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Carbon and nitrogen in soil and vine roots in harrowed and grass-covered vineyards

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ABSTRACT

To examine the effects of vineyard soil management on soil C and N content and quality, we studied harrowed and grass-covered vineyards on a soil developed on plio-pleistocene, marine sediments. A soil naturally covered by grasses adjacent to the vineyards served as control. To reach this goal, we assessed (1) the distribution of C and N and their ^{13}C and ^{15}N signatures in different soil organic matter pools, (2) the amount of C and N as live and dead vine fine roots and their ^{13}C, ^{15}N and ^{14}C signatures, and (3) the stocks of C and N forms accumulated at two soil-depth intervals (0–50 and 50–100 cm).

Independent of the soil management, the vines increased the total organic C and total N content in the deeper soil horizons because of root turnover and rhizodeposition processes. In the upper horizons, a greater organic matter accumulation was fostered by the presence of the grass cover and the absence of tillage. The grass cover favoured the organic C storage mainly in the form of particulate and highly stabilised organic matter (humic acids and humin), and reduced the soil N content by plant uptake, whereas the harrowing produced a greater abundance of fulvic acids, which were mainly ascribed to oxidative processes enhanced by the soil tillage. In both vineyard soils, decaying vine roots represented an important source of organic C and N, especially in the deepest horizons. Indeed, isotope analyses revealed a more intense degradation of the dead vine roots in the deeper soil portion, where they likely constituted the main substrate for soil microorganisms. In the deepest horizons of the grass-covered vineyard, the greater mean residence time of the decaying vine roots and the lower root production were attributed to the easily available energetic substrates supplied by grass root turnover and rhizodeposition, which were preferentially used by microorganisms. This fact fostered a larger C accumulation in the grass-covered than in the harrowed vineyard.

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1. Introduction

Soil properties are directly linked up to soil organic matter (SOM) content and quality, which are controlled by climate, vegetation, soil type and management (e.g., Guo and Gifford, 2002; Seddaiu et al., 2013). In agro-ecosystems, practices such as crop choice, tillage and machinery, organic inputs, fertilisers and xenobiotics usage also affect the content and the characteristics of SOM (Campbell et al., 1999; Lal, 2004). The transition from traditional farming to intensive agriculture, coupled with the use of large amounts of chemical fertilizers, has led to a loss of SOM from cultivated soils (Miller et al., 2004). Over the last 150 years, the land use and land-cover changes contributed to about 33% of the total anthropogenic carbon emissions (Houghton, 1999), with a consequent worsening of soil fertility and quality (Lal, 2004) and a significant contribution to global warming (IPCC, 2000; Burney et al., 2010). The growing interest in the sustainable use of soil, environmental quality and long-term productivity of agro-ecosystems (UNFCCC, 2008) has led to increasing use of agricultural practices that favour the conservation or increase of SOM (Paustian et al., 1997; Lal, 2004).

While there are numerous studies dealing with the influence of agricultural practices on the content and dynamics of SOM in annual cropping systems (e.g., Doran, 2002; Tilman et al., 2002), little is known for perennial cropping systems (Carlisle et al., 2006), which can be defined as the cultivations that last for more than two

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growing seasons and whose economic life spans for several years. Perennial woody crops cover large areas worldwide. For example, in the twenty seven-European Union Member States about 23,778 km^2 are occupied by fruit and berry plantations, 41,375 km^2 by olive trees, and 33,103 km^2 by vineyards (Eurostat, 2008). Vineyards also cover large areas outside Europe, with about 33,000 km^2 in the year 2011; of these, 21.9% are in Asia and 20.9% in USA and Southern Hemisphere (OIV, 2012).

Considering the worldwide diffusion of grape cultivation, there is an increasing need for sustainable management practices increasing SOM levels and consequently soil fertility and functioning. In this regards, a great debate exists on which is the best vineyard soil management in order to obtain the finest grape quality and to reduce costs and ecosystem impact of vine cultivation (Ripoche et al., 2010; Guerra and Steenwerth, 2012). Tillage is considered the best way to conserve water and control weeds, although it may favour erosion and stimulate the oxidation of SOM following the disruption of soil aggregates (Balesdent et al., 2000; Steenwerth et al., 2010). In contrast, growing grass in the vineyard alleys reduces erosion in the hilly environments and favours the traffic of farm machinery (Corti et al., 2011), but increases competition for nutrients and water (Goulet et al., 2004). With respect to SOM, Goulet et al. (2004) observed an increase of the organic C in the topsoil of vineyards from Champagne (France) subjected to organic-mulch or bluegrass cover over 9 years. Ruiz-Colmenero et al. (2013), after 4 years, found an increase of soil organic C in a grass-covered vineyard located in the Henares River basin (Spain) and attributed the increase mostly to incorporation of vegetative residues and decomposition of grass roots. Conversely, in vineyard soils from a semi-arid Mediterranean environment (Sardinia, Italy), Seddaiu et al. (2013) found that, after 20 years, tillage and grass-cover have had a similar effect on the organic C content. From these reports one might argue that the influence of soil management on SOM storage in vineyard is site-specific. Further, as SOM is made of a mixture of heterogeneous compounds that have specific stabilisation mechanisms and turnover rates (von Lützow et al., 2007), its fractionation by physical and/or chemical methods into pools and their quantification and characterisation can help to better understand the dynamics of organic C in agro-ecosystems. Following a chemical fractionation of SOM, Seddaiu et al. (2013) assessed that low levels of humic acids and a low stabilisation degree of SOM in tilled vineyard was due to disturbances produced by harrowing and nitrogen fertilisation, while higher content of humin was accumulated in annual cropping systems with grass cover and reduced tillage. Yang et al. (2004) reported an increase of humic acids and humin in the presence of grass cover or rotation with clover or rye grass.

The aim of the study was to examine how soil management (harrowed or grass-covered) affected the different SOM pools in a vineyard soil derived from fine-textured plio-pleistocene marine sediments under Mediterranean climate (central Italy). Specifically, we tested the hypotheses that, with respect to harrowing, grass-cover (1) favours the accumulation of stabilised forms of SOM; (2) reduces vine root production; (3) enhances soil C and N storage. The above hypotheses were tested through the assessment of the distribution of C and N in different SOM pools (water extractable organic matter, humic and fulvic acids, humin) and their ^{13}C and ^{15}N isotopic signatures, the distribution of C and N as live and dead vine roots with their ^{13}C, ^{15}N and ^{14}C isotopic signatures, and the stock of organic C and N accumulated in two soil-depth intervals.

2. Materials and methods

2.1. Study site, soil morphology and soil sampling

The study was conducted in a vineyard of the experimental farm of the Polytechnic University of Marche located in Agugliano (Ancona, Italy) (Fig. 1). The mean annual precipitation of the area is 780 mm, concentrated during autumn and winter and with a summer drought; the mean annual air temperature is 13.3 °C, with July and August as the warmest months and January as the coldest one. The soil, developed from plio-pleistocene marine sediments, is subalkaline to alkaline (pH values range from 7.5 to 8.6) and is classified as fine-loamy, mixed, mesic, Vertic Haplustept (Soil Survey Staff, 2010).

The vineyard occupies an area of about 1 ha with South-Southeast exposure on a 5–6% slope. The soil was cropped with cereals for 60–70 years prior to the vineyard establishment. The vineyard was planted in 1993 with vines (*Vitis vinifera* L.) of the *cv* Montepulciano on a Kober 5BB rootstock after a breaking up (about 70 cm) of the soil. The distance between rows was established at 2.8 m. The vineyard was experimentally designed as differently managed randomised blocks, with the inter-rows harrowed or grass-covered. All plots received a fertilisation of about 30 kg ha^{-1} $year^{-1}$ of N in form of ammonium nitrate or urea only for the first 3 years after the vine planting. Later on, no fertilisation had been done. The harrowed plots were ploughed to 25–30 cm of depth for the first 3 years after the planting, but thereafter only superficial tillage (5–8 cm) was performed using disc, teeth harrow or small ploughs. The grass-covered plots were ploughed 25–30 cm depth for the first 3 years and then left to spontaneous colonisation of herbaceous species; the grass-covered alleys were mown twice per year and the herbage left in place. In both tilled and grass-covered alleys, a strip of soil of about 60 cm wide under the vine trunks was kept free of vegetation by 1–2 herbicide treatments per year with Glyphosate®.

As a control, we selected a site adjacent the vineyard (10 m from the border), naturally covered by grasses and that was part of the field cropped with cereals prior to vine planting. The control was mown twice per year and the herbage left in place.

Soil samples were collected during the early spring of 2003, when the vines were at the beginning of the bud break phase, namely when significant photosynthetic activity and rhizodeposition were limited. For each soil management (harrowed and grass-covered) two plots were considered and, in each plot, one soil trench was opened from row to row. These four trenches were at least 10 m apart. Two trenches were also opened in the control soil at about 18 m apart. The soil profiles were described (see Appendix I) according to Schoeneberger et al. (2002), and sampled by horizons in duplicate. Once in the laboratory, the soil samples were air-dried, sieved at 2 mm, and fine roots removed with the help of a magnifying lens.

2.2. Determination of C and N, and organic matter fractionation

The total organic C (TOC) content was estimated by the Springer and Klee method, and total N content was determined by a Carlo Erba EA1110 dry combustion analyzer.

The organic matter pools were extracted from the samples by sequential fractionation. Briefly, 100 g of sample was placed into a plastic container with water (solid:liquid ratio 1:10) and shaken overnight at room temperature. This allowed recovery of the free particulate organic matter (POM) and water extractable organic matter (WEOM) with minimum disturbance to the remaining organic pools (Jandl and Sollins, 1997; Ghani et al., 2003). The suspension was allowed to stand for 24 h, then the supernatant was collected by sieving at 53 μm. The coarser than 53 μm fraction was washed with water over the sieve, then washed with 0.5 M HCl to eliminate carbonates, washed again with water and then dried at 40 °C; this fraction represented the POM and consisted of large, undecomposed and partly decomposed root and plant fragments (Golchin et al., 1994). The solution used to wash the POM was added to the suspension passed through the 53 μm sieve. After

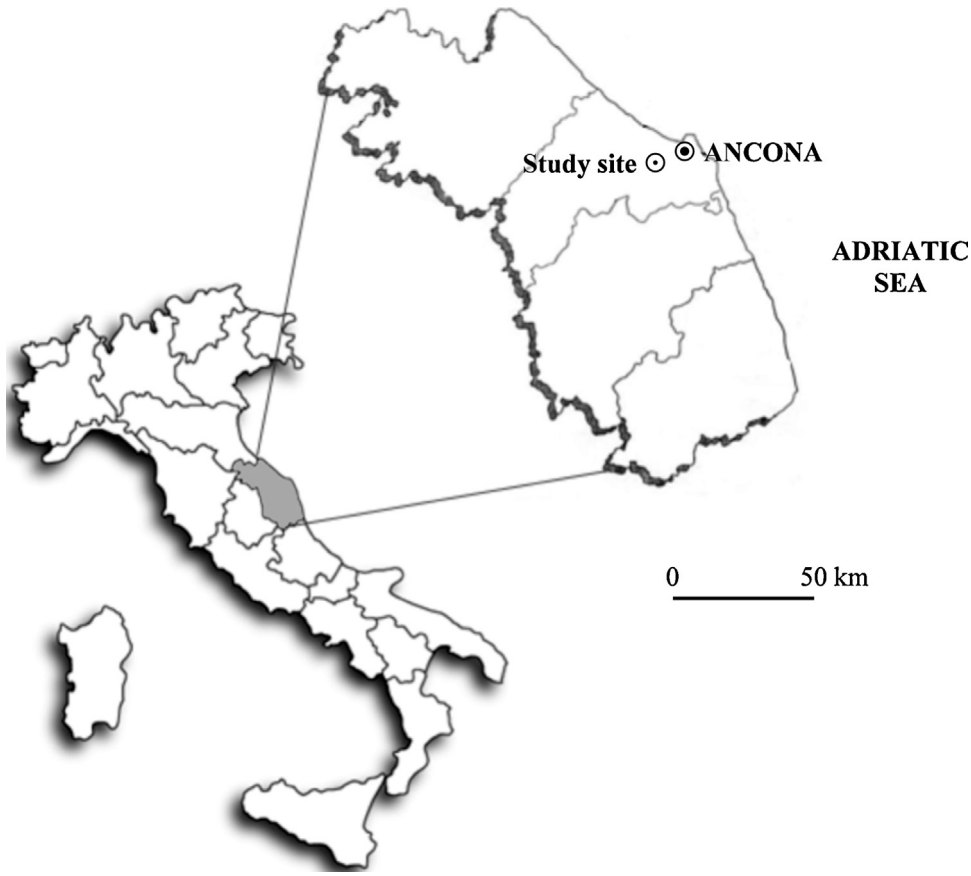

Fig. 1. Map of Italy with magnification of the Marche region and indication of the study site.

sedimentation, the supernatant was collected, acidified to pH 3.5 with 0.5 M HCl to eliminate inorganic C, filtered through Whatman 42 filter paper and freeze-dried. This fraction represented the WEOM, namely the more mobile and labile organic matter pool. Both POM and WEOM fractions were weighed and their C and N content was determined by a Carlo Erba EA1110 dry combustion analyzer. Humic and fulvic acids were extracted and purified according the IHSS procedure (Swift, 1996). The humic and fulvic fractions, once freeze-dried, were weighed and analysed for their C and N content by dry combustion analyzer. The extraction residue containing the non-extractable organic matter (NEOM) was washed several times with a 0.5 M HCl and freeze-dried; on this residue, the C and N content was also determined by dry combustion.

During the organic matter fractionation, some losses occurred (about 4–9% of the TOC) probably during the purification of the humic and fulvic acids.

2.3. Root sampling

A second set of soil samples was collected in duplicate from each horizon by cylindrical steel cores of a known volume (502.6 mL) to retrieve vine roots. The only exceptions were the Ap horizons, where the samples were collected by a volume basis according to the irregular hole method (Blake and Hartge, 1986). Within 2 days from the sampling, vine roots were isolated from each sample by immersion in water and gentle shaking. Once isolated from the soil, the fine vine roots (diameter < 2 mm) were separated into "live roots" and "dead roots" (at the moment of sampling) by using tweezers under a magnifying lens. Finally, the roots were washed with 0.05 M HCl solution, dried at 105 °C and weighed. A representative aliquot of roots was subdivided into two size classes (1–2 mm and <1 mm in diameter) and analysed for radiocarbon natural abundance; the rest of the <2 mm roots was powdered under liquid nitrogen and analysed for their natural abundance of ^{13}C and ^{15}N.

2.4. Isotopic analyses

Aliquots of WEOM, humic acids, fulvic acids, NEOM, and vine roots were analysed for evaluating $\delta^{13}C$ and $\delta^{15}N$ content by using a Carlo Erba NA2000 analyser (CE Instruments, Wigan, UK) coupled with a SerCon 20–22 isotope ratio mass spectrometer (SerCon Ltd., Crewe, UK) at Rothamsted Research – North Wyke (UK). Wheat flour (1.91% N, 41.81% C, 4.80 $\delta^{15}N$ and −26.1 $\delta^{13}C$), calibrated against IAEA-N-1 by Iso-Analytical (Crewe, UK), was used as a reference standard and analysed after every ten samples. This resulted in an analytical precision of 0.1% and 0.2% of the $\delta^{13}C$ and $\delta^{15}N$ units obtained, respectively.

The radiocarbon (^{14}C) content in the vine roots was determined at the Accelerator Mass Spectrometry Laboratory at the Max-Planck Institute for Biogeochemistry in Jena, Germany. Radiocarbon data are expressed in $\Delta^{14}C$, the ‰ deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950 (Modern) corrected to a common $\delta^{13}C$ of −25‰, which removes the influence of mass dependent isotopic fractionation effects (Stuiver and Polach, 1977). On the basis of the ^{14}C values, the mean residence time (MRT) of the roots was calculated according to the model of Trumbore et al. (1996) and Gaudinski et al. (2000), using the radiocarbon atmospheric data reported by Levin and Kromer (2004) and Xu and Levin (personal communication).

By assuming that the fine root pool was at the steady state, root production equals the rate of loss of the dead roots. Hence, according to Gaudinski et al. (2001), it was possible to estimate the root production (g C_{root} m^{-2} $year^{-1}$) as:

$$\text{root production} = \text{dead root biomass}\, C \cdot k,$$

where k is the decomposition rate, which can be obtained by 1/MRT.

2.5. Calculation of the organic C and N stocks

The stocks of organic C and N fractions accumulated in each horizon were calculated taking into consideration its C and N concentration, bulk density and thickness by:

$$\text{Stock}\ (\text{kg m}^{-2}) = [\text{concentration}\ (\text{g kg}^{-1}) \cdot \text{bulk density}\ (\text{kg dm}^{-3}) \cdot \text{thickness}\ (\text{m})]$$

To compute the C and N stored in the 0–50 and 50–100 cm soil depth intervals it was sufficient to add the stock of the various horizons (or a portion of it when the horizon thickness crossed the 50 or the 100 cm) to the required depth.

2.6. Replicates and statistics

For each trench, the two results obtained for every single horizon were averaged. In the tables, the values reported for each horizon were the means of the 2 averages obtained from the two trenches belonging to the same soil management, and the standard deviation was calculated accordingly ($n = 2$). For the isotopic measurements of WEOM, humic acids, fulvic acids, NEOM and roots one single analysis was performed on a composite sample obtained by mixing the separates recovered from the 4 samples collected from each horizon.

After evaluation of the normal distribution of the data, statistical analyses to compare differences among data-sets were performed by the two-way ANOVA with Fisher's LSD test by using XLSTAT software. The trends with soil depth were evaluated by the linear trend post-test (GraphPad Instat 3.1 software).

3. Results

3.1. Total and particulate organic C and total N

The TOC concentration decreased with depth ($P < 0.0001$) in the control (CTR) and the harrowed vineyard soil (HV), while the grass-covered vineyard soil (GCV) showed an increase below the BC1 horizon (Fig. 2). Harrowing produces lower TOC contents than grass-covering (both GCV and CTR) in the Ap, Bw1 and Bw2 horizons ($P < 0.05$), while in the BC horizons the vine cultivation increases the TOC level ($P < 0.05$). It is noteworthy that in these lower horizons TOC concentration of GCV was more than twice that of CTR. The POC content, ranging from 0.1 to 4.8 g kg^{-1}, was similar in the Ap horizons, while for the Bw1 horizons the largest quantity was found in GCV (Fig. 2). The highest POC concentration was in the Bw2 and Bw3 horizons of CTR where it represented about 40 and 28% of the TOC, respectively. The largest TOC content observed in the BC horizons of the vineyards was partly due to an increase of POC with respect to CTR.

The total N content tended to gradually decrease with depth in the vineyard soils, while in CTR it showed a sharp decrease from Ap to Bw horizons (Fig. 2). With the exception of the Ap horizon, HV showed the highest amounts of total N ($P < 0.05$) throughout the soil.

In summary therefore: (i) grass-covering of the vineyard increases organic matter content all throughout the soil; (ii) vine cultivation increases POM content at depth; (iii) vine cultivation, especially with harrowing, increases N content in the sub-superficial horizons.

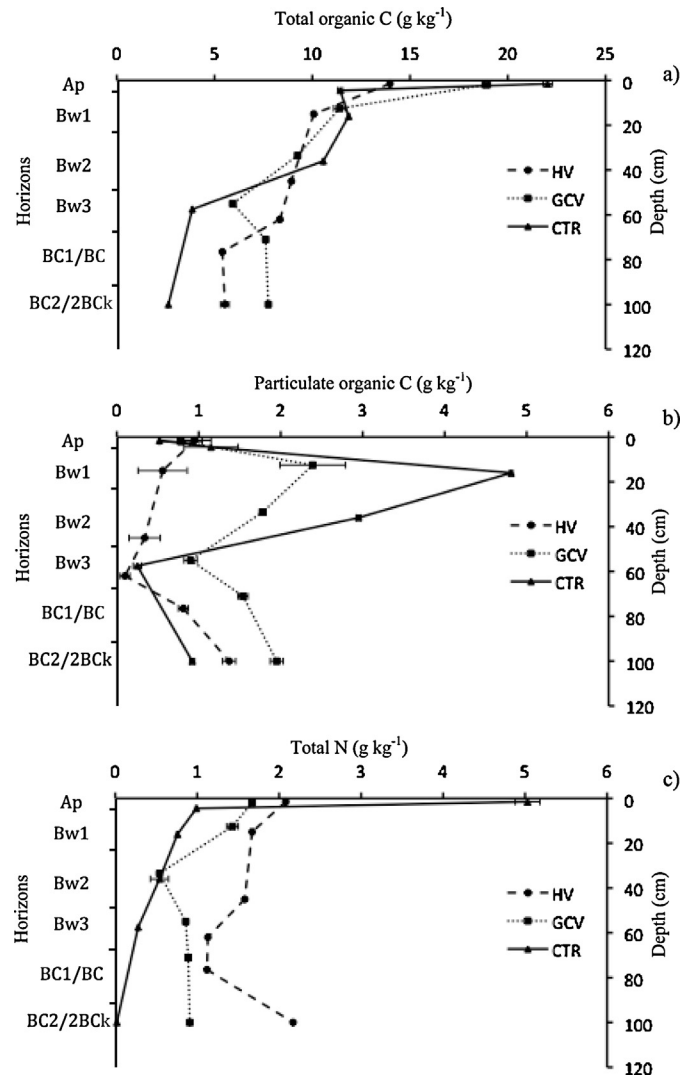

Fig. 2. Contents of (a) total organic C, (b) particulate organic C, and (c) total N of the samples collected from the harrowed and grass-covered vineyard soils and from the control soil. Agugliano experimental farm (Ancona, Italy). Error bars are the standard deviations ($n = 2$).

3.2. Forms of organic C and their $\delta^{13}C$ signature

The organic C content as WEOM (WEOC) decreased with depth in all profiles ($P < 0.02$), showing the highest concentrations in the Ap horizon of CTR and in the Bw horizons of HV and GCV (Table 1).

The C present as humic acids (HA-C) showed different distribution in the three soils (Table 1). The HA-C contents of the Ap horizons from GCV and CTR were similar but markedly higher than that of HV. For the Bw horizons, the highest content of HA-C was in CTR, while no significant difference was detected between the two vineyards. The lowest amount of HA-C was found in the 2BCk horizon of CTR. In general HA-C decreased with depth for all the soils ($P < 0.01$).

The amount of C present as fulvic acids (FA-C) was more abundant in HV than in the other two soils (Table 1). Both GCV and CTR had generally similar FA-C concentrations down the profiles, except for Ap, Bw1 and Bw2 horizons where some difference occurred. The

Table 1
Amount of water extractable organic carbon (WEOC), humic and fulvic acids carbon (HA-C and FA-C), non-extractable organic carbon (NEOC), and their relative ^{13}C signatures for the vineyard (harrowed and grass-covered) and control soils. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 2$).

	WEOC		HA-C		FA-C		NEOC	
	g C kg^{-1} soil	$\delta^{13}C$ (‰)	g C kg^{-1} soil	$\delta^{13}C$ (‰)	g C kg^{-1} soil	$\delta^{13}C$ (‰)	g C kg^{-1} soil	$\delta^{13}C$ (‰)
Harrowed								
Ap	0.17 (0.04)^{b}	−26.4	0.52 (0.01)^{def}	−27.6	5.56 (0.06)^{a}	−27.9	5.91 (0.02)^{e}	−24.2
Bw1	0.14 (0.04)^{cd}	−26.0	0.46 (0.01)^{efg}	−26.4	3.71 (0.01)^{c}	−25.4	4.32 (0.17)^{h}	−23.6
Bw2	0.13 (0.04)^{e}	−22.0	0.32 (0.00)^{fgh}	−26.1	3.87 (0.09)^{b}	−25.5	3.60 (0.14)^{i}	−23.2
Bw3	0.13 (0.00)^{ef}	−21.5	0.60 (0.06)^{de}	−25.1	3.20 (0.12)^{d}	−25.0	3.88 (0.02)^{i}	−22.8
BC1	0.14 (0.04)^{cd}	−20.7	0.24 (0.00)^{gh}	−24.9	0.84 (0.00)^{g}	−24.5	2.98 (0.01)^{j}	−23.2
BC2	0.08 (0.07)^{k}	−21.4	0.14 (0.08)^{h}	−24.7	0.53 (0.02)^{i}	−24.0	3.04 (0.19)^{j}	−23.0
Grass-covered								
Ap	0.14 (0.04)^{c}	−28.0	3.87 (0.01)^{a}	−27.9	1.73 (0.08)^{e}	−26.3	11.11 (0.10)^{b}	−26.4
Bw1	0.14 (0.02)^{cd}	−27.4	0.70 (0.07)^{de}	−26.5	0.36 (0.08)^{j}	−26.2	7.11 (0.50)^{c}	−25.2
Bw2	0.13 (0.07)^{de}	−27.0	0.59 (0.02)^{def}	−26.3	0.64 (0.10)^{hi}	−25.6	5.67 (0.05)^{ef}	−25.1
Bw3	0.12 (0.04)^{fg}	−27.1	0.49 (0.01)^{defg}	−25.3	0.16 (0.04)^{kl}	−23.9	3.92 (0.14)^{i}	−23.7
BC1	0.11 (0.04)^{gh}	−26.5	0.75 (0.05)^{d}	−25.2	0.20 (0.11)^{kl}	−23.2	4.64 (0.04)^{gh}	−23.3
BC2	0.10 (0.03)^{j}	−27.2	0.60 (0.05)^{de}	−25.1	0.15 (0.06)^{kl}	−23.7	4.55 (0.03)^{gh}	−23.2
Control								
Ap	0.33 (0.07)^{a}	−29.5	4.13 (0.49)^{a}	−28.7	1.40 (0.03)^{f}	−28.4	14.20 (0.29)^{a}	−26.2
Bw1	0.10 (0.04)^{j}	−27.7	1.98 (0.03)^{b}	−27.0	0.62 (0.00)^{hi}	−27.1	6.48 (0.14)^{d}	−23.2
Bw2	0.11 (0.04)^{gh}	−27.6	1.13 (0.13)^{c}	−26.1	0.28 (0.10)^{jk}	−26.0	4.75 (0.10)^{g}	−22.3
Bw3	0.08 (0.04)^{k}	−27.1	1.04 (0.13)^{c}	−25.8	0.17 (0.00)^{kl}	−25.6	5.50 (0.07)^{f}	−21.9
BC	0.10 (0.04)^{ij}	−27.7	0.53 (0.08)^{def}	−25.3	0.24 (0.00)^{jk}	−25.3	2.44 (0.01)^{k}	−22.0
2BCk	0.11 (0.04)^{hi}	−22.3	0.10 (0.01)^{h}	−25.0	0.08 (0.01)^{l}	−25.4	1.22 (0.01)^{l}	−23.6

For each column, mean values with different letters significantly differ for $P < 0.05$.

FA-C evenly decreased with depth in HV, while a sharp decrease occurred from the Ap to the Bw1 horizon of GCV and CTR.

As observed with TOC, in the Ap, Bw1 and Bw2 horizons harrowing gave rise to reduce C as NEOM (NEOC) than the green-covering, whereas for the BC horizons the concentration of NEOC was higher in the vineyards. NEOC was the greatest part of the humic C pool in the GCV and CTR soils, while it was comparable to FA-C in the Ap and Bw horizons of HV.

The $\delta^{13}C$ signature of WEOC in CTR (Table 1) increased from Ap to Bw1 horizon (−29.5‰ and −27.1‰, respectively), had a rather constant values until the BC horizon (−27.7‰), and was followed by a steep increase in the 2BCk horizon (−22.3‰). In HV, the $\delta^{13}C$ of WEOC markedly raised from Bw1 (−26.0‰) to Bw2 (−22.0‰) horizons, and slightly increased in the BC1 and BC2 horizons (−20.7‰ and −21.4‰, respectively). These values were the highest among all the fractions. The WEOC $\delta^{13}C$ showed small variations throughout GCV profile, ranging between −26.5‰ and −28.0‰. The $\delta^{13}C$ values of both humic and fulvic acids generally increased by 3–4‰ from the Ap to the BC horizons of the three soils. In the vineyard soils, the $\delta^{13}C$ of the fulvic acids was generally higher than that of the humic acids, with the highest ^{13}C content in the Bw3 and BC horizons of GCV. The $\delta^{13}C$ values of NEOC were up to 4‰ higher than those of humic and fulvic acids.

In summary therefore: (i) grass-covering preferentially produces greater abundance of HA and NEOM, whereas harrowing fosters the formation of FA; (ii) in the two vineyards, the $\delta^{13}C$ tends to increase from HA to FA to NEOM; (iii) harrowing produces a WEOM with a higher content of ^{13}C than the respective humic and fulvic acids and, in the subsoil, NEOM.

3.3. Forms of N and their $\delta^{15}N$ signature

The amount of water extractable N (WEN) was very low and similar for the three soils, and generally diminished with depth (Table 2). The N associated to humic acids (HA-N) showed the largest concentration in the Ap and Bw horizons of CTR. This form of N displayed no significant difference between the two vineyard soils, with the exception of the Ap horizon of GCV, which had the highest amount (0.33 g kg^{-1}). The N content as fulvic acids (FA-N) was higher in HV than in the other two soils. The non-extractable N (NEN) of the Ap horizons was abundant in CTR, followed by HV and then GCV (Table 2). In the sub-superficial horizons HV had generally the highest NEN contents, while CTR showed the lowest ones.

Lower $\delta^{15}N$ values of WEN were obtained in GCV than HV and CTR for the Ap, Bw1 and Bw2 horizons (Table 2). In the deeper horizons the $\delta^{15}N$ was generally higher in the two vineyard soils than in CTR. The $\delta^{15}N$ of HA-N, FA-N and NEN tended to increase with increasing depth in all the soils.

In summary therefore: (i) harrowing produces higher FA-N than grass-covering; (ii) vine cultivation promotes the formation of NEN at depth.

3.4. Live and dead fine roots, their C and N content, $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$ signatures

Living vine root biomass was higher throughout the soil in HV compared to GCV (Table 3). For both vineyard soils, the highest concentration of live roots was found in the Bw2 horizon. Also the largest amount of dead vine roots (Table 4) was recovered from the Bw2 horizon of both soils, with greater concentration in GCV than in HV (52.5 and 42.9 mg kg^{-1} soil, respectively).

The C and N content of live and dead roots (Tables 3 and 4) tended to decrease with increasing depth ($P < 0.05$). In all the horizons, dead roots had a greater C and N concentration than the live ones ($P < 0.01$), except for the BC2 horizon of GCV and Bw2 and BC1 horizons of HV.

In both vineyard soils, live roots showed $\delta^{13}C$ values ranging from −28.5‰ to −25.7‰ (Table 3), while dead roots had $\delta^{13}C$ values ranging from −29.1‰ to −25.7‰ (Table 4), with a significant increase with depth ($P < 0.001$). Live roots $\delta^{15}N$ values ranged from 0.9‰ to 3.2‰ in both soils, with no trend with depth. The $\delta^{15}N$ values of dead roots ranged from 1.8‰ to 5.9‰, increasing with depth in both HV and GCV ($P < 0.05$). The $\Delta^{14}C$ values of the live and dead roots were all positive (Tables 3 and 4). In both soils, the live roots had an estimated MRT (that in this case can be roughly considered as age) of less than 1 year (Table 3), whereas the dead roots ranged

Table 2
Amount of water extractable nitrogen (WEN), humic and fulvic acids nitrogen (HA-N and FA-N), and non-extractable nitrogen (NEN), and their relative ^{15}N signatures, for the vineyard (harrowed and grass-covered) and control soils. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 2$).

	WEN		HA-N		FA-N		NEN	
	g N kg^{-1} soil	$\delta^{15}N$ (‰)	g N kg^{-1} soil	$\delta^{15}N$ (‰)	g N kg^{-1} soil	$\delta^{15}N$ (‰)	g N kg^{-1} soil	$\delta^{15}N$ (‰)
Harrowed								
Ap	0.06 (0.01)^{b}	4.6	0.07 (0.00)^{e}	4.1	0.49 (0.01)^{a}	4.0	1.35 (0.05)^{c}	4.9
Bw1	0.05 (0.00)^{c}	6.0	0.04 (0.02)^{fg}	5.7	0.15 (0.00)^{d}	4.3	1.30 (0.01)^{c}	4.7
Bw2	0.03 (0.00)^{e}	5.6	0.04 (0.00)^{fg}	6.1	0.23 (0.02)^{b}	5.0	1.17 (0.01)^{de}	5.5
Bw3	0.02 (0.00)^{f}	6.2	0.04 (0.00)^{fg}	7.9	0.09 (0.00)^{f}	6.4	0.93 (0.04)^{f}	6.3
BC1	0.04 (0.00)^{d}	4.5	0.01 (0.00)^{h}	8.1	0.03 (0.00)^{hij}	7.6	0.97 (0.01)^{f}	5.8
BC2	0.01 (0.00)^{h}	4.9	0.01 (0.00)^{h}	8.2	0.07 (0.00)^{g}	9.2	1.94 (0.01)^{b}	5.9
Grass-covered								
Ap	0.06 (0.01)^{b}	1.6	0.33 (0.01)^{b}	3.1	0.10 (0.00)^{e}	3.6	1.11 (0.01)^{e}	4.0
Bw1	0.03 (0.00)^{e}	2.3	0.04 (0.00)^{fg}	4.6	0.02 (0.01)^{ijkl}	5.1	1.27 (0.06)^{cd}	4.4
Bw2	0.02 (0.00)^{f}	2.7	0.03 (0.00)^{g}	5.0	0.03 (0.01)^{hi}	5.2	0.44 (0.07)^{i}	4.9
Bw3	0.01 (0.00)^{h}	6.2	0.03 (0.00)^{g}	7.6	0.01 (0.00)^{lm}	7.4	0.76 (0.01)^{g}	6.4
BC1	0.01 (0.00)^{h}	6.1	0.04 (0.00)^{fg}	7.5	0.01 (0.00)^{kl}	7.7	0.79 (0.01)^{g}	6.5
BC2	0.01 (0.00)^{h}	4.4	0.05 (0.00)^{f}	7.7	0.01 (0.00)^{kl}	8.0	0.79 (0.00)^{g}	5.6
Control								
Ap	0.07 (0.01)^{a}	4.2	0.69 (0.02)^{a}	1.9	0.17 (0.00)^{c}	2.2	3.83 (0.13)^{a}	1.9
Bw1	0.01 (0.00)^{h}	5.0	0.13 (0.01)^{c}	6.7	0.04 (0.00)^{h}	5.5	0.74 (0.01)^{g}	4.2
Bw2	0.01 (0.00)^{h}	4.8	0.09 (0.01)^{d}	5.9	0.02 (0.01)^{jkl}	5.9	0.59 (0.02)^{h}	4.4
Bw3	0.02 (0.00)^{fg}	4.5	0.08 (0.00)^{de}	5.9	0.02 (0.00)^{ijk}	5.9	0.38 (0.11)^{i}	5.2
BC	0.01 (0.00)^{h}	1.9	0.04 (0.00)^{fg}	6.4	0.01 (0.00)^{kl}	7.9	0.20 (0.01)^{j}	4.8
2BCk	<0.01 (–)	3.9	<0.01 (–)	6.1	<0.01 (–)	7.4	<0.01 (–)	4.8

For each column, mean values with different letters significantly differ for $P < 0.05$.

from 0.7 to 7.4 years, with the 1–2 mm roots older than the <1 mm ones (Table 4). From the MRT, it appeared that the dead roots of the deepest horizons of GCV remained longer than those of HV. The root production estimated for the <1 mm roots was greater in HV than in GCV all throughout the soil, with the only exception of the Bw1 horizon (Table 5). For the 1–2 mm roots, the major production was in the Bw2 horizon of HV and Bw1 of GCV. The <1 mm root production increased with depth from the Bw3 to the BC2 horizons for both HV and GCV, although a less marked trend was observed for the latter.

In summary therefore: (i) harrowing promotes a greater living vine root biomass than grass-covering; (ii) dead vine roots have greater C and N concentration than the live ones; (iii) $\delta^{13}C$ and $\delta^{15}N$ of the dead roots increase with depth; (iv) in the deepest horizons, dead roots of GCV have longer turnover time than those of HV; (v) harrowing induces a greater root production than grass-covering.

3.5. Stocks of C and N forms

In the upper 50 cm of soil, vine cultivation seemed to have reduced the TOC content (Table 6) with respect to CTR (9.15 kg m^{-2}), with a higher value in GCV than HV (8.32 and 7.52 kg m^{-2}, respectively). In contrast, in the underlying 50–100 cm, the two vineyard soils had more than twice the TOC content of CTR (2.24 kg m^{-2}), with higher concentration in GCV (5.75 kg m^{-2}) than in HV (5.14 kg m^{-2}). For both depth intervals, the TOC stock was mainly comprised of NEOC, which was found in largest amount in GCV, where it accounted for about 61% of the TOC in the whole soil (Table 6). The harrowing produced the

Table 3
Amount of the live vine roots with a less than 2 mm diameter, their elemental composition (C and N), ^{13}C, ^{15}N, ^{14}C signatures, and mean residence time (MRT) for the harrowed and grass-covered vineyards. Agugliano experimental farm (Ancona, Italy). For the radiocarbon measurements the roots were distinctly analysed according to their diameter. Numbers in parentheses are the standard deviations ($n = 2$).

	<2 mm vine live roots								
	Amount	Elemental composition		$\delta^{13}C$	$\delta^{15}N$	$\Delta^{14}C$		MRT	
		C	N			<1 mm	1–2 mm	<1 mm	1–2 mm
	mg kg^{-1} soil	mg g^{-1}		‰		‰		Years	
Harrowed									
Ap	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bw1	46.0 (0.5)^{c}	330.2 (1.2)^{a}	15.1 (0.6)^{b}	−28.2	2.0	55.6	53.8	<1	<1
Bw2	91.1 (0.7)^{a}	218.6 (1.1)^{g}	10.4 (0.5)^{f}	−27.0	0.9	n.d.	n.d.	n.d.	n.d.
Bw3	35.5 (2.3)^{d}	204.2 (2.6)^{h}	8.0 (0.4)^{gh}	−28.4	2.7	n.d.	n.d.	n.d.	n.d.
BC1	21.9 (2.2)^{e}	316.3 (2.7)^{b}	8.8 (0.5)^{g}	−28.5	1.3	n.d.	n.d.	n.d.	n.d.
BC2	17.8 (1.0)^{f}	265.1 (1.6)^{c}	13.5 (0.5)^{c}	−26.6	2.6	n.d.	n.d.	n.d.	n.d.
Grass-covered									
Ap	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d
Bw1	19.3 (2.3)^{f}	267.0 (2.0)^{c}	11.6 (0.32)^{e}	−28.3	2.1	63.7	58.8	<1	<1
Bw2	72.6 (1.3)^{b}	251.1 (2.1)^{d}	17.0 (0.7)^{a}	−25.7	3.0	60.4	58.3	<1	<1
Bw3	4.9 (1.5)^{h}	253.0 (3.4)^{d}	12.8 (0.2)^{d}	−26.5	2.1	57.1	n.d.	<1	n.d.
BC1	7.6 (0.7)^{g}	236.7 (2.5)^{e}	11.3 (0.2)^{e}	−27.7	2.9	n.d.	n.d.	n.d.	n.d.
BC2	8.9 (3.4)^{g}	220.4 (1.8)^{f}	8.4 (0.6)^{g}	−28.4	3.2	n.d.	n.d.	n.d.	n.d.

In each column, mean values with different letters significantly differ for $P < 0.05$.
n.d.: not determined.

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Table 4
Amount of the dead vine roots with a less than 2 mm diameter, their elemental composition (C and N), ^{13}C, ^{15}N, ^{14}C signatures, and mean residence time (MRT) for the harrowed and grass-covered vineyards. Agugliano experimental farm (Ancona, Italy). For the radiocarbon measurements the roots were distinctly analysed according to their diameter. Numbers in parentheses are the standard deviations ($n = 2$).

	<2 mm vine dead roots								
	Amount	Elemental composition		$\delta^{13}C$	$\delta^{15}N$	$\Delta^{14}C$		MRT	
		C	N			<1 mm	1–2 mm	<1 mm	1–2 mm
	mg kg^{-1} soil	mg g^{-1}		‰		‰		Years	
Harrowed									
Ap	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bw1	17.9 (1.0)^{g}	376.4 (4.0)^{b}	18.6 (1.0)^{a}	−29.1	1.8	77.3	80.7	2.8	3.6
Bw2	42.9 (1.7)^{b}	383.6 (3.9)^{a}	17.2 (1.6)^{ab}	−27.7	3.8	78.5	84.1	3.1	4.3
Bw3	20.9 (1.5)^{f}	333.5 (3.2)^{ef}	12.4 (0.6)^{de}	−27.8	3.7	74.8	100.4	2.0	6.7
BC1	32.9 (3.0)^{d}	340.5 (7.3)^{e}	12.9 (1.1)^{de}	−27.1	4.6	83.9	91.8	4.2	5.5
BC2	26.6 (6.8)^{e}	324.7 (5.0)^{g}	13.4 (1.0)^{d}	−25.7	4.3	73.7	83.6	1.7	4.2
Grass-covered									
Ap	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bw1	9.5 (3.2)^{h}	364.1 (4.3)^{c}	19.6 (1.0)^{a}	−28.4	2.5	80.1	85.6	3.4	4.5
Bw2	52.5 (2.4)^{a}	269.6 (3.4)^{h}	15.5 (1.0)^{c}	−27.1	3.3	65.6	82.5	0.7	4.0
Bw3	10.4 (0.8)^{h}	348.8 (4.2)^{d}	15.0 (1.1)^{c}	−27.2	4.5	91.8	99.7	5.5	6.6
BC1	22.9 (3.0)^{f}	258.8 (5.3)^{i}	9.1 (0.8)^{f}	−27.7	4.6	96.6	106.1	6.2	7.4
BC2	36.2 (3.1)^{c}	322.2 (4.2)^{g}	13.8 (1.6)^{d}	−25.8	5.9	87.3	98.6	4.8	6.5

In each column, mean values with different letters significantly differ for $P < 0.05$.
n.d.: not determined.

Table 5
Estimation of the root production according to their diameter (<1 mm and 1–2 mm) for the harrowed and grass-covered vineyards. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 2$).

Horizons	Vine root production	
	<1 mm	1–2 mm
	g C_{root} m^{-2} year^{-1}	
Harrowed		
Ap	n.d.	n.d.
Bw1	783.6 (37.1)^{e}	143.0 (6.8)^{d}
Bw2	1907.4 (67.3)^{a}	458.4 (16.2)^{a}
Bw3	148.1 (8.9)^{g}	19.0 (1.1)^{e}
BC1	769.4 (5.9)^{e}	251.8 (9.7)^{b}
BC2	1335.7 (22.2)^{c}	220.8 (3.7)^{c}
Grass-covered		
Ap	n.d.	n.d.
Bw1	1089.0 (28.0)^{d}	443.0 (11.4)^{a}
Bw2	1667.6 (92.9)^{b}	143.7 (9.3)^{d}
Bw3	43.9 (6.7)^{h}	18.0 (0.2)^{e}
BC1	551.2 (15.1)^{f}	136.6 (3.4)^{d}
BC2	711.2 (17.5)^{e}	139.6 (3.4)^{d}

In each column, mean values with different letters significantly differ for $P < 0.05$.

largest proportion of FA-C, which constituted about 39 and 24% of the TOC in the upper and lower soil thickness, respectively. The HA-C stock was the largest in the upper 50 cm of CTR and in the 50–100 cm portion of GCV. The proportion of HA-C with respect to the TOC did not vary in the two depth intervals of the vineyard soils (about 4.1% for HV and 8.4–8.5% for GCV), while it decreased in the deeper part of CTR (from about 11.5% in the upper layer to 8.0% in the lower layer). The grass-covering (CTR and GCV) produced the largest stocks of POC in the upper layer and, for both soil portions, the highest percentage of POC referred to TOC (Table 6). Further, in the vineyard soils the POC contribution increased from the upper to the lower layer, while the contrary was true in CTR. The amount of WEOC was very small and similar in all soils.

In the upper 50 cm of soil (Table 7), harrowing produced the greatest total N stock (1.27 kg m^{-2}), whereas grass-covered soils stored a similar amount of it (0.76 kg m^{-2}). In the deeper layer, the storage of total N showed only a slight decrease for both the vineyard soils, but reached a very low value in CTR (0.07 kg m^{-2}). For the three soils, the most common N form was NEN, which accounted for about 84% and 87% of the total N in the lower layer of HV and GCV, respectively. For HV, the FA-N represented a great proportion of the total N stored in the 0–50 cm, much higher than that of GCV (12.5% and 3.9%, respectively). The control soil displayed the largest abundance of HA-N in the 0–50 cm layer (0.10 kg m^{-2}), followed by GCV and HV.

In summary therefore: (i) vineyard promotes organic matter, POM and total N storage at depth; (ii) grass-covering produces a larger stock of organic matter, mainly as HA and NEOM, than harrowing; (iii) harrowing produces a greater stock of N than grass-covering.

Table 6
Content of total organic C (TOC), particulate organic C (POC), water extractable organic C (WEOC), humic acids C (HA-C), fulvic acids C (FA-C), and not-extractable organic C (NEOC) in the 0–50 and 50–100 cm depth intervals for the vineyard (harrowed and grass-covered) and control soils. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 2$).

	TOC (kg m^{-2})	POC (kg m^{-2})	WEOC (kg m^{-2})	HA-C (kg m^{-2})	FA-C (kg m^{-2})	NEOC (kg m^{-2})
0–50 cm						
Harrowed	7.52 (0.07)^{c}	0.37 (0.18)^{e}	0.10 (0.01)^{a}	0.31 (0.02)^{d}	2.97 (0.05)^{a}	3.15 (0.14)^{d}
Grass-covered	8.32 (0.01)^{b}	1.45 (0.12)^{b}	0.11 (0.00)^{a}	0.70 (0.03)^{b}	0.47 (0.07)^{c}	5.12 (0.18)^{a}
Control	9.15 (0.02)^{a}	2.57 (0.04)^{a}	0.09 (0.0)^{b}	1.05 (0.01)^{a}	0.27 (0.04)^{d}	4.50 (0.02)^{b}
50–100 cm						
Harrowed	5.14 (0.03)^{e}	0.64 (0.02)^{d}	0.10 (0.00)^{a}	0.21 (0.02)^{e}	1.25 (0.01)^{b}	2.58 (0.12)^{e}
Grass-covered	5.75 (0.02)^{d}	1.22 (0.04)^{c}	0.09 (0.00)^{b}	0.49 (0.02)^{c}	0.13 (0.03)^{e}	3.51 (0.10)^{c}
Control	2.24 (0.01)^{f}	0.58 (0.02)^{de}	0.09 (0.00)^{b}	0.18 (0.02)^{e}	0.10 (0.01)^{e}	1.27 (0.06)^{f}

In each column, mean values with different letters significantly differ for $P < 0.05$.

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Table 7
Content of total N, water extractable nitrogen (WEN), humic acids N (HA-N), fulvic acids N (FA-N), and not-extractable N (NEN) in the 0–50 and 50–100 cm depth intervals for the vineyard (harrowed and grass-covered) and control soils. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 2$).

	Total N (kg m^{-2})	WEN (kg m^{-2})	HA-N (kg m^{-2})	FA-N (kg m^{-2})	NEN (kg m^{-2})
0–50 cm					
Harrowed	1.27 (0.00)^{a}	0.03 (0.00)^{a}	0.03 (0.01)^{c}	0.15 (0.01)^{a}	0.95 (0.01)^{a}
Grass-covered	0.76 (0.04)^{c}	0.02 (0.00)^{bc}	0.05 (0.00)^{b}	0.03 (0.00)^{c}	0.67 (0.01)^{b}
Control	0.76 (0.01)^{cd}	0.01 (0.00)^{cd}	0.10 (0.01)^{a}	0.03 (0.00)^{c}	0.55 (0.03)^{d}
50–100 cm					
Harrowed	1.17 (0.00)^{b}	0.02 (0.00)^{b}	0.02 (0.00)^{d}	0.07 (0.00)^{b}	0.98 (0.01)^{a}
Grass-covered	0.71 (0.00)^{d}	0.01 (0.00)^{de}	0.03 (0.00)^{c}	0.01 (0.00)^{cd}	0.62 (0.01)^{c}
Control	0.07 (0.00)^{e}	0.01 (0.00)^{e}	0.01 (0.00)^{d}	0.00 (0.00)^{d}	0.05 (0.00)^{e}

In each column, mean values with different letters significantly differ for $P < 0.05$.

4. Discussion

4.1. Effect of soil management on the content of different C and N forms

In 10 years the vine cultivation strongly affected pedogenesis, with the development of thick Bw horizons, and support the observation for Mediterranean environment reported by Cuniglio et al. (2009). The acceleration of soil evolution, especially at depth, was attributed mainly to the vine through rooting and organic matter incorporation. In fact, as reported by many authors, a high incorporation of SOM is favoured by the presence and turnover of fine roots (Gale and Cambardella, 2000; Cocco et al., 2013).

In the soils understudy, the expected decreasing trend with depth of the organic matter pools did not occur for POC. This was possibly because of the *vertic* properties of the soils (Appendix I), as the formation of cracks during the summer season favoured the transfer of organic debris from the surface to the sub-superficial horizons. In the upper horizons (from Ap to Bw3) CTR had more TOC than the two vineyard soils, indicating that cultivation reduces the organic C storage (Stockmann et al., 2013), and grass vegetation contributes to organic C accumulation (McLauchlan et al., 2006). In the BC horizons, the larger amount of TOC and total N in the vineyards than in CTR was attributed to the vine continual rhizodeposition and root turnover. An estimation of TOC stored in the 50–100 cm soil layer during 10 years of vine cultivation amounted to 3–3.5 kg m^{-2} (Table 6), a quantity compatible with the calculated production of fine vine roots in the BC horizons: about 2.5 and 1.5 kg m^{-2} year^{-1} for HV and GCV, respectively (Table 5). Although these latter values appear elevated for an accumulation of about 3 kg m^{-2} TOC in 10 years, one has to consider that (1) root production varies during the lifespan of the plants (López et al., 2001), (2) in the early years of plantation the deepest soil horizons were poorly colonised by vine roots, (3) part of the root biomass undergoes degradation.

The N content of cropland soils surrounding our vineyards ranged from 0.49 to 0.65 kg m^{-2} in the 0–50 cm layer, and from 0.26 to 0.36 kg m^{-2} in the 50–100 cm layer (Roggero, 2008). If one considers that our vineyards did not receive fertilisers for 7 years before sampling, the N content in the 50–100 cm layer of the vineyard soils (Table 7) was surprisingly higher than croplands and was attributed to two counteracting processes: (1) grass redistributes N from belowground to aboveground; (2) vine increases N content at depth through root turnover and rhizodeposition. In the two vineyard soils, differences in concentration and distribution of N were attributed to the different soil management. In HV, the high N concentration found in the Bw and BC horizons was ascribed to the absence of grass and to tillage. This latter, through enhancing oxidative processes, favoured degradation of organic matter with possible formation of N-bearing low molecular weight humic substances (Szajdak et al., 2003; Ohno et al., 2009) and release of small amounts of NO_3^- (Corti et al., 2011). This might explain the higher content of fulvic acids in harrowed than in grass-covered soils. These organic compounds were probably leached to depth and (i) used by the microbial biomass (Jones et al., 2004), (ii) incorporated in organic compounds such as humic acids and humin, (iii) precipitated as Ca-organic complexes (Schoenau and Bettany, 1988), (iv) adsorbed on mineral surfaces (Kothawala and Moore, 2009). In the case of GCV and CTR, the much smaller rate of soil oxygenation due to the absence of tillage probably reduced decomposition, while the permanent grass cover favoured the absorption of the inorganic N forms derived from SOM mineralisation. Further, the abundance of exchangeable basic cations such as Ca and Mg (Corti et al., 2011) and the presence of a grass cover with a large belowground biomass should have promoted the accumulation of stable and stationary humic acids and NEOM. The positive effect of the zero tillage and the herbaceous cover on SOM stabilisation has been widely recognised as a successful strategy to increase soil organic C (West and Post, 2002; McConkey et al., 2003).

The amount of WEOC tended to decrease with depth as generally found by many authors (e.g., Kalbitz et al., 2000; Corvasce et al., 2006), although our values were higher than those reported for agricultural soils, where they range from 0.01 to 0.06 g kg^{-1} (e.g., Gregorich et al., 2000; Corvasce et al., 2006). However, the amount of dissolved organic C depends on land use and vegetation (Chantigny, 2003) and on sequestration/release cycles that, in turns, are controlled by the soil matrix (Don and Schulze, 2008).

The WEN is made of organic and inorganic N, and their proportion can be affected by the extraction protocol (Christou et al., 2005; Jones and Willet, 2006; Ros et al., 2009), but includes water extractable organic N (Ros et al., 2009), which is considered sensitive to cropping systems and management practices (Haynes, 2005). Further, thanks to its dual organic and inorganic nature, WEN (1) continuously interchanges among different organic and inorganic N forms, (2) is affected by microbial activity and processes such as fixation in organic compounds, mineralisation of organics, nitrification/denitrification, solubilisation, plant absorption, adsorption/desorption processes on organic and inorganic colloids (Kalbitz et al., 2000; Nannipieri and Eldor, 2009), and (3) is restored by organic matter inputs and root exudates. Because of all the processes affecting the dynamics of the soluble N compounds, the absence of fertilisation can be considered the main driver explaining the low and similar content of WEN in our soils.

Although the different vineyard soil managements did not affect markedly the amount of WEOM, the generally low enrichment in ^{13}C of WEOC from GCV and CTR indicated the recent formation of a large part of this organic pool. This result was expected because of the presence of a grass cover, which was probably the main source of soluble organic compounds in both soils. In HV, the high C isotope fractionation of WEOC, generally higher than that of the respective humic and fulvic acids, was ascribed to decomposition of fresh residues and microbial recycling of stabilised humic moieties (Sanderman et al., 2008, and references herein). In the Bw2, Bw3 and BC horizons, the low $\delta^{13}C$ values of WEOC indicated that

the main source of soluble organic compounds was the recycling of humic substances.

The largest C and N pools for all the three soils were NEOC and NEN, although in the deepest horizons of CTR they were lower than in the vineyard soils. This fact was mainly attributed to the presence of vine roots, which supplied organic C and N at depth through root turnover and rhizodeposition (Stockmann et al., 2013), and likely supported a microbial community active in mineralisation and humification (Fierer et al., 2003; Agnelli et al., 2004).

In the vineyard soils, the δ^{13}C had a general increasing trend from HA-C to FA-C and, then, to NEOC, which is considered the most recalcitrant and physically protected organic C form in soil (Lorenz et al., 2007). The generally higher abundance of ^{13}C in fulvic than in humic acids suggested that fulvic acids mostly represented a degradation product of humic acids. A ^{13}C enrichment has been interpreted as the occurrence of isotopic fractionation due to decomposer organisms (Boutton, 1996), which could have been favoured by the presence of vine roots and the microbial community living in the rhizosphere. Indeed, rhizodeposits, mostly containing highly labile compounds (Toal et al., 2000), act as a microbial substrate (Anderson et al., 1993), especially in the deeper horizons. Here, the rhizodeposited labile moieties enhanced the cycling of the stabilised SOM probably through a "priming effect" (Fontaine et al., 2003; Sanderman et al., 2010). This explanation is supported by the absence of a marked isotopic fractionation between humic and fulvic acids in CTR where the vine roots were absent.

4.2. Effect of soil management on the C and N storage

In the 0–50 cm soil layer the TOC stock was mostly due to NEOC, POC, and HA-C, and followed the sequence CTR > GCV > HV; according to Balesdent et al. (2000), this result would indicate that the presence of the grass cover and reduced tillage foster the accumulation of organic matter. The more than six-fold larger amount of FA-C in HV than in GCV indicated that the degradation of organic matter favoured by harrowing promotes a large formation of low molecular weight humic substances. In the 50–100 cm soil layer the TOC stock was mostly comprised of NEOC, and followed the sequence GCV > HV > CTR. This fact demonstrated that the vine fosters the accumulation of organic matter at depth. With respect to the vineyard soils, notwithstanding in this layer the harrowing induced a greater vine root production, the TOC stock was higher in GCV than HV probably because of the organic inputs derived from the below-ground grass biomass. The high storage of FA-C in the 50–100 cm layer of HV would indicate the mobility of the fulvic acids through the profile, even though ^{13}C signatures suggested that fulvic acids represent a degradation product of humic acids throughout the soil.

The total N stock, was higher in HV than in GCV and CTR, confirming the role of the grass cover in the N retention, both through direct uptake of inorganic N and by supporting a microbial population with a high enzymatic capacity for N mineralisation (Jackson, 2000; Steenwerth and Belina, 2008). The greater amount of N, mainly made of NEN, in the deeper layers of the vineyard soils was attributed to the vine roots presence and activity (Stockmann et al., 2013).

4.3. Vine root distribution and turnover

Considering the vine root mass distribution throughout the profile, the lower amount of live fine roots in GCV than in HV was attributed to the presence of the grass cover. Also Morlat and Jaquet (2003) found a negative correlation between the mass of grass roots and the number of vine roots in the upper soil layer (0–45 cm), and concluded that permanent grass cover inhibits the growth of vine roots. In our case, competition between grass and vine roots was observed until the BC2 horizon, at a depth of about 110 cm. For both soil managements, the greater concentration of C in the dead compared to the live roots was attributed to the degradation of sugars and starches and to the indirect accumulation of lignin, which contains about 50% more C per unit weight than the polysaccharides (Benner et al., 1987). Other than C, N was also present in higher concentration in the dead than in the live roots as a result of N immobilisation likely due to links between lignin and various N forms (Berg and Theander, 1984; Camiré et al., 1991). The indirect increase of lignin during root decomposition and the formation of N-lignin derivative compounds should result in a low N availability even in N-rich decaying roots. For example, with respect to the live roots, the amount of N retained during root decay was from 23% to 65% in HV (with the exception of the BC2 horizon where live and dead roots had the same N concentration), and from 17% to 69% in GCV (with the exception of the Bw2 and BC1 horizons where dead roots contained less N than the live roots). Similar results were obtained by Camiré et al. (1991), who found 69% higher N concentration than the initial values in decaying fine roots of black alder after 462 days decomposition experiment, and Gale and Cambardella (2000) who reported of an increase N concentration of 130% in decaying oat roots after an incubation experiment of 360 days. In our case, the indirect enrichment of C and N in the dead roots was likely obtained also because we sampled during the early spring, when dead roots had already experienced a period of decomposition. With regard to the decrease of N during root decay in the Bw2 and BC1 horizons of GCV, we do not offer any explanation. However, Arunachalam et al. (1996) found that N concentration in fine roots, following an initial increase after the first 240 days, decreased during a 600 days-long decomposition experiment in a 16 years old sub-humid forest in India.

The decreasing trend with depth of C and N concentration in the dead roots was attributed to a greater microbial utilisation of the decaying roots as, in the deeper horizons, a scarce amount of easily degradable substrates are available. This was supported by the increase with depth of the δ^{13}C and δ^{15}N of the dead roots and their inverse relationship with C and N concentration (Fig. 3). The low regression coefficient of GCV line in the C vs. δ^{13}C graph (Fig. 3a) was ascribed to the grass root-derived organics that represent a more easily degradable substrate for microbes compared to vine roots.

The comparison of the δ^{13}C signatures between live and dead roots further supported the different utilisation of the decaying roots by the microorganisms in the upper and deeper horizons of the two vineyard soils. Indeed, in the upper horizons (Bw1 and Bw2 for HV, and Bw1–Bw3 for GCV) the dead roots had a lower δ^{13}C than the respective live roots. This behaviour was attributed to the loss from the decaying roots of easily decomposable compounds such as polysaccharides and the indirect enrichment of lignin, which is depleted of ^{13}C by 4–7% relative to cellulose (Benner et al., 1987). The paucity of easily degradable substances at depth should cause a more intense degradation of the dead roots and a consequent strong isotopic fractionation.

As expected, both live and dead roots had positive Δ^{14}C values, indicating the presence of "bomb carbon" in the CO_2 fixed through photosynthesis (Trumbore, 2000) by vines. However, the decaying roots from the Bw3 and BC horizons of GCV were constructed from C fixed earlier than those of HV. We hypothesised that the greater MRT of the GCV dead vine roots was due to a soil microbial community adapted to diverse energetic substrates such as those supplied by grass root turnover and rhizodeposition. The presence of these easily degradable compounds probably allowed the woody vine roots to persist longer. This hypothesis did not agree with Fontaine et al. (2007), who investigated the stability of C in a soil under grassland (Massif Central, France) and proposed that a lack of supply of fresh plant-derived C may hinder the mineralisation of the stabilised organic matter in deep soil layers.

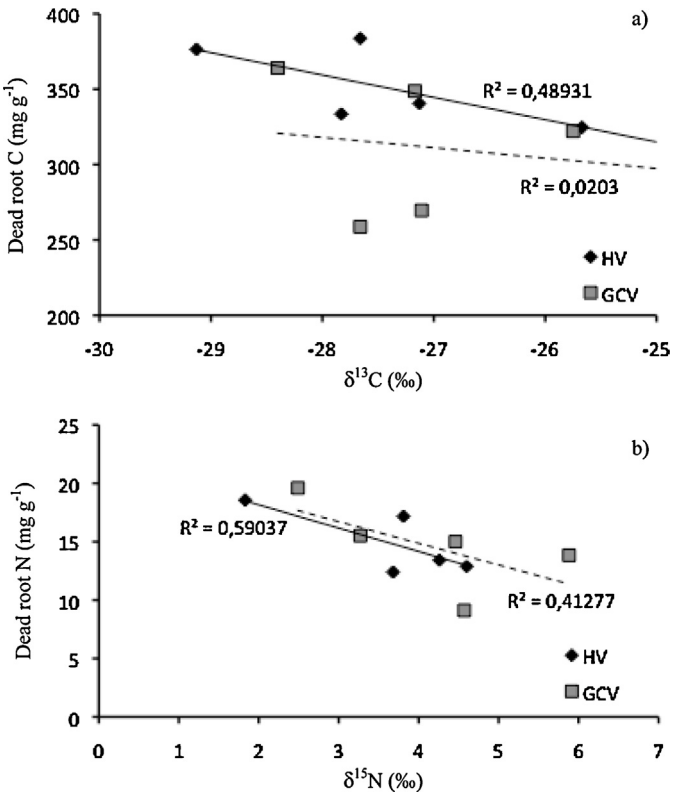

Fig. 3. Relationships between (a) C content and δ^{13}C, and (b) N content and δ^{15}N of the dead roots from harrowed (solid line) and grass-covered (dotted line) vineyards. Agugliano experimental farm (Ancona, Italy).

The different MRT and amount of dead fine root biomass in the two vineyards influenced the estimation of annual root production. The greater vine root production in the Bw1 horizon of GCV was ascribed to the competition of grape roots for nutrients and water. Bouma et al. (2001) and Volder et al. (2005), studying apple, orange and vine, demonstrated that the availability of soil nutrients or the resources expended in uptake and root maintenance influenced the fine root efficiency and lifespan. According to López et al. (2001), we hypothesised that competition with the herbaceous species for nutrients and water in the Bw1 horizon of GCV could have increased the rate of mortality of the grape fine roots resulting in a stimulation of their production. In the horizons below the Bw1, a general greater production of vine fine roots occurred in HV and was attributed to the effect of tillage that, reducing evapotranspiration, probably favoured a better conservation of soil moisture. However, as reported by Strand et al. (2008), many edaphic factors controlling the degradation rate of the vine roots may affect the calculated root production results. For example, in the deepest horizons of HV and GCV, the different availability of easily degradable substrates greatly affected the turnover time of the root-C and, in turn, the estimation of root production.

5. Conclusions

In the upper soil layer (0–50 cm), a greater organic matter stock was favoured more by the presence of a grass cover and the absence of tillage rather than vines. In the lower soil layer (50–100 cm), the presence of vines, independent of the soil management, increased organic matter and total N content mainly through accumulated rhizodeposition and vine root turnover.

By considering the distribution of the different organic pools (summarised in Fig. 4), GCV accumulated organics mainly in form of POM, HA and NEOM, while harrowing produced a greater stock of FA by enhancing oxidative processes. As indicated by the ^{13}C data, the two soil managements affected the SOM evolution pathway (Fig. 5). Indeed, although in both vineyard soils FA generally represented a degradation product of more stabilised compounds (HA and NEOM), the WEOM sources were mainly the grass in GCV and the recycling of humic substances in HV.

In both vineyard soils, decaying vine roots represented an important source of organic C and N, especially in the deepest horizons. Here, an intense degradation of the dead vine roots occurred

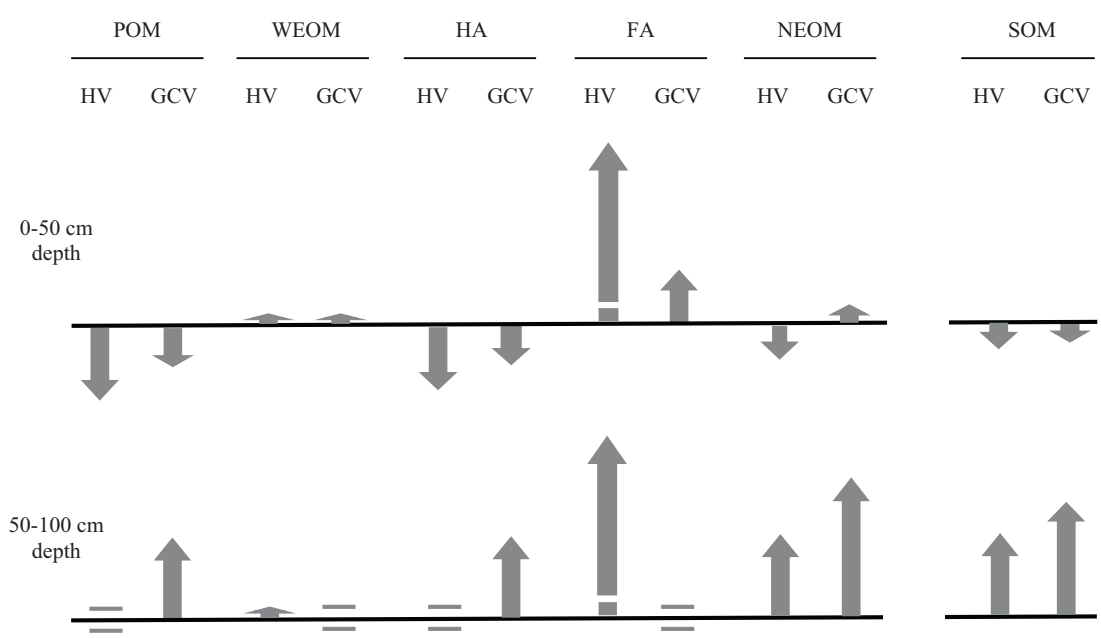

Fig. 4. Schematic representation of the effect of soil management on organic matter pools in vineyards. Scheme is based on C stock data, and arrow lengths are proportional to control soil data. Agugliano experimental farm (Ancona, Italy). *Legend*: POM – particulate organic matter; WEOM – water extractable organic matter; HA – humic acids; FA – fulvic acids; NEOM – non-extractable organic matter; SOM – soil organic matter.

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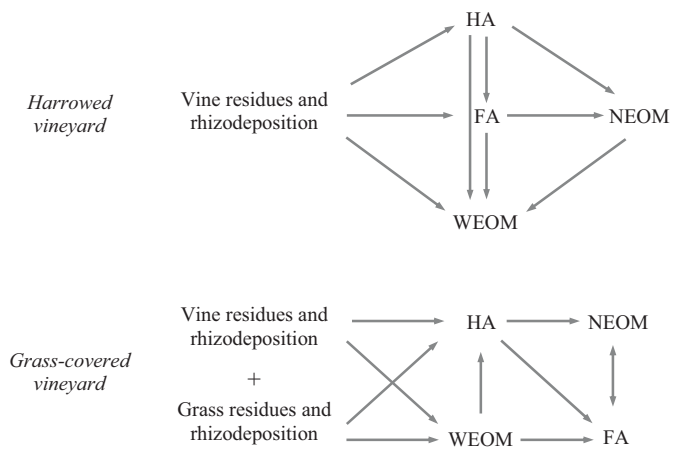

Fig. 5. Schematic representation of soil organic matter evolution pathways in harrowed and grass-covered vineyards. Scheme is based on ^{13}C data. Augugliano experimental farm (Ancona, Italy). *Legend*: WEOM – water extractable organic matter; HA – humic acids; FA – fulvic acids; NEOM – non-extractable organic matter.

as they likely represented the main microbial substrate. However, the generally greater age of the decaying vine roots in the deeper horizons of GCV, and the consequent lower root production, were ascribed to preferential microbial use of labile substrates supplied by grass root turnover and rhizodeposition. This fact fostered a larger organic matter accumulation in GCV than in HV.

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Appendix I.

Morphological description of the two trenches opened in each soil (harrowed, grass-covered, control). Augugliano experimental farm (Ancona, Italy). For symbols see legend. When differences between the two trenches were consistent, both conditions are reported.

	Depth[a] (cm)	Colour[b]	Texture[c]	Structure[d]	Consistency and plasticity[e]	Vine roots[f]	Boundary[g]	Thickness (cm)	Other observations[h]
Harrowed									
Ap	0–3	10YR 6/4	sl	3f,m cr + abk + sbk	mfr, wss, wps-p	$v_1$mi,vf,f 1mi,vf,f	ci	1–7	sk <1%; vertic cracks
Bw1	3–30	2.5Y 4/3 2.5Y 4/4	sl	2m,tk pl → f,m,c sbk 2m pl → f sbk	mfr-fi, wss, wp	1mi,vf,f,m 2mi,vf,f,m; 1co	cw	18–28	sk <1%; vertic cracks; 1f Fe–Mn ndl
Bw2	30–60	2.5Y 5/4 2.5Y 5/6	sl	2m,tk pl → f abk 2m pl → f,m abk	mfr-fi, wss, wps	3mi,vf,f,m,co	cw cs	26–31	sk <1%; 1f Fe–Mn ndl; erthw
Bw3	60–64	2.5Y 4/3 2.5Y 5/3	scl	2m pl → f sbk	mfr, wss, wps	2mi,vf,f,m,co 2mi,vf,f	cw cs	4–5	sk 1–3%; 1f Fe–Mn ndl; 1f cc
BC1	64–89	2.5Y 6/4 2.5Y 5/4	scl	2th,m pl 2m pl	mfr, wss, wps	2mi,vf,f,m,co	cs	23–28	sk 5–8%; 1 gyp cnc; 2f Fe–Mn ndl; 1f cc
BC2	89–112+	2.5Y 4/3	scl	2f pl	mfr, wss, wps	1mi,vf,f,m; $v_1$co 2mi,vf,f	–	–	sk 5–8%; 2 gyp cnc; 1f Fe–Mn ndl; 1f cc
Grass covered									
Ap	0–4	2.5Y 4/4 2.5Y 4/2	sl	3f,m cr 3c,vc pr → f,m pl	mfr, wss, wps	1mi,vf,f 2mi,vf,f	cw	3–4	sk <1%; vertic cracks; erthw
Bw1	4–21	10YR 5/32.5Y 5/3	sl	3c,vc cpr → m abk 2m,c pr → f,m sbk	mfi, wss, wps	2mi,vf,f,m; 1co 3mi,vf,f,m	cw	15–20	sk <1%; vertic cracks; erthw
Bw2	21–46	2.5Y 5/3 2.5Y 5/4	sl	2m pl → f,m sbk	mfr, wss, wps	3mi,vf,f,m 3mi,vf,f; 1m,co	cw cb	0–28	sk <1%; 1 gyp cnc; 1f Fe–Mn ndl; erthw
Bw3	46–64	2.5Y 4/3 2.5Y 4/4	sl	2m pl → m sbk	mfr, wss, wps	1mi,vf,f,m,co	cw	18–40	sk <1%; 1 gyp cnc
BC1	64–78	2.5Y 5/3	scl	2m pl → f,m abk	mfr, wss, wps	1mi,vf,f,m,co	cw ab	0–18	sk = 5%; 1 gyp cnc; 1f cc
BC2	78–113+	2.5Y 4/3 2.5Y 4/4	scl	2th pl → f,m sbk	mfr, wss, wp	1mi,vf,f,m,co	–	–	sk 5–8%; 1 gyp cnc; 1f Fe–Mn ndl; 1f cc
Control									
Ap	0–3	2.5Y 3/2 2.5Y 3/3	sl	3f cr + 3f,m sbk 3f,m cr	mfr, wss, wps	0	cs	2–3	sk <1%; vertic cracks; erthw
Bw1	3–6	2.5Y 4/4 2.5Y 4/3	sl	3f,m sbk + 3f cr 3f,m sbk	mfi, wss, wps	0	cs	3–5	sk <1%; vertic cracks; erthw
Bw2	6–26	2.5Y 5/4 2.5Y 5/6	sl	2m,c pr → m sbk 2f,m sbk	mfi, wss, wps	0	cs	17–21	sk <1%; vertic cracks; erthw
Bw3	26–46	2.5Y 4/4 2.5Y 5/4	sl	2m,c pr → m sbk	mfi, wss, wps	0	cs	18–22	sk <1%; 1f Fe–Mn ndl; erthw
BC	46–69	2.5Y 5/4 2.5Y 5/3	scl	2m,c pr → m sbk	mfi-vfi, wss, wps	0	as	22–25	sk 3–5%; 1 gyp cnc; 1f Fe–Mn ndl; 1f cc
2BCk	69–104+	2.5Y 5/3	scl	2m pr → th,m pl	mfr, wss, wp	0	–	–	sk 3–5%; 1 gyp cnc; 1f Fe–Mn ndl; 1f cc

[a] The depth of each horizon is the average of the different conditions observed in the two trenches.
[b] Moist and crushed, according to the Munsell Soil Color Charts, 1992 Edition.
[c] sl = silty loam, scl = silty clay loam.
[d] 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse, vc = very coarse, th = thin, tk = thick; cr = crumb, abk = angular blocky, sbk = sub-angular blocky, pl = platy, cpr = cuneiform prism; pr = prism; → breaking into.
[e] m = moist, w = wet; fr = friable, fi = firm, vfi = very firm; ss = slightly sticky, s = sticky; ps = slightly plastic, p = plastic.
[f] 0 = absent, v_1 = very few, 1 = few, 2 = plentiful, 3 = abundant; mi = micro, vf = very fine, f = fine, m = medium, co = coarse.
[g] c = clear, a = abrupt; i = irregular, w = wavy, s = smooth, b = broken.
[h] sk = skeleton; 1 = few, 2 = plentiful; f = fine; ndl = nodules; erthw = earthworms; cc = clay cutans; gyp cnc = gypsum concretions.

Appendix II.

Bulk density values of the samples collected from the harrowed and grass-covered vineyard soils, and from the control soil. Agugliano experimental farm (Ancona, Italy). Numbers in parentheses are the standard deviations ($n = 4$).

	Bulk density (kg dm^{-3})
Harrowed	
Ap	1.46 (0.20)
Bw1	1.49 (0.24)
Bw2	1.60 (0.08)
Bw3	1.52 (0.02)
BC1	1.65 (0.04)
BC2	1.61 (0.04)
Grass-covered	
Ap	1.61 (0.30)
Bw1	1.61 (0.02)
Bw2	1.56 (0.08)
Bw3	1.59 (0.14)
BC1	1.60 (0.04)
BC2	1.60 (0.04)
Control	
Ap	1.85 (0.26)
Bw1	1.65 (0.04)
Bw2	1.61 (0.04)
Bw3	1.56 (0.02)
BC	1.59 (0.04)
2BCk	1.61 (0.04)

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