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Abstract

Vineyard soils are typically characterised by poor development, low organic matter content, steep slopes, and consequently a limited capacity of conservation of the organic matter which is weakly bound to the mineral soil phase. In such conditions, permanent grass may help the conservation of the soil quality. The aim of this work was to evaluate the effects of permanent grass vs. single autumn tillage on soil structure and organic matter dynamics in a hilly vineyard. In the periods 1994-1996 and 2010-2012 soil samples were collected three times per year, in different seasons. Aggregate stability analyses and organic matter fractionation were performed. The effects of grass cover on soil recovery capacity after tillage disturbance were slow. Slight increases in aggregate resistance and organic matter contents were visible after three years, and only after long-lasting permanent grass the two plots (permanent grass/previously tilled) showed great decrease of aggregate losses and increase of organic matter. Even a single tillage produced however an immediate decrease in aggregate resistance, while the amounts of organic matter remained unaffected. Organic matter, however, showed marked seasonal dynamics, which involved not only recently added organic matter fractions but also the mineral-associated pool. Tillage altered organic matter dynamics by preventing the addition of new material into the mineral-associated organic fractions and limiting the stabilization of aggregates.

Keywords: organic matter fractions, porosity, structure, Entisol.

INTRODUCTION

Soils carry out a large number of functions such as primary production and biodiversity conservation, as described by Berendse et al. (2015), Brevik et al. (2015), De Graaf et al. (2015). They also control the hydrological and biogeochemical cycles, acting as a filter for groundwater (Keesstra et al. 2012), storing organic matter and nutrients (e.g. Batjes et al. 2014; Jaiarree et al. 2014; Köchy et al. 2015 a b), protecting land from hydrogeological hazards such as water erosion.Soil organic matter (SOM) protection and soil structure are key factors for soil conservation and sustainable land management (e.g. Cerdà et al. 1996, Wick et al. 2014), and for carbon sequestration and fertility (Gosling et al. 2013 and references within). They are mutually related: aggregates provide protection of SOM against microbial decomposition by occlusion, i.e. physical protection, and by association of organic matter to clay and silt fractions, i.e. physico-chemical protection (Tisdall and Oades 1982; Golchin et al. 1994; 1995; Jagadamma and Lal 2010; Vogel et al. 2015). In turn, SOM bound to mineral particles leads to the formation of stable aggregates that are thus fundamental to reduce C turnover. Aggregate complexity typically increases with soil evolution, therefore poorly developed soils, such as Entisols, retain several characteristics of the parent material in aggregate hierarchy (Falsone et al. 2012). The role of SOM in the aggregation processes is then even more important.

The importance of SOM management and conservation, and the effects of land use on SOM and soil structure have been studied by several authors in a variety of environments (e.g. Munoz-Rojas et al. 2012; De Moraes Sá et al. 2013; Gelaw et al. 2013; Parras-Alcántara et al. 2013, 2014; Srinivasarao et al. 2014; Yu and Jia 2014; Behera et al. 2015; Peng 2015). Agriculture can strongly limit SOM accumulation, influencing soil aggregate formation and turnover (Jastrow 1996; Cerdà, 2000; Jacobs et al. 2010; Gosling et al. 2013). In particular, tillage can increase the turnover of macroaggregates (Six et al. 2000), consequently limiting the physical protection of SOM, hence the formation of microaggregates inside macroaggregates. Panettieri et al. (2015) confirmed that in agricultural soils the formation of organo-mineral interactions can provide for an effective protection of SOM, while Comeau et al. (2013) highlighted the important contribution of fresh organic matter inputs like crop residues to stabilized SOM. Minimum-till, no-till and cover crops can enhance aggregation and promote SOM protection (Bronick and Lal 2005).

Vineyard soils are often highly sensitive to SOM loss and topsoil degradation due to their intrinsic properties such as limited soil development, coarse texture and low capacity to protect SOM by binding to soil minerals (Le Bissonnais et al. 2007; Martinez-Casasnovas and Ramos 2009; Tarolli et al. 2015). These soils often show different properties with respect to other agricultural soils, e.g limited SOM contents, hilly morphology and sloping topography (Ramos and

Martinez-Casasnovas 2004; Novara et al. 2011). These characteristics make vineyard soils more susceptible to erosion, thus improper management may result in permanent soil degradation (Ruiz-Colmenero et al. 2013; Novara et al. 2013; Lieskovský et al. 2014).

Most improvements in soil management in viticulture currently aim at enhancing SOM contents and aggregation by increasing C inputs (Bustamante et al. 2011; Guerra and Steenwerth 2012), whereas practices that limit soil disturbance and mitigate C loss are marginally taken into account. Besides favouring soil erosion, traditional tillage has been reported to be unsustainable in terms of SOM and nutrient depletion (Ruiz-Colmenero et al. 2013). Conversely, the combination of no-tillage and permanent grass cover in the inter-row may represent an optimal solution for improving aggregation and SOM protection, that is also feasible as the competition between the herbaceous cover and grapevines often does not reduce yields even in rain-fed Mediterranean environments (Marques et al. 2010; Agnelli et al. 2014; Mercenaro et al. 2014). Grasses contribute to organic matter input while no-tillage reduces soil macroaggregate turnover, which is crucial for C sequestration in stable microaggregates. For example, Marques et al. (2010) and Ruiz-Colmenero et al. (2013) observed lower soil losses in cover cropped rows than in tilled rows in Spanish vineyards. Despite the general consensus on the positive role of organic matter inputs and reduced tillage in vineyards (Goulet et al. 2004; Steenwerth and Belina 2008; Peregrina et al. 2012; Simansky et al. 2013), only a few works have related these practices to aggregation and SOM dynamics and evaluated the time scale of soil quality improvement or decline. Moreover, in most experiments permanent grass has been compared to situations where tillage was carried out several times per year resulting in strong structure degradation. To mitigate the negative effects of tillage, while avoiding excessive grass competition with vines, vineyard soil management may involve a single tillage operation in the inter-row per year.

The aim of this work was to evaluate the effects of permanent grass vs. single autumn tillage on soil structure and SOM dynamics in a calcareous vineyard soil in hilly environment. These soils show common characteristics in important Italian viticultural areas: they are poorly developed and highly susceptible to erosion. We hypothesized that the characteristics of these soils make them particularly sensitive to even minor soil management practices with important consequences on aggregation and organic matter dynamics even in the short term. To test this hypothesis, a first comparison between a single tillage disturbance and permanent grass cover was carried out for 3 years during the ‘90s. Then, grass was allowed to cover the whole vineyard for 13 years and, in 2009, tillage disturbance was reintroduced and the comparison repeated. Our specific objectives were (i) to evaluate the effects of short and medium-to-long term inter-row grass cover on aggregate stability and organic matter dynamics; (ii) to assess the sensitivity of the soil to the reintroduction of a single tillage after 13 years of permanent grass; (iii) to study the mechanisms relating organic matter turnover and aggregate stability through soil porosity evaluation and physical fractionation of SOM.

MATERIALS AND METHODS

Study site, experimental setup and soil sampling

The study site is located in a hilly area of NW-Italy at the experimental farm “Tenuta Cannona” (44°40′59.35″N, 8°37′36.85″E, total farm surface 54 ha, Figure 1), close to the Ligurian Appennine, where a Mediterranean influence in the thermal and rainfall regime is present. The average air temperature is 13°C and the average annual rainfall is 850 mm (30 years record from 1967 to 1997; Cagnazzi and Marchisio, 1998). In 2010 an automatic weather station was installed to monitor rainfall and temperature data (Figure 2); average (2010-2012) rainfall and temperature were 900 mm and 13°C, quite similar therefore to the earlier records.

The vineyard selected for this study (0.71 ha) is rain fed. It was deep tilled (0.5-0.6 m) in 1988 and planted with *Vitis vinifera* L. cv. Chardonnay, grafted on Kober 5BB rootstock (5000 vines ha^{-1}) with 0.80 m between vines and 2.50 m between rows. The vines were vertically shoot positioned trained and pruned according to the Guyot system. The slope of the vineyard is homogeneous (14%) with south-east aspect, and rows are oriented downslope (NW-SE). The soil (Table 1) develops on marls and is a homogeneously distributed Typic Ustorthent, fine-loamy, mixed, calcareous, mesic (Soil Survey Staff 2014), as confirmed by several control pits made during field survey. Between 1989 and 1992 the whole vineyard was managed with a rototiller (0.15 m, 3 times per year) and in 1993 it was subdivided into six experimental plots to evaluate the effect of grass cover. The first experiment was carried out for 3 years (1994-1996, Experiment I) and aimed at the comparison between permanent grass and a single autumn tillage. From 1997 to 2009 the whole vineyard was managed with permanent grass, allowing the development of the autochthonous seed bank and natural cover. In autumn 2009 a second experiment (Experiment II), aimed at evaluating the effect of tillage reintroduction (i.e. permanent grass vs. single autumn tillage), was carried out, and is still going on to date (Figure 1).

The experimental design (Figure 1) thus included two treatments: permanent grass (PG) and single autumn tillage (AT), with three replicate plots (a, b, c) per treatment. Each plot consisted of an inter-row 60 m long and 2.5 m wide (75 vines). AT and PG treatments were separated by one non-treated buffer row.

In the PG the soil surface was covered by autochthonous grasses, dominated by Poaceae (40%) and Fabaceae (20%), including *Avena fatua* L., *Bromus spp*., *Cynodon dactylon* L., *Festuca* *spp.*, *Hordeum spp.*, *Lathyrus spp.*, *Lolium multiflorum* Lam., *Lolium perenne* L., *Lotus corniculatus* L., *Medicago spp.*, *Melilotus officinalis* L., *Poa spp.*, *Trifolium repens* L., *Trifolium pratense* L., *Vicia sativa* L.. The rows were mowed twice a year (May, July) at an approximate height of 10 cm and the residues left on the soil, which was therefore permanently covered by vegetation and grass residues.

In AT the rows were tilled in November with a rototiller to 10-15 cm, and mowed twice a year (May, July) as in PG. Thus the soil was uncovered after tillage but resident vegetation was allowed to grow till April and covered the soil until the next autumn tillage.

In both treatments no fertilizers were supplied. The strip beneath vines (0.3 m each side of the row) was treated with herbicides (post-emergence glyphosate, applied manually on a limited surface). All other practices were similar for the two treatments: disease and pest control, and canopy management were performed mechanically, whereas pruning, shoot positioning, leaf removal and harvesting were done manually.

In experiment I and II soil samples were collected three times per year, in spring (April), summer (July) and autumn prior to tillage (November). At each sampling time, one composite topsoil sample (0-5 cm depth, i.e. the most prone to degradation) was collected from each of the 6 rows (Figure 1), always from the center of the row to avoid soil disturbance by machinery. Each composite sample was made of 3 sub-samples, taken from the top, medium and bottom part of the row, then mixed. The resulting sample number at each sampling time was therefore 6. All samples were air-dried and sieved to <2 mm.

Aggregate stability

An aliquot of each soil sample (<2 mm) was dry sieved to obtain the 1-2 mm aggregate fraction required for evaluating the wet aggregate stability. Ten grams of these aggregates were placed in a 0.2 mm sieve and allowed to rotate at 60 rpm in beakers containing deionized water for sieving times of 5, 10, 15, 20, 40, and 60 minutes. After sieving, the remaining material >0.2 mm (i.e. aggregates and coarse sand) was oven dried and weighed. The coarse sand fraction remaining in the sieve was determined after treatment with H_2O_2 (Gee and Bauder 1986), and subsequently the loss of aggregates was determined as follows (Kemper and Rosenau 1986):

$$\mathbf{Aggregate\ loss\ (AL, \%) = 100 - \frac{100(weight\ retained - weight\ of\ coarse\ sand)}{total\ sample\ weight - weight\ of\ coarse\ sand}} \quad [\text{Eq.1}]$$

The aggregate breakdown kinetics was fitted with the exponential model by Zanini et al. (1998):

$$\mathbf{y(t) = a + b(1 - e^{\frac{-t}{c}})} \qquad \text{[Eq. 2]}$$

where *y* is the aggregate loss in %, *t* is the sieving time (min), *a* the aggregate breakdown by water saturation (g 100 g^{-1}), i.e. the initial loss, *b* the maximum estimated abrasion loss (g 100 g^{-1}), and *c* (min) is a parameter that links the rate of aggregate breakdown to wet sieving time. The total aggregate loss can be computed as (a+b) (e.g. Stanchi et al. 2015).

Organic carbon and nitrogen content and density fractionation of SOM

Organic C content (OC) was determined by dry combustion (NA2100, CE Instruments, Rodano, Italy) after pre-treatment with HCl for the removal of inorganic C (Harris et al. 2001). The total N content was determined by combustion method, too. The density fractionation of SOM was performed only on the samples collected in 2011, following Cerli et al. (2012). Briefly, 125 ml of Na-polytungstate (NaPT) solution with density 1.6 g cm^{-3} was added to 25 g of soil. The suspension was then gently hand shaken to ensure complete soil wetting, while avoiding aggregate disruption, and allowed to settle for 1 h. After centrifugation at 5600 g for 20 min, the light fraction (free particulate organic matter, *f*POM) was collected on a 0.7-µm glass microfibre filter, rinsed with deionized water, air dried and finely ground. The remaining soil was resuspended in fresh NaPT solution and treated ultrasonically with 275 J ml^{-1} using a probe-type sonicator (Sonoplus HD 2200, Bandelin electronic GmbH & Co. KG, Berlin, Germany). After centrifugation at 5600 g for 20 min, the light fraction released from aggregate breakdown (occluded particulate organic matter, *o*POM) was separated, washed, dried and finely ground as described above. Preliminary tests were performed to select the appropriate sonication energy to release the whole pool of *o*POM, while avoiding contamination by minerals and release of organic-mineral complexes (Cerli et al. 2012). The remaining soil (mineral-associated organic matter, MOM) was washed until free from salts with deionized water, dried and ground. All fractions were characterized for their mass yields as well as organic C and N contents as described above.

Aggregate porosity

Soil porosity was evaluated by Hg-intrusion (Porosimeter 200 WS equipped with a Macropore unit 120, CE Instruments, Rodano, Italy) on the 1-2 mm aggregate fraction. Analysis was performed by step-increasing the pressure (up to 200 MPa) and measuring the volume of intruded Hg at each step. Total porosity was subdivided into three pore size classes according to Brewer (1964): 30-5 µm, 5-0.1 µm and < 0.1 µm. From Hg-intrusion data, the density of the aggregates was also calculated. Additionally, to better interpret the data, we also followed the approach proposed by Bruand and Prost (1987), and recently applied to poorly developed soils by Falsone et al. (2012). This method allowed for the determination of the most represented pore class (i.e. the modal pore class), from the curve of intruded-Hg volume as a function of the pore radius. The slope of the curve is calculated as:

$$\boldsymbol{slope_{ij}} = \frac{\boldsymbol{Vi - Vj}}{\boldsymbol{\log_{10} Rj - \log_{10} Ri}} \qquad \text{[Eq. 3]}$$

where the volumes *Vi* and *Vj* correspond to the radii *Ri* and *Rj* at two successive positions *i* and *j*. The relative maxima of the slope curve indicated the most represented classes of pores. Each peak thus corresponded to a radius that was taken directly from the graph. This radius was termed modal radius (µm) and the volume of each modal class of pores was, thus, included between two minimum values of the slope, which defined the limits of the class.

Statistical analyses

The analysis of variance (ANOVA), using treatments as factor variable, was carried out for soil properties. In Experiment II a two-way ANOVA was performed to evaluate the effects of treatment, season and their interaction. Differences were tested against a Fischer's distribution. The correlation between variables was evaluated using the Pearson's coefficient (two-tailed), after visual inspection of the data to verify that the dependence relationship was linear. All statistical analyses were performed using SPSS 20.

RESULTS

Experiment I: introduction of permanent grass cover

At the beginning of experiment I, the contents of OC were extremely low (5.0±1.7 g kg^{-1}) (Table 1) without any difference between treatments and remained rather low in 1994 and 1995 (Figure 3A). In 1996, the average OC contents in PG were significantly higher than in AT (11.8 and 5.0 g kg^{-1} respectively; Figure 3A, left), although the variability in the former was relatively high. Aggregates were rather unstable (Figure 3B, left) with total losses always exceeding 90%. However, significant differences between treatments were already visible in 1994, as PG showed a lower total aggregate loss than AT (92.2±5.0 and 98.0±2.1%, respectively). During experiment I the total aggregate loss remained almost constant in AT, while it was more variable in PG (Figure 3B, left). A significant negative correlation between total aggregate losses and OC content was present in PG, although rather weak ($r = -0.484$, $p<0.01$). Although aggregate breakdown was caused by both initial losses and abrasion (i.e., *a* and *b* coefficients in Eq. 2), in PG we observed a significant decrease in the former in 1996 (from 56-61% in 1994-1995 to 44%; Figure 3C, left). In contrast, no differences in the prevailing mechanisms during the years of the first experiment were observed for AT (Figure 3C, left).

Experiment II: reintroduction of a single tillage

After 13 years of permanent grass cover, the OC contents increased significantly ($p<0.01$) with respect to 1996. Before the beginning of experiment II (i.e. in 2009), the average content of OC across treatments was 21.5±1.5 g kg^{-1}. Aggregate stability was also enhanced with respect to the end of experiment I, as the average total and initial aggregate losses across both treatments in 2009 decreased significantly ($p<0.01$) to 72.2±6.9 and 13.5±7.8, respectively.

Three years of tillage following the 13 years of permanent grass did not have any effect on OC (Figure 3A, right). However, tillage rapidly induced an increase in both total and initial aggregate loss. Significantly higher total losses of aggregates were observed for AT with respect to PG ($p<0.01$) in 2010 and 2011 (Figure 3B, right), as well as higher initial losses ($p<0.05$ in 2010 and $p<0.01$ in 2011, Figure 3C, right). No significant correlations were found between OC and aggregate stability for both treatments. The differences in aggregate breakdown between AT and PG were mainly due to the loss of aggregates upon water saturation (Figure 3C, right) that, on average, represented 3% of total loss for PG and 20% for AT.

In experiment II, the OC content was significantly affected both in terms of year and seasonal dynamics ($p<0.01$). In all years, the lowest OC contents were observed in spring sampling (Figure 4A), with lowest values obtained in 2011 (15.4.0±2.1 g kg^{-1}). The seasonal effect was also important for aggregate stability, influenced by both season ($p<0.01$) and the year × season interaction ($p<0.05$, Figure 4B, 4C). The lowest total losses were almost always found in summer, although they did not always correspond to the lowest initial losses.

Organic matter fractionation and aggregate porosity

The SOM density fractionation showed that the MOM was always the dominant fraction, with average values of 10.59±2.55 and 9.63±1.58 g C kg^{-1} soil for PG and AT respectively, whereas, the OC contents in *f*POM and *o*POM fractions were much lower (Table 2). Regarding the seasonal distribution of organic fractions in PG, the *f*POM increased from spring to summer then decreased slightly in autumn ($p=0.06$, at the limit of significance), while the *o*POM fraction increased from spring to summer and autumn ($p<0.01$). Differently, in AT no differences between seasons were visible in *f*POM and *o*POM. In PG the mineral-associated OC fraction was particularly high in spring (85% of soil OC), it decreased down to 55% in summer, and increased again in autumn (Table 2). Conversely, the proportion of OC in MOM varied less markedly in AT, with similar percentages in spring and summer samples (64.46±6.52% and 62.14±6.64%, respectively) and decreased in autumn (55.86±1.99%). The proportion of total OC in the *f*POM fraction was affected by season and by the interaction soil management × season, while *o*POM and MOM showed an effect of both factors, as well as a significant interaction (Table 3).

The C/N ratio differed among fractions (Table 2) with lowest values in the MOM fraction (9.7 on average) and the highest in *f*POM (17 on average). Only the C/N ratio of MOM showed a significant effect of sampling time, whereas C/N of *o*POM and *f*POM fractions were not affected by either soil management or sampling time, although a significant interaction effect was present for *o*POM (Table 3). The C/N ratio of MOM was high in spring and showed a clear decreasing trend during the year, while the ratios of both *o*POM and *f*POM were more or less constant during the year.

The Hg-porosimetry (Table 4) indicated that the 1-2 mm aggregates had on average 34% porosity and, consequently a relatively high density (range 1.64 - 1.79 g cm^{-3}). Ultramicropores (5 - 0.1μm) represented between 64 and 69% of total porosity, followed by cryptopores (<0.1 μm) and micropores (5 - 30 μm), being 17 and 16%, respectively. The total porosity and the pore size classes did not show any effect of soil management or sampling time. The modal coarser pores had an average radius across treatments falling within the ultramicropore class (0.826±0.287 μm) and sharply differed between AT and PG for summer samples (Table 4), although no significant season or treatment effects were found by the ANOVA (Table 5). The finer modal class was much less abundant, had an average ratio falling within the cryptopores, it was significantly affected by season ($p<0.05$) and differed between AT and PG ($p<0.01$, Table 5).

DISCUSSION

Two experiments each, with a duration of three years, were carried out in the studied vineyard to investigate the effects of cover crops on aggregate stability, porosity, and SOM dynamics. Two treatments were considered, i.e. permanent grass (PG) vs. single autumn tillage (AT). The two experiments were separated by thirteen years of permanent grass, which allowed to evaluate the effect of the re-introduction of tillage on soil aggregate stability and organic matter stabilization, and to assess the mechanisms linking aggregate and SOM turnover.

The topsoil of the studied vineyard was rather vulnerable to aggregate breakdown in both treatments (Figure 3), as often observed in hilly areas of the Mediterranean, where OC contents as low as 20 g kg^{-1} are not unusual (Jones et al. 2005) due to erosion (García-Ruiz et al. 2013; Novara et al. 2015) and poor soil development (Novara et al. 2015). Moreover, deep tillage before vine planting could have also contributed to reducing OC contents due to the combined effects of (i) mixing of deep horizons with the topsoil, as often happens after severe disturbances (e.g., Stanchi et al. 2012; Curtaz et al. 2014), and (ii) enhanced C mineralization due to a greater accessibility of SOM for microbial degradation.

Permanent grass induced a significant increase in OC content at the end of experiment I (Figure 3). The higher input of organic residues under grasses (Post and Kwon 2000) led to the accumulation of a significantly greater amount of OC with respect to AT after three years. Even if grasses also partially covered the inter-row in the AT treatment, single tillage disturbance during autumn nonetheless limited OC accumulation. Moreover, soil management had a rapid effect on aggregate loss that was already observed in the first year of treatment. In fact, PG soils showed greater aggregate stability than AT (Figure 3). Whereas tillage disturbance is known to accelerate aggregate turnover (Yang and Wander 1998; Andruschkewitsch et al. 2014), in the absence of such disturbance permanent grass may have reduced macroaggregate turnover due to enhanced stability resulting from a stronger association between organic matter and the mineral phase (Six et al. 2000).

Further thirteen years of permanent grass greatly contributed to OC accumulation and aggregate stability, inducing a four-fold increase in OC, while total and initial aggregate losses were reduced by 20 and 31%, respectively, with respect to 1996 (Figure 3). As reported by Six et al. (2000; 2004), fresh organic matter inputs from decaying grass leaves and roots favour aggregate formation by enhancing microbial activity. Subsequently, fragmented SOM residues and partially decomposed organic compounds can be associated to stable microaggregates. However, OC accumulation did not continue indefinitely. In fact, no further increase in OC contents was observed in PG over the 2010-2012 period. This suggests that, after having been covered with grasses since 1994 the soil could have reached OC saturation levels (Six et al. 2002), even though the OC contents were still rather low (Figure 4). Surprisingly, we did not observe significant linear correlations between aggregate stability and soil OC contents. The OC contents were however comparable to those reported for low aggregate stability soils by Le Bissonnais et al. (2007), where clay significantly contributed in explaining stability variations.

In contrast, introduction of autumn tillage after thirteen years of permanent grass resulted in an immediate deterioration of aggregate stability with higher initial losses in AT than in PG, even after one year (Figure 3). The increased aggregate breakdown after tillage is in agreement with Peregrina et al. (2010) who, however, did not distinguish between the different loss mechanisms. Initial losses are mainly due to slaking produced by water pressure into small pores, but also due to clay swelling and dispersion (Le Bissonnais 1996). SOM may occlude the smallest pores and increase the surface roughness of the pore system, thus impeding water diffusion, and consequently decreasing the effects of slaking (Zaher et al. 2007). A good positive correlation (r=0.752, p<0.05) was indeed found between initial losses and MRF that in PG was about one half that of AT, thus indicating much smaller pores and consequently lower initial losses of aggregates.

The re-introduction of autumn tillage after thirteen years of permanent grass did not reduce OC contents, as instead commonly observed (e.g., Goulet et al. 2004; Peregrina et al. 2012). However, the tillage intensity adopted in our experiment (a single autumn tillage) was lower than that usually reported in literature, and therefore allowed the development of some grass cover. In addition the large seasonal variability observed in experiment II could have masked any minor loss of OC due to tillage over the three year experimental period (Figure 4). The observed seasonal dynamics in total aggregate loss seemed to be related to rainfall distribution with lower losses under dry, summer conditions, and higher losses in spring and autumn with higher soil water content (Figure 2, 4). This seasonal pattern was in line with numerous field studies where greater aggregate stability was recorded in summer with respect to autumn and early spring (Bullock et al. 1988; Blackman 1992; Chan et al. 1994; Dimoyiannis 2009; Algayer et al. 2014).

The seasonal variability in OC contents (Figure 4) clearly reflected the annual balance between organic matter inputs and degradation and was probably enhanced by the low structure complexity of this soil (Falsone et al. 2012), poorly effective in buffering disturbances. In fact, OC contents increased through the growing season, showing lower values in the following spring. Seasonal changes in SOM density fractions helped elucidate these variations. The inputs of fresh organic matter during the initial period of grass growth in PG deeply changed the relative proportion of SOM fractions from spring to summer, feeding the *f*POM and *o*POM pools with highest C/N ratios (Table 2). Although the input of fresh organic material presumably continued till autumn, the relative decrease in these fractions (Table 2) suggested a faster mineralization of recently added organic matter and/or stabilization into the mineral phase.

In agricultural soils most organic C is associated to minerals (e.g. John et al. 2005; Curaqueo et al. 2010; Cerli et al. 2012), and this was also true in our study (Table 2). The MOM fraction is generally considered as the most stable since the chemical interaction between organic compounds and the mineral soil phases protects OM from degradation (e.g. Turchenek and Oades 1979; Baldock and Skjemstad, 2000). Therefore strong seasonal variations should not be expected. In our case, a seasonal variability in the MOM fraction was observed, particularly in PG. Moreover, the relatively higher C/N ratio observed for MOM in spring (C/N = 12.2, Table 2) in PG is in agreement with a contribution of relatively fresh organic compounds from the previous year, while the decreasing trend with time (C/N = 9.2 and 8.4 in summer and autumn respectively, Table 2) suggests that even this relatively stable fraction undergoes some annual turnover (Hooker and Stark

2012). It is worthy to note that in calcareous soils, even when carbonates are lithogenic and therefore do not play an important role in aggregate stabilization by grain coating and pore clogging (Catoni et al. 2012), calcium cations may contribute to the binding of particulate organic matter on minerals through weak chemical bonds, that may have nonetheless resisted disruption at the relatively low sonication energy applied (Cerli et al. 2012). We thus hypothesised that in the PG treatment a fraction of partly degraded SOM might be bound to clay particles through Ca^{2+} bridges, resulting in relatively labile associations not able to preserve OM from degradation (Mikutta et al. 2007). The same process cannot be totally excluded in AT, because of the development of some herbaceous cover in the inter-row, but its intensity is certainly lower. In fact, in AT the C/N values of MOM in spring (Table 2) were more similar to those obtained in summer and autumn, suggesting that even a single tillage influences SOM dynamics by accelerating decomposition processes and reducing the differences in the turnover of the three fractions.

AT and PG porosities differed only in terms of MRF (Table 4). Bruand and Prost (1987) demonstrated that fine porosity is related to the packing of the clay fraction, therefore a lower MRF in PG can be linked to the dynamics of the organic fraction adsorbed onto clay surfaces (MOM) favouring a closer packing. Indeed, the highest was the MOM proportion, the smallest the radius ($r=-0.697$, $p<0.01$). In addition the dynamics of organic fractions also helped in explaining the higher sensitivity of aggregate stability than OC to soil management. The amount of MOM was negatively related to the initial aggregates loss ($r=-0.674$, $p<0.001$). The presence of relatively poorly decomposed inputs into the MOM fraction limited the slaking process as suggested by the negative correlation between a and C/N ratio ($r=-0.558$, $p<0.05$). On the contrary, the higher the *f*POM fractions, the higher were the initial losses ($r=0.661$, $p<0.01$). The organic matter fraction occluded into aggregates actively prevented abrasion losses, as evidenced by the negative correlation between *o*POM fraction and b ($r=-0.578$, $p<0.05$). Organic matter fractions were therefore more related to aggregate stability than total organic carbon. All together these relationships highlight that physical (i.e. *o*POM) and chemical (i.e. MOM) interaction mechanisms of OM with mineral phase play a pivotal role in governing aggregate stability, acting on both breakdown processes.

CONCLUSIONS

The results of this work highlight how, in hilly vineyards characterized by poorly developed soils, even a single tillage operation may negatively influence soil aggregate stability and SOM dynamics. Grass cover may contribute to increasing the resistance of aggregates to breakdown and organic matter accumulation with respect to autumn tillage, with measurable differences within just three years. However, the effects of grass cover on soil recovery capacity after stress were slow, and even after thirteen years of permanent grass, the effects of previous tillage on aggregate loss were still significant. Conversely, re-introduction of tillage after permanent grass had more rapid effects, with an almost immediate decrease in aggregate resistance, although OC contents remained almost unaffected. Organic matter, however, showed marked seasonal dynamics which not only involved those organic matter fractions containing young, recently added organic matter but also the mineral-associated SOM pool, generally considered to have relatively long turnover times. Tillage altered SOM dynamics by preventing the addition of new material into the mineral-associated organic fractions and, thus, limiting the stabilization of aggregates. These results also suggest that the resistance to light disturbances becomes progressively lower thus potentially enhancing soil losses and erosion risks, but this needs to be further investigated.

Acknowledgements

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Figure 1: localization, experimental design and time scale.

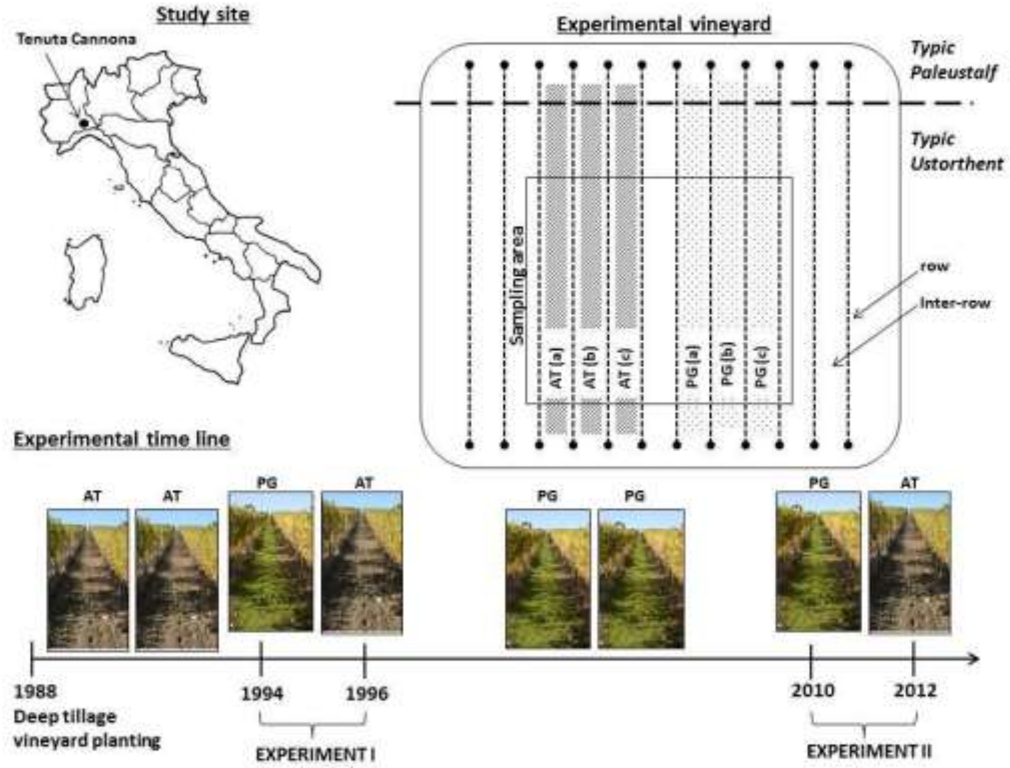

Figure 2: average rainfall and temperature recorded in 2010, 2011 and 2012. Error bars represent the standard error of the mean.

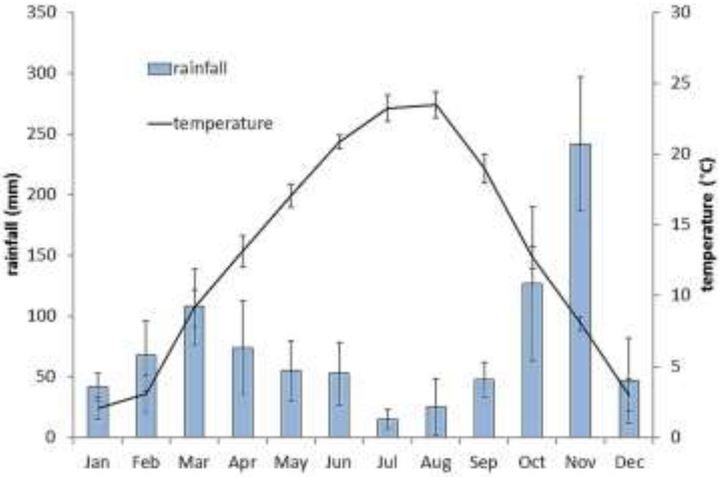

Figure 3: A) OC contents, B) total aggregate loss (a+b), and C) initial aggregate loss (a) during experiment I (1994-1996) and II (2010-2012). Bars represent the standard error of the mean (n=9) and letters indicate significant differences (p<0.05) between treatments in the same year. Letters are reported only where significant differences are present.

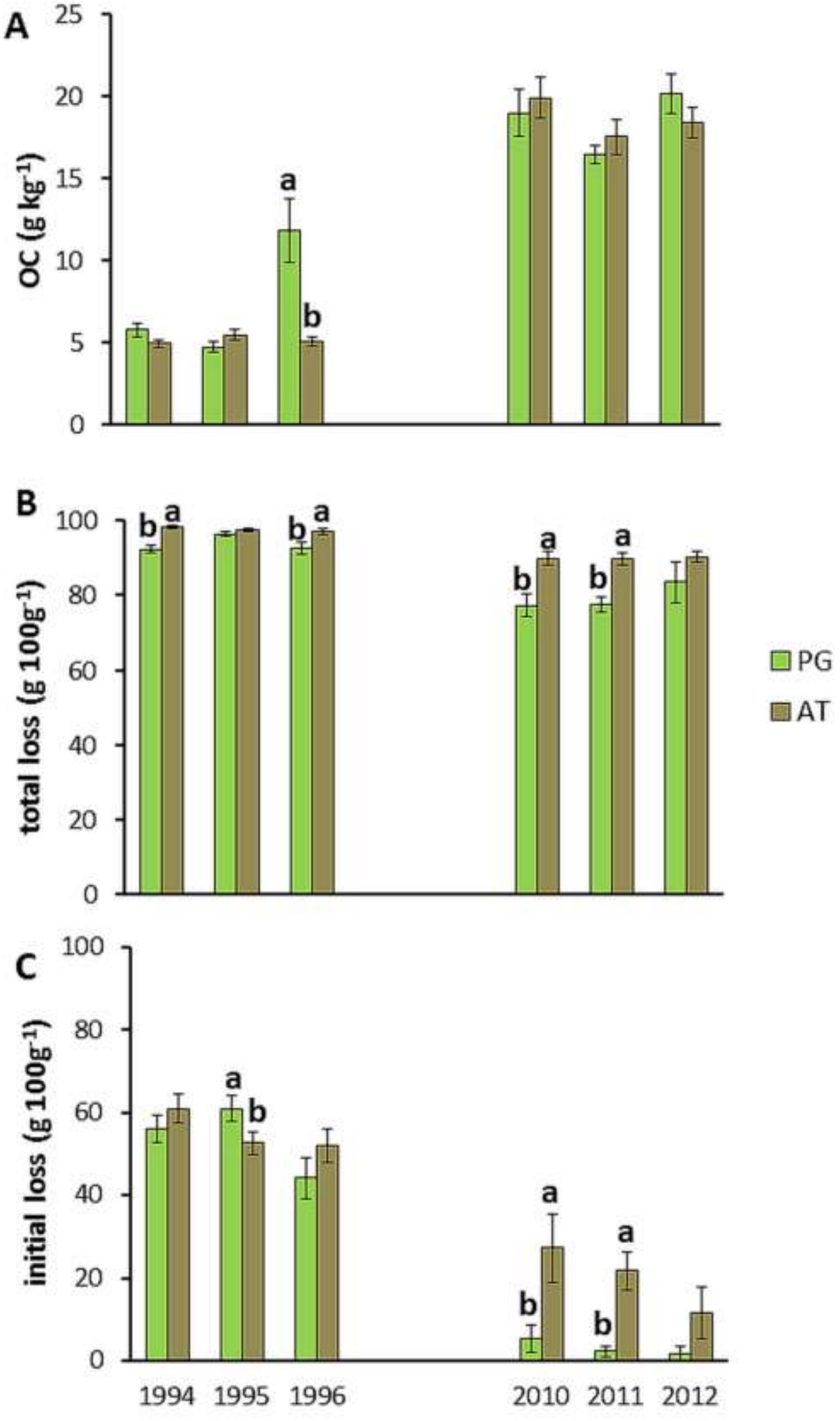

Figure 4: A) OC contents, B) total aggregate loss (*a*+*b*), and C) initial aggregate loss (*a*) for permanent grass (PG) vs. autumn tillage (AT) treatments in spring, summer and autumn for 2010, 2011 and 2012 sampling. Bars represent standard error of the mean (n=3).

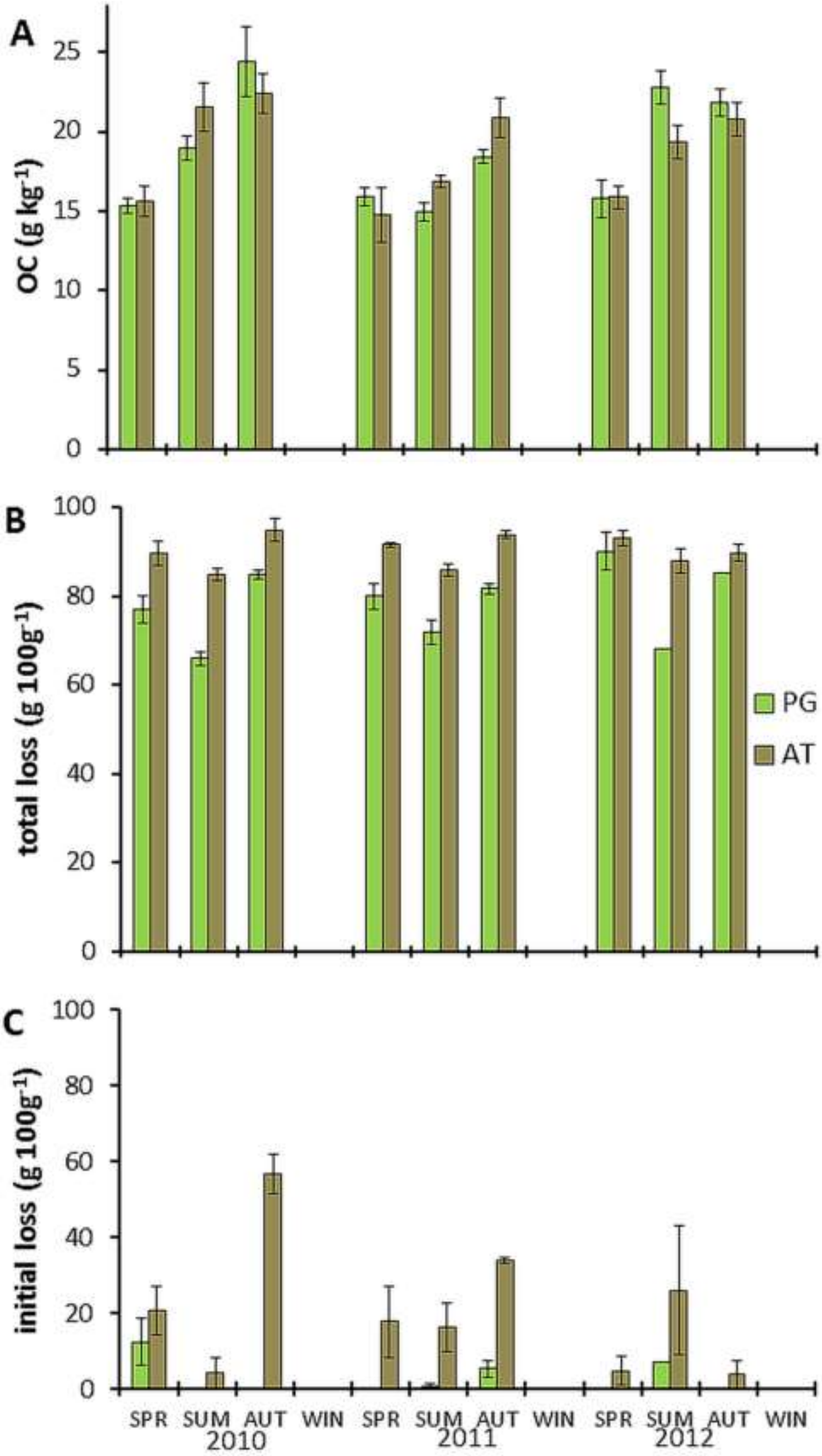

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Table 1: Means and standard deviation of properties of surface soil (0-5 cm depth, n=6).

Soil characteristics[1]		
Bulk density	kg dm^{-3}	1.34 ± 0.04
Coarse sand (2-0.2 mm)	%	13.3 ± 4.3
Fine sand (0.2-0.05 mm)	%	27.4 ± 4.2
Coarse silt (0.05-0.02 mm)	%	17.9 ± 2.2
Fine silt (0.02-0.002 mm)	%	26.3 ± 3.7
Clay (<0.002 mm)	%	15.0 ± 3.3
CEC	$cmol_c\ kg^{-1}$	17.67 ± 2.40
K_{ex}	$cmol_c\ kg^{-1}$	0.78 ± 0.18
Mg_{ex}	$cmol_c\ kg^{-1}$	1.80 ± 0.25
Ca_{ex}	$cmol_c\ kg^{-1}$	19.35 ± 2.70
pH		7.8 ± 0.11
$CaCO_3$	g kg^{-1}	175 ± 10
OC	g kg^{-1}	5.0 ± 1.65
Total N	g kg^{-1}	0.51 ± 0.08
C/N		9.8 ± 0.9

[1] K_{ex}, Ca_{ex}, Mg_{ex}: exchangeable K, Ca and Mg; OC: organic C

Table 2: Organic C concentrations, percentages, and C/N ratio (± standard deviation) in SOM density fractions (fPOM, oPOM and MOM) for permanent grass (PG) and autumn tillage (AT) treatments in spring, summer and autumn and overall average for 2011 sampling. Different letters indicate significant differences between treatments for each sampling time. Letters are reported only when significant differences were present ($p<0.05$).

parameters	PG				AT			
	spring (n=3)	summer (n=3)	autumn (n=3)	2011 (n=9)	spring (n=3)	summer (n=3)	autumn (n=3)	2011(n=9)
*f*POM (g C kg^{-1})	1.64±0.46	2.77±0.60	2.28±0.24	2.23±0.63	2.19±0.29	2.46±0.73	3.36±.65	2.67±0.74
*o*POM (g C kg^{-1})	0.75±0.19 **b**	3.91±0.96	3.34±0.46	2.67±1.56	3.02±0.82 **a**	3.96±0.69	3.66±0.87	3.55±0.81
MOM (g C kg^{-1})	13.44±0.85	8.26±2.01	10.07±0.75	10.59±2.55	9.58±2.54	10.47±0.88	8.83±0.87	9.63±1.58
*f*POM (% OC)	10.32 ± 2.89	18.69 ± 4.72	14.52 ± 1.40 **b**	14.51 ± 4.62	15.03 ± 2.03	14.50 ± 3.93	21.23 ± 2.94 **a**	16.92 ± 4.19
*o*POM (% OC)	4.75 ± 1.11 **b**	26.27 ± 6.76	21.32 ± 3.04	17.45 ± 10.46	20.51 ± 4.84 **a**	23.36 ± 3.11	22.91 ± 3.20	22.26 ± 3.55
MOM (% OC)	84.93 ± 1.85 **a**	55.04 ± 11.00	64.16 ± 4.29 **a**	68.04 ± 14.55	64.46 ± 6.52 **b**	62.14 ± 6.64	55.86 ± 1.99 **b**	60.82 ± 6.12
C/N *f*POM	17.1 ± 2.2	17.0 ± 0.6	17.0 ± 1.7	17.0 ± 1.4	16.8 ± 1.3	16.1 ± 1.2	17.7 ± 0.2	16.9 ± 1.1
C/N *o*POM	14.6 ± 0.9	13.6 ± 0.6	13.7 ± 0.2 **b**	14.0 ± 0.7	13.1 ± 0.4	13.5 ± 0.7	14.6 ± 0.2 **a**	13.7 ± 0.8
C/N MOM	12.2 ± 0.6 **a**	9.2 ± 0.9	8.4 ± 0.3	9.9 ± 1.8	10.3 ± 1.0 **b**	9.3 ± 1.4	8.7 ± 0.2	9.4 ± 1.1

Table 3: ANOVA p-values showing the effects of season and vineyard soil management on OC concentrations, percentages with respect to OC and C/N ratio in SOM density fractions (*f*POM, *o*POM and MOM) in 2011 sampling. Seasons are spring, summer and autumn and treatments are permanent grass (PG) and autumn tillage (AT).

	season	treatment	season × treatment
*f*POM (g C kg^{-1})	0.028	NS	NS
*o*POM (g C kg^{-1})	0.001	0.024	0.040
MOM (g C kg^{-1})	0.047	NS	0.014
*f*POM (% OC)	0.038	NS	0.027
*o*POM (% OC)	0.001	0.028	0.005
MOM (% OC)	0.001	0.030	0.008
C/N *f*POM	NS	NS	NS
C/N *o*POM	NS	NS	0.009
C/N MOM	0.000	NS	NS

Table 4: Data (± standard deviation) of porosity, pore size distribution, modal radius and volume of coarse (MRC and MVC) and fine (MRF and MVF) pores of the 1-2 mm aggregate fractions. Different letters indicate significant differences between treatments for each sampling time and for average annual data. Letters are reported only when significant differences were present ($p<0.05$).

	PG				AT			
	spring (n=3)	summer (n=3)	autumn (n=3)	2011 (n=9)	spring (n=3)	summer (n=3)	autumn (n=3)	2011 (n=9)
Total pore volume (mm^3)	196.42 ± 5.08	201.22 ± 7.66	203.58 ± 15.23	200.41 ± 9.44	196.44 ± 8.43	212.53 ± 14.61	196.92 ± 9.94	201.96 ± 12.60
Total porosity (%)	32.45 ± 0.76 **b**	34.10 ± 0.83	35.92 ± 2.99	34.16 ± 2.19	35.15 ± 1.19 **a**	34.74 ± 1.72	33.77 ± 0.92	34.55 ± 1.29
Aggregate density (kg dm^{-3})	1.65 ± 0.08 **b**	1.70 ± 0.08	1.77 ± 0.11	1.71 ± 0.09	1.79 ± 0.02 **a**	1.64 ± 0.06	1.72 ± 0.04	1.71 ± 0.08
Micropores (30-5 μm, %)	16.09 ± 3.32	15.45 ± 1.59	16.24 ± 0.61	15.93 ± 1.90	16.41 ± 1.32	15.34 ± 2.28	16.33 ± 0.40	16.03 ± 1.43
Ultramicropores (5-0.1 μm, %)	67.51 ± 3.64	67.17 ± 2.05	68.60 ± 1.80	67.76 ± 2.36	67.99 ± 1.14	63.56 ± 6.40	68.89 ± 1.97	66.81 ± 4.20
Cryptopores (<0.1 μm, %)	16.39 ± 0.37	17.34 ± 0.80	15.17 ± 2.33	16.30 ± 1.56	15.59 ± 1.94	21.10 ± 5.75	14.78 ± 1.61	17.16 ± 4.33
MRC (μm)	0.810 ± 0.331	1.184 ± 0.259 **a**	0.636 ± 0.319	0.877 ± 0.358	0.804 ± 0.235	0.650 ± 0.111 **b**	0.874 ± 0.236	0.776 ± 0.202
MVC (mm^3)	187.1 ± 4.8	182.0 ± 3.7	192.6 ± 17.1	187.3 ± 10.2	179.9 ± 7.8	180.9 ± 8.9	181.6 ± 8.6	180.8 ± 7.4
MRF (μm)	0.006 ± 0.001	0.013 ± 0.004	0.010 ± 0.001	0.010 ± 0.004 **b**	0.015 ± 0.005	0.026 ± 0.007	0.014 ± 0.008	0.018 ± 0.008 **a**
MVF (mm^3)	4.9 ± 0.9	13.7 ± 6.6	7.5 0 ± 3.8	8.7 ± 5.5	13.6 ± 5.6	20.8 ± 9.4	8.3 ± 1.0	14.2 ± 7.7

Table 5: ANOVA p-values for effects of season and vineyard soil management treatment on modal radii and modal volumes of coarse pore (MRC, MVC) and fine pore (MRC, MRF) in 2011 sampling. Seasons are spring, summer and autumn and treatments are permanent grass (PG) and autumn tillage (AT).

	season	treatment	season × treatment
MRC (μm)	NS	NS	NS
MVC (mm^3)	NS	NS	NS
MRF (μm)	0.022	0.005	NS
MVF (mm^3)	0.025	NS	NS

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