in spring-summer 2013 and 2014 and eight
167 times in 2015 by measuring the SPAD values in five leaves opposite to basal clusters on each
168 plant artificially-infested with *P. ficus* mealybugs.

169 The grapevine growth and productivity was evaluated in the central 20 vines of each
170 experimental row. The supernumerary shoots were thinned after bud break, and the number of
171 shoots per vine was then determined. Each year, the evolution of fruit composition was
172 assessed from veraison to harvest in 600 berries per plot randomly collected approximately
173 every two weeks starting from the stage of ‘50% veraison’, corresponding to 60, 72 and 74
174 days after anthesis (DAA) in 2013, 2014 and 2015, respectively. Berries were weighed and
175 crushed, and total soluble solids (°Brix), pH and titratable acidity of juice were determined in
176 accordance with the procedures of the Organisation Internationale de la Vigne et du Vin
177 (O.I.V., 2006). Total anthocyanins and polyphenols were evaluated by spectrophotometry,
178 measuring ultraviolet absorption at 520 nm and 700 nm, respectively (Di Stefano and
179 Cravero, 1991). All the grapevines investigated were harvested on the same dates: 3 October
180 2013 (130 DAA), 7 October 2014 (127 DAA) and 12 October 2015 (137 DAA). Vine yield
181 and yield composition (cluster and berry weights, and number of clusters per vine) were
182 determined by weighing ten clusters randomly chosen for each replicate and ten berries
183 randomly picked from each cluster. The weight of the dry pruning wood was recorded during
184 the dormant season in order to estimate the vegetative growth and calculate the Ravaz index
185 (determined as the ratio between crop yield and pruning wood).

186

187 *2.4 Vine mealybug biological parameters*

188

189 The response of *P. ficus* to different floor management systems was investigated in artificial
190 cohorts established on grapevines. Mealybugs were obtained from a mass-rearing colony
191 maintained on sprouted potato placed inside Plexiglas cages (30 × 30 × 30 cm) with two sides
192 covered with mesh for ventilation. The culture was maintained at 26 ± 1 °C, 60-70% RH, in
193 constant darkness. In order to obtain eggs of the same age, a number of ovipositing females
194 were placed with a sable-hair brush (gauge 000) in 2 × 2 cm strips of cardboard and allowed
195 to oviposit for 24 hours, after which females were removed. Eggs were counted under a
196 dissecting microscope and held in a growth chamber at 25 °C for seven days. Batches of 500
197 hatching eggs were used to infest one shoot from each of three separate plants per plot by
198 securing the cardboard strips to the abaxial surface of a median leaf in order to minimize *P.*
199 *ficus* handling.

200 Experimental plants were inspected before the study to ensure the absence of wild populations
201 of mealybugs in the canopy and under the bark. Trials started on 14 June 2013, 30 May 2014
202 and 3 June 2015 (egg release) and ended on 5 August 2013, 16 July 2014 and 20 July 2015
203 (count of remaining females). During their development, mealybugs were confined by
204 covering 3-4 leaves of the artificially-infested shoot with a cage of spun-bonded
205 polypropylene fabric (Agribon AG-15, 18.65 g m⁻², 90% light transmission) secured at both
206 ends with elastic bands. Cages protected mealybugs from natural enemies and prevented the
207 spread of *P. ficus* immatures within the canopy, which would have dramatically increased the
208 time and effort required for a daily check of the experimental plants.

209 Starting three weeks after egg release, all leaves, petioles and stems inside the cages were
210 inspected daily, and the first 20 females at the onset of oviposition were collected with a
211 sable-hair brush (gauge 00) and placed inside plastic containers. Ovipositing females were
212 stored in a cooler at ~10 °C during the transport back to the laboratory. The dates of collection
213 were recorded in order to determine the development time from egg eclosion to ovipositing

214 female. All the mealybugs from the different treatments were stored under the same
215 laboratory conditions and allowed to complete oviposition inside the containers, upon which
216 the fecundity was determined under a dissecting microscope by counting the number of first
217 instar nymphs and unhatched eggs. In 2014 and 2015, the fertility was also calculated as the
218 percentage of hatched first-instar nymphs. The survival to adulthood was estimated in each
219 plant by counting adult females since males could not be counted due to their small size and
220 short lifespan. The mealybug survival was estimated as follows: [adult females/(released eggs
221 \times percentage of female eggs released)] \times 100, assuming a percentage of female eggs of 60.3%
222 (Cocco et al., 2015).

223

224 *2.5 Data analysis*

225

226 The cover crop dry matter yield, the grapevine growth and yield variables, and the mealybug
227 development and reproductive parameters were compared using a generalized linear mixed
228 model (PROC GLIMMIX, SAS Institute 2008) with cover crops as fixed and blocks as
229 random effects. In order to compare parameters among years, the treatment factor 'year' was
230 included as a fixed effect (Giese et al., 2014). In the model, numerical and percentage data
231 were assumed to follow normal and binomial distributions, respectively. The patterns of
232 SPAD values and cover crop soil covering rates during the experiments were compared with
233 the same treatment factors previously described (i.e. cover crops and year) and separated
234 among treatments by analysis of variance with a repeated-measures design (PROC MIXED,
235 SAS Institute 2008). Treatments and treatment interactions were compared by Tukey's post
236 hoc test at the significance level of 0.05. When the interaction was significant, differences
237 among cover crops were further investigated within each year. When needed, letter displays
238 indicating significant treatment difference were generated with the %MULT macro within

239 PROC GLIMMIX (Piepho, 2012). Data from plants affected by esca disease were not
240 included in the statistical analyses.

241

242 **3. Results**

243

244 *3.1 Cover crop covering and composition*

245

246 Both NC and GM cover crops established quickly and provided consistent and similar cover
247 through seasons and among years (>77%) (Fig. 1). On the other hand, LM failed to re-
248 establish in the autumn of the first year, resulting in a significantly lower covering rate than
249 NC and GM in 2013. After the over-sowing in February 2014, LM had similar covering rate
250 to other treatments.

251 Growth of cover crops, and thus the mowing frequency, varied by year due to climate
252 conditions. Plots were mowed once in 2013 and 2014 and three times in 2015 (Fig. 2). The
253 production of dry matter differed significantly by mowing date and year, and main effect
254 interactions were also significant. NC produced significantly less dry matter than LM in 2013
255 and less than both LM and GM in 2014. In the last year of the study, LM and NC were in
256 general more productive than GM. Seeded species dominated the stands of LM and GM with
257 >61% and >85% of DMY, respectively. The most common weeds were: *Plantago lanceolata*
258 L., *Conyza canadensis* (L.) Cronq., *Senecio vulgaris* L., *Avena sterilis* L., *Poa annua* L.,
259 *Sonchus oleraceus* L.

260

261 *3.2 Grapevine leaf nitrogen content, vegetative growth and crop yield*

262

263 The different floor management systems significantly affected the leaf nitrogen content of
264 grapevines, assessed as SPAD values, in all three years of the survey (Table 1). In 2013 and
265 2015, ST and LM treatments exhibited higher leaf nitrogen content (averaged across season)
266 than GM and NC, while the nitrogen concentration in 2014 differed in all treatment groups
267 (ST>LM>GM>NC, $P < 0.05$).

268 The number of shoots per vine did not vary across treatments in any of the years (Table 2) as
269 a consequence of the removal of supernumerary shoots. Relative to the grapevine vigor, the
270 GM treatment in the first year showed statistically lower pruning weights than all the other
271 treatments. In 2014 and 2015, ST grapevines produced significantly more pruning wood than
272 other treatments, while GM vines exhibited the lowest values confirming the observation of
273 the first year. The Ravaz index varied significantly by year but it was not affected by the
274 different floor management systems (Table 2).

275 Grapevine yield differed significantly among treatments during the trial. Soil tillage promoted
276 higher grape production than cover crops in all experimental years except in 2013 (Table 3).
277 Focusing on the various floor covers, yield in GM was consistently lower than that in NC and
278 LM plots in all three years of observations. Regardless of the treatment, the yield harvested in
279 2013 was higher and almost twice that of the following year, while in 2015 the production
280 was intermediate compared with 2013 and 2014 (Table 3).

281 In relation to yield components, the number of clusters per vine was lower in GM plots than
282 in other treatments, with significant differences in 2014 and 2015, suggesting that the lower
283 production depended on a lower number of clusters per vine (Table 3). Relative to ST, cover
284 crop effects on cluster weight were not consistent among years (cover crop \times year interaction
285 $P < 0.05$), but tended to reduce the weight of clusters. These effects were most consistent in
286 GM plots. In 2013, cluster weight was similar in ST, NC and LM and greater than GM. ST
287 produced heavier clusters than GM and LM in 2014 and than all other treatments in 2015.

288 Berry weight was not affected by either soil tillage or cover crops in 2013, while it tended to
289 be lower in LM and higher in NC vines in the following years.

290 The floor management significantly influenced most of the fruit composition parameters at
291 harvest (Figs. 3 and 4), except for total acidity and pH (data not shown). However, the must
292 quality changed significantly from vintage to vintage. Overall, the 2013 vintage was
293 characterized by grapes with lower soluble solids content and higher acidity than the other
294 two vintages, while the highest sugar levels at harvest were achieved in 2014 regardless of
295 soil management. Focusing on differences in the phenolic component among vintages, the
296 total anthocyanins were the lowest in 2013 and highest in 2015. Conversely, the total
297 polyphenols were less influenced by vintage, and were significantly lower than in previous
298 years only in 2015.

299 Effects of cover crop treatments on the sugar content were not consistent among years (cover
300 crop \times year interaction $P < 0.05$). No effects were observed at harvest in the first year of the
301 study but significant differences were found among treatments in the final two years. In 2014,
302 the sugar level detected on GM vines (22.7 °Brix) was higher than on LM vines (20.7 °Brix),
303 while soluble solids in 2015 were significantly higher on GM than on ST vines (20.8 and 18.9
304 °Brix, respectively) (Fig. 3). The total acidity was influenced by treatments only in the first
305 sampling dates of each season, while at harvest no differences among cover crops were
306 recorded (Fig. 3).

307 The color intensity, measured as total anthocyanins, generally increased along with the
308 ripening process in all treatments (Fig. 4). At harvest, the anthocyanin content of grapes in
309 NC was consistently the lowest, while other treatments had similar concentrations to each
310 other in the first two years. In 2015, anthocyanins in GM were higher than in LM and ST. The
311 concentration of total polyphenols in NC, LM and GM plots increased in the first weeks of
312 ripening and then declined slowly until harvest, except in 2013 on LM vines (Fig. 4).

313 Conversely, vines subjected to traditional soil tillage showed a steady increase in total
314 polyphenols from veraison to harvest in 2013 and 2014. The statistical analysis indicates that
315 at harvest 2013, the polyphenol content was higher in ST and LM grapes than GM, which in
316 turn was higher than NC. In 2014, LM showed a lower concentration of polyphenols at
317 harvest compared to the other treatments. In the last harvest, a higher accumulation of
318 polyphenols was observed on GM and ST than NC berries, with LM grapes showing the
319 lowest polyphenol content.

320

321 *3.3 Vine mealybug biological parameters*

322

323 All the vine mealybug biological parameters investigated were significantly affected by
324 ground covers, especially in 2014 and 2015 (Table 4). In 2013, the development time from
325 egg hatching to ovipositing female was shorter in mealybugs collected in ST and LM plots
326 than in NC plots, while ST values in 2014 differed from all cover crop treatments. In 2015,
327 mealybugs on ST and LM plants developed faster than those in NC and GM plots. The pest
328 survival was highly variable in the first two years of the survey, when differences were not
329 significant. Conversely, mealybug survival was higher in LM plots than in other treatments in
330 2015. In 2013, the floor management systems did not affect the fecundity of *P. ficus* females,
331 while the fecundity in 2014 was higher in mealybugs developed in ST and LM grapevines
332 compared with those reared in NC. In 2015, the number of eggs oviposited by mealybugs in
333 LM was higher than that observed in ST treatment, which in turn was higher than that
334 recorded in NC and GM plots. The fertility was statistically higher in LM (2014 and 2015)
335 and in ST plots (2015) compared to NC and GM.

336

337 **4. Discussion**

338

339 Control of fruit composition during ripening can be achieved through oenological and cultural
340 practices. The increase of sugar content and color intensity is commonly obtained through
341 cluster thinning, especially for ‘appellation of origin’ wines that require crop yield limits.
342 Although undoubtedly effective, thinning is also time consuming and expensive (Berkey et
343 al., 2011; Preszler et al., 2013). Other practices that increase nutritional and water
344 competition, such as cover crops, are also effective in avoiding excessive crop yield and are
345 more economically sustainable compared to cluster thinning. In addition, cover crops have a
346 number of beneficial effects on the vineyard agro-ecosystem, including all-year-round
347 accessibility for time-sensitive cultural practices (e.g. harvest, fungicide applications) (Pardini
348 et al., 2002).

349 In our experiment, all the complete floor cover crops investigated promoted lower yields
350 compared to conventional soil tillage from the second year of the study, most likely due to the
351 competition for water and nutrients. However, not all cover crops competed in the same
352 manner with vines, as only grass cover crop (GM) had a negative impact on the following
353 year's grape production. Conversely, in our previous experiment carried out for five years in
354 the same vineyard, inter-row GM did not affect grape yield and its components (Mercenaro et
355 al., 2014). This was probably due to insufficient competition of grass in inter-rows since the
356 soil areas of maximum root water and nutrient uptake are located near the vine trunk (Fuentes
357 et al., 2008).

358 Few studies have been conducted to evaluate complete floor cover crops in vineyards. Our
359 results confirm the findings of a four-year experiment carried out in France by Gontier et al.
360 (2011), all of which observed a reduced crop yield and vigor and an increased sugar and
361 polyphenolic content in grapevines subjected to complete grass cover cropping. In contrast,
362 Giese et al. (2014a, 2014b) found no depressive effect on productivity caused by complete

363 floor covers in a Cabernet Sauvignon vineyard located in North Carolina. Giese et al. (2014a)
364 also reported a significant effect of complete grass cover on reducing canopy density as well
365 as pruning weight. The latter outcome is in accordance with our trial, in which a general
366 reduction in the weight of pruning wood was observed during the three experimental years in
367 all cover crop plots compared with traditional floor management. All cover crops except
368 legume mixture (LM) established well in the first year. However, the over-sowing in LM
369 plots in early 2014 ensured a satisfactory soil covering similar to GM and natural covering
370 (NC). Afterwards, the density of all the investigated ground covers ensured a good control of
371 the grapevine vigor, in accordance with findings of Pou et al. (2011) in a Manto negro
372 vineyard in the Balearic Islands (Spain). Therefore, changes in vegetative growth and yield in
373 2014 and 2015 represent the response of grapevines to mature complete floor covers.

374 Floor management may also contribute to improve the must quality. In the present study, GM
375 increased sugar concentrations at harvest relative to ST in the final year of the study. Cover
376 crop treatments also affected concentrations of anthocyanins and polyphenols relative to
377 standard tillage, but effects were most consistent in the final two years. Grass cover produced
378 concentrations that were higher than or similar to ST, while NC reduced anthocyanin
379 concentrations and LM reduced polyphenol concentrations relative to ST in most years. In our
380 previous study (Mercenaro et al., 2014), the only significant change in the must composition
381 involved the total anthocyanin content, with higher values in the grass treatment. Several
382 studies have tested the between-row cover crop strategy, showing that the choice of an
383 appropriate cover crop led to, for instance, higher sugar (Lavezzi et al., 2005) and total
384 polyphenol (Lopes et al., 2008) content in the berries and improved wine quality (Xi et al.,
385 2011). Conversely, cover crops did not influence the must composition over a three-year
386 period in an intercropped vineyard (Ingels et al., 2005), whereas grape ripeness improved
387 from the fourth year of observations on vines managed with a permanent complete floor cover

388 (Tescic et al., 2007). These results suggest a greater influence of cover crops on vegetative
389 growth and yield than on must quality, especially in the first years of ground cover
390 establishment, and indicate the importance of long-term studies to highlight changes in the
391 grape composition due to floor management practices.

392 Currently, the vine mealybug control mostly relies on chemical applications, although this
393 method is often unsatisfactory as mealybugs prefer concealed locations under the bark or in
394 the roots. From the perspective of a more sustainable agriculture and integrated pest
395 management, active ingredients with novel modes of action and more sustainable control
396 strategies have been tested with promising results (Mansour et al., 2010; Karamaouna et al.,
397 2013; Cocco et al., 2014). Cover crops should additionally be considered in integrated pest
398 management programs. In fact, floor management systems affected all the investigated
399 biological parameters of *P. ficus*, in particular development time, fecundity and fertility.
400 Development and reproductive performances of mealybugs developed on LM grapevines
401 were overall similar to the reference treatment (ST) and higher than those of mealybugs
402 reared on GM and NC plots. Differences among treatments became more evident in 2014 and
403 2015 and were generally consistent in both years. Because the ovipositing mealybugs
404 collected from the experimental plots were kept under the same conditions of temperature,
405 relative humidity and photoperiod, differences in the reproductive output of mealybugs are
406 attributable to their nutritional status and feeding history at the time of the onset of
407 oviposition.

408 Our findings show that all the tested floor cover treatments affected - through a bottom-up
409 regulation process - the development and reproductive parameters of *P. ficus*. In particular,
410 GM and NC reduced grape growth and nitrogen content relative to ST, resulting in a negative
411 effect on mealybug performance. Improved *P. ficus* development and reproduction was
412 consistently observed in grapevines with a higher leaf nitrogen content and vigor (ST and

413 LM), in accordance with prior studies on mealybugs (Hogendorp et al., 2006; Cocco et al.,
414 2015). Competition of cover crops for water and nutrients can alter the phenology of host
415 plants, reducing their nutritional quality and, thereby, pest development (Costello and Daane
416 2003; Schmidt et al., 2007). However, response of pests to changes in host quality cannot be
417 generalized, as stressed plants can enhance the performance of some pests and in contrast
418 reduce the density of others (Bukovinszky et al., 2004). The effectiveness of a bottom-up
419 integrated pest management program based on habitat management, cultural practices and
420 minimum use of pesticides was also demonstrated in a long-term trial conducted in a
421 commercial apple orchard (Prokopy, 2003).

422 Further aspects need to be considered in order to fully understand the influence of cover crops
423 in regulating mealybug populations, such as the top-down effects that could help to reduce
424 pest density via the enhancement of the natural enemy complex (Landis et al., 2000). In fact,
425 cover crops also play an important ecological role, as they can influence the development of
426 insect populations by harboring and sheltering beneficials, such as generalist predators (Daane
427 and Costello, 1998; Nicholls et al., 2000) or pests (Meagher and Meyer, 1990; Bone et al.,
428 2009). Moreover, untilled soil in vineyards indirectly favors higher *P. ficus* infestation by
429 promoting the establishment of ant colonies that disrupt the activity of the vine mealybug
430 parasitoid complex (Serra et al., 2006; Mgocheki and Addison, 2010). Finally, the choice of
431 cover crop species should also consider their potential harboring of stolbur phytoplasma (bois
432 noir), as a number of potential cover crop species have been successfully inoculated by the
433 vector *Hyalesthes obsoletus* Signoret (Hemiptera: Cixiidae) (Maixner et al., 2001).
434 Conversely, competitive cover crops could suppress *H. obsoletus* host species, hence reducing
435 the pest population density (Maixner, 2007).

436

437 **5. Conclusions**

438

439 Our findings highlight that complete vineyard floor cover cropping significantly influences
440 grapevine growth, yield and must composition and, when optimized, represents a sustainable
441 tool to improve the quality of wines. Making generalizations about the most suitable floor
442 management system in vineyards is difficult, as response to cover crop is site-specific and
443 variety-dependent due to differences in terms of soil, plant vigor, level of production and
444 oenological objectives. Therefore, the choice of cover crops strongly depends on the wine
445 grape cultivar and cultivation site. The viticultural terroir investigated in this study was
446 characterized by a Mediterranean climate, fertile soil and a productive and vigorous cultivar
447 (Carignano). In this context, complete grass cover is recommended in order to limit excessive
448 vegetative growth and improve must quality, especially the phenolic content.

449 In addition, complete grass mixture and natural covering negatively influenced the vine
450 mealybug development, creating unfavorable conditions for pest development. However, total
451 ground cover does not effectively reduce *P. ficus* populations as a stand-alone control strategy
452 but should instead be integrated in sustainable control programs. This study indicates the
453 importance of floor management systems for the trophic system grapevine – *P. ficus* and
454 suggests, in addition to other factors, the inclusion of cover cropping in pest management
455 programs.

456

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643 **Table and figure captions**

644

645 **Table 1**

646 SPAD values (mean \pm SE) on grapevine leaves in spring-summer under different floor
 647 management systems: soil tillage (ST); natural covering (NC); grass mixture (GM); legume
 648 mixture (LM).

Year	SPAD value ^a			
	ST	NC	GM	LM
2013	41.62 \pm 0.81 a	35.11 \pm 0.86 b	36.48 \pm 0.61 b	42.06 \pm 1.09 a
2014	45.71 \pm 0.82 a	37.13 \pm 0.80 d	40.86 \pm 1.08 c	43.71 \pm 0.83 b
2015	47.54 \pm 0.72 a	43.23 \pm 0.71 b	43.82 \pm 0.85 b	47.71 \pm 0.56 a
Significance ^b				
Cover crop		**		
Year		**		
Cover crop \times year		**		

649 ^a Values within rows followed by different letters are not significantly different ($P < 0.05$) by

650 Tukey's test.

651 ^b * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

652

653 **Table 2**654 Grapevine growth parameters (mean \pm SE) under different floor management systems: soil

655 tillage (ST); natural covering (NC); grass mixture (GM); legume mixture (LM).

Year	Shoots/vine (no.) ^a			
	ST	NC	GM	LM
2013	9.8 \pm 1.4	10.4 \pm 1.2	9.9 \pm 1.4	10.5 \pm 0.9
2014	9.3 \pm 1.2	9.1 \pm 0.6	9.3 \pm 0.9	10.6 \pm 0.9
2015	10.5 \pm 0.6	9.7 \pm 1.2	9.7 \pm 0.8	9.2 \pm 1.3
Significance ^b				
Cover crop		ns		
Year		ns		
Cover crop \times year		ns		
Year	Pruning weight/vine (kg) ^a			
	ST	NC	GM	LM
2013	1.04 \pm 0.19 a	1.00 \pm 0.13 a	0.85 \pm 0.18 b	1.06 \pm 0.16 a
2014	0.80 \pm 0.14 a	0.62 \pm 0.07 b	0.52 \pm 0.09 c	0.68 \pm 0.11 b
2015	1.05 \pm 0.04 a	0.97 \pm 0.25 b	0.72 \pm 0.22 c	0.93 \pm 0.20 b
Significance ^b				
Cover crop		*		
Year		**		
Cover crop \times year		**		
Year	Ravaz index (kg yield/kg pruning weight) ^a			
	ST	NC	GM	LM
2013	5.5 \pm 1.0	6.3 \pm 0.6	4.9 \pm 0.7	5.4 \pm 0.9
2014	4.4 \pm 0.5	4.3 \pm 1.1	3.8 \pm 0.9	3.8 \pm 0.4
2015	4.7 \pm 0.3	3.7 \pm 0.3	4.2 \pm 0.5	3.9 \pm 0.4
Significance ^b				
Cover crop		ns		
Year		**		
Cover crop \times year		*		

656 ^a Values within rows followed by different letters are not significantly different ($P < 0.05$) by

657 Tukey's test.

658 ^b * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

659 **Table 3**

660 Grapevine yield parameters (mean \pm SE) under different floor management systems: soil
 661 tillage (ST); natural covering (NC); grass mixture (GM); legume mixture (LM).

Year	Yield/vine (kg) ^a			
	ST	NC	GM	LM
2013	5.7 \pm 0.6 a	6.3 \pm 0.4 a	4.2 \pm 0.2 b	5.7 \pm 0.6 a
2014	3.6 \pm 0.5 a	2.7 \pm 0.5 b	2.0 \pm 0.3 c	2.6 \pm 0.7 b
2015	4.9 \pm 0.4 a	3.6 \pm 0.3 b	3.0 \pm 0.3 c	3.6 \pm 0.4 b
Significance ^b				
Cover crop		*		
Year		**		
Cover crop \times year		*		
Year	Clusters/vine (no.) ^a			
	ST	NC	GM	LM
2013	16.9 \pm 1.9	19.0 \pm 1.2	14.6 \pm 1.9	17.8 \pm 1.3
2014	9.0 \pm 0.9 a	9.4 \pm 0.6 a	7.3 \pm 0.6 b	9.1 \pm 0.3 a
2015	14.0 \pm 0.8 a	13.7 \pm 0.7 a	10.2 \pm 0.8 b	13.8 \pm 0.6 a
Significance ^b				
Cover crop		*		
Year		**		
Cover crop \times year		**		
Year	Cluster weight (g) ^a			
	ST	NC	GM	LM
2013	442.1 \pm 29.5 a	420.4 \pm 50.5 a	361.4 \pm 36.9 b	414.0 \pm 31.0 a
2014	365.0 \pm 24.8 a	328.0 \pm 56.4 ab	269.7 \pm 49.8 b	266.0 \pm 38.0 b
2015	339.0 \pm 13.5 a	277.8 \pm 39.5 b	262.8 \pm 45.1 b	264.0 \pm 31.7 b
Significance ^b				
Cover crop		*		
Year		**		
Cover crop \times year		**		
Year	Berry weight (g) ^a			
	ST	NC	GM	LM
2013	2.94 \pm 0.25	2.63 \pm 0.34	2.59 \pm 0.18	2.88 \pm 0.11
2014	2.36 \pm 0.30 b	2.83 \pm 0.22 a	2.30 \pm 0.27 b	1.96 \pm 0.19 c
2015	2.62 \pm 0.12 ab	2.82 \pm 0.09 a	2.62 \pm 0.12 ab	2.49 \pm 0.10 b

Significance^b

Cover crop *

Year *

Cover crop × year *

662 ^a Values within rows followed by different letters are significantly different ($P < 0.05$) by

663 Tukey's test.

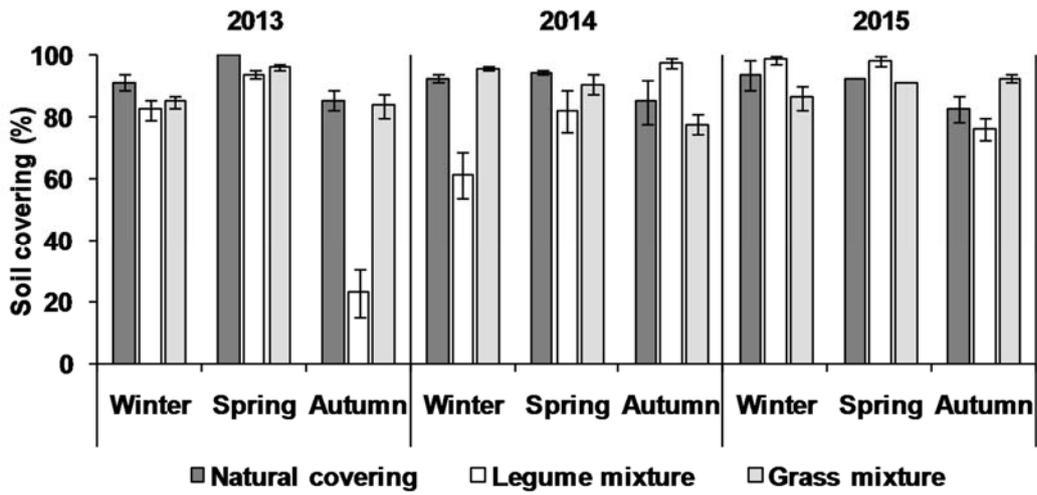
664 ^b * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

665

666 **Table 4**

667 Biological parameters (mean ± SE) of *Planococcus ficus* on vines under different floor
 668 management systems: soil tillage (ST); natural covering (NC); grass mixture (GM); legume
 669 mixture (LM).

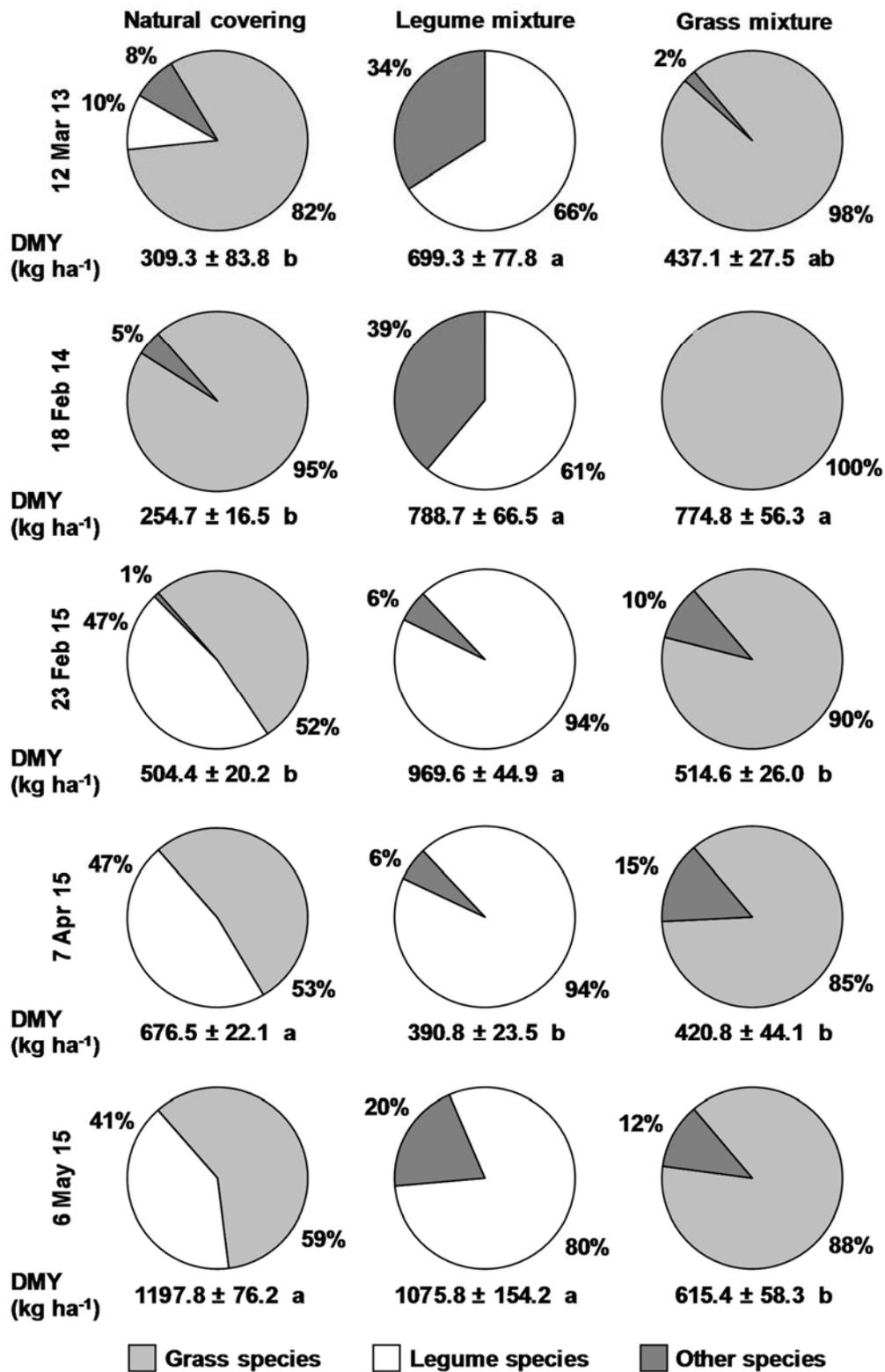
Year	Development time (d) ^a			
	ST	NC	GM	LM
2013	34.07 ± 0.23 b	35.71 ± 0.27 a	34.62 ± 0.24 ab	33.82 ± 0.22 b
2014	33.82 ± 0.16 c	35.57 ± 0.20 a	34.95 ± 0.23 ab	34.82 ± 0.18 b
2015	33.26 ± 0.15 b	34.36 ± 0.15 a	34.52 ± 0.16 a	32.96 ± 0.17 b
Significance ^b				
Cover crop		**		
Year		**		
Cover crop × year		**		
Year	Survival (%) ^a			
	ST	NC	GM	LM
2013	13.93 ± 6.13	12.84 ± 5.31	14.60 ± 8.68	12.03 ± 4.77
2014	26.85 ± 2.91	26.52 ± 2.01	28.87 ± 2.04	26.37 ± 2.82
2015	26.66 ± 2.71 b	27.24 ± 2.44 b	27.03 ± 2.23 b	30.49 ± 3.39 a
Significance ^b				
Cover crop		*		
Year		**		
Cover crop × year		**		
Year	Fecundity (no. eggs) ^a			
	ST	NC	GM	LM
2013	133.66 ± 6.55	124.57 ± 6.53	119.76 ± 4.91	133.52 ± 7.19
2014	178.95 ± 4.55 a	138.57 ± 3.14 c	162.69 ± 4.95 b	172.34 ± 4.67 ab
2015	126.89 ± 2.52 b	116.18 ± 2.68 c	108.00 ± 2.44 c	141.82 ± 3.47 a
Significance ^b				
Cover crop		**		
Year		**		
Cover crop × year		**		
Year	Fertility (%) ^a			
	ST	NC	GM	LM
2014	97.12 ± 0.20 b	96.23 ± 0.28 c	96.18 ± 0.32 c	97.32 ± 0.21 a
2015	91.81 ± 0.69 a	90.18 ± 0.71 b	90.47 ± 0.71 b	92.39 ± 0.58 a



674

675 **Fig. 1.** Percentage soil cover by natural covering legume mixture and grass mixture during the
 676 survey (2013-2015).

677

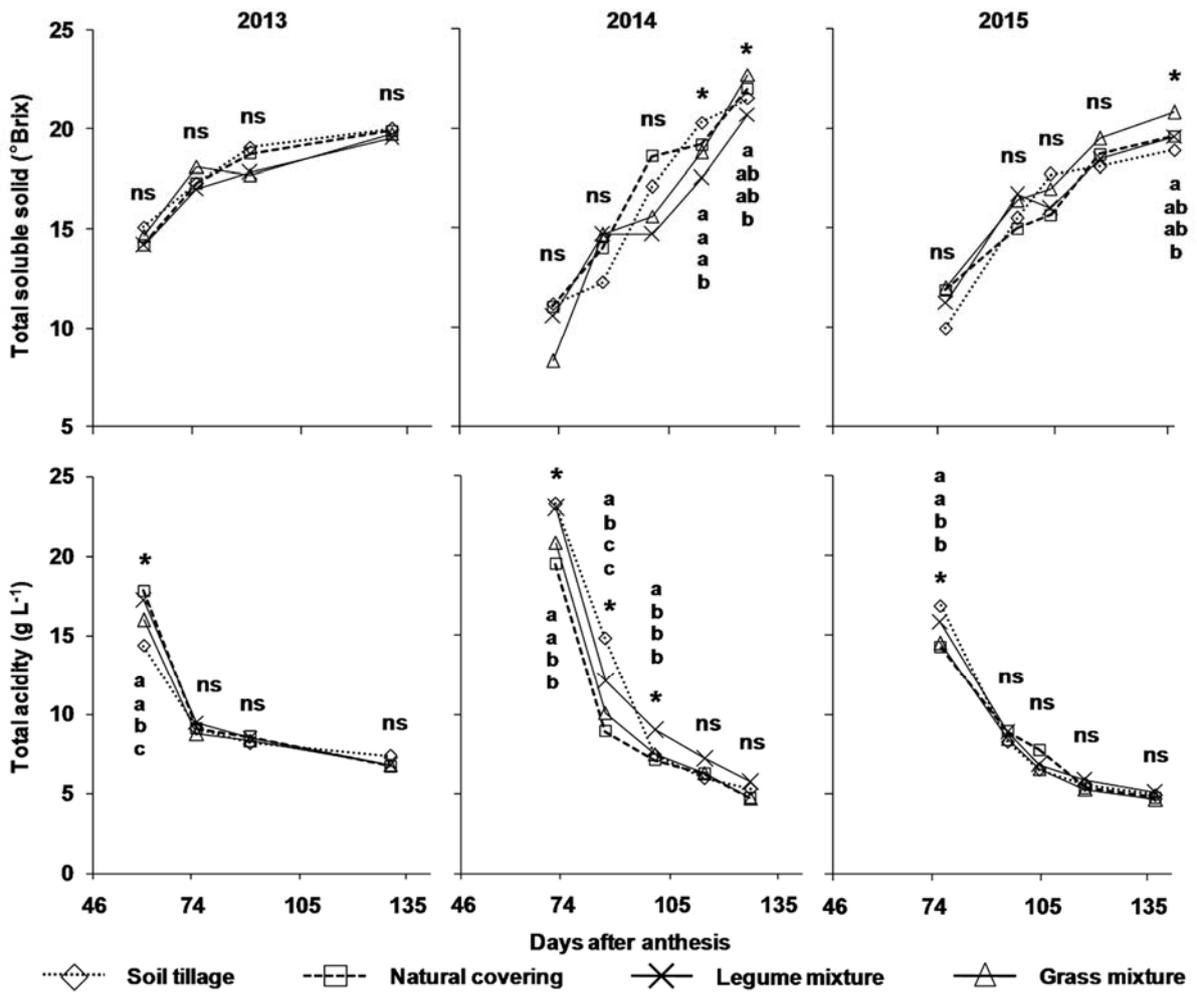


678

679 **Fig. 2.** Dry matter yield (DMY) and percentage species contribution to dry matter production

680 for each cut during the survey. DMY values within each cut bearing the same letters were not

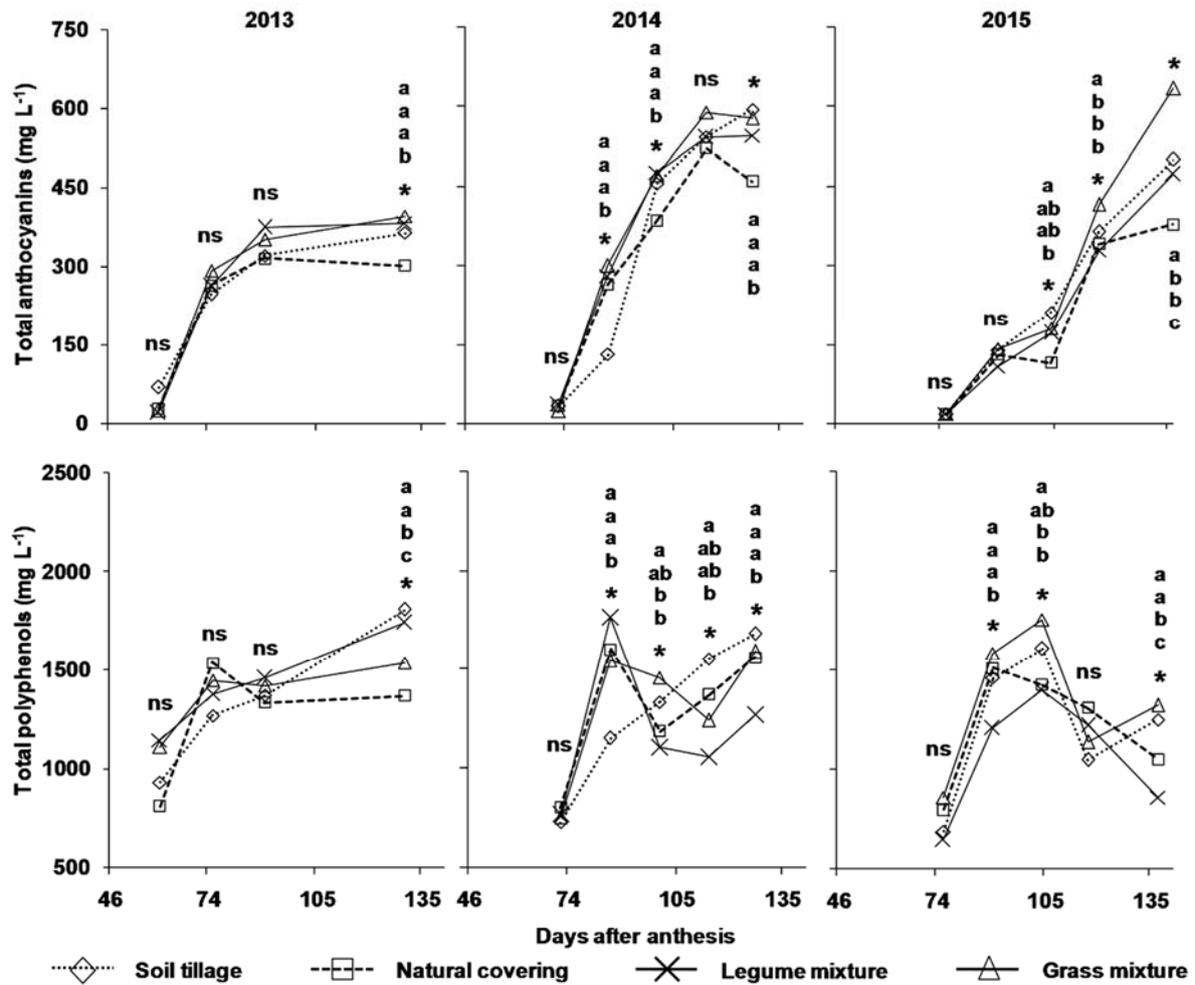
681 significantly different ($P < 0.05$) by Tukey's test.



682

683 **Fig. 3.** Total soluble solids and total acidity of must from veraison to harvest under different
 684 floor management systems. Levels of significance are denoted by * = $P < 0.05$ or ns = not
 685 significant. Different letters within each sampling date indicate significant differences among
 686 means by Tukey's test. Note the different axis scales.

687



688

689 **Fig. 4.** Total polyphenol and total anthocyanin content on must from veraison to harvest under
 690 different floor management systems. Levels of significance are denoted by * = $P < 0.05$ or ns
 691 = not significant. Different letters within each sampling date indicate significant differences
 692 among means by Tukey's test. Note the different axis scales.