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**Effects of permanent grass versus tillage on aggregation and organic matter dynamics in a poorly developed vineyard soil**

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(Article begins on next page)

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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

1 Soils carry out a large number of functions such as primary production and biodiversity  
2 conservation, as described by Berendse et al. (2015), Brevik et al. (2015), De Graaf et al. (2015).  
3 They also control the hydrological and biogeochemical cycles, acting as a filter for groundwater  
4 (Keesstra et al. 2012), storing organic matter and nutrients (e.g. Batjes et al. 2014; Jaiarree et al.  
5 2014; Köchy et al. 2015 a b), protecting land from hydrogeological hazards such as water  
6 erosion. Soil organic matter (SOM) protection and soil structure are key factors for soil conservation  
7 and sustainable land management (e.g. Cerdà et al. 1996, Wick et al. 2014), and for carbon  
8 sequestration and fertility (Gosling et al. 2013 and references within). They are mutually related:  
9 aggregates provide protection of SOM against microbial decomposition by occlusion, i.e. physical  
10 protection, and by association of organic matter to clay and silt fractions, i.e. physico-chemical  
11 protection (Tisdall and Oades 1982; Golchin et al. 1994; 1995; Jagadamma and Lal 2010; Vogel et  
12 al. 2015). In turn, SOM bound to mineral particles leads to the formation of stable aggregates that  
13 are thus fundamental to reduce C turnover. Aggregate complexity typically increases with soil  
14 evolution, therefore poorly developed soils, such as Entisols, retain several characteristics of the  
15 parent material in aggregate hierarchy (Falsone et al. 2012). The role of SOM in the aggregation  
16 processes is then even more important.

17         The importance of SOM management and conservation, and the effects of land use on SOM  
18 and soil structure have been studied by several authors in a variety of environments (e.g. Munoz-  
19 Rojas et al. 2012; De Moraes Sá et al. 2013; Gelaw et al. 2013; Parras-Alcántara et al. 2013, 2014;  
20 Srinivasarao et al. 2014; Yu and Jia 2014; Behera et al. 2015; Peng 2015). Agriculture can strongly  
21 limit SOM accumulation, influencing soil aggregate formation and turnover (Jastrow 1996; Cerdà,  
22 2000; Jacobs et al. 2010; Gosling et al. 2013). In particular, tillage can increase the turnover of  
23 macroaggregates (Six et al. 2000), consequently limiting the physical protection of SOM, hence the  
24 formation of microaggregates inside macroaggregates. Panettieri et al. (2015) confirmed that in  
25 agricultural soils the formation of organo-mineral interactions can provide for an effective  
26 protection of SOM, while Comeau et al. (2013) highlighted the important contribution of fresh  
27 organic matter inputs like crop residues to stabilized SOM. Minimum-till, no-till and cover crops  
28 can enhance aggregation and promote SOM protection (Bronick and Lal 2005).

29         Vineyard soils are often highly sensitive to SOM loss and topsoil degradation due to their  
30 intrinsic properties such as limited soil development, coarse texture and low capacity to protect  
31 SOM by binding to soil minerals (Le Bissonnais et al. 2007; Martinez-Casasnovas and Ramos  
32 2009; Tarolli et al. 2015). These soils often show different properties with respect to other  
33 agricultural soils, e.g. limited SOM contents, hilly morphology and sloping topography (Ramos and

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

37 Most improvements in soil management in viticulture currently aim at enhancing SOM  
38 contents and aggregation by increasing C inputs (Bustamante et al. 2011; Guerra and Steenwerth  
39 2012), whereas practices that limit soil disturbance and mitigate C loss are marginally taken into  
40 account. Besides favouring soil erosion, traditional tillage has been reported to be unsustainable in  
41 terms of SOM and nutrient depletion (Ruiz-Colmenero et al. 2013). Conversely, the combination of  
42 no-tillage and permanent grass cover in the inter-row may represent an optimal solution for  
43 improving aggregation and SOM protection, that is also feasible as the competition between the  
44 herbaceous cover and grapevines often does not reduce yields even in rain-fed Mediterranean  
45 environments (Marques et al. 2010; Agnelli et al. 2014; Mercenaro et al. 2014). Grasses contribute  
46 to organic matter input while no-tillage reduces soil macroaggregate turnover, which is crucial for C  
47 sequestration in stable microaggregates. For example, Marques et al. (2010) and Ruiz-Colmenero et  
48 al. (2013) observed lower soil losses in cover cropped rows than in tilled rows in Spanish vineyards.  
49 Despite the general consensus on the positive role of organic matter inputs and reduced tillage in  
50 vineyards (Goulet et al. 2004; Steenwerth and Belina 2008; Peregrina et al. 2012; Simansky et al.  
51 2013), only a few works have related these practices to aggregation and SOM dynamics and  
52 evaluated the time scale of soil quality improvement or decline. Moreover, in most experiments  
53 permanent grass has been compared to situations where tillage was carried out several times per  
54 year resulting in strong structure degradation. To mitigate the negative effects of tillage, while  
55 avoiding excessive grass competition with vines, vineyard soil management may involve a single  
56 tillage operation in the inter-row per year.

57 The aim of this work was to evaluate the effects of permanent grass vs. single autumn tillage  
58 on soil structure and SOM dynamics in a calcareous vineyard soil in hilly environment. These soils  
59 show common characteristics in important Italian viticultural areas: they are poorly developed and  
60 highly susceptible to erosion. We hypothesized that the characteristics of these soils make them  
61 particularly sensitive to even minor soil management practices with important consequences on  
62 aggregation and organic matter dynamics even in the short term. To test this hypothesis, a first  
63 comparison between a single tillage disturbance and permanent grass cover was carried out for 3  
64 years during the '90s. Then, grass was allowed to cover the whole vineyard for 13 years and, in  
65 2009, tillage disturbance was reintroduced and the comparison repeated. Our specific objectives  
66 were (i) to evaluate the effects of short and medium-to-long term inter-row grass cover on aggregate  
67 stability and organic matter dynamics; (ii) to assess the sensitivity of the soil to the reintroduction of

68 a single tillage after 13 years of permanent grass; (iii) to study the mechanisms relating organic  
69 matter turnover and aggregate stability through soil porosity evaluation and physical fractionation  
70 of SOM.

71

## 72 **MATERIALS AND METHODS**

### 73 *Study site, experimental setup and soil sampling*

74 The study site is located in a hilly area of NW-Italy at the experimental farm “Tenuta Cannona”  
75 (44°40'59.35"N, 8°37'36.85"E, total farm surface 54 ha, Figure 1), close to the Ligurian Appennine,  
76 where a Mediterranean influence in the thermal and rainfall regime is present. The average air  
77 temperature is 13°C and the average annual rainfall is 850 mm (30 years record from 1967 to 1997;  
78 Cagnazzi and Marchisio, 1998). In 2010 an automatic weather station was installed to monitor  
79 rainfall and temperature data (Figure 2); average (2010-2012) rainfall and temperature were 900  
80 mm and 13°C, quite similar therefore to the earlier records.

81 The vineyard selected for this study (0.71 ha) is rain fed. It was deep tilled (0.5-0.6 m) in  
82 1988 and planted with *Vitis vinifera* L. cv. Chardonnay, grafted on Kober 5BB rootstock (5000  
83 vines ha<sup>-1</sup>) with 0.80 m between vines and 2.50 m between rows. The vines were vertically shoot  
84 positioned trained and pruned according to the Guyot system. The slope of the vineyard is  
85 homogeneous (14%) with south-east aspect, and rows are oriented downslope (NW-SE). The soil  
86 (Table 1) develops on marls and is a homogeneously distributed Typic Ustorthent, fine-loamy,  
87 mixed, calcareous, mesic (Soil Survey Staff 2014), as confirmed by several control pits made  
88 during field survey. Between 1989 and 1992 the whole vineyard was managed with a rototiller  
89 (0.15 m, 3 times per year) and in 1993 it was subdivided into six experimental plots to evaluate the  
90 effect of grass cover. The first experiment was carried out for 3 years (1994-1996, Experiment I)  
91 and aimed at the comparison between permanent grass and a single autumn tillage. From 1997 to  
92 2009 the whole vineyard was managed with permanent grass, allowing the development of the  
93 autochthonous seed bank and natural cover. In autumn 2009 a second experiment (Experiment II),  
94 aimed at evaluating the effect of tillage reintroduction (i.e. permanent grass vs. single autumn  
95 tillage), was carried out, and is still going on to date (Figure 1).

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

100 In the PG the soil surface was covered by autochthonous grasses, dominated by Poaceae  
101 (40%) and Fabaceae (20%), including *Avena fatua* L., *Bromus spp.*, *Cynodon dactylon* L., *Festuca*

102 *spp.*, *Hordeum spp.*, *Lathyrus spp.*, *Lolium multiflorum* Lam., *Lolium perenne* L., *Lotus*  
103 *corniculatus* L., *Medicago spp.*, *Melilotus officinalis* L., *Poa spp.*, *Trifolium repens* L., *Trifolium*  
104 *pratense* L., *Vicia sativa* L.. The rows were mowed twice a year (May, July) at an approximate  
105 height of 10 cm and the residues left on the soil, which was therefore permanently covered by  
106 vegetation and grass residues.

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

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

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

122

### 123 *Aggregate stability*

124 An aliquot of each soil sample (<2 mm) was dry sieved to obtain the 1-2 mm aggregate fraction  
125 required for evaluating the wet aggregate stability. Ten grams of these aggregates were placed in a  
126 0.2 mm sieve and allowed to rotate at 60 rpm in beakers containing deionized water for sieving  
127 times of 5, 10, 15, 20, 40, and 60 minutes. After sieving, the remaining material >0.2 mm (i.e.  
128 aggregates and coarse sand) was oven dried and weighed. The coarse sand fraction remaining in the  
129 sieve was determined after treatment with H<sub>2</sub>O<sub>2</sub> (Gee and Bauder 1986), and subsequently the loss  
130 of aggregates was determined as follows (Kemper and Rosenau 1986):

$$131 \text{ **Aggregate loss (AL, \%)} = 100 - \frac{100(\text{weight retained} - \text{weight of coarse sand})}{\text{total sample weight} - \text{weight of coarse sand}} \quad [\text{Eq.1}]**$$

132

133 The aggregate breakdown kinetics was fitted with the exponential model by Zanini et al.  
134 (1998):

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

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

140  
141 *Organic carbon and nitrogen content and density fractionation of SOM*

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

160  
161 *Aggregate porosity*

162 Soil porosity was evaluated by Hg-intrusion (Porosimeter 200 WS equipped with a Macropore unit  
163 120, CE Instruments, Rodano, Italy) on the 1-2 mm aggregate fraction. Analysis was performed by  
164 step-increasing the pressure (up to 200 MPa) and measuring the volume of intruded Hg at each step.  
165 Total porosity was subdivided into three pore size classes according to Brewer (1964): 30-5  $\mu\text{m}$ , 5-  
166 0.1  $\mu\text{m}$  and  $< 0.1 \mu\text{m}$ . From Hg-intrusion data, the density of the aggregates was also calculated.  
167 Additionally, to better interpret the data, we also followed the approach proposed by Bruand and  
168 Prost (1987), and recently applied to poorly developed soils by Falsone et al. (2012). This method

169 allowed for the determination of the most represented pore class (i.e. the modal pore class), from  
170 the curve of intruded-Hg volume as a function of the pore radius. The slope of the curve is  
171 calculated as:

$$172 \textit{slope}_{ij} = \frac{V_i - V_j}{\log_{10} R_j - \log_{10} R_i} \quad [\text{Eq. 3}]$$

173 where the volumes  $V_i$  and  $V_j$  correspond to the radii  $R_i$  and  $R_j$  at two successive positions  $i$  and  $j$ .  
174 The relative maxima of the slope curve indicated the most represented classes of pores. Each peak  
175 thus corresponded to a radius that was taken directly from the graph. This radius was termed modal  
176 radius ( $\mu\text{m}$ ) and the volume of each modal class of pores was, thus, included between two minimum  
177 values of the slope, which defined the limits of the class.

178

### 179 *Statistical analyses*

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

186

## 187 **RESULTS**

### 188 *Experiment I: introduction of permanent grass cover*

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

201 the prevailing mechanisms during the years of the first experiment were observed for AT (Figure  
202 3C, left).

203

#### 204 *Experiment II: reintroduction of a single tillage*

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

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

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

224

#### 225 *Organic matter fractionation and aggregate porosity*

226 The SOM density fractionation showed that the MOM was always the dominant fraction, with  
227 average values of  $10.59 \pm 2.55$  and  $9.63 \pm 1.58 \text{ g C kg}^{-1}$  soil for PG and AT respectively, whereas, the  
228 OC contents in *f*POM and *o*POM fractions were much lower (Table 2). Regarding the seasonal  
229 distribution of organic fractions in PG, the *f*POM increased from spring to summer then decreased  
230 slightly in autumn ( $p = 0.06$ , at the limit of significance), while the *o*POM fraction increased from  
231 spring to summer and autumn ( $p < 0.01$ ). Differently, in AT no differences between seasons were  
232 visible in *f*POM and *o*POM. In PG the mineral-associated OC fraction was particularly high in  
233 spring (85% of soil OC), it decreased down to 55% in summer, and increased again in autumn  
234 (Table 2). Conversely, the proportion of OC in MOM varied less markedly in AT, with similar

235 percentages in spring and summer samples ( $64.46\pm 6.52\%$  and  $62.14\pm 6.64\%$ , respectively) and  
236 decreased in autumn ( $55.86\pm 1.99\%$ ). The proportion of total OC in the *f*POM fraction was affected  
237 by season and by the interaction soil management  $\times$  season, while *o*POM and MOM showed an  
238 effect of both factors, as well as a significant interaction (Table 3).

239 The C/N ratio differed among fractions (Table 2) with lowest values in the MOM fraction  
240 ( $9.7$  on average) and the highest in *f*POM ( $17$  on average). Only the C/N ratio of MOM showed a  
241 significant effect of sampling time, whereas C/N of *o*POM and *f*POM fractions were not affected by  
242 either soil management or sampling time, although a significant interaction effect was present for  
243 *o*POM (Table 3). The C/N ratio of MOM was high in spring and showed a clear decreasing trend  
244 during the year, while the ratios of both *o*POM and *f*POM were more or less constant during the  
245 year.

246 The Hg-porosimetry (Table 4) indicated that the 1-2 mm aggregates had on average 34%  
247 porosity and, consequently a relatively high density (range  $1.64 - 1.79 \text{ g cm}^{-3}$ ). Ultramicropores (5 -  
248  $0.1\mu\text{m}$ ) represented between 64 and 69% of total porosity, followed by cryptopores ( $<0.1 \mu\text{m}$ ) and  
249 micropores (5 -  $30 \mu\text{m}$ ), being 17 and 16%, respectively. The total porosity and the pore size classes  
250 did not show any effect of soil management or sampling time. The modal coarser pores had an  
251 average radius across treatments falling within the ultramicropore class ( $0.826\pm 0.287 \mu\text{m}$ ) and  
252 sharply differed between AT and PG for summer samples (Table 4), although no significant season  
253 or treatment effects were found by the ANOVA (Table 5). The finer modal class was much less  
254 abundant, had an average ratio falling within the cryptopores, it was significantly affected by season  
255 ( $p<0.05$ ) and differed between AT and PG ( $p<0.01$ , Table 5).

256

## 257 **DISCUSSION**

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

264 The topsoil of the studied vineyard was rather vulnerable to aggregate breakdown in both  
265 treatments (Figure 3), as often observed in hilly areas of the Mediterranean, where OC contents as  
266 low as  $20 \text{ g kg}^{-1}$  are not unusual (Jones et al. 2005) due to erosion (García-Ruiz et al. 2013; Novara  
267 et al. 2015) and poor soil development (Novara et al. 2015). Moreover, deep tillage before vine  
268 planting could have also contributed to reducing OC contents due to the combined effects of (i)

269 mixing of deep horizons with the topsoil, as often happens after severe disturbances (e.g., Stanchi et  
270 al. 2012; Curtaz et al. 2014), and (ii) enhanced C mineralization due to a greater accessibility of  
271 SOM for microbial degradation.

272 Permanent grass induced a significant increase in OC content at the end of experiment I  
273 (Figure 3). The higher input of organic residues under grasses (Post and Kwon 2000) led to the  
274 accumulation of a significantly greater amount of OC with respect to AT after three years. Even if  
275 grasses also partially covered the inter-row in the AT treatment, single tillage disturbance during  
276 autumn nonetheless limited OC accumulation. Moreover, soil management had a rapid effect on  
277 aggregate loss that was already observed in the first year of treatment. In fact, PG soils showed  
278 greater aggregate stability than AT (Figure 3). Whereas tillage disturbance is known to accelerate  
279 aggregate turnover (Yang and Wander 1998; Andruschkewitsch et al. 2014), in the absence of such  
280 disturbance permanent grass may have reduced macroaggregate turnover due to enhanced stability  
281 resulting from a stronger association between organic matter and the mineral phase (Six et al.  
282 2000).

283 Further thirteen years of permanent grass greatly contributed to OC accumulation and  
284 aggregate stability, inducing a four-fold increase in OC, while total and initial aggregate losses were  
285 reduced by 20 and 31%, respectively, with respect to 1996 (Figure 3). As reported by Six et al.  
286 (2000; 2004), fresh organic matter inputs from decaying grass leaves and roots favour aggregate  
287 formation by enhancing microbial activity. Subsequently, fragmented SOM residues and partially  
288 decomposed organic compounds can be associated to stable microaggregates. However, OC  
289 accumulation did not continue indefinitely. In fact, no further increase in OC contents was observed  
290 in PG over the 2010-2012 period. This suggests that, after having been covered with grasses since  
291 1994 the soil could have reached OC saturation levels (Six et al. 2002), even though the OC  
292 contents were still rather low (Figure 4). Surprisingly, we did not observe significant linear  
293 correlations between aggregate stability and soil OC contents. The OC contents were however  
294 comparable to those reported for low aggregate stability soils by Le Bissonnais et al. (2007), where  
295 clay significantly contributed in explaining stability variations.

296 In contrast, introduction of autumn tillage after thirteen years of permanent grass resulted in  
297 an immediate deterioration of aggregate stability with higher initial losses in AT than in PG, even  
298 after one year (Figure 3). The increased aggregate breakdown after tillage is in agreement with  
299 Peregrina et al. (2010) who, however, did not distinguish between the different loss mechanisms.  
300 Initial losses are mainly due to slaking produced by water pressure into small pores, but also due to  
301 clay swelling and dispersion (Le Bissonnais 1996). SOM may occlude the smallest pores and  
302 increase the surface roughness of the pore system, thus impeding water diffusion, and consequently

303 decreasing the effects of slaking (Zaher et al. 2007). A good positive correlation ( $r=0.752$ ,  $p<0.05$ )  
304 was indeed found between initial losses and MRF that in PG was about one half that of AT, thus  
305 indicating much smaller pores and consequently lower initial losses of aggregates.

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

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

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

337 2012). It is worthy to note that in calcareous soils, even when carbonates are lithogenic and  
338 therefore do not play an important role in aggregate stabilization by grain coating and pore clogging  
339 (Catoni et al. 2012), calcium cations may contribute to the binding of particulate organic matter on  
340 minerals through weak chemical bonds, that may have nonetheless resisted disruption at the  
341 relatively low sonication energy applied (Cerli et al. 2012). We thus hypothesised that in the PG  
342 treatment a fraction of partly degraded SOM might be bound to clay particles through  $\text{Ca}^{2+}$  bridges,  
343 resulting in relatively labile associations not able to preserve OM from degradation (Mikutta et al.  
344 2007). The same process cannot be totally excluded in AT, because of the development of some  
345 herbaceous cover in the inter-row, but its intensity is certainly lower. In fact, in AT the C/N values  
346 of MOM in spring (Table 2) were more similar to those obtained in summer and autumn, suggesting  
347 that even a single tillage influences SOM dynamics by accelerating decomposition processes and  
348 reducing the differences in the turnover of the three fractions.

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

365

## 366 CONCLUSIONS

367 The results of this work highlight how, in hilly vineyards characterized by poorly developed soils,  
368 even a single tillage operation may negatively influence soil aggregate stability and SOM dynamics.  
369 Grass cover may contribute to increasing the resistance of aggregates to breakdown and organic  
370 matter accumulation with respect to autumn tillage, with measurable differences within just three

371 years. However, the effects of grass cover on soil recovery capacity after stress were slow, and even  
372 after thirteen years of permanent grass, the effects of previous tillage on aggregate loss were still  
373 significant. Conversely, re-introduction of tillage after permanent grass had more rapid effects, with  
374 an almost immediate decrease in aggregate resistance, although OC contents remained almost  
375 unaffected. Organic matter, however, showed marked seasonal dynamics which not only involved  
376 those organic matter fractions containing young, recently added organic matter but also the mineral-  
377 associated SOM pool, generally considered to have relatively long turnover times. Tillage altered  
378 SOM dynamics by preventing the addition of new material into the mineral-associated organic  
379 fractions and, thus, limiting the stabilization of aggregates. These results also suggest that the  
380 resistance to light disturbances becomes progressively lower thus potentially enhancing soil losses  
381 and erosion risks, but this needs to be further investigated.

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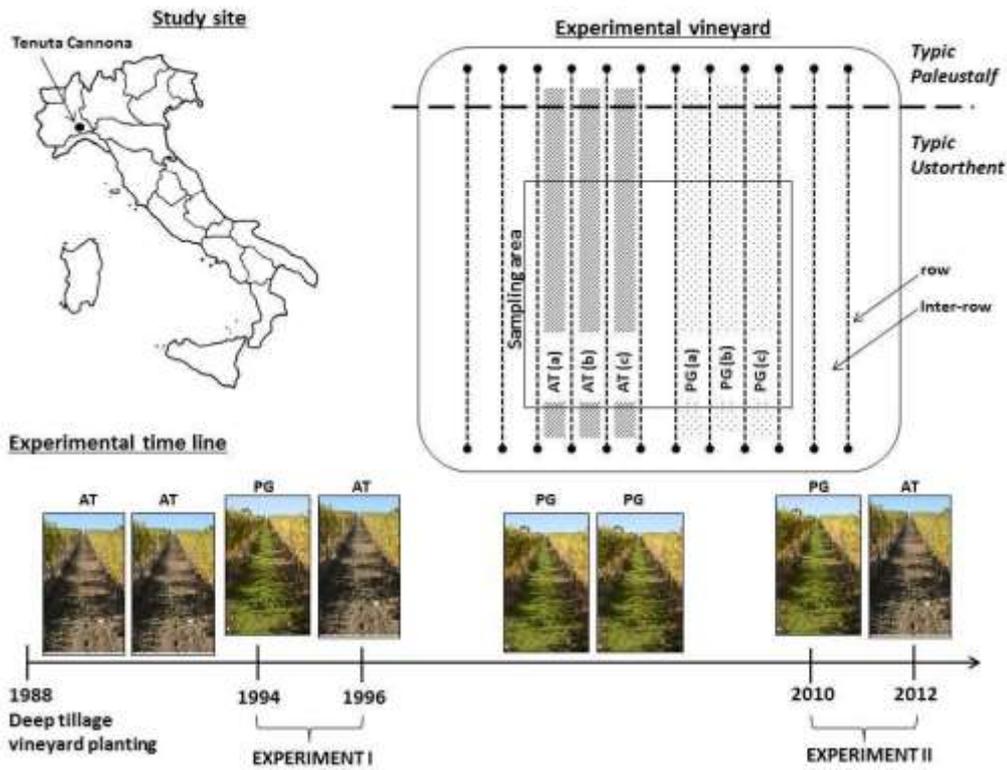
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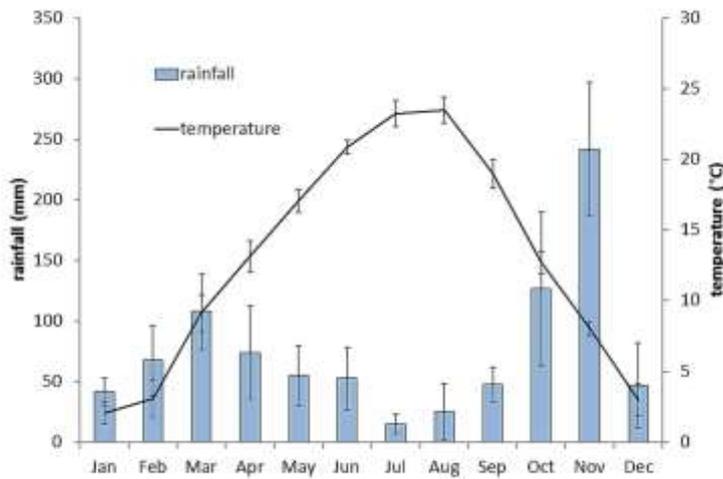
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**Figure 1: localization, experimental design and time scale.**



**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.**

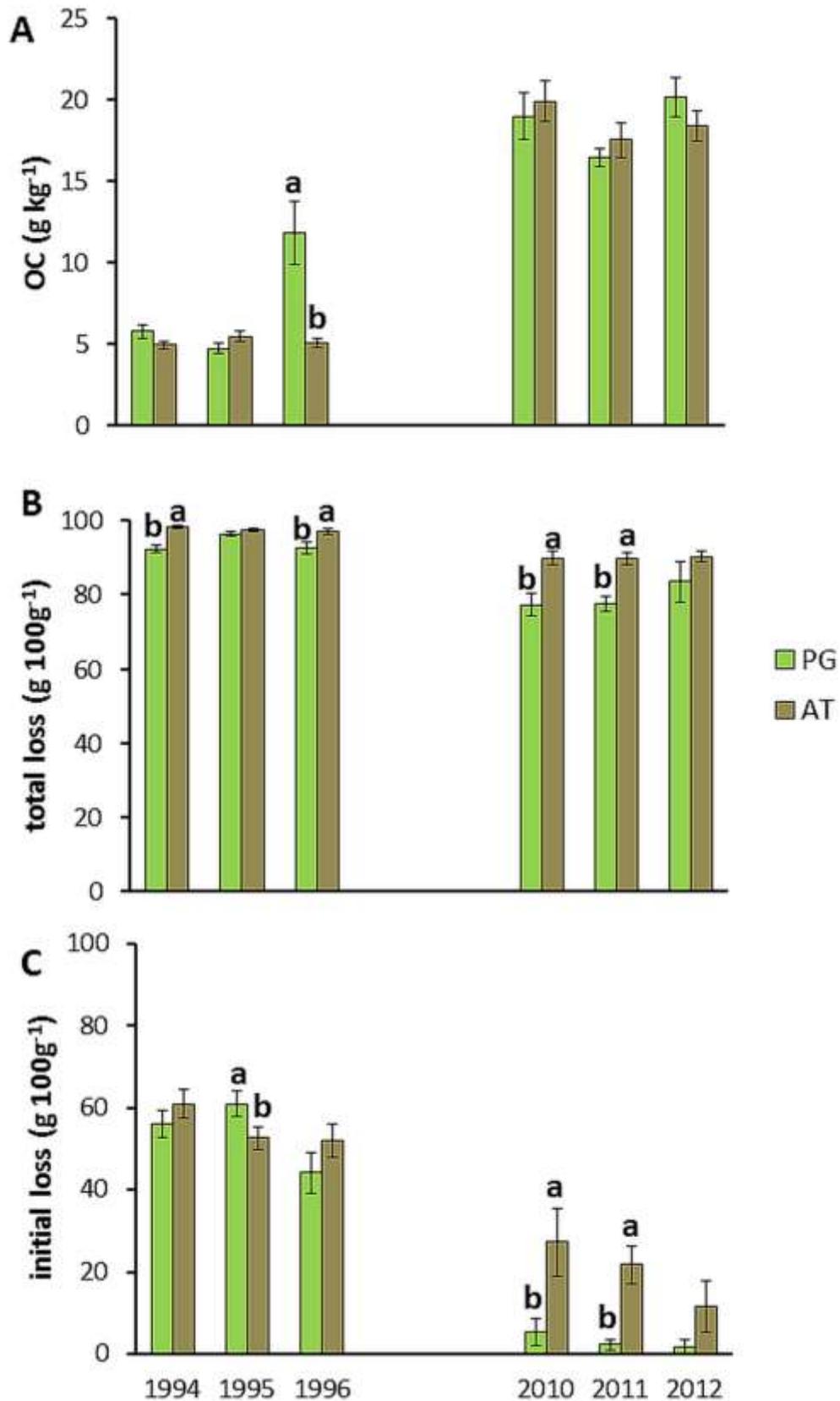
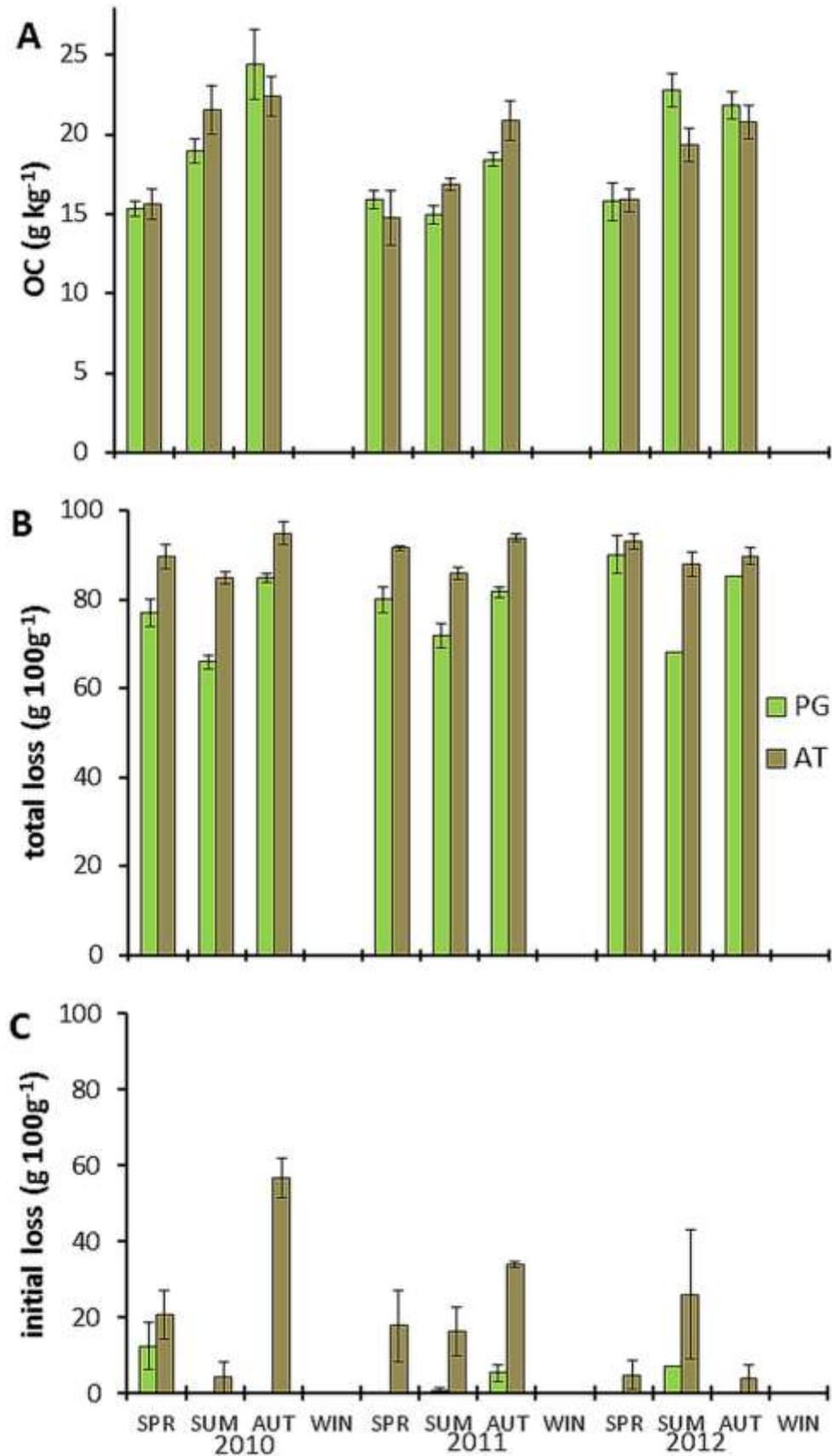


Figure 4: A) OC contents, B) total aggregate loss ( $a+b$ ), and C) initial aggregate loss ( $a$ ) for permanent grass (PG) vs. autumn tillage (AT) treatments in spring, summer and autumn for 2010, 2011 and 2012 sampling. Bars represent standard error of the mean ( $n=3$ ).



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

Soil characteristics <sup>1</sup>		
Bulk density	kg dm <sup>-3</sup>	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 <sub>c</sub> kg <sup>-1</sup>	17.67 ± 2.40
K <sub>ex</sub>	cmol <sub>c</sub> kg <sup>-1</sup>	0.78 ± 0.18
Mg <sub>ex</sub>	cmol <sub>c</sub> kg <sup>-1</sup>	1.80 ± 0.25
Ca <sub>ex</sub>	cmol <sub>c</sub> kg <sup>-1</sup>	19.35 ± 2.70
pH		7.8 ± 0.11
CaCO <sub>3</sub>	g kg <sup>-1</sup>	175 ± 10
OC	g kg <sup>-1</sup>	5.0 ± 1.65
Total N	g kg <sup>-1</sup>	0.51 ± 0.08
C/N		9.8 ± 0.9

<sup>1</sup>K<sub>ex</sub>, Ca<sub>ex</sub>, Mg<sub>ex</sub>: exchangeable K, Ca and Mg; OC: organic C

**Table 2: Organic C concentrations, percentages, and C/N ratio ( $\pm$  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)
fPOM (g C kg <sup>-1</sup> )	1.64 $\pm$ 0.46	2.77 $\pm$ 0.60	2.28 $\pm$ 0.24	2.23 $\pm$ 0.63	2.19 $\pm$ 0.29	2.46 $\pm$ 0.73	3.36 $\pm$ .65	2.67 $\pm$ 0.74
oPOM (g C kg <sup>-1</sup> )	0.75 $\pm$ 0.19 <b>b</b>	3.91 $\pm$ 0.96	3.34 $\pm$ 0.46	2.67 $\pm$ 1.56	3.02 $\pm$ 0.82 <b>a</b>	3.96 $\pm$ 0.69	3.66 $\pm$ 0.87	3.55 $\pm$ 0.81
MOM (g C kg <sup>-1</sup> )	13.44 $\pm$ 0.85	8.26 $\pm$ 2.01	10.07 $\pm$ 0.75	10.59 $\pm$ 2.55	9.58 $\pm$ 2.54	10.47 $\pm$ 0.88	8.83 $\pm$ 0.87	9.63 $\pm$ 1.58
fPOM (% OC)	10.32 $\pm$ 2.89	18.69 $\pm$ 4.72	14.52 $\pm$ 1.40 <b>b</b>	14.51 $\pm$ 4.62	15.03 $\pm$ 2.03	14.50 $\pm$ 3.93	21.23 $\pm$ 2.94 <b>a</b>	16.92 $\pm$ 4.19
oPOM (% OC)	4.75 $\pm$ 1.11 <b>b</b>	26.27 $\pm$ 6.76	21.32 $\pm$ 3.04	17.45 $\pm$ 10.46	20.51 $\pm$ 4.84 <b>a</b>	23.36 $\pm$ 3.11	22.91 $\pm$ 3.20	22.26 $\pm$ 3.55
MOM (% OC)	84.93 $\pm$ 1.85 <b>a</b>	55.04 $\pm$ 11.00	64.16 $\pm$ 4.29 <b>a</b>	68.04 $\pm$ 14.55	64.46 $\pm$ 6.52 <b>b</b>	62.14 $\pm$ 6.64	55.86 $\pm$ 1.99 <b>b</b>	60.82 $\pm$ 6.12
C/N fPOM	17.1 $\pm$ 2.2	17.0 $\pm$ 0.6	17.0 $\pm$ 1.7	17.0 $\pm$ 1.4	16.8 $\pm$ 1.3	16.1 $\pm$ 1.2	17.7 $\pm$ 0.2	16.9 $\pm$ 1.1
C/N oPOM	14.6 $\pm$ 0.9	13.6 $\pm$ 0.6	13.7 $\pm$ 0.2 <b>b</b>	14.0 $\pm$ 0.7	13.1 $\pm$ 0.4	13.5 $\pm$ 0.7	14.6 $\pm$ 0.2 <b>a</b>	13.7 $\pm$ 0.8
C/N MOM	12.2 $\pm$ 0.6 <b>a</b>	9.2 $\pm$ 0.9	8.4 $\pm$ 0.3	9.9 $\pm$ 1.8	10.3 $\pm$ 1.0 <b>b</b>	9.3 $\pm$ 1.4	8.7 $\pm$ 0.2	9.4 $\pm$ 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
<i>f</i> POM (g C kg <sup>-1</sup> )	0.028	NS	NS
<i>o</i> POM (g C kg <sup>-1</sup> )	0.001	0.024	0.040
MOM (g C kg <sup>-1</sup> )	0.047	NS	0.014
<i>f</i> POM (% OC)	0.038	NS	0.027
<i>o</i> POM (% OC)	0.001	0.028	0.005
MOM (% OC)	0.001	0.030	0.008
C/N <i>f</i> POM	NS	NS	NS
C/N <i>o</i> POM	NS	NS	0.009
C/N MOM	0.000	NS	NS

**Table 4: Data ( $\pm$  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 ( $\text{mm}^3$ )	196.42 $\pm$ 5.08	201.22 $\pm$ 7.66	203.58 $\pm$ 15.23	200.41 $\pm$ 9.44	196.44 $\pm$ 8.43	212.53 $\pm$ 14.61	196.92 $\pm$ 9.94	201.96 $\pm$ 12.60
Total porosity (%)	32.45 $\pm$ 0.76 <b>b</b>	34.10 $\pm$ 0.83	35.92 $\pm$ 2.99	34.16 $\pm$ 2.19	35.15 $\pm$ 1.19 <b>a</b>	34.74 $\pm$ 1.72	33.77 $\pm$ 0.92	34.55 $\pm$ 1.29
Aggregate density ( $\text{kg dm}^{-3}$ )	1.65 $\pm$ 0.08 <b>b</b>	1.70 $\pm$ 0.08	1.77 $\pm$ 0.11	1.71 $\pm$ 0.09	1.79 $\pm$ 0.02 <b>a</b>	1.64 $\pm$ 0.06	1.72 $\pm$ 0.04	1.71 $\pm$ 0.08
Micropores (30-5 $\mu\text{m}$ , %)	16.09 $\pm$ 3.32	15.45 $\pm$ 1.59	16.24 $\pm$ 0.61	15.93 $\pm$ 1.90	16.41 $\pm$ 1.32	15.34 $\pm$ 2.28	16.33 $\pm$ 0.40	16.03 $\pm$ 1.43
Ultramicropores (5-0.1 $\mu\text{m}$ , %)	67.51 $\pm$ 3.64	67.17 $\pm$ 2.05	68.60 $\pm$ 1.80	67.76 $\pm$ 2.36	67.99 $\pm$ 1.14	63.56 $\pm$ 6.40	68.89 $\pm$ 1.97	66.81 $\pm$ 4.20
Cryptopores (<0.1 $\mu\text{m}$ , %)	16.39 $\pm$ 0.37	17.34 $\pm$ 0.80	15.17 $\pm$ 2.33	16.30 $\pm$ 1.56	15.59 $\pm$ 1.94	21.10 $\pm$ 5.75	14.78 $\pm$ 1.61	17.16 $\pm$ 4.33
MRC ( $\mu\text{m}$ )	0.810 $\pm$ 0.331	1.184 $\pm$ 0.259 <b>a</b>	0.636 $\pm$ 0.319	0.877 $\pm$ 0.358	0.804 $\pm$ 0.235	0.650 $\pm$ 0.111 <b>b</b>	0.874 $\pm$ 0.236	0.776 $\pm$ 0.202
MVC ( $\text{mm}^3$ )	187.1 $\pm$ 4.8	182.0 $\pm$ 3.7	192.6 $\pm$ 17.1	187.3 $\pm$ 10.2	179.9 $\pm$ 7.8	180.9 $\pm$ 8.9	181.6 $\pm$ 8.6	180.8 $\pm$ 7.4
MRF ( $\mu\text{m}$ )	0.006 $\pm$ 0.001	0.013 $\pm$ 0.004	0.010 $\pm$ 0.001	0.010 $\pm$ 0.004 <b>b</b>	0.015 $\pm$ 0.005	0.026 $\pm$ 0.007	0.014 $\pm$ 0.008	0.018 $\pm$ 0.008 <b>a</b>
MVF ( $\text{mm}^3$ )	4.9 $\pm$ 0.9	13.7 $\pm$ 6.6	7.5 0 $\pm$ 3.8	8.7 $\pm$ 5.5	13.6 $\pm$ 5.6	20.8 $\pm$ 9.4	8.3 $\pm$ 1.0	14.2 $\pm$ 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 (MRF, 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 ( $\mu\text{m}$ )	NS	NS	NS
MVC ( $\text{mm}^3$ )	NS	NS	NS
MRF ( $\mu\text{m}$ )	0.022	0.005	NS
MVF ( $\text{mm}^3$ )	0.025	NS	NS

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