

RESEARCH ARTICLE

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The short term influence of aboveground biomass cover crops on C sequestration and β -glucosidase in a vineyard ground under semiarid conditions

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Abstract

Tillage and semiarid Mediterranean climatic conditions accelerate soil organic matter losses in Spanish vineyards. Previous studies showed that cover crops can increase soil organic carbon (SOC) in Mediterranean vineyards. The objectives of this study were to evaluate the influence of two different cover crops in the short term on soil C sequestration in a semiarid vineyard and to study the potential use of both β -glucosidase enzymatic activity (GLU) and the GLU/SOC ratio in order to assess the SOC increase. The experiment was carried out in a cv. Tempranillo (*Vitis vinifera* L.) vineyard on a *Oxyaquic Xerorthent* soil in Rioja winegrowing region (NE, Spain). The experimental design was established in 2009 with three treatments: conventional tillage; sown barley cover crop (*Hordeum vulgare*, L.); sown Persian clover cover crop (*Trifolium resupinatum* L.). Carbon in the aboveground biomass with each cover crop was monitored. Soil was sampled in June 2011 and June 2012, and SOC, GLU and the GLU/SOC ratio were determined. After 3 years both cover crops increased SOC at soil surface with C sequestration rates of 0.47 and 1.19 t C ha⁻¹ yr⁻¹ for BV and CV respectively. GLU and GLU/SOC ratio increased in both cover crops at 0-5 cm soil depth. The C sequestration rates and GLU were related to the cover crops aboveground biomass. In consequence, in semiarid vineyards under cover crops GLU could be an appropriate indicator to assess the increase of SOC and the soil quality improvement in the short-term (2-3 years).

Additional key words: conservation agriculture; soil enzymatic activity; soil quality; vineyard soil management.

Introduction

Spain, with 1,032,000 ha, has the largest vineyard area worldwide. Its vineyards are mainly under Mediterranean semiarid conditions and, traditionally, tillage is the common management method used to prevent weeds competing with vines for soil water. Soil tillage buries residues, disrupts macroaggregates, increases aeration, stimulates microbial breakdown of soil organic matter (SOM) (Reeves, 1997) and accelerates soil organic matter decomposition and C loss from soil to the atmosphere as CO₂.

Conservation agriculture techniques such as minimum tillage, no-till and cover crops enhance soil C

sequestration (Grandy & Roberston, 2007); carbon sequestered in the SOC pool can help in the mitigation of problems due to greenhouse gas emissions (Lal, 2004).

The Appellation d'Origine Contrôlée (AOC) Rioja has a vineyard surface of 60,000 ha, and its vineyard soils are generally tilled and have a low SOM content (<1%) (Peregrina *et al.*, 2010a). Recently, the use of cover crops as a tool to avoid soil erosion (Novara *et al.*, 2011) and to reduce soil N-NO₃⁻ availability, which in excess can have a negative impact on grape juice and wine, has been studied in Mediterranean vineyards (Peregrina *et al.*, 2012; Pérez-Álvarez *et al.*, 2013). A previous study in the AOC Rioja showed

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Abbreviations used: BV (barley cover crop); CT (conventional tillage); CV (clover cover crop); GLU (β -glucosidase enzymatic activity); SOC (soil organic C); SOM (soil organic matter).

that 4 years after the establishment of a resident vegetation cover crop, SOC had increased at a rate of $1.1 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ (Peregrina *et al.*, 2010b). However, under semiarid climatic conditions, little information exists about the soil C sequestration rates for cover crop species with different biomass production capacity. On the other hand, under semiarid climatic conditions (where the SOM rate of decomposition is high and soils usually have low SOM content), soil organic C (SOC) determination alone might not be the best indicator to assess soil quality as SOC changes slowly.

Several studies have attempted to evaluate the impact of soil management practices on soil quality using microbial parameters as indicators (Schloter *et al.*, 2003). Microbial communities and their enzymes are important for nutrient cycles, soil structure stabilisation, organic waste decomposition and organic matter formation (Nannipieri *et al.*, 2002). Thus, some enzymatic activities have also been considered as early indicators of soil changes caused by different soil management practices (Paz-Ferreiro *et al.*, 2009).

β -glucosidase activity (GLU) is involved in the C cycle hydrolyzing b-D-glucoside into glucose, which is an important energy source for soil microorganisms (Eivazi & Tabatabai, 1988). Moreover, GLU is sensitive to different soil management practices in a semiarid vineyard (Peregrina *et al.*, 2014) and it is related to CO_2 respiration under Mediterranean climatic conditions (Mariscal-Sancho *et al.*, 2010). In addition, Stot *et al.* (2010) found, in a compilation of different soils, that the higher GLU to SOC ratios occurred in soils where the recently altered management was characterized by an increase of the SOC content. These findings show, in consequence, that the GLU to SOC ratio could be sensitive to the C sequestration under cover crops in semiarid vineyards. Therefore, under these climatic conditions, the determination of biochemical properties such as GLU, which is closely related to SOM transformations and nutrient cycling, may be interesting in order to reveal how soil management affects soil quality.

The aim of the study was to evaluate, in the short term (2 and 3 years), the relationship between the soil C sequestration rates and the aboveground biomass under two different cover crops, one gramineous (barley) and another leguminous (Persian clover) in a semiarid vineyard; as well as to evaluate the GLU and the GLU to SOC ratio, as indicators of both the soil C

sequestration and the soil quality improvement under cover crops.

Material and methods

Site description, experimental layout and treatments

The experiment was set up in 2009 in a vineyard plot located on a terrace of the Najerilla river in the municipality of Nájera (La Rioja, Spain) (Latitude $42^\circ 26' 34'' \text{N}$; Longitude $2^\circ 43' 32'' \text{W}$). The field slope was about 0.2% facing west. The soil was classified as *Oxyaquic Xerorthent* (Soil Survey Staff, 2006) and contained 185 g kg^{-1} clay, 432 g kg^{-1} silt, and 383 g kg^{-1} sand, 15% coarse elements ($>2 \text{ mm}$), 12.4 g kg^{-1} organic matter, 42 g kg^{-1} carbonates, with pH 8.50, electrical conductivity 0.11 dS m^{-1} (25°C) at the Ap horizon (0–20 cm). The climate in the area is semiarid according to the UNESCO aridity index (UNESCO, 1979).

The vineyard had been planted in 1999 with *Vitis vinifera* L., cv. Tempranillo, grafted on 110-Ritcher rootstock. Vine and row spacing were 1.3 m and 2.7 m, respectively, rows had a west-east orientation and the planting density was $2,850 \text{ plants ha}^{-1}$. Vines were trained to a vertical shoot position trellis system on a double cordon Royat with twelve buds per vine. All water shoots were removed in the spring.

The experimental design was a randomized complete block with three treatments and three replications per treatment. Each replicate (plot) had four rows with 20 vines in each. The soil treatments were: CT, conventional tillage between rows; BV, cover crop of barley *Hordeum vulgare* L. cv. "Naturel" without any tillage; CV, cover crop of Persian clover (*Trifolium resupinatum* L.) without any tillage. Both BV and CV cover crops were sown (*i.e.* $50 \text{ kg seed ha}^{-1}$) with a rotary seed drill in February 2009, and they were re-sown in February 2011 and February 2012. In general cover crops were not mowed, but CV was mowed in April 2010 to avoid frost damage to the grapevine buds.

The treatments had not been N-fertilised since 2000. In the CT treatment, soil tillage was carried out approximately once every 4 to 6 weeks, at 0–15 cm depth. In all the treatments a 0.8 m-wide strip under the vines was kept using herbicide thus allowing a cover crop width of 1.7 m.

Aboveground biomass sampling and C determination

The cover crop biomass and the C content that it had added to the soil were estimated using its aboveground biomass. In June 2009, June 2010, and June 2011 when both cover crops had reached the maximum biomass development and in April 2010 prior to the clover cover crop was mowed: Plant material was cut at soil level and, once in the laboratory, was washed with tap water, then rinsed with distilled water and lastly dried at 60°C for 72 hours. The dry biomass was weighed and then ground through a 0.5 mm sieve with an ultracentrifugal mill (Retsch ZM1). Carbon concentration in the biomass was determined by combustion analysis with a CNS analyzer elemental (TruSpec CN LECO Inc.). We considered that the C supplied by aboveground biomass from cover crops previously to June 2011 corresponding to the sum of C biomass from June 2009, April 2010 and June 2010, and the C supplied previously to June 2012 corresponding to the sum of C biomass from samplings of June 2009, April 2010, June 2010 and June 2011.

Soil sampling procedure

Soil sampling was carried out in June 2011 and June 2012 at 0-5, 5-15, and 15-30 cm depth. In each plot, six soil cores were taken to make a composite sample representative of each plot and depth. At the 0-5 cm depth, soil cores were taken with stainless steel cylinders (height 51 mm, diameter 50 mm, volume 100 cm³). These cylinders were used to collect undisturbed soil samples for bulk density determination by the core method (Grossman & Reinsch, 2002). For both 5-15 and 15-30 cm soil depths, a composite sample of six subsamples was collected with an Edelman type auger.

Soil sample preparation and laboratory analyses

Once in the laboratory, the 0-5 cm soil cores were weighed for soil bulk density determination dry at 105°C. Visible plant residues were removed from the soil samples and then the samples, at field moist, were ground and sieved (2 mm), the percentage of soil >2 mm was determined and the soil <2 mm divided into two subsamples. One was immediately stored at

4°C and analyzed within 4 weeks. The other was air-dried for chemical analysis.

Soil organic carbon (SOC) was determined by the Walkley and Black wet oxidation method of (Nelson & Sommers, 1982). SOC concentrations in the 0-5 cm depth were expressed as a mass per area unit by calculating the product of SOC concentration in the 0- to 5-cm soil depth, the bulk dry density, the thickness, and the percentage of soil <2 mm.

The method used to determine measure β -glucosidase was based on the colorimetric determination of saligenin released by β -glucosidase where 5 g of soil were incubated for 3 hours at 37°C with an acetate buffer (pH 6.2), and salicin (β -glucosido-saligenin) (Tabatabai, 1994). For each analysis of enzymatic activity, two subsamples and one control were carried out. Results were reported on an oven-dry-weight basis, determined by drying the soils for 24 h at 105°C.

Statistical analysis

Treatment effects on measured variables were tested using ANOVA (univariate linear model), and comparisons between treatment means were made using the least significant difference (LSD) multiple range test calculated at $p < 0.05$. Pearson correlations coefficients were used to assess the relationships between the studied soil parameters and the C biomass supplied by the cover crops. Statistical procedures were carried out with the software program Statgraphics Plus for Windows (1998).

Results

The C input to the soil from aboveground biomass of cover crops, before June 2011 and June 2012, was higher in CV than BV (Table 1).

At 0-5 cm soil depth, CV have greater SOC content than CT and BV) two and three years after cover crop establishment; and, at the same soil depth in June 2012, three years after cover crop establishment, BV had a higher SOC than CT. Moreover, in June 2012, CV had increased SOC with respect to CT and BV at 5-15 cm soil depth (Fig. 1).

Soil organic C content for the 0-5 cm layer was converted from g kg⁻¹ to Mg ha⁻¹ making use of the soil bulk density, its thickness and the percentage of soil particles smaller than 2 mm; values shown in Table 2.

Table 1. Carbon content of barley (BV) and Persian clover (CV) aboveground biomass before soil samplings

Cover crop treatment	C in aboveground biomass supplies to the soil (Mg C ha ⁻¹)	
	June 2011	June 2012
BV	0.87b	1.52b
CV	2.42a	3.12a
95% LSD	1.01	1.19

Means in a column followed by the same letter are not significantly different at $p < 0.05$.

When comparing the measurements of the soil C stock for the 0-5 cm soil depth in the different treatments expressed as mass per area unit, CV increased the soil C stock with respect to CT two years after the cover crop establishment and both cover crops CV and BT had higher values than CT after three years (Table 2).

Regarding to the C sequestration ratios respect to CT, those was higher in CV than BV in June 2012 (Table 2). Moreover, C sequestration ratios tend to increase in June 2012 respect to June 2011 in both cover crops treatments. In addition, in June 2011 and June 2012 soil samplings, we observed a strong relationship between the SOC stock at 0-5 cm soil depth and the aboveground biomass (Fig. 2).

With respect to the microbiologic properties, Fig. 1 shows that at both 0-5 cm and 5-15 cm soil depths: (i) β -glucosidase activity was higher for CV than CT and BV; and (ii) BV showed higher GLU values than CT.

Our results showed a strong relationship between GLU at 0-5 cm soil depth and C content in the aboveground biomass of each cover crop (Fig. 2).

With respect to the GLU/SOC ratio, it was higher in CV than CT at both 0-5 cm and 5-15 cm soil depths for the two years sampled. Also, BV had a higher GLU to SOC ratio with respect to CT in the 0-5 cm soil depth for the years 2011 and 2012, and in the 5-15 cm soil depth in the year 2012 (Fig. 1).

Discussion

The higher C input to the soil surface from aboveground biomass of CV than BV is consequence of the higher production of aboveground biomass in CV respect to BV and this can be explained by two factors: 1) in our field conditions, clover withered later (early-

August) than barley (late-June); and 2) with no N fertilization, clover fixes N from the atmosphere and therefore has higher N availability than barley and, consequently, higher development.

Respect to the increase of SOC under both CV and BV cover crops, higher SOC at the soil surface has also been observed in vineyards with cover crops under Mediterranean climates in Spain (Peregrina *et al.*, 2010b, 2012) and France (Celette *et al.*, 2009) as well as in California (Smith *et al.*, 2008; Steenwerth & Belina, 2008).

Cover crop practices reduce the soil disturbance and leave a higher quantity of plant residues on the soil surface. The reduced contact of the plant residues with the soil reduce the interactions with soil heterotrophic microorganisms leading to an accumulation of SOC in the upper soil layer (Woods, 1989; Reicosky *et al.*, 1995). Thus, the use of a cover crop of barley or Persian clover may be an effective strategy to increase SOC and, in consequence, enhance soil quality.

The SOC sequestration rates (*i.e.* the SOC increment with respect to CT divided by the number of years with cover crop) for CV after three years were higher than those reported in an experiment with NT-cereal systems in a semiarid area of Spain, where the SOC sequestration rates were 0.50 ± 0.16 Mg C ha⁻¹ yr⁻¹ after five years (Hernández *et al.*, 2009), our rates were also higher to data compiled by Franzluebbbers (2005) from publications comparing CT to NT in South-eastern USA offering sequestration rates of 0.42 ± 0.46 Mg C ha⁻¹ yr⁻¹.

However, SOC sequestration rates for CV in our experiment were similar to those after 5 years of grass establishment, with a rate of 1.40 Mg C ha⁻¹ yr⁻¹ (Franzluebbbers *et al.*, 2001). Using a total of 12 comparisons, Franzluebbbers (2005) reported that the average rate of SOC sequestration with grass was 2.6 times higher than non-tilled crops in south-eastern USA. Furthermore, the C sequestration rates for CV were similar to these reported by Peregrina *et al.* (2010b) under resident vegetation after 5 years in semiarid conditions; in contrast, the C sequestration rates for BV were lower.

After three years, the higher C sequestration rate in CV than in BV could be due to that transformation rate from C biomass to soil organic C would be similar under both cover crops. Therefore the principal factor that affected to the C sequestration rate would be the C input to the soil. In addition, the tend to increase the C sequestration ratios after three years with cover crops

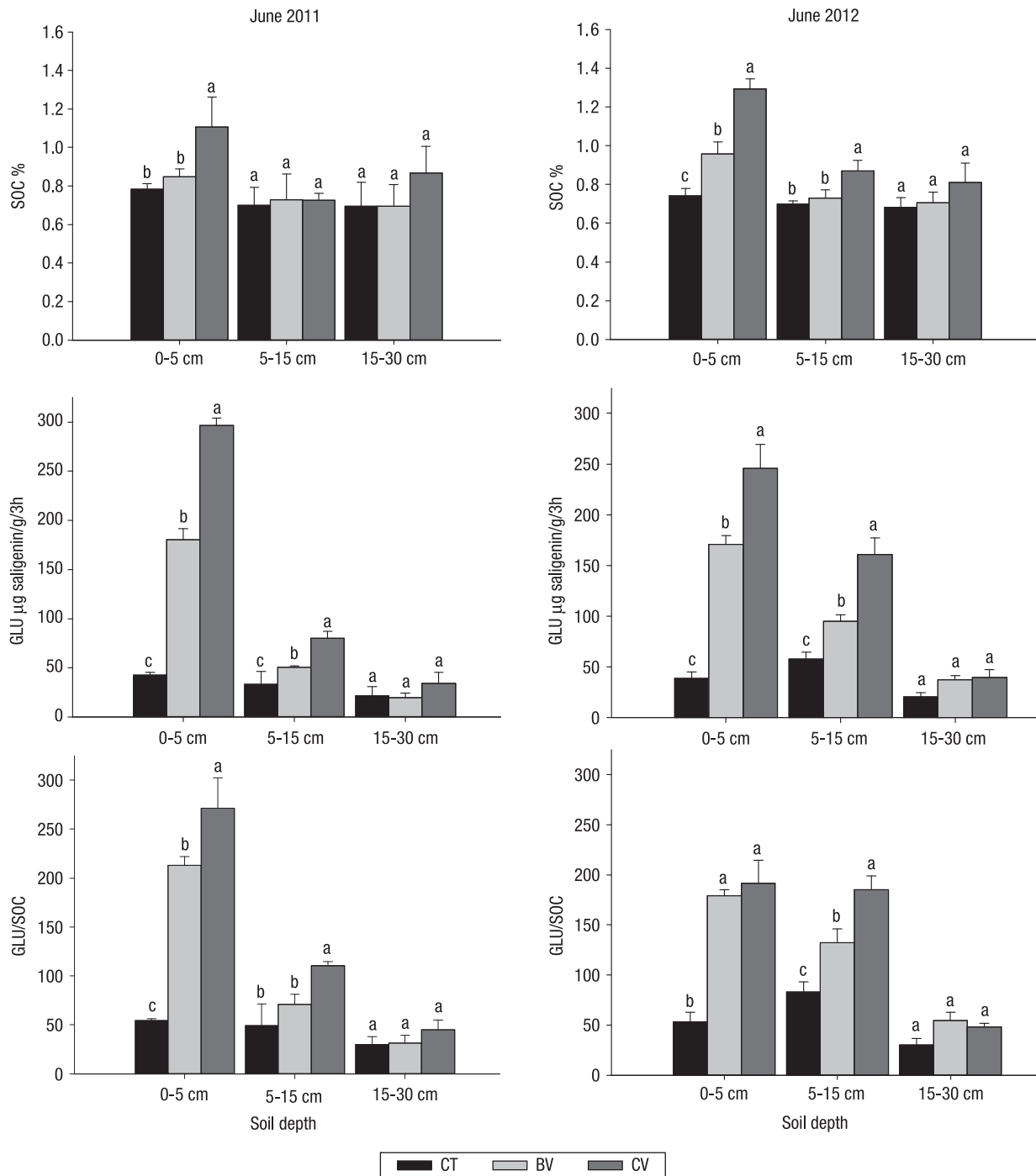


Figure 1. Soil organic carbon (SOC), β -glucosidase enzymatic activity (GLU), β -glucosidase activity to soil organic carbon ratio (GLU/SOC) values at different soil depths of each treatment in June of 2011 and June 2012: conventional tillage (CT), barley cover crop (BV) and Persian clover cover crop (CV). The letters represent the comparisons by LSD test ($p < 0.05$) among treatments within each depth. Bars represent standard error ($n = 3$).

would indicate that the steady state of C sequestration would be not reached after three years under cover crops. This is according with Peregrina *et al.* (2010c),

who found that the C sequestration did not reach the steady state after 5 years under cover crops in a semiarid vineyard.

Table 2. Bulk density, whole soil organic carbon content, in conventional tillage (CT), in barley cover crop (BV), and in Persian clover cover crop (CV), and C sequestration rate in comparison to CT, at 0–5 cm soil depth after 2 years (June 2012) and 3 years (June 2013) of the cover crop establishment

Soil management	Bulk density (g cm ⁻³)	C content per unit area (Mg ha ⁻¹)	C sequestration rate respect to CT (Mg ha ⁻¹ yr ⁻¹)
June 2012			
CT	1.50a	4.96b	
BV	1.51a	5.40b	0.22a ± 0.08 [†]
CV	1.50a	7.05a	1.03a ± 0.56
95% LSD	0.03	1.21	0.91
June 2013			
CT	1.50a	4.76c	
BV	1.51a	6.14b	0.47b ± 0.25
CV	1.51a	8.30a	1.19a ± 0.11
95% LSD	0.04	1.15	0.44

Means in a column followed by the same letter are not significantly different at $p < 0.05$. [†] Standard deviation

Therefore, in the short term (2 and 3 years), the C sequestration rates were substantially influenced by different biomass amounts supplied to the soil by each cover crop. This effect could explain, therefore, the lower C sequestration rates of a barley cover crop with respect to a resident vegetation cover crop (Peregrina *et al.*, 2010b) or a grass establishment (Franzluebbers *et al.*, 2001).

However, Franzluebbers (2005) reported that the SOC sequestration rates were relatively unaffected by the quantity of residue and that the main cause of SOC accumulation under a no tillage system was the reduced contact between the plant residue and the soil. The reason for this disparity could be that the results shown by Franzluebbers (2005) were for a long-term land use, where SOC could have reached the steady state of SOC sequestration.

Respect to GLU, our results agreed with the increase in GLU found at the soil surface under cover crops in a vineyard (Virto *et al.*, 2012), a rainfed olive grove (Moreno *et al.*, 2009) and an almond orchard (Ramos *et al.*, 2011), in all cases under Mediterranean semiarid climatic conditions.

As the GLU reflects the ability of soil microorganisms to decompose cellulose, the cellulose present in the aboveground biomass would explain the increase in GLU at soil surface and the relationship between

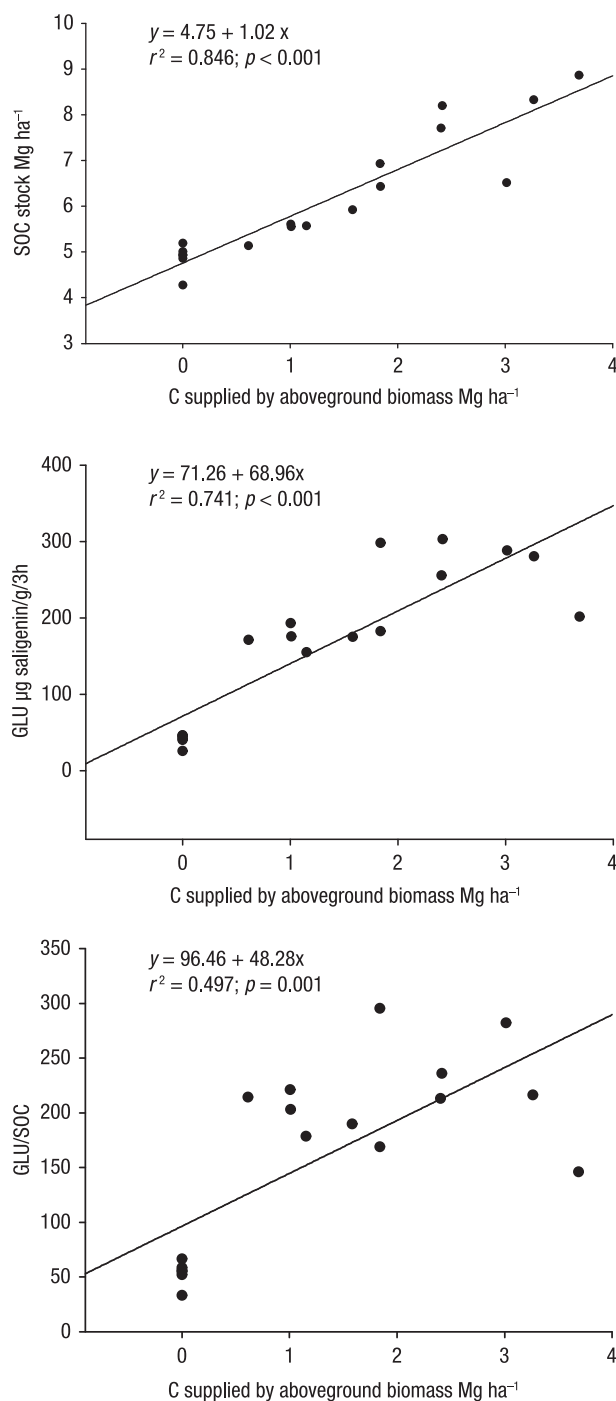


Figure 2. Linear correlations between soil organic C (SOC), β -glucosidase activity (GLU), and β -glucosidase activity to soil organic C ratio (GLU/SOC) at 0–5 cm soil depth with C from aboveground biomass ($n = 18$).

the GLU and the aboveground biomass supplied by the cover crops. In addition, at 5–15 cm soil depth, the GLU increase under both cover crops could be due to

the biomass supplied by the cover crop roots. In consequence, the higher GLU values for CV with respect to CT could be used as indicators of the higher SOC content for CV at 5-15 cm soil depth.

β -glucosidase is one of the most important glycosidases in soils since it catalyzes the hydrolysis of carbohydrates with β -D-glucosidase-bonds, such as cellobiose. As a result, this enzyme contributes to the mineralization of cellulose, the main organic C compound in nature. It also shows the state of decomposition of organic matter (Knight & Dick, 2004). Therefore, according to our results cover crops can enhance soil quality, as shown by the increase in GLU which indicates a more active C cycle with a higher amount of derivatives from cellulose, such as glucose, which can support diverse microbial populations.

Further, the values of GLU at soil surface under BV and CV were respectively 3 and 6 times higher than under CT, although SOC under BV and CV were respectively 1.2 and 1.7 times higher than CT. This showed that GLU is quantitatively more sensitive to the variation of aboveground biomass than the SOC increases measured using the oxidation method.

The effect of cover crops on GLU/SOC ratio are in accordance with Stott *et al.* (2010), who showed higher GLU/SOC ratios in soils with management systems that increased SOC content (for instance no-till soils or soils being converted to pasture) than in soils with management types characterized by SOC losses. The higher GLU/SOC ratios under both BV and CV cover crops would indicate that the new SOC incorporated into soil has a higher content of cellulosic substrates than the SOC present under CT.

In June 2011, BV cover crop did not increase SOC with respect to CT at soil surface, whereas GLU and GLU/SOC increased in BV with respect to CT. This result was similar to that obtained under CV and it would indicate that under BV, the supplies of cover crop biomass increase SOC, but to a lesser extent than CV due to the lower amount of biomass supplied.

The GLU to SOC ratio is linked to the C biomass supplied by the cover crop, as is the case of GLU, but the ratio has a lower correlation coefficient than GLU (Fig. 2). In consequence, GLU was quantitatively more sensitive than GLU/SOC to SOC changes but, in spite of this GLU/SOC showed, from a qualitative point of view, that under both cover crops conditions for the increase of SOC existed.

In summary, in the short term (3 years) in a semiarid vineyard, both barley and Persian clover

cover crops can increase soil organic C (SOC) at the soil surface (0-5 cm soil depth) with C sequestration rates of 0.47 and 1.19 Mg C ha⁻¹ yr⁻¹ respectively. These C sequestration rates under cover crop were related to the different aboveground biomass supplied to the soil from each cover crop. Thus, the species chosen for the cover crop can affect the C sequestration capacity at the short term (3 years). Therefore the vine growers must select for the cover crop those plant species that have the higher vegetative development in the climatic and edaphic conditions of his vineyard. The higher β -glucosidase enzymatic activity (GLU) under both cover crops indicated a soil quality improvement with respect to conventional tillage in the short term. Moreover, GLU was a more sensitive indicator than SOC for both the biomass supplied by the cover crop and the SOC increase under cover crops. GLU/SOC ratio also was a good qualitative indicator of the SOC increase under cover crops, but with a weaker relationship to C biomass than the GLU.

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