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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ABSTRACT

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

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

1. Introduction

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

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

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

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

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

In vineyards, cover crops have had variable effects on pest densities. For example, competition for water and nutrients caused lower plant vigor and reduced leafhopper density due to a poorer host quality (Costello and Daane, 2003). On the other hand, a higher abundance of the vine mealybug, *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae), was observed as a consequence of the suppression of tillage which promoted the development of ant populations and, therefore, the disruption of its natural enemies (Serra et al., 2006; Mgocheki and Addison, 2010; Mansour et al., 2012). *P. ficus* is a key widespread pest in the main grape growing areas which severely reduces the economic yield of table grape and the quality of wine grape, in addition to being a vector of several viruses and diseases (Daane et al., 2012).

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

2. Materials and methods

2.1 Study site and experimental design

The experiment was carried out between 2013 and 2015, in a 17-year-old vineyard, cv. Carignano, located at 40 m a.s.l. in northwestern Sardinia (Italy, 40°33’28”44 N; 08°19’19”56 E). Prior to this study, the site was used for a separate cover crop trial (Mercenaro et al., 2014). The cultivar Carignano is widely cultivated in Sardinia, Spain (known as Cariñena and Mazuela) and southern France (Carignan noir), and it is a highly productive and vigorous cultivar when cultivated in fertile soils (Christensen et al., 2003). Vines were grafted onto 779 P rootstock, trained by a spur-pruned cordon (commonly with five spurs with two buds each) and spaced 2.7 m between rows and 1.0 m within rows. The site has a relatively uniform calcareous alluvial soil, with an average depth of 60-70 cm, and the following physico-chemical characteristics: sand 51.0%, clay 24.9%, silt 24.1%; pH = 7.44; organic matter content = 16 g kg^{-1}. Vines were drip-irrigated three times per year from late June to mid August (corresponding to about 700 m^3 ha^{-1} $year^{-1}$). The experimental vineyard is characterized by a typical central Mediterranean climate, with mild winters and hot dry summers, and precipitations concentrated between October and May (560 mm average total annual rainfall). Daily temperature, relative humidity and rainfall during the survey were recorded by a weather station positioned in the vineyard. In 2013, annual and spring rainfall were higher compared with 2014 and 2015, while summer precipitations were generally scarce, especially in 2014 when the dry season lasted from June to October. Temperatures varied among years. 2015 had a relatively colder winter and hotter summer, resulting in increased abiotic stress for plant growth.

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

Cover crops were seeded along LM and GM rows in mid November 2012 at a rate of 30 kg ha^{-1}, and plots were rolled immediately afterwards. Since the re-establishment of LM in autumn 2013 was unsatisfactory due to adverse weather conditions, an over-sowing was performed in mid February 2014 at the rate of 20 kg ha^{-1}. No herbicides or fertilizers were used on cover crop plots during the trial. The only exception was on LM plots where the non-residual herbicide glyphosate (Roundup Power 2.0, Monsanto, Milano, Italy) was sprayed once in late October

2012 at the rate of 2.5 L ha^{-1} before LM sowing in order to remove a severe infestation of annual and perennial grasses. Glyphosate is most effective against perennial weeds and less costly than pre-emergence herbicides or soil tillage (Monteiro and Moreira, 2004; Tourte et al., 2008).

2.2 Cover crop assessment

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

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

2.3 Grapevine leaf nitrogen content, vegetative growth and crop yield

The content of nitrogen on leaves was estimated with the SPAD 502 Chlorophyll Meter (Minolta, Osaka, Japan), which is a non-destructive portable tool to measure the chlorophyll concentration in leaves (Shaahan et al., 1999; Porro et al., 2001). The nitrogen content in grapevine leaves is closely related with SPAD readings ($r^2 = 0.989$) (Cocco et al., 2015). The leaf nitrogen content was estimated on six dates in spring-summer 2013 and 2014 and eight times in 2015 by measuring the SPAD values in five leaves opposite to basal clusters on each plant artificially-infested with *P. ficus* mealybugs.

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

2.4 Vine mealybug biological parameters

The response of *P. ficus* to different floor management systems was investigated in artificial cohorts established on grapevines. Mealybugs were obtained from a mass-rearing colony maintained on sprouted potato placed inside Plexiglas cages (30 × 30 × 30 cm) with two sides covered with mesh for ventilation. The culture was maintained at 26 ± 1 °C, 60-70% RH, in constant darkness. In order to obtain eggs of the same age, a number of ovipositing females were placed with a sable-hair brush (gauge 000) in 2 × 2 cm strips of cardboard and allowed to oviposit for 24 hours, after which females were removed. Eggs were counted under a dissecting microscope and held in a growth chamber at 25 °C for seven days. Batches of 500 hatching eggs were used to infest one shoot from each of three separate plants per plot by securing the cardboard strips to the abaxial surface of a median leaf in order to minimize *P. ficus* handling. Experimental plants were inspected before the study to ensure the absence of wild populations of mealybugs in the canopy and under the bark. Trials started on 14 June 2013, 30 May 2014 and 3 June 2015 (egg release) and ended on 5 August 2013, 16 July 2014 and 20 July 2015 (count of remaining females). During their development, mealybugs were confined by covering 3-4 leaves of the artificially-infested shoot with a cage of spun-bonded polypropylene fabric (Agribon AG-15, 18.65 g m^{-2}, 90% light transmission) secured at both ends with elastic bands. Cages protected mealybugs from natural enemies and prevented the spread of *P. ficus* immatures within the canopy, which would have dramatically increased the time and effort required for a daily check of the experimental plants.

Starting three weeks after egg release, all leaves, petioles and stems inside the cages were inspected daily, and the first 20 females at the onset of oviposition were collected with a sable-hair brush (gauge 00) and placed inside plastic containers. Ovipositing females were stored in a cooler at ~10 °C during the transport back to the laboratory. The dates of collection were recorded in order to determine the development time from egg eclosion to ovipositing female. All the mealybugs from the different treatments were stored under the same laboratory conditions and allowed to complete oviposition inside the containers, upon which the fecundity was determined under a dissecting microscope by counting the number of first instar nymphs and unhatched eggs. In 2014 and 2015, the fertility was also calculated as the percentage of hatched first-instar nymphs. The survival to adulthood was estimated in each plant by counting adult females since males could not be counted due to their small size and short lifespan. The mealybug survival was estimated as follows: [adult females/(released eggs × percentage of female eggs released)] × 100, assuming a percentage of female eggs of 60.3% (Cocco et al., 2015).

2.5 Data analysis

The cover crop dry matter yield, the grapevine growth and yield variables, and the mealybug development and reproductive parameters were compared using a generalized linear mixed model (PROC GLIMMIX, SAS Institute 2008) with cover crops as fixed and blocks as random effects. In order to compare parameters among years, the treatment factor ‘year’ was included as a fixed effect (Giese et al., 2014). In the model, numerical and percentage data were assumed to follow normal and binomial distributions, respectively. The patterns of SPAD values and cover crop soil covering rates during the experiments were compared with the same treatment factors previously described (i.e. cover crops and year) and separated among treatments by analysis of variance with a repeated-measures design (PROC MIXED, SAS Institute 2008). Treatments and treatment interactions were compared by Tukey’s post hoc test at the significance level of 0.05. When the interaction was significant, differences among cover crops were further investigated within each year. When needed, letter displays indicating significant treatment difference were generated with the %MULT macro within PROC GLIMMIX (Piepho, 2012). Data from plants affected by esca disease were not included in the statistical analyses.

3. Results

3.1 Cover crop covering and composition

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

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

3.2 Grapevine leaf nitrogen content, vegetative growth and crop yield

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

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

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

In relation to yield components, the number of clusters per vine was lower in GM plots than in other treatments, with significant differences in 2014 and 2015, suggesting that the lower production depended on a lower number of clusters per vine (Table 3). Relative to ST, cover crop effects on cluster weight were not consistent among years (cover crop × year interaction $P < 0.05$), but tended to reduce the weight of clusters. These effects were most consistent in GM plots. In 2013, cluster weight was similar in ST, NC and LM and greater than GM. ST produced heavier clusters than GM and LM in 2014 and than all other treatments in 2015. Berry weight was not affected by either soil tillage or cover crops in 2013, while it tended to be lower in LM and higher in NC vines in the following years.

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

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

The color intensity, measured as total anthocyanins, generally increased along with the ripening process in all treatments (Fig. 4). At harvest, the anthocyanin content of grapes in NC was consistently the lowest, while other treatments had similar concentrations to each other in the first two years. In 2015, anthocyanins in GM were higher than in LM and ST. The concentration of total polyphenols in NC, LM and GM plots increased in the first weeks of ripening and then declined slowly until harvest, except in 2013 on LM vines (Fig. 4). Conversely, vines subjected to traditional soil tillage showed a steady increase in total polyphenols from veraison to harvest in 2013 and 2014. The statistical analysis indicates that at harvest 2013, the polyphenol content was higher in ST and LM grapes than GM, which in turn was higher than NC. In 2014, LM showed a lower concentration of polyphenols at harvest compared to the other treatments. In the last harvest, a higher accumulation of polyphenols was observed on GM and ST than NC berries, with LM grapes showing the lowest polyphenol content.

3.3 Vine mealybug biological parameters

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

4. Discussion

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

In our experiment, all the complete floor cover crops investigated promoted lower yields compared to conventional soil tillage from the second year of the study, most likely due to the competition for water and nutrients. However, not all cover crops competed in the same manner with vines, as only grass cover crop (GM) had a negative impact on the following year's grape production. Conversely, in our previous experiment carried out for five years in the same vineyard, inter-row GM did not affect grape yield and its components (Mercenaro et al., 2014). This was probably due to insufficient competition of grass in inter-rows since the soil areas of maximum root water and nutrient uptake are located near the vine trunk (Fuentes et al., 2008). Few studies have been conducted to evaluate complete floor cover crops in vineyards. Our results confirm the findings of a four-year experiment carried out in France by Gontier et al. (2011), all of which observed a reduced crop yield and vigor and an increased sugar and polyphenolic content in grapevines subjected to complete grass cover cropping. In contrast, Giese et al. (2014a, 2014b) found no depressive effect on productivity caused by complete floor covers in a Cabernet Sauvignon vineyard located in North Carolina. Giese et al. (2014a) also reported a significant effect of complete grass cover on reducing canopy density as well as pruning weight. The latter outcome is in accordance with our trial, in which a general reduction in the weight of pruning wood was observed during the three experimental years in all cover crop plots compared with traditional floor management. All cover crops except legume mixture (LM) established well in the first year. However, the over-sowing in LM plots in early 2014 ensured a satisfactory soil covering similar to GM and natural covering (NC). Afterwards, the density of all the investigated ground covers ensured a good control of the grapevine vigor, in accordance with findings of Pou et al. (2011) in a Manto negro vineyard in the Balearic Islands (Spain). Therefore, changes in vegetative growth and yield in 2014 and 2015 represent the response of grapevines to mature complete floor covers.

Floor management may also contribute to improve the must quality. In the present study, GM increased sugar concentrations at harvest relative to ST in the final year of the study. Cover crop treatments also affected concentrations of anthocyanins and polyphenols relative to standard tillage, but effects were most consistent in the final two years. Grass cover produced concentrations that were higher than or similar to ST, while NC reduced anthocyanin concentrations and LM reduced polyphenol concentrations relative to ST in most years. In our previous study (Mercenaro et al., 2014), the only significant change in the must composition involved the total anthocyanin content, with higher values in the grass treatment. Several studies have tested the between-row cover crop strategy, showing that the choice of an appropriate cover crop led to, for instance, higher sugar (Lavezzi et al., 2005) and total polyphenol (Lopes et al., 2008) content in the berries and improved wine quality (Xi et al., 2011). Conversely, cover crops did not influence the must composition over a three-year period in an intercropped vineyard (Ingels et al., 2005), whereas grape ripeness improved from the fourth year of observations on vines managed with a permanent complete floor cover (Tesic et al., 2007). These results suggest a greater influence of cover crops on vegetative growth and yield than on must quality, especially in the first years of ground cover establishment, and indicate the importance of long-term studies to highlight changes in the grape composition due to floor management practices.

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

Our findings show that all the tested floor cover treatments affected - through a bottom-up regulation process - the development and reproductive parameters of *P. ficus*. In particular, GM and NC reduced grape growth and nitrogen content relative to ST, resulting in a negative effect on mealybug performance. Improved *P. ficus* development and reproduction was consistently observed in grapevines with a higher leaf nitrogen content and vigor (ST and LM), in accordance with prior studies on mealybugs (Hogendorp et al., 2006; Cocco et al., 2015). Competition of cover crops for water and nutrients can alter the phenology of host plants, reducing their nutritional quality and, thereby, pest development (Costello and Daane 2003; Schmidt et al., 2007). However, response of pests to changes in host quality cannot be generalized, as stressed plants can enhance the performance of some pests and in contrast reduce the density of others (Bukovinszky et al., 2004). The effectiveness of a bottom-up integrated pest management program based on habitat management, cultural practices and minimum use of pesticides was also demonstrated in a long-term trial conducted in a commercial apple orchard (Prokopy, 2003).

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

5. Conclusions

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

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

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Table and figure captions

Table 1

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

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

[a] Values within rows followed by different letters are not significantly different ($P < 0.05$) by Tukey's test.

[b] * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

Table 2

Grapevine growth parameters (mean ± SE) under different floor management systems: soil tillage (ST); natural covering (NC); grass mixture (GM); legume mixture (LM).

Year	Shoots/vine (no.)[a]			
	ST	NC	GM	LM
2013	9.8 ± 1.4	10.4 ± 1.2	9.9 ± 1.4	10.5 ± 0.9
2014	9.3 ± 1.2	9.1 ± 0.6	9.3 ± 0.9	10.6 ± 0.9
2015	10.5 ± 0.6	9.7 ± 1.2	9.7 ± 0.8	9.2 ± 1.3
Significance[b]				
Cover crop		ns		
Year		ns		
Cover crop × year		ns		

Year	Pruning weight/vine (kg)[a]			
	ST	NC	GM	LM
2013	1.04 ± 0.19 a	1.00 ± 0.13 a	0.85 ± 0.18 b	1.06 ± 0.16 a
2014	0.80 ± 0.14 a	0.62 ± 0.07 b	0.52 ± 0.09 c	0.68 ± 0.11 b
2015	1.05 ± 0.04 a	0.97± 0.25 b	0.72 ± 0.22 c	0.93 ± 0.20 b
Significance[b]				
Cover crop		*		
Year		**		
Cover crop × year		**		

Year	Ravaz index (kg yield/kg pruning weight)[a]			
	ST	NC	GM	LM
2013	5.5 ± 1.0	6.3 ± 0.6	4.9 ± 0.7	5.4 ± 0.9
2014	4.4 ± 0.5	4.3 ± 1.1	3.8 ± 0.9	3.8 ± 0.4
2015	4.7 ± 0.3	3.7 ± 0.3	4.2 ± 0.5	3.9 ± 0.4
Significance[b]				
Cover crop		ns		
Year		**		
Cover crop × year		*		

[a] Values within rows followed by different letters are not significantly different (*P* < 0.05) by Tukey's test.

[b] * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

Table 3

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

Year	Yield/vine (kg)[a]			
	ST	NC	GM	LM
2013	5.7 ± 0.6 a	6.3 ± 0.4 a	4.2 ± 0.2 b	5.7 ± 0.6 a
2014	3.6 ± 0.5 a	2.7 ± 0.5 b	2.0 ± 0.3 c	2.6 ± 0.7 b
2015	4.9 ± 0.4 a	3.6 ±0.3 b	3.0 ± 0.3 c	3.6 ± 0.4 b
Significance[b]				
Cover crop		*		
Year		**		
Cover crop × year		*		

Year	Clusters/vine (no.)[a]			
	ST	NC	GM	LM
2013	16.9 ± 1.9	19.0 ± 1.2	14.6 ± 1.9	17.8 ± 1.3
2014	9.0 ± 0.9. a	9.4 ± 0.6 a	7.3 ± 0.6 b	9.1 ± 0.3 a
2015	14.0 ± 0.8 a	13.7 ± 0.7 a	10.2 ± 0.8 b	13.8 ± 0.6 a
Significance[b]				
Cover crop		*		
Year		**		
Cover crop × year		**		

Year	Cluster weight (g)[a]			
	ST	NC	GM	LM
2013	442.1 ± 29.5 a	420.4 ± 50.5 a	361.4 ± 36.9 b	414.0 ± 31.0 a
2014	365.0 ± 24.8 a	328.0 ± 56.4 ab	269.7 ± 49.8 b	266.0 ± 38.0 b
2015	339.0 ± 13.5 a	277.8 ± 39.5 b	262.8 ± 45.1 b	264.0 ± 31.7 b
Significance[b]				
Cover crop		*		
Year		**		
Cover crop × year		**		

Year	Berry weight (g)[a]			
	ST	NC	GM	LM
2013	2.94 ± 0.25	2.63 ± 0.34	2.59 ± 0.18	2.88 ± 0.11
2014	2.36 ± 0.30 b	2.83 ± 0.22 a	2.30 ± 0.27 b	1.96 ± 0.19 c
2015	2.62 ± 0.12 ab	2.82 ± 0.09 a	2.62 ± 0.12 ab	2.49 ± 0.10 b

Significance[b]	
Cover crop	*
Year	*
Cover crop × year	*

[a] Values within rows followed by different letters are significantly different ($P < 0.05$) by Tukey's test.

[b] * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

Table 4

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

Significance[b]	
Cover crop	**
Year	**
Cover crop × year	*

[a] Values within rows followed by different letters are significantly different ($P < 0.05$) by Tukey’s test.

[b] * = $P < 0.05$; ** = $P < 0.01$; ns = not significant

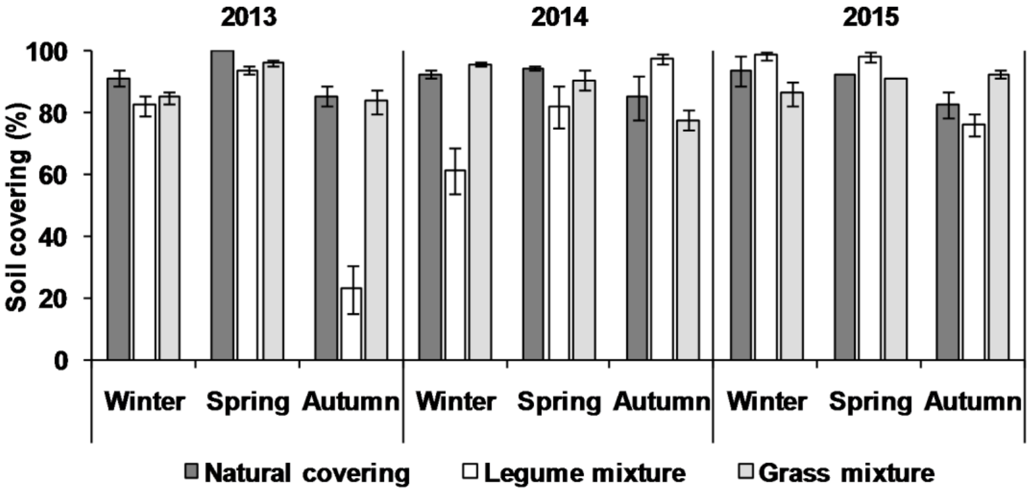

Fig. 1. Percentage soil cover by natural covering legume mixture and grass mixture during the survey (2013-2015).

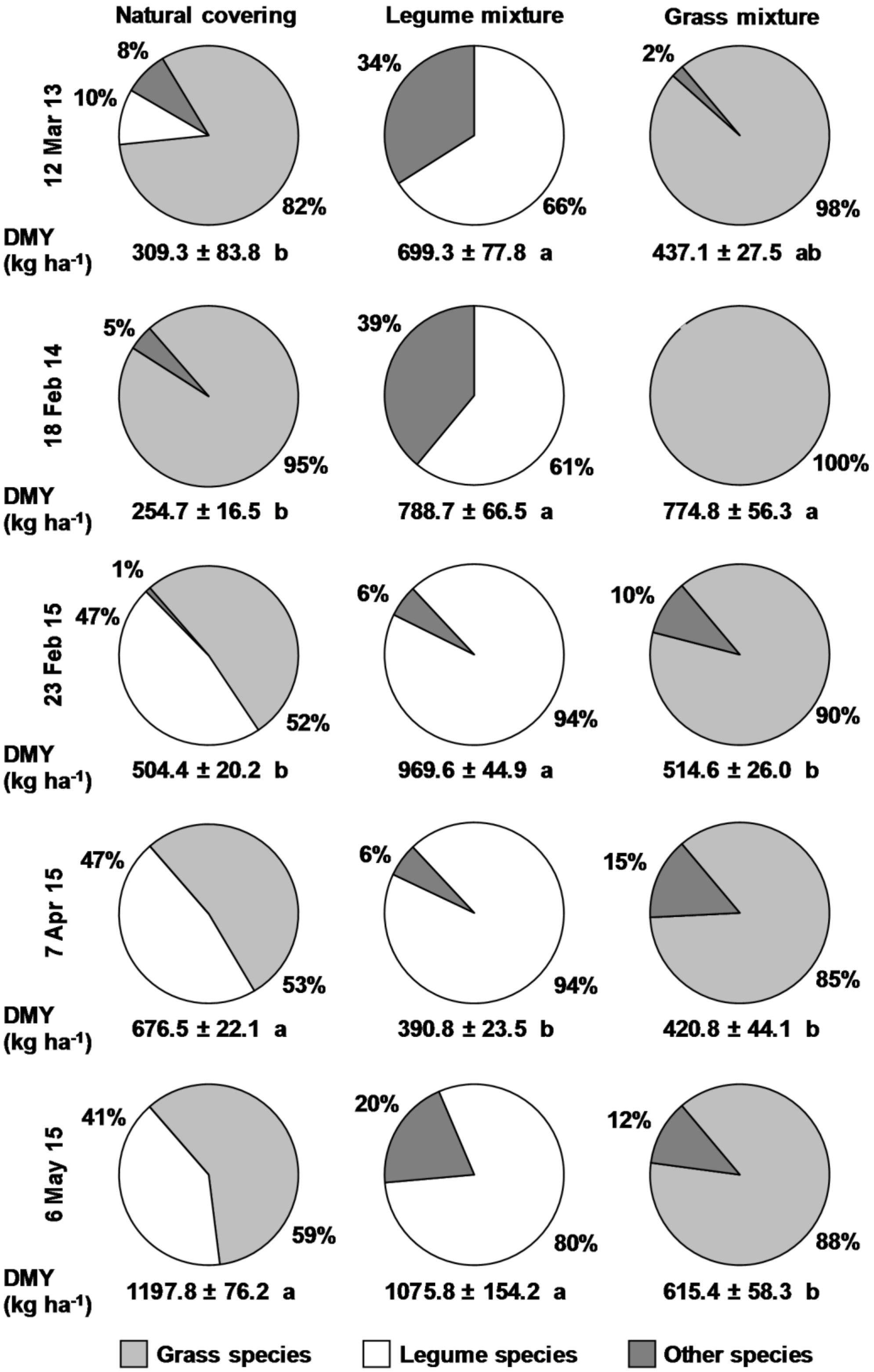

Fig. 2. Dry matter yield (DMY) and percentage species contribution to dry matter production for each cut during the survey. DMY values within each cut bearing the same letters were not significantly different (*P* < 0.05) by Tukey's test.

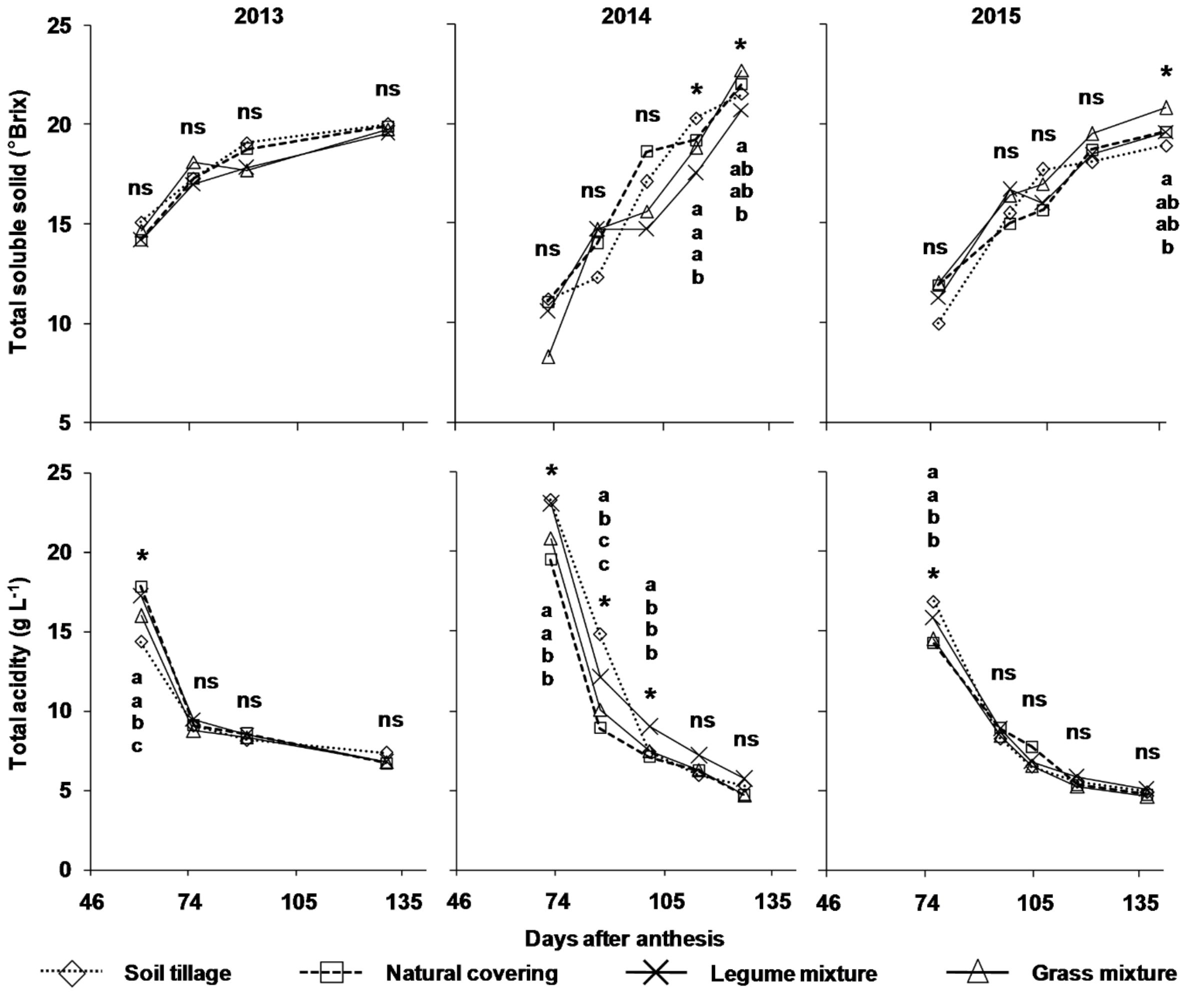

Fig. 3. Total soluble solids and total acidity of must from veraison to harvest under different floor management systems. Levels of significance are denoted by * = P < 0.05 or ns = not significant. Different letters within each sampling date indicate significant differences among means by Tukey's test.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

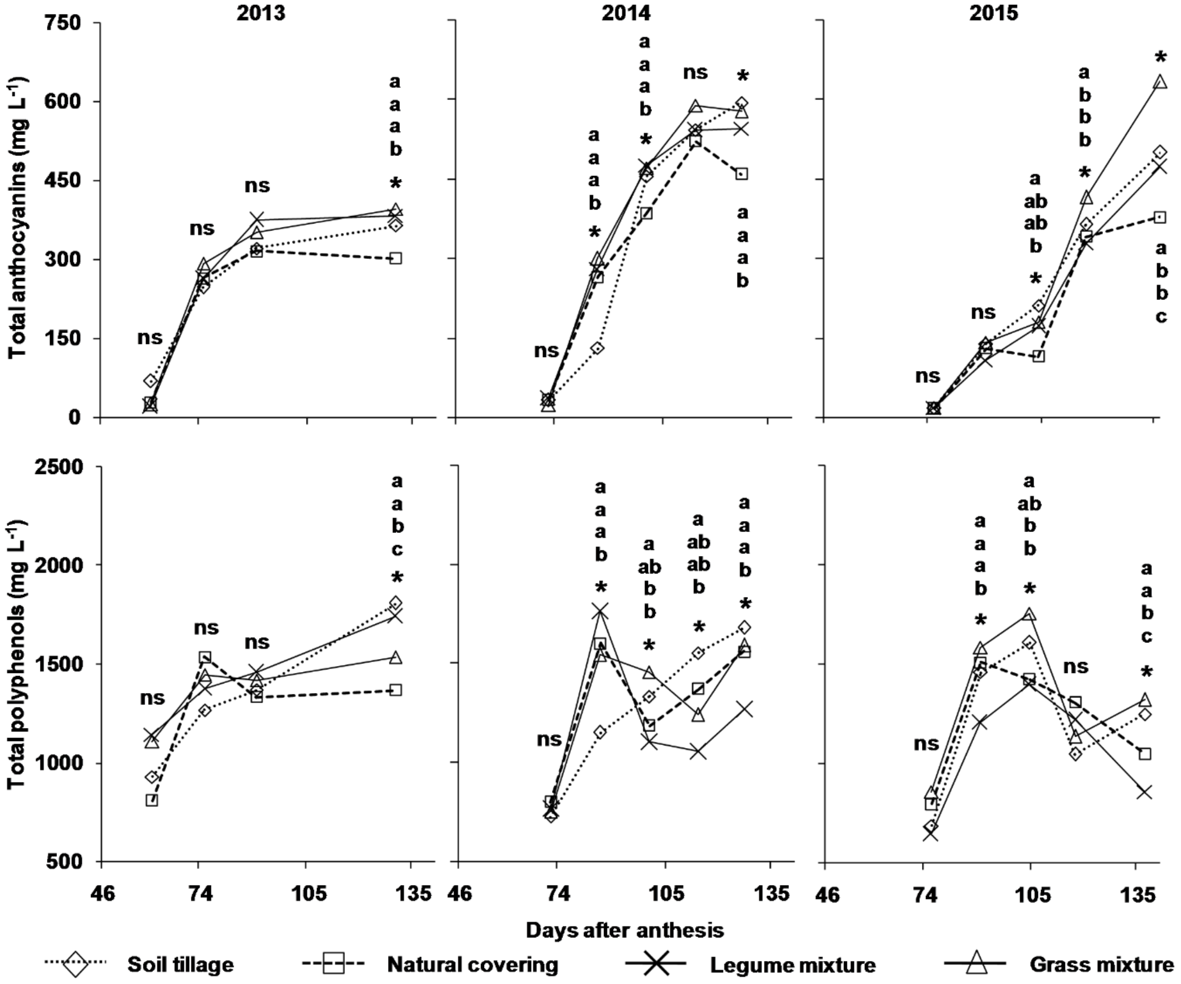

Fig. 4. Total polyphenol and total anthocyanin content on must from veraison to harvest under different floor management systems. Levels of significance are denoted by * = P < 0.05 or ns = not significant. Different letters within each sampling date indicate significant differences among means by Tukey's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