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RESEARCH ARTICLE

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Influence of cover crop treatments on the performance of a vineyard in a humid region

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Abstract

Vineyards are usually managed by tilling the inter-rows to avoid competition from other plants for soil water and nutrients. However, in humid and sub-humid climates, such as that of NW Spain, cover crops may be an advantage for controlling vine vegetative growth and improving berry composition, while reducing management costs. The current study was conducted over three consecutive growing seasons (2012-2014) to assess the effects of establishing three permanent cover crop treatments on water relations, vine physiology, yield and berry composition of a vineyard of the red cultivar 'Mencía' (*Vitis vinifera* L.) located in Leiro, Ourense. Treatments consisted of four different soil management systems: ST, soil tillage; NV, native vegetation; ER, English ryegrass (*Lolium perenne* L.); and SC, subterranean clover (*Trifolium subterraneum* L.). Midday stem water potential was more negative in the native vegetation treatment, causing significant reductions in leaf stomatal conductance on certain dates. Total vine leaf area and pruning weight was reduced in the cover crop treatments in the last year of the experiment. Yield was unaffected by the presence of a cover crop. No significant differences among treatments were observed for berry composition; however, wines were positively affected by the SC treatment (higher tannin content and colour intensity and lower malic acid concentration when compared with ST). Wines from the cover crop treatments were preferred by taste panelists. These results indicate that in humid climates cover crop treatments can be useful for reducing vine vegetative growth without compromising yield and berry quality.

Additional key words: berry composition; cover crops; Mencía grapevine; tillage; sustainability; wine composition.

Abbreviations used: AU (absorbance units); CCI (chlorophyll content index); Chl *a* (chlorophyll *a*); ER (English ryegrass); ETR (electron transport rate); F_s (the steady-state fluorescence yield); FTIR (Fourier transform infrarred spectrometry); F_v/F_m (maximum quantum efficiency of PSII); NPQ (non-photochemical quenching); NV (native vegetation); PQ (photochemical quenching); PSII (photosystem II); PW (pruning weight); SA (surface area); SC (subterranean clover); ST (soil tillage); Φ_{PSII} (photochemical efficiency of PSII).

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Introduction

The adoption of cover crops as soil management systems in Mediterranean rain-fed vineyards has been limited due to the concern of excessive water and nutrient competition between these crops and the vines (Lopes *et al.*, 2008). However, a number of environmental and agronomical benefits could be obtained from cover crops in these agrosystems: soil protection, improvements in physical and biological properties of soils, increases in plant species diversity, enhancement of berry quality, reductions in vine vigour, etc. (Morlat & Jacquet, 2003; Peregrina *et al.*, 2010; Ruiz-Colmenero *et al.*, 2011; Virto *et al.*, 2012). Despite these advantages, vineyards in Spain are usually managed through tillage in the inter-row and herbicides in the vine row (Ibáñez Pascual, 2013).

In Galicia (NW Spain), favourable temperatures and soil water availability during springtime enable a fast canopy establishment in the vineyards. This may induce a dense canopy, creating unbalanced vines and unfavourable microclimate at the cluster zone, which can be deleterious for berry health and ripening (Smart & Robinson, 1991), which is the case of Galicia due to high spring rainfall amounts and mild temperatures. Those highly vigorous vines need more intensive canopy management (shoot trimming and defoliation), increasing management costs. In these situations, the use of cover crops may provide a useful tool for reducing grapevine vegetative growth since soil water and nutrients can be extracted by those cover crops and thus induce a mild stress in the vines (Celette *et al.*, 2008, 2009).

Nevertheless, research on competition for water resources between grapevines and cover crops has led to contradictory results, likely due to differing pedoclimatic conditions. Some studies showed a high water stress affecting grapevines when they were grown with a cover crop (Maigre, 1996; Monteiro & Lopes, 2007), whereas others reported that intercropped vineyards did not always exhibit higher water stress than those under bare soil (Celette *et al.*, 2005; Lopes *et al.*, 2008). Moreover, vineyards need a moderate water stress to produce the grape quality necessary for wine production (Dry & Loveys, 1998), and the characteristics usually considered for grape quality (total soluble solids content, titratable acidity, pH, malic and tartaric acid contents) have been reported to improve or not to be altered by the use of cover crops (Monteiro & Lopes, 2007; Lopes *et al.*, 2008; Marques *et al.*, 2010), although grapevine vigour and yield can be affected (Ruiz-Colmenero *et al.*, 2011).

Therefore, more research is needed in order to characterize the effects that permanent cover crops may have on grapevine yields and quality, as well as in soil water and soil quality dynamics under different pedoclimatic conditions. In the case of Galicia and its traditional grapevine cultivars such as 'Mencía', the main problems that the use of cover crops may help to solve are the excess of vegetative growth and unfavourable microclimate at the cluster zone which, favoured by autumn rainfall, causes rotten harvests. Moreover, 'Mencía' is a cultivar with a low colour potential that may be increased by the use of cover crops. Although erosion is not a major problem in Galicia as compared to Mediterranean regions, under certain circumstances such as bare soil and heavy autumn rainfalls, soil losses may reach values similar to those observed in Mediterranean conditions (Mirás-Avalos *et al.*, 2009); this problem would be reduced by the use of cover crops as soil management. Finally, tractor fuel and time consumption would decrease by the use of cover crops, reducing air pollution.

In this regard, the present study aimed to assess the effects of four different soil management systems in the vineyard, including soil tillage and three different permanent cover crops (native vegetation, sown ryegrass, and subterranean clover), on water relations, grapevine physiology, vegetative growth, yield, berry composition and wine attributes of 'Mencía' cultivar, the main red grapevine cultivar from Galicia (a subhumid region in NW Spain). For this purpose, experiments were carried out over a period of 3 years with contrasting rainfall regimes.

Material and methods

Site description

The experiment was carried out during three consecutive seasons (2012-2014) in a 0.1-ha rain-fed vineyard (*Vitis vinifera* L. cv. 'Mencía') located in the experimental field of the Estación de Viticultura e Enoloxía de Galicia (EVEGA), in Leiro (42º 21.6' N, 8º 7.02' W, elevation 115 m), Ourense, Spain. The vineyard was planted in 2007 on 196-17C rootstock at a spacing of 2.3×1.25 m (3,478 vines/ha) and vines were trained to a vertical trellis on a single cordon system (10-12 buds/vine) oriented in the East-West direction.

The soil at the site was an Inceptisol with sandy-loam texture (68% sand, 19.4% silt, 12.6% clay), acidic (pH 5.8), and with a high organic matter content (4%). The soil has a rather shallow profile (≈ 1 m), and available water capacity is about 100 mm/m.

Climate of this region has been classified as temperate, humid with cool nights (Fraga *et al.*, 2014), with average annual rainfall of 900 mm of which about 70% falls during the dormant period. Weather conditions during the experiment (Table 1) were recorded with an automated meteorological station located 250 m from the vineyard.

Experimental design

Treatments consisted of four different soil management systems in the inter-rows (approximately, 85% of the inter-row): i) soil tillage (ST); ii) native vegetation (NV); iii) English ryegrass (*Lolium perenne* L.) sown at 40 kg/ha (ER); and iv) subterranean clover (*Trifolium subterraneum* L.) sown at 30 kg/ha (SC). Treatments with cover crops were mowed three times per year, when vegetation reached 20 cm height, whereas the control

Table 1. Mean temperature, potential evapotranspiration (ET_0) and rainfall during the growing season (April to harvest) and annual rainfall, ET_0 and mean temperature of each year.

Year	Growing season rainfall (mm)	Annual rainfall (mm)	Annual ET_0 (mm)	Growing season ET_0 (mm)	Growing season mean temperature (ºC)	Annual mean temperature (ºC)	Growing season degree days (ºC day)
2012	313	841	914	698	17.2	13.1	1491
2013	163	1283	955	745	17.8	13.5	1511
2014	185	1301	963	739	18.0	14.2	1509

ºC day = Growing degree days basis 10ºC.

under tillage was kept with no vegetation through cultivation. Cover crop residues were left on the soil surface after mowing. Soil was worked the same number of times under ST than under the cover crop treatments.

With the exception of soil use, vineyard management practices were the same for all treatments. In spring, shoot thinning was carried out. Trimming was performed in order to control vegetative growth, once prior to veraison, on the same day in all treatments.

The treatments were replicated three times in a complete randomized block design. Each replicate consisted of three rows with 7 vines per row. The five vines in the center of the middle row were used for measurements and the rest acted as buffers.

Field determinations

Stem water potential was assessed fortnightly on one mature and healthy leaf of two plants per replicate (thus, 6 plants/treatment), using a pressure chamber (SoilMoisture Inc., Santa Barbara, CA, USA). Stem water potential was measured at midday on non-transpiring leaves that had been bagged with both plastic sheet and aluminium foil for at least 1 hour before measurements (Choné *et al.*, 2001). This modality of leaf water potential has been proven useful for estimating the vine water status of Galician grapevine cultivars (Mirás-Avalos *et al.*, 2014).

The water stress integral (MPa-days) was calculated from the stem water potential data according to the following equation (Myers, 1988):

$$S_\Psi = \left| \sum_{i=0}^{i=t} (\bar{\Psi}_{i,i+1} - c)n \right|$$

where $\Psi_{i,i+1}$ is the average stem water potential for any time interval; c is the value of the maximum stem water potential measured during the study; and n is the number of days in the interval.

Stomatal conductance was measured fortnightly on one leaf per plant and two plants per replicate (6 plants/treatment) using a leaf porometer (Model SC1 Decagon Devices, WA, USA). The leaves were healthy, mature and fully exposed to sunlight.

Chlorophyll *a* (Chl *a*) fluorescence attributes were measured *in situ* with a pulse-amplitude-modulated fluorometer (FMS 2, Hansatech Instruments, Norfolk, UK) as described by Moutinho-Pereira *et al.* (2012), on the same leaves where stomatal conductance was measured. Leaves were dark-adapted for at least 10 minutes using dark-adapting leaf-clips. Several photosystem II (PSII) attributes were obtained (Maxwell & Johnson, 2000): the maximum quantum efficiency of PSII (F_v/F_m), the photochemical efficiency of PSII (Φ_{PSII}), the electron transport rate (ETR), photochemical quenching (PQ), non-photochemical quenching (NPQ) and the steady-state fluorescence yield (F_s).

Chl *a* fluorescence measurements were performed between 1130 and 1330 h, at canopy maturation (beginning or mid-August). In 2013, these determinations were not taken, due to equipment malfunctioning.

Chlorophyll concentration index (CCI) was also estimated non-destructively using a CCM-200 portable chlorophyll meter (Opti-Sciences, Tyngsboro, MA, USA), which calculates a unitless CCI value from the ratio of optical absorbance at 655 nm to that at 940 nm. These measurements were performed on 3 leaves/plant and 6 plants/treatment. Major veins and areas of obvious visual damage or disease were avoided. CCI values have been reported to be correlated with total foliar extractable chlorophyll (van den Berg & Perkins, 2004; Steele *et al.*, 2008).

Yield was assessed at harvest (date was determined by berry sugar content, although rainfall forecast was also taken into account) on each of the internal rows (5 vines/row) of each replicate and treatment. The number of clusters/vine was also recorded. Average cluster weight was computed by dividing yield per plant by the number of clusters. Berry weight was determined on random samples of about 150 berries per replicate.

Pruning weight (PW) was determined in five vines per replicate. Surface area (SA) was estimated in 6 vines/treatment, after veraison, when shoot growth had ceased, following the method proposed by Sánchez de Miguel *et al.* (2010), in which the width and height of the canopy are collected at five different spots along the vine using a measurement tape.

Furthermore, in 2014, total leaf area per plant was estimated in 6 plants/treatment. For doing this, a relationship between leaf main nerve and leaf area was established in 100 leaves using a leaf area meter (AM350, ADC Bioscientific Ltd., UK). Finally, we obtained the following relationship: Leaf area = 0.9984 × (Leaf main nerve)$^{2.1121}$, with an R^2 of 0.92 ($p< 0.01$). Then, the veins of all the leaves from four shoots per plant were measured and the leaf area for an average shoot was obtained. Then, this value was multiplied by the number of shoots to obtain the total leaf area of each vine.

Must and wine quality determinations

Basic attributes of musts (total soluble solids, pH, titratable acidity, tartaric and malic acid concentrations) and wines (alcohol, pH, titratable acidity, tartaric and malic acids concentrations) were determined by Fourier transform infrarred spectrometry (FTIR) using a WineScan FT120 analyzer (FOSS Electric, Barcelona, Spain) calibrated according to the official methods (OIV, 2009).

Wine colour attributes, including colour intensity, total phenolics index, anthocyanins and tannins contents were determined using the methodology described by Glories (1984) and Zamora (2003) using an ultraviolet-visible Themo Helios Zeta spectrophotometer. Total polyphenol index was directly measured after diluting 1% the wines. Total anthocyanins were quantified according to the decolouration experimented by adding metabisulphite and were expressed as mg/L; total tannins were determined after wine treatment with concentrated hydrochloric acid.

Microvinifications procedure and consumer tests

Grapes from the different treatments were manually harvested on the same day and were transported to the experimental winery in field boxes. Vinifications were performed at EVEGA separately on samples of about 35 kg from each treatment. Due to limitations on grape production and fermentation tanks and to fulfill the requirements of a bigger project, only one vinification per treatment was carried out.

Grapes were mechanically destemmed and transferred to 35-L stainless steel containers. During grape processing, 50 mg/L of SO_2 were added to the mass. Fermentations were carried out at room temperature (22-24ºC). Excellence XR (Lamothe-Abiet, Bordeaux, France) yeast was added following manufacturer's instructions. The wine lots were punched down daily until the end of alcoholic fermentation (8 days). Then, they were pressed, racked into new tanks and kept at room temperature for a couple of days. Then, wines were kept at 4ºC in a chamber for a period of approximately one month for cold stabilization. After this period, wines were filtered, bottled and stored.

Analytical determinations in the wines were performed just after alcoholic fermentation. Finally, after three months of storage under the same temperature and humidity conditions, the wines from the different treatments were tasted each year by, approximately, 50 consumers ranging from 21 to 65 years (55, 51 and 51 consumers in 2012, 2013 and 2014, respectively). The proportion of males and females was different each year but, on average, 60% of them were males. These tasters had different wine consumption habits and diverse experiences with wines; hence, a broad spectrum of opinion was expected. Wines from each treatment were presented to consumers in transparent glasses, so they could compare them visually, olfactory and tasting. Consumers were asked to rank the wines from first to fourth, according to their preferences.

Statistical analysis

Analysis of variance was performed on the agronomic data using the "aov" procedure of the R statistical software (v. 2.11.1; R Development Core Team, 2010). Means were separated using the Tukey's Honest Significant Difference test. Across years, data were analyzed with soil management treatment, block, year and their interaction as factors. Differences were considered significant when $p<0.05$. In the case of wines, since we only had one data per year and treatment, we performed the statistical analysis using year as replication. Therefore, we present data on wine attributes per treatment as an average for the three years considered. Data from the consumer tests was analyzed through correspondence analysis (Benzécri, 1992).

Results

Climatic conditions and plant water relations

During the experimental period (2012-2014), the mean air temperature varied between 13 and 14ºC (Table 1). Precipitation was different between years, the lowest amount of rainfall was observed in 2012 (841 mm), whereas in 2013 and 2014 annual rainfall was similar (1283 and 1301 mm, respectively). However, the growing season of 2012 was wetter than those of 2013 and 2014 (Table 1).

Plant water status, as measured by midday stem water potential, showed a decreasing trend throughout the growing season for all the four soil management practices considered in this study (Fig. 1). In 2012, vines under ST showed a less negative stem water potential by the end of the season. In 2013, plants under NV showed more negative values for stem water potential from mid-season till harvest, whereas in 2014, both plants under NV and ST showed more negative values than those under ER and SC by harvest date.

Water stress integral values (Fig. 2) differed between years, being greater in 2013. This water status indicator was significantly different among treatments in 2013 and 2014 but not in 2012. Significantly higher water stress integral values were found under the NV treatment and lower values were observed under SC.

Stomatal conductance showed a similar evolution over the growing season for all the treatments considered in this study (Fig. 1). Due to the high variability in stomatal conductance readings, no significant differences between treatments were observed in most of the measurement dates.

The attributes of Chl *a* at the ripeness stage reflected significant differences among treatments (Table 2), although their behaviour was different in 2012 and 2014. In both seasons, F_v/F_m did not present significant differences among treatments. In 2012, no significant differences were observed for Φ_{PSII} and NPQ; however, ETR was greater in ST and NV vines than in those under ER and SC, PQ was lower under NV and F_s was higher under NV than under the other treatments. In 2014, ER vines presented higher values for Φ_{PSII} and PQ and lower values

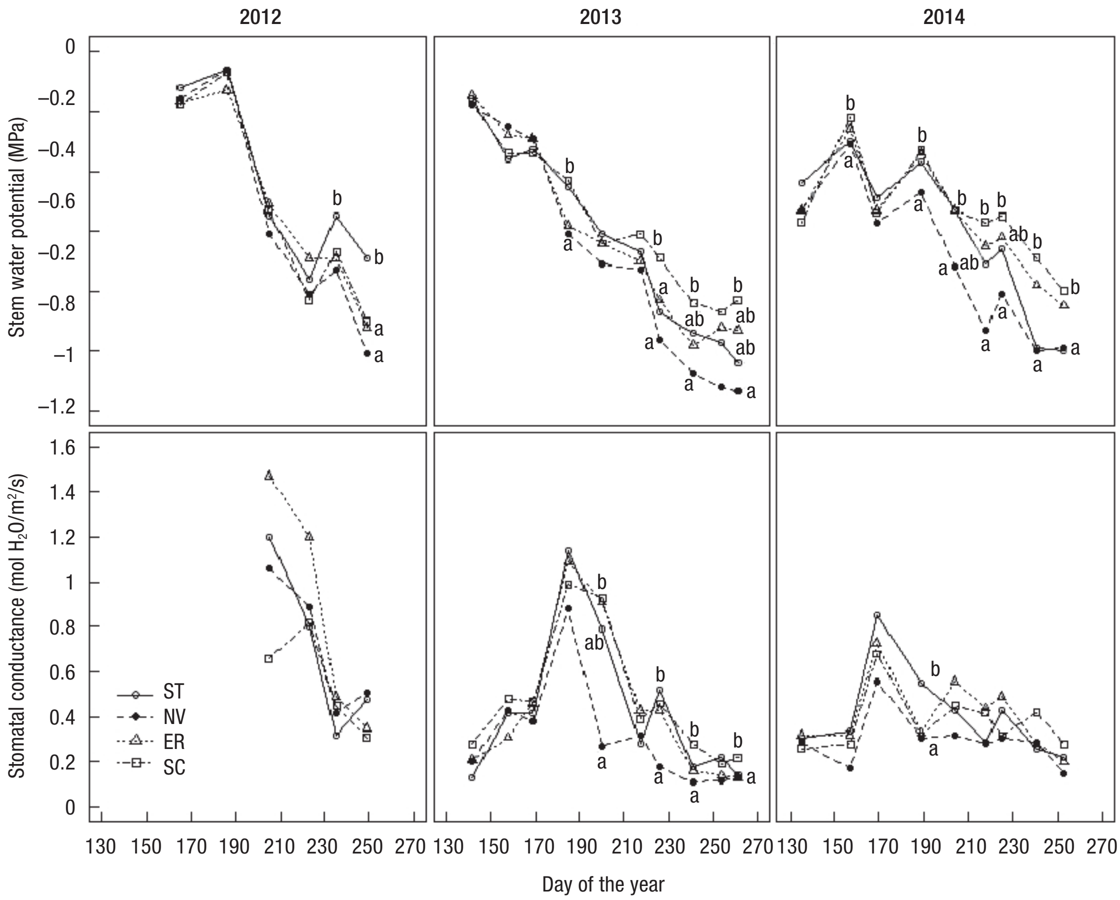

Figure 1. Effect of four soil management strategies (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) on stem water potential and stomatal conductance during the growing season (2012, 2013 and 2014). Points are the average of six measurements. Different letters indicate significant differences (p<0.05) among treatments.

Table 2. Maximum (F_v/F_m) and effective (Φ_{PSII}) quantum efficiency of photosystem II, apparent electron transport rate (ETR), photochemical (PQ) and non-photochemical (NPQ) fluorescence quenching and fluorescence in steady-state (F_s) in 'Mencía' grapevine attached leaves as a function of soil management determined on ripeness stage (10 August 2012 and 6 August 2014).

Treatment[1]	F_v/F_m	Φ_{PSII}	ETR	PQ	NPQ	F_s
			10 August 2012			
ST	0.76	0.46	141.02 b	0.50 b	0.85	288.43 a
NV	0.82	0.42	133.38 b	0.44 a	0.82	354.10 b
ER	0.80	0.50	92.71 a	0.52 b	0.85	286.80 a
SC	0.81	0.50	82.62 a	0.56 b	0.85	305.47 ab
	ns	ns	*	*	ns	*
			6 August 2014			
ST	0.87	0.48 a	91.06 b	0.49 a	0.79 b	353.60 b
NV	0.87	0.43 a	97.59 b	046 a	0.58 a	234.43 a
ER	0.86	0.62 b	77.21 a	0.65 b	0.86 b	209.07 a
SC	0.85	0.50 a	99.33 b	0.55 ab	0.79 b	211.77 a
	ns	*	*	*	*	*

[1] ST: soil tillage; NV: native vegetation; ER: English ryegrass; SC: subterranean clover. ns = non-significant, * = significant at $p<0.05$. Different letters in the column indicate significant differences between treatments at $p<0.05$.

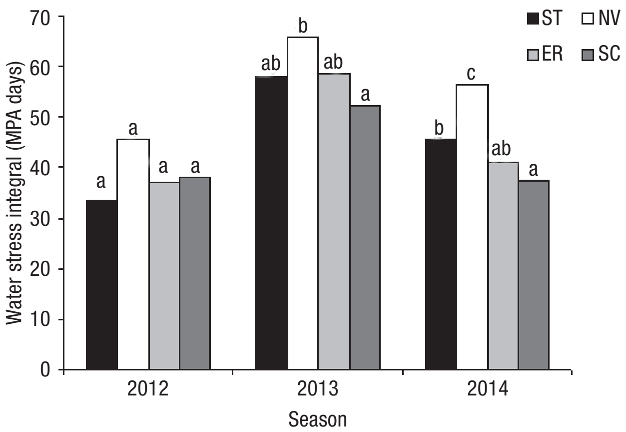

Figure 2. Effect of four soil management strategies (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) on the water stress integral over the three studied growing seasons. Different letters on the columns indicate significant differences among treatments ($p<0.05$).

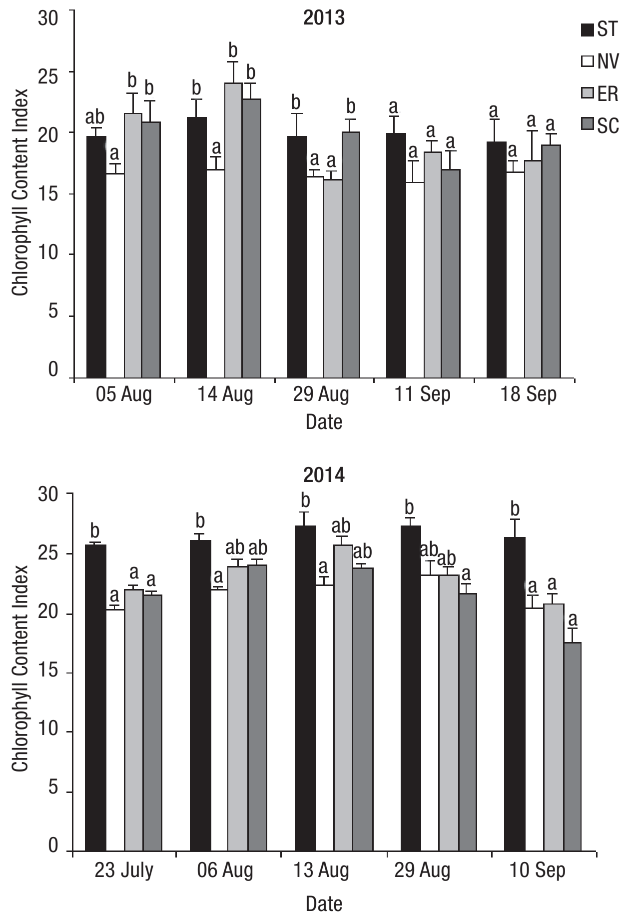

Figure 3. Effect of four soil management strategies (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) on the relative chlorophyll content index during the 2013 and 2014 growing seasons, from ripeness stage to harvest. Bars indicate the standard error of the mean. Different letters on the columns indicate significant differences ($p<0.05$) for each date.

of ETR than those from the other treatments; whereas NPQ was higher under NV and F_s was greater under ST.

In the 2013 growing season, CCI values were lower under NV except in dates near harvest, when no significant differences were observed between treatments (Fig. 3). In 2014, leaves from vines under ST showed greater CCI values than those from the rest of the treatments.

Vegetative growth, yield and crop load

Overall, vegetative growth and yield were mostly unaffected by soil management except for pruning and berry weight (Table 3). Year exerted a significant influence on pruning weight, yield, cluster number/vine and average cluster weight. The interaction between treatment and year factors exerted a significant influence on pruning weight and average cluster weight.

When each year was considered separately, the vegetative growth and yield attributes behaved differently (Table 3). Surface area was lower in SC than in ER in 2012 and lower than ST in 2014. Total leaf surface, measured in 2014 (Table 3), was significantly greater in vines under ST than those from the cover crop treatments. Pruning weight was lower in NV and ER than ST in 2014. Yield was lower in SC than in ER in 2013. The number of clusters per vine was unaffected by the soil management treatment in the three seasons considered. Cluster weight was greater in ST than in ER and SC in 2012, and higher than in NV in 2014. Finally, berry weight was greater in ST than in the other treatments the three years considered (Table 3).

Surface area values (Table 3) are within the range of those considered optimal for traditional vineyards and could ensure the optimal maturation up to 9-12 t/ha, considering a minimum of 1 m^2/kg of grape (Schneider, 1989). Our yield values ranged from 3.7 to 9 t/ha so no much differences in must composition are expected. Despite some seasonal differences in SA between treatments (Table 3), the statistical analysis, considering "year" as a factor, turned out almost no differences between treatments ($p>0.05$). SA and pruning weight are tightly related and thus, in this case, significant differences were obtained when studying the 3-year response (Table 3). Probably, the bigger sample collected for pruning weight (15 vines/treatment) respect to SA (6 vines/treatment) allowed for decreasing field variability and obtaining more consistent results. ST had the highest pruning weight while no differences arised among cover crops. Factor "Year" was also significant (Table 3) being average PW

Table 3. Surface area, pruning weight and yield components of the different soil management treatments (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) during each season. The significance of the factors (treatment and year) and their interaction is also presented.

Parameter	Year	ST	NV	ER	SC	Statistical significance	Treatment	Year	Treatment × Year
Surface area (m^2/m^2)	2012	1.03 ab	1.04 ab	1.20 b	0.98 a	*			
	2013	0.90	0.89	0.98	0.87	ns	ns	ns	ns
	2014	1.22 b	1.11 ab	1.10 ab	1.00 a	*			
Total leaf area (m^2/vine)	2014	6.52 b	5.26 a	5.23 a	4.97 a	*	*	–	–
Pruning weight (kg/vine)	2012	0.65	0.54	0.77	0.87	ns			
	2013	0.66	0.47	0.63	0.65	ns	*	*	*
	2014	1.36 b	0.87 a	0.87 a	1.06 ab	*			
Yield (kg/vine)	2012	2.55	2.17	2.37	1.95	ns			
	2013	1.43 ab	1.40 ab	1.66 b	1.08 a	*	ns	*	ns
	2014	1.97	1.52	1.68	1.85	ns			
Clusters/vine	2012	15	13	17	15	ns			
	2013	9	10	10	8	ns	ns	*	ns
	2014	18	17	16	17	ns			
Cluster weight (g)	2012	178.06 b	163.88 ab	138.44 a	125.27 a	*			
	2013	150.98	135.31	164.14	144.52	ns	ns	*	*
	2014	110.47 b	86.36 a	107.15 b	104.41 ab	*			
Berry weight (g)	2012	1.96 b	1.86 ab	1.72 a	1.69 a	*			
	2013	1.65 b	1.26 a	1.43 a	1.30 a	*	*	ns	ns
	2014	2.29 b	1.93 a	1.82 a	1.84 a	*			

Different letters in the row indicate significant differences between treatments at $p<0.05$. ns: not significant; * significant at $p<0.05$.

higher in 2014 than 2012 and 2013. Season 2014 was warmer (Table 1) with temperatures closer to the optimal range of vegetative growth. Rainfall was more than enough to fulfill vineyard and cover crop water needs and soil water availability can guarantee the water supply during the short dry period (compare rainfall and ET_0 values in Table 1).

Must and wine composition, consumers' perception

In this experiment, no significant differences were observed in any of the must attributes considered (Table 4). Alcohol content, titratable acidity, pH, tartaric acid, total phenolics index and anthocyanins

Table 4. Attributes of must quality of the different soil management treatments (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) during each season.

Attribute	Year	ST	NV	ER	SC
Total soluble solids (ºBrix)	2012	21.6	21.6	21.3	21.1
	2013	24.0	23.2	23.5	22.9
	2014	22.9	22.5	21.7	19.1
Titratable acidity (g/L tartaric acid)	2012	4.2	4.2	4.6	4.6
	2013	3.5	3.5	4.0	4.0
	2014	4.5	4.3	4.8	5.9
pH	2012	3.76	3.74	3.69	3.69
	2013	3.96	3.86	3.83	3.81
	2014	3.84	3.77	3.70	3.46
Tartaric acid (g/L)	2012	4.8	5.0	5.1	5.3
	2013	5.2	4.9	5.2	5.1
	2014	4.9	5.0	5.0	5.2
Malic acid (g/L)	2012	3.1	3.5	3.1	3.0
	2013	3.6	2.7	3.5	3.0
	2014	3.6	3.1	3.5	3.8

Table 5. Attributes of wine quality of the different soil management treatments (ST, soil tillage; NV, native vegetation; ER, English ryegrass; SC, subterranean clover) averaged for the three seasons studied.

Attribute	ST	NV	ER	SC	Statistical significance
Wine alcohol (% vol)	13.1	12.9	12.6	12.1	ns
Titratable acidity (g/L tartaric acid)	4.3	4.2	4.3	4.0	ns
Wine pH	4.39	4.31	4.24	4.25	ns
Tartaric acid (g/L)	2.3	2.4	2.3	2.3	ns
Malic acid (g/L)	1.9 b	2.0 b	1.6 ab	1.2 a	*
Total phenolics index (AU)	56.7	55.7	58.3	57.7	ns
Anthocyanins (mg/L)	569.9	570.8	605.8	561.8	ns
Colour intensity (AU)	9.5 a	10.1 ab	9.1 a	10.8 b	*
Tannins (g/L)	2.0 b	1.8 a	2.1 b	2.1 b	*

Different letters in the row indicate significant differences between treatments at $p<0.05$. ns: not significant; * significant at $p<0.05$. AU: absorbance units.

content were very similar in the wines from the four treatments studied (Table 5). Malic acid content tended to be higher in wines from the NV treatment. Colour intensity was lower in ST and ER wines. Finally, tannins content was lower in NV wines.

When data from the three years studied were pooled, correspondence analysis of consumer rankings of the wines revealed a slight preference of the tasters for the wines from the SC treatment, whereas those wines from ST were mainly ranked in the fourth place (Fig. 4). The two dimensions displayed in Figure 4 accounted for 87.7% of the total variance in the data.

Discussion

Results of this 3-year study of soil management systems indicate that cover crops can be used as a tool for reducing vegetative vigour in vineyards located in humid and sub-humid regions. The three crops used in this study presented different plant coverages throughout the growing seasons; resident vegetation and sown ryegrass covered almost 70% of inter-row surface, whereas subterranean clover only reached 45% of soil coverage. This coverage may protect soil against water erosion, which in this region can be considerable (Mirás-Avalos *et al.*, 2009); hence, cover crops should be favoured against soil tillage since they would not only protect soil against erosion but also reduce the high vegetative vigour of the vines in a humid climate (Caspari *et al.*, 1997; Wheeler *et al.*, 2005) such as the one of this region.

The decreasing pattern of the midday stem water potential along the season reflects the fall in soil water availability (Monteiro & Lopes, 2007). However, differences of vine water status among treatments (Fig. 1)

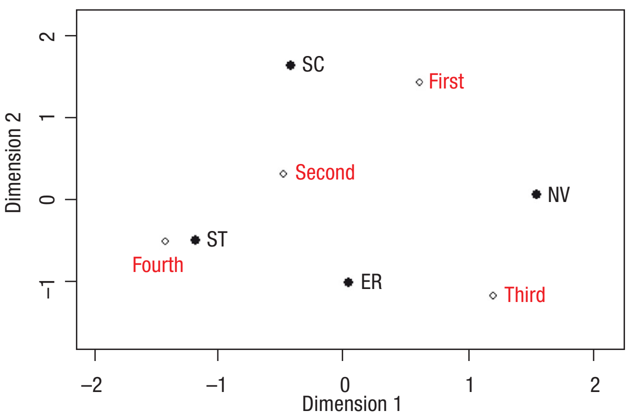

Figure 4. Plot for the correspondence analysis of the consumer ranking data of the wines from the four soil management treatments considered. Data are the average from the 2012, 2013 and 2014 wines.

were not very clear in 2012, which was the driest year but the wettest growing season, and reflected slightly higher values of midday stem water potential in vines under ST at the end of the season, due to the lack of competition from cover crops. However, in 2013 and 2014, with similar rainfall amounts, significant differences in water status were detected on several dates. Surprisingly, the less negative stem water potential values were observed in vines under the SC and ER treatments (Fig. 2), which may be due to the reduction of total leaf area that may have caused a lower plant transpiration in these treatments when compared with ST, as observed in some irrigation studies (Intrigliolo & Castel, 2010).

Despite the observed differences in water status, vine stomatal conductance was only slightly affected by the use of cover crops, indicating an adequate physiological status of the vines in each treatment (Fig. 1). Anyway, vines in the NV treatment showed lower stomatal conductance values, suggesting a greater competition between cover crop and vines in this treatment, probably due to the presence of broad-leaved species (Monteiro *et al.*, 2008) and the fact that NV and ER covered around 70% of the interrow, whereas SC only covered 45% of the interrow, approximately. Stomatal conductances measured in our study were much greater than those reported by other authors (Williams & Araujo, 2002; Intrigliolo & Castel, 2009). These results might have been caused by sufficient soil water availability and a high relative humidity in the atmosphere (higher than 45% over the study period) during our study. Apparently, no water restrictions were experienced by the vines and they had normal vegetative and transpirative behaviour, which was close to the optimal value for stomatal conductance reported by Buckley *et al.* (2014). In addition, most of the published data come from studies carried out in semi-arid or Mediterranean regions (*e.g.* de Souza *et al.*, 2003; Intrigliolo & Castel, 2009), where vines are subjected to greater water restrictions than those observed in our study; thus, we expected to obtain higher stomatal conductances. Moreover, stomatal density in grapevine leaves depends on the cultivar (Rogiers *et al.*, 2009), which might have also exerted an effect when considering the high conductance values observed. Furthermore, Williams & Trout (2005) and Teszlák *et al.* (2013) found peak values of stomatal conductance greater than 0.8 mol $H_2O/m^2/s$ in well-irrigated 'Thompson Seedless' and 'Riesling' vines (similar to those observed for Mencía in the current study). A recent study in Italy reported values greater than 1 mol $H_2O/m^2/s$ in four Sicilian grapevine cultivars (Inzerillo *et al.*, 2014). In fact, stem water potential and stomatal conductances measured in the current study are over the optima (-1.25 to -1.4 MPa and 0.12 to 0.15 mol $H_2O/m^2/s$, respectively) suggested by Romero *et al*. (2010) for Monastrell cultivar in semi-arid conditions, which might have been the reason for the lack of differences among treatments in the present study.

At the photochemical level, it was found that F_v/F_m was unaffected by soil management strategies in this cultivar, suggesting that this parameter is not an ideal indicator to evaluate the influence of cover crops on photosynthesis under field conditions. In addition, its values were close to 0.8, the threshold suggested for healthy terrestrial plants (Cavender-Bares & Bazzaz, 2004). Nevertheless, the measurements done in leaves previously exposed to sunlight demonstrated that Φ_{PSII}, ETR and PQ were significantly altered by soil management in our study. In agreement with these Chl *a* results, CCI measurements indicated a significant reduction of chlorophyll content in leaves from grapevines of the cover crop treatments. This suggests that leaf photosynthetic composition is sensitive to the competition exerted by cover crops, as it is for other stresses (Moutinho-Pereira *et al.*, 2012), confirming that this index may be useful for ecophysiological studies (van den Berg & Perkins, 2004).

Vegetative growth attributes were similar among treatments in the first two seasons of the experiment. However, in the third season (2014), total leaf area and pruning weight were reduced by the cover crops. This effect of the sward treatments on grapevine vegetative growth reduction is a consequence of the competition for water and nutrients exerted by the sown and resident plant species that constitute the cover crops, as reported by other authors (Caspari *et al.*, 1997; Wheeler *et al.*, 2005; Lopes *et al.*, 2008). In high vigor vineyards, such as the Galician ones, this effect can be beneficial to grape health and berry composition since it induces a more favourable balance between vegetative and reproductive growth, and allows a better microclimate in the cluster zone (Dokoozlian & Kliewer, 1996), which may reduce rotten harvest when there are rainfalls by end of summer and beginning of autumn.

The absence of significant effects of the cover crops on grapevine yield may be attributed to high water supply by rainfall, which was enough for fulfilling the water requirements of both the vineyard and the cover crops. However, some yield components, such as berry weight, were significantly reduced by the cover crops, in accordance with reports by other authors (Maigre & Aerny, 2001; Marques *et al.*, 2010).

Berry composition was not significantly affected by the cover crop treatments, and the values observed for total soluble solids and titratable acidity under these treatments were very similar to those of the tillage system. These observations may indicate an excess of source compared to sink demand. However, they are different from those observed in vineyards from other regions under semi-arid conditions, where sink demands were not covered by source availability (Lopes *et al.*, 2008; Marques *et al.*, 2010), proving that Galician climate conditions might favour the use of cover crops for soil management in vineyards without compromising berry quality.

Nevertheless, wines from the cover crop treatments tended to show slightly higher contents in anthocyanins and colour intensity than those from the ST treatment. This trend could be related to the lower berry sizes observed under the cover crop treatments, which increased the relative skin exposure and color extraction during maceration. However, differences in alcoholic grade and titratable acidity among wines were very low. In spite of these slight differences, consumers tended to prefer wines from the cover crop treatments (especially those from SC) over those from ST, perhaps due to a better balance in its components (alcohol/acidity ratio). This suggests that cover crops had a positive effect on wine quality, and is in agreement with previous studies (Wheeler *et al.*, 2005; Marques *et al.*, 2010). These authors reported that wines from vineyards managed through cover crop treatments were mainly preferred due to a greater varietal intensity than those from bare soil.

In summary, soil management systems using cover crops can provide a useful tool for reducing vegetative vigour in vineyards located in humid and sub-humid regions such as Galicia (NW Spain) without compromising grapevine yield, as reflected by this study. Significant reductions on vine vegetative growth on the cover crop treatments were observed, whereas no significant reductions in yield were detected. Vine physiology was slightly affected by the cover crops even though stem water potential was more positive under ST. Must composition was not affected by cover crops; however, wines from the SC treatment showed a lower concentration in malic acid and higher values of colour intensity and tannins. Moreover, wines from the cover crop treatments were preferred by consumers compared with those from ST.

These results enable us to conclude that native vegetation may be the best choice for a cover crop under the studied conditions, since it does not need to be sown and, therefore, its implementation is cheaper. However, attention must be given to the species that constitute this vegetation (which might include many broad-leaved species) in order not to obtain an excessive competition for soil water and nutrients.

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