Effect of Long-Term Soil Management on the Mutual Interaction Among Soil Organic Matter, Microbial Activity and Aggregate Stability in a Vineyard

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ABSTRACT

Vineyard management practices to enhance soil conservation principally focus on increasing carbon (C) input; whereas mitigating impacts of disturbance through reduced tillage has been rarely considered. Furthermore, information is lacking on the effects of soil management practices adopted in the under-vine zone on soil conservation. In this work, we evaluated the long-term effects (22 years) of alley with a sown cover crop and no-tillage (S + NT), alley with a sown cover crop and tillage (S + T), and under-vine zone with no vegetation and tillage (UV) on soil organic matter (SOM), microbial activity and aggregate stability in a California vineyard in USA. Their mutual interactions were also considered. Vegetation biomass, microbial biomass and activity, organic C and nitrogen (N) pools, and SOM size fractionation and aggregate stability were analysed. Soil characteristics only partially reflected the differences in vegetation biomass input. Organic C and N pools and microbial biomass/activity in S + NT were higher than those in S + T, while the values in UV were intermediate between the two other treatments. Furthermore, S + NT also exhibited higher particulate organic matter C (POM-C). No differences were found in POM-C between S + T and UV, but the POM fraction in S + T was characterized by fresher material. Aggregate stability was observed in the order S + NT > UV > S + T. Tillage, even if shallow and performed infrequently, has a negative effect on organic C and N pools and aggregate stability. Consequently, the combination of a sown cover crop and reduced tillage still limits SOM accumulation and reduces aggregate stability in the surface soil layer of vineyards suggesting relatively lower resistance of soils to erosion compared to no-till systems.

Key Words: loamy Ultisol, microbial biomass, permanent grass, soil organic matter fractionation, soil respiration, tillage, vegetation biomass

INTRODUCTION

Microorganisms, soil organic matter (SOM), and aggregate stability are key factors of soil conservation and functionality and sustainable land management (Gosling et al., 2013), and they are mutually related. Microorganisms are the drivers of SOM mineralization and a principal source of aggregating agents (Allison, 1968; Cosentino et al., 2006). Aggregates protect SOM against microbial decomposition by occlusion, i.e. physical protection, and by chemical interaction of organic matter with clay and silt fractions, i.e. chemical protection (Tisdall and Oades, 1982; Golchin et al., 1994, 1995; Jagadamma and Lal, 2010; Vogel et al., 2015). In turn, SOM bound to mineral particles leads to the formation of stable aggregates, which favor air and water diffusion into soil (Dörner et al., 2010) and root development, and promote a favourable rhizospheric environment characterized by an active microbial community (Hayat et al., 2010). The equilibrium among SOM turnover, microbial activity, and aggregate stability is thus essential to guarantee proper soil functionality in terms of physical, biological, and chemical properties that support soil fertility. Agricultural practices can strongly affect this equilibrium (Jastrow, 1996; Jacobs et al., 2010; Gosling et al., 2013). In particular, tillage can increase the turnover of macroaggregates (Six et al., 2000), consequently limiting the physical and chemical protection of SOM against microbial mineralization. Furthermore, agricultural practices affect the biomass and activity of microorganisms through modification of organic matter (OM) input both quantitatively and qualitatively. This occurs directly by amendment or fertilization (Lazcano et al., 2013; Stevenson et al., 2014; Sánchez-García et al., 2016) and indirectly by changes in plant

growth, litter and root decomposition, and root exudates (Corneo et al., 2013; Loeppmann et al., 2016). These effects are evident in vineyards where soils are often highly sensitive to SOM loss and topsoil degradation because of their intrinsic properties, such as limited soil development, coarse texture, and low capacity to protect SOM binding to soil minerals (Le Bissonnais et al., 2007; Martínez-Casasnovas and Ramos, 2009). Other properties that increase the vulnerability of vineyard soils are related to limited SOM contents, hilly morphology, and sloping topography (Ramos and Martínez-Casasnovas, 2004; Novara et al., 2011). These characteristics make vineyard soils, even those at slopes of less than 2 percent, more susceptible to erosion. Therefore, improper management may result in permanent soil degradation (Smith et al., 2008; Novara et al., 2013; Ruiz-Colmenero et al., 2013; Lieskovský and Kenderessy, 2014).

Nevertheless, in recent efforts to enhance soil conservation in vineyards, most practices mainly focus on increasing carbon (C) input (Bustamante et al., 2011; Guerra and Steenwerth, 2012), whereas mitigating impacts of disturbance through tillage reduction is marginally considered. Furthermore, only a few studies in vineyards focus on the effect of soil management on the relationships among SOM dynamics, microbial activity, and aggregate turnover and stability (López-Piñeiro et al., 2013; Zehetner et al., 2015). In this context, no information is available on the effects of soil management practices adopted in the alley compared to the under-vine zone, where herbicides are often used in combination with tillage to control weed establishment and which represents nearly 30% of vineyard floor (Karl et al., 2016). This current work thus is aimed at evaluating the long-term effects (22 years) of permanent vegetation cover with no-tillage in the alley in comparison to a sown cover crop with tillage in the alley and a bare soil region in the vine row. We hypothesized that microbial activity, SOM dynamics, and aggregate stability were strongly and reciprocally influenced by the type and intensity of disturbance. To test this hypothesis, we evaluated the effects of the different soil management practices in a loamy Ultisol in a California vineyard in USA by determining vegetation cover biomass, microbial biomass and activity, as well as SOM size distribution and aggregate stability.

MATERIALS AND METHODS

Study site and experimental design

This study was conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA (38°13’12.03” N; 112°21’21.05” W). The climate in the Napa Valley is described as Mediterranean, while the mesoclimate in Los Carneros is slightly more moderate as the region is cooled by marine breezes. Los Carneros has an average annual precipitation of 506 mm (with extreme drought conditions in the 2012-2014 period) and an average annual temperature of 13.3 °C (Fig. 1). The conditions are typically dry during the growing season, and precipitation occurs mostly between January and April, and October and December.

The study site was part of the Napa County Resource Conservation District’s Sustainable Agriculture Demonstration Vineyard, which was established in 1991 with Vitis vinifera L. cv. Pinot Noir (clone UCD 2A) on 1103 Paulsen rootstock (V. berlandieri Planch. × V. rupestris Scheele). Vines were vertically shoot positioned, trained, and cordon spur pruned. The space was 1.5 m between vines and 2.4 m between alleys. The vineyard slope ranged from 0%--1%, and rows were oriented north-south. The soil series was Haire loam (fine, mixed, superactive, thermic Typic Haploxerult) (Soil Survey Staff, 2014). Selected soil characteristics are shown in Table I.

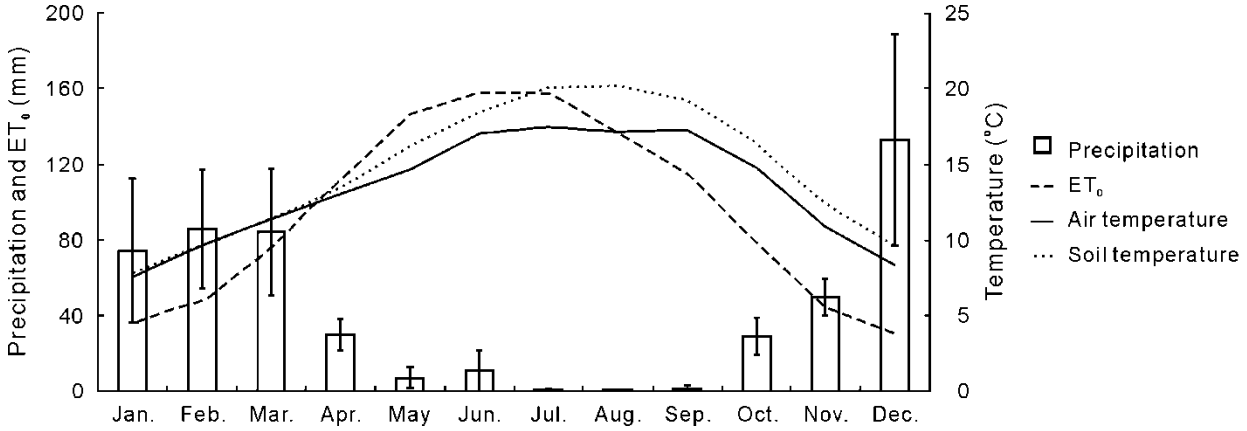

Fig. 1 Precipitation, reference crop evapotranspiration (ET0), and temperature in 2010--2014 of the study site in the Los Carneros American Viticultural Area of Napa Valley, California, USA, recorded by the California Irrigation Management Information System. Precipitation values are the means with standard errors shown by the vertical bars (n = 5).

Table I Selected characteristics of the studied soil (0--5 cm) in the Los Carneros American Viticultural Area of Napa Valley, California, USA

Soil characteristic[a)]	Value
pH	6.00 ± 0.55[b)]
Sand (%)	26.2 ± 2.2
Silt (%)	51.5 ± 2.4
Clay (%)	22.3 ± 1.4
TOC (g kg^{-1})	21.7 ± 3.87
TN (g kg^{-1})	1.90 ± 0.29
K^{+}_{ex} ($cmol_{+}$ kg^{-1})	0.65 ± 0.11
Mg^{2+}_{ex} ($cmol_{+}$ kg^{-1})	4.39 ± 0.58
Ca^{2+}_{ex} ($cmol_{+}$ kg^{-1})	9.93 ± 1.12
Na^{+}_{ex} ($cmol_{+}$ kg^{-1})	0.24 ± 0.17
P-Olsen (mg kg^{-1})	23.6 ± 10.9

[a)]TOC = total organic C; TN = total N; K^{+}_{ex}, Mg^{2+}_{ex}, Ca^{2+}_{ex}, and Na^{+}_{ex} = exchangeable K^{+}, Mg^{2+}, Ca^{2+}, and Na^{+}, respectively.

[b)]Means ± standard deviation (*n* = 15).

The experimental design for the study was a complete randomized block, with three treatments and five blocks (one treatment replicate per block and a total of five replicates per treatment) (Fig. 2). The treatments were established in 1993 and included: alley with a sown cover crop and no-tillage (S + NT); alley with a sown cover crop and tillage (S + T); and under-vine zone with no-grass cover (by applying a herbicide) and tillage (UV). In S + NT, soil surface in the alley was not tilled and left covered with vegetation. It was only seeded in 1993 with Vulpia myuros (L.) C. C. Gmel, Bromus hordeaceus L., Trifolium hirtum All., and Trifolium pratense L., and in the subsequent years, it was not re-seeded. Non-native annual grasses and forbs also grew among the cover crops, and the vegetation composition at the sampling time was: 45% Ranunculus muricatus L., 25% Trifolium spp., 15% B. hordeaceus L., 10% V. myuros (L.) C. C. Gmel, 4% Lactuca spp., and 1% Geranium dissectum L.. The vegetation in S + NT alley was mowed thrice a year in

March, May, and July to an approximate height of 10 cm, and the plant residues were left on the surface. The S + T alley was seeded every year after harvest at the end of September with a cover crop Vicia faba L., Pisum sativum L., Triticum aestivum L., and Secale cereale L. The vegetation composition at the sampling time was 95% T. aestivum L. and/or S. cereale L., 3% V. faba L., and 2% P. sativum L.. The S +T alley was tilled thrice a year. The first tillage pass in September was shallow (ca. 10 cm depth) using a ring roller to prepare the soil bed for seeding the cover crop; the second pass (20 cm depth) was in mid-March using discs to incorporate the cover crop into the soil; and the third pass (20 cm depth) with discs was in mid-April. Thus, the soil was uncovered from the spring tillage until fall. The UV alley was managed with a glyphosate herbicide (48.7% a.i.; distribution rate: 4.8 L ha-1; a.i. distribution rate: 2.3 kg a.i. ha-1) and two cultivations per year with a Clemens® vineyard cultivator during the late spring. Here, the soil was uncovered permanently (bare soil).

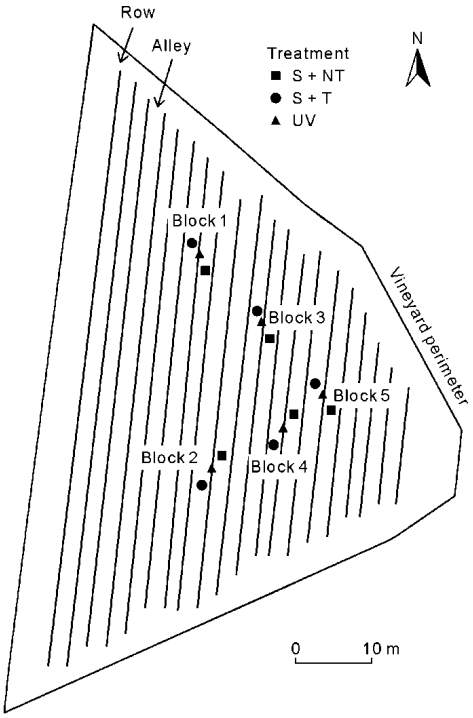

Fig. 2 Experimental design of this study containing five blocks, each with three treatments in the Los Carneros American Viticultural Area of Napa Valley, California, USA. S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

All other agricultural practices were the same for the entire vineyard: disease, pest control, and canopy management were performed mechanically, whereas pruning, shoot positioning, and harvesting were performed manually. Drip irrigation was applied from June to September each year (330 L vine-1 year-1; 915 m3 ha-1 in 2015).

Sampling and sample preparation

From each treatment replicate, one soil sample was taken from a 30 cm × 30 cm soil pit in March 2015, just before the second tillage pass in the S + T alleys. The sampling depth was 5 cm. Soil was collected from the middle of the alleys under S + T and S + NT and between two adjacent vines under UV. Soil samples were immediately placed on ice and stored overnight at 4 °C. All the soil samples were gently broken by hand,

passed through an 8-mm sieve and divided in to two subsamples: one subsample was stored at 4 °C for field-moist soil analyses, whereas the other subsample was air dried.

Shoot and root biomass (5 cm deep) samples were taken adjacent to the soil sampling area from a 0.15 m2 area using a rectangular quadrant (0.5 m × 0.3 m). Shoots were cut at ground level with a pair of scissors; and root was separated by hand from the soil in the lab and carefully washed with water. Plant biomass was dried at 60 °C, weighed (dry weight), and analysed for C and nitrogen (N) contents by dry combustion (LECO FP-528 and TruSpec CN Analyzers, LECO Corporation, Saint Joseph, USA).

Soil C and N forms

Total C and N (TN) contents of the bulk soil subsamples (< 0.5 mm) were measured as described above. As the soil sample was free of carbonate, the total C was considered as total organic C (TOC). Dissolved organic C (DOC), total dissolved N (TDN), and inorganic N pools—NO3--N and NH4+-N— were extracted with 0.5 mol L-1 K2SO4 from field-moist soil samples. Afterwards, DOC was measured by platinum-catalyzed, high-temperature combustion (680 °C), followed by infrared detection of CO2 (Shimadzu TOC-VCSH, Shimadzu Scientific Instruments, Columbia, USA), and TDN was measured by persulfate oxidation (Williams et al., 1995), followed by colorimetric determination of nitrate (Miranda et al., 2001), while the inorganic N pools were determined colourimetrically (Kempers and Kok, 1989; Miranda et al., 2001). Dissolved organic N (DON) was calculated as the difference between TDN and the sum of all inorganic N forms (NO3--N and NH4+-N). To measure microbial biomass C (MBC) and N (MBN), fresh samples were fumigated overnight with chloroform and extracted with 0.5 mol L-1 K2SO4 parallel with non-fumigated samples (Williams and Sparling, 1988), and MBC and MBN were calculated from the flushes of extractable C and N using the recovery factor of 0.45 for C (Sarathchandra et al., 1989) and 0.54 for N (Brookes et al., 1985; Vance et al., 1987).

Potential net N mineralization (potential N) of field-moist soil was measured by anaerobic incubation at 40 °C for 7 d (Waring and Bremner, 1964; Soon et al., 2007). Aerobic microbial respiration was measured by placing field-moist soil (equivalent to100 g dry weight) adjusted to 40% water filled pore space in sealed jars (946 cm3). The headspace CO2-C concentration was measured during 1-week aerobic incubation at 25 °C in the dark (Potthoff et al., 2005). Briefly, soil sample was placed in a sealed mason jar, flushed with air for 30 s, and the headspace sample was collected after the jar was closed for 1 min (t0 sample). The headspace was sampled 24 h after the initiation until day 7 at 2 d intervals. After each sampling, the jars were opened and flushed with air before being closed again to ensure aerobic conditions. Finally, potential microbial respiration (cumulative CO2-C evolved during the 7-d incubation, ΣCO2-C), CO2-C production per unit of TOC (ΣCO2-C/TOC), and metabolic quotient (qCO2), i.e., µg CO2-C produced per mg MBC per hour (µg CO2-C mg-1 MBC h-1) were calculated.

SOM fractionation

In order to evaluate the processes protecting different organic pools against mineralization, SOM was separated into coarse particulate organic matter (POM , coarse POM), fine particulate organic matter (fine POM) and < 53 µm organic matter (< 53 µm OM) (Cambardella and Elliott, 1992). Briefly, 30 g dry soil sample was dispersed in 0.5% Na-hexametaphosphate solution (100 mL) for 18 h and passed through a series of sieves (250 µm, 53 µm, and catch pan). All fractions were thoroughly rinsed and transferred to pre-weighed pans, oven-dried at 65 °C, weighed, ground, and stored at room temperature. Three size fractions were isolated: > 250 µm (coarse POM), 53--250 µm (fine POM), and < 53 µm (< 53 µm OM), and then total organic C and N contents of each fraction were determined by dry combustion (LECO FP-528 and

TruSpec CN Analyzers, LECO Corporation, Saint Joseph, USA). To evaluate the POM (i.e., fine POM and coarse POM) C/N ratio, due to the low contents of C (POM-C) and N (POM-N) in soil, they were calculated indirectly as the difference between TOC and TN and C (< 53 µm OM-C) and N (< 53 µm OM-N) in the fraction < 53 µm, respectively. Furthermore, distribution of the coarse POM-C, fine POM-C, and < 53 µm OM-C in TOC was calculated.

Aggregate stability

Aggregate stability was measured by wet sieving. Briefly, dry soil was gently sieved using 0.25-mm sieve to remove the particles smaller than the size of the sieve used for wet sieving. Ten grams of this soil fraction were placed in a 0.2-mm sieve and allowed to rotate at 60 r min-1 for 10 min in 600 mL beakers containing 400 mL deionized water. After wet sieving, the weight of the soil retained in the sieve (> 0.2--0.25 mm) was measured after drying (Wsr). Aggregate loss (%) was determined according to Kemper and Rosenau (1986) as follows:

$$\text{Aggregate loss} = 100 - \frac{100 \times (Wsr - Wcs)}{Wts - Wcs} \qquad (1)$$

where Wts is the weight of total soil sample and Wcs is the weight of coarse sand.

Wcs was determined after H2O2 oxidation (Gee and Bauder, 1986) of the 0.25--8 mm soil fraction.

Amounts of exchangeable cations (Ca2+ex, Mg2+ex, K+ex, and Na+ex) were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, iCAP 6500, Thermo Scientific, Cambridge, United Kingdom) after extraction with ammonium acetate solution buffered to pH 7.0. Bioavailable P was determined by the Olsen method (P-Olsen) (Olsen and Sommers, 1982).

Statistical analyses

A one-way analysis of variance (ANOVA) using treatment as the fixed factor was carried out to determine treatment effects on soil analyses. A two-way ANOVA using fractions and treatment as fixed factors was performed for the SOM fractionation. The mean separation was performed with the Tukey's honest significant difference (HSD) mean separation test. All statistical analyses were conducted using SPSS version 20 statistical software.

RESULTS

Vegetation biomass and soil C and N forms

Shoot biomass was not affected by tillage in the alley since there were no differences between S + NT and S + T and the corresponding C and N contents (Table II). In contrast, herbicide application and tillage strongly reduced shoot biomass in UV, which had significantly lower C and N contents. Root biomass was the highest in S + NT, which was 3- and 30-fold higher than S + T and UV, respectively ($P \leq 0.05$). Consequently, the root to shoot biomass ratio was much higher in S + NT than in S + T, while the C and N contents in the

roots were similar in S + NT and S + T but higher in UV. Although the C and N contents varied among treatments, no differences were observed in the corresponding shoot, root, and overall biomass C/N ratios.

Table II. Plant biomass, C and N contents and stocks, and root/shoot biomass ratio (R/S ratio) under different treatments[a)] in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA

Variable	S + NT	S + T	UV	*P* value
Biomass (g dry weight m^{-2})				
Shoot	195.6a[b)]	147.4a	4.7b	0.000
Root	208.9a	64.1b	5.9c	0.001
C content (g C kg^{-1})				
Shoot	41.24a	41.46a	36.63b	0.000
Root	32.60b	35.36b	40.74a	0.000
N content (g N kg^{-1})				
Shoot	2.84a	2.56ab	1.89b	0.040
Root	1.31b	1.22b	1.70a	0.014
C/N ratio (g g^{-1})				
Shoot	15.1	16.2	18.8	NS[c)]
Root	26.0	29.1	24.0	NS
Shoot + Root	18.3	18.9	19.0	NS
C stock (g C m^{-2})				
Shoot	80.17a	61.44a	1.50b	0.000
Root	69.44a	23.31b	2.41c	0.001
N stock (g N m^{-2})				
Shoot	5.25a	3.43b	0.11c	0.000
Root	2.18a	0.79b	0.10c	0.000
R/S ratio	1.2a	0.4b	1.5ab	0.010

[a)]S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

[b)]Means (n = 5) followed by different letters are significantly different using one-way analysis of variance and Tukey's honestly significant difference mean separation at $P \leq 0.05$.

[c)]Not significant.

Different vegetation biomass input strongly affected soil TOC and TN contents, with higher contents in S + NT ($P \leq 0.01$, Table III). However, no significant differences in the TOC/TN ratio among treatments were revealed. Despite the extremely low shoot and root biomass in UV (Table II), both TOC and TN contents did not significantly differ from those of S + T (Table III). NH4+-N content in UV was relatively lower than S + NT and S + T, whereas NO3--N content was the highest in UV. The DON content showed no difference among

treatments, while the DOC content decreased in the order: S + NT > S + T = UV. Soil MBC and MBN were 1.8- and 1.9-fold higher in S + NT than the other two treatments ($P \leq 0.01$), and the potential N mineralization decreased in the order S + NT > UV > S + T. The amount of exchangeable cations was generally higher in UV treatment than S + NT and S + T. Available P (P-Olsen) was similar between S + NT and S + T, being 2-fold higher in UV.

Table III. Selected soil characteristics under different treatments[a)] in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA

Soil characteristic[b)]	S + NT	S + T	UV	*P* value
TOC (g kg^{-1})	26.64a[c)]	17.75b	19.28b	0.000
TN (g kg^{-1})	2.45a	1.70b	1.86b	0.000
TOC/TN (g g^{-1})	10.8	10.6	11.0	NS[d)]
NH_4^+-N (mg kg^{-1})	2.75a	2.53a	1.22b	0.009
NO_3^--N (mg kg^{-1})	1.18b	1.56b	9.69a	0.001
DOC (mg kg^{-1})	42.59a	27.58b	35.49ab	0.007
DON (mg kg^{-1})	3.83	3.26	3.40	NS
MBC (mg kg^{-1})	106.42a	59.43b	58.24b	0.000
MBN (mg kg^{-1})	13.94a	8.17b	6.71b	0.000
MBC/MBN	7.3	7.4	10.1	NS
MBC/TOC (mg g^{-1})	4.00a	3.31b	3.02c	0.000
MBN/TOC (mg g^{-1})	0.53a	0.47a	0.32b	0.004
PNM (mg kg^{-1} $week^{-1}$)	74.02a	33.85c	51.19b	0.000
K^+_{ex} ($cmol_+$ kg^{-1})	0.68ab	0.55b	0.72a	0.033
Mg^{2+}_{ex} ($cmol_+$ kg^{-1})	4.42a	3.80b	4.96a	0.000
Ca^{2+}_{ex} ($cmol_+$ kg^{-1})	9.91b	8.71c	11.15a	0.000
Na^+_{ex} ($cmol_+$ kg^{-1})	0.14b	0.16b	0.41a	0.011
P-Olsen (mg kg^{-1})	16.38b	16.30b	36.68a	0.000

[a)]S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

[b)]TOC = total organic C; TN = total N; DOC = dissolved organic C; DON = dissolved organic N; MBC = microbial biomass C; MBN = microbial biomass N; PNM = potential N mineralization; K^+_{ex}, Mg^{2+}_{ex}, Ca^{2+}_{ex}, and Na^+_{ex} = exchangeable K^+, Mg^{2+}, Ca^{2+}, and Na^+, respectively.

[c)]Means (n = 5) followed by different letters are significantly different using one-way analysis of variance and Tukey's honestly significant difference mean separation at $P \leq 0.05$.

[d)]Not significant.

After one week of aerobic incubation, there was a significant treatment effect on the potential microbial respiration indicated by ΣCO2-C (Fig. 3), with higher values in S + NT than in S + T and UV. However, significant differences in CO2-C production per unit of TOC (ΣCO2-C/TOC) were observed between S + T and UV, and the lowest values were found in UV. No differences in the metabolic quotient (qCO2) were detected.

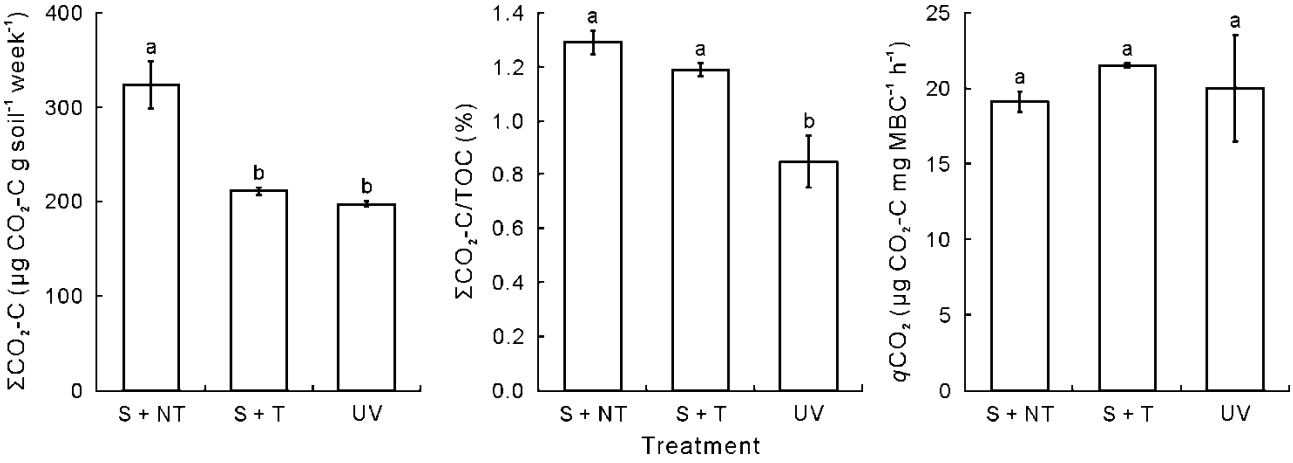

Fig. 3 Potential microbial respiration (Σ CO2-C), CO2-C production per unit of TOC (ΣCO2-C/TOC), and metabolic quotient (qCO2) under different treatments in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA. Values are the means with standard errors shown by the vertical bars (n = 5). Bars with different letters are significantly different using one-way analysis of variance and Tukey's honest significant difference mean separation at P ≤ 0.05. S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

Soil organic matter fractionation and aggregate stability

In all the three OM fractions, C and N contents were higher in S + NT than S + T and UV, with differences between the latter two treatments only present in the fine POM fraction (Table IV). The two-way ANOVA indicated significant effects of fraction, treatment, and fraction × treatment interactions (P ≤ 0.01, data not shown). The POM-C content was 2-fold higher in S + NT than S + T and UV, while POM-N decreased in the order S + NT > UV > S + T. This resulted in a much higher C/N ratio in S + T compared to the other treatments. The < 53 µm OM-C was always the most abundant (Fig. 4). In S + NT, the < 53 µm OM-C represented 68% of TOC, while fine POM-C and coarse POM-C represented 15.8% and 15.5% of TOC, respectively. Conversely, in S + T, the contribution of < 53 µm OM-C increased to 81.1%, while fine and coarse POM-C decreased to 10.1% and 8.5%, respectively. Significant differences in < 53 µm OM-C (P ≤ 0.01), coarse POM-C (P ≤ 0.01), and fine POM-C (P ≤ 0.05) were found between S + NT and S + T. In UV, an intermediate trend was observed, with < 53 µm OM-C, fine POM-C, and coarse POM-C representing 74.2%, 12.4%, and 13.5% of TOC, respectively.

Table IV

C and N contents and C/N ratio in different organic matter (OM) fractions, particulate organic matter (POM) containing coarse POM and fine POM and < 53 µm OM under different treatments[a)] in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA

Parameter	S + NT	S + T	UV	*P* value

coarse POM-C (g kg^{-1})	4.15a[b)]	1.51b	2.49ab	0.015
fine POM-C (g kg^{-1})	4.15a	1.83c	2.32b	0.000
< 53 µm OM-C (g kg^{-1})	17.81a	14.35b	15.05b	0.001
coarse POM-N (g kg^{-1})	0.22a	0.04b	0.05b	0.033
fine POM-N (g kg^{-1})	0.29a	0.11c	0.18b	0.000
< 53 µm OM-N (g kg^{-1})	1.95a	1.57b	1.56b	0.000
POM-C (g kg^{-1})	7.93a	3.34b	4.59b	0.000
POM-N (g kg^{-1})	0.50a	0.12c	0.23b	0.000
C/N ratio in POM	15.0b	27.2a	18.1b	0.000
C/N ratio in < 53 µm OM	9.5a	9.2b	9.4ab	0.004

[a)]S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.[b)]Means (n = 5) followed by different letters are significantly different using one-way analysis of variance and Tukey's honestly significant difference mean separation at $P \leq 0.05$.

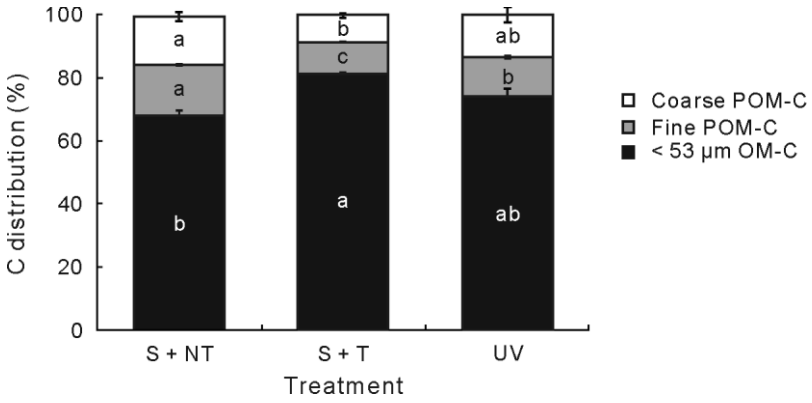

Fig. 4 Distribution of C in different organic matter (OM) fractions, particulate organic matter (POM) containing coarse POM (coarse POM-C) and fine POM (fine POM-C) and < 53 µm OM (< 53 µm OM-C) in total organic C under different treatments in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA. Values are the means with standard errors shown by the vertical bars (n = 5). Bars with different letters are significantly different for a given OM fraction using one-way analysis of variance and Tukey's honest significant difference mean separation at P ≤ 0.05. S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

Aggregate loss 10 min-after wet sieving showed a significant treatment effect (P ≤ 0.01) in the order: S + NT < UV < S + T (Fig. 5).

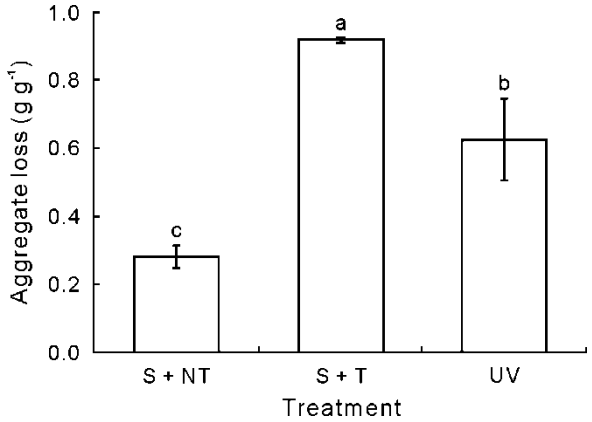

Fig. 5 Aggregate loss under different treatments in a field experiment conducted in the Los Carneros American Viticultural Area of Napa Valley, California, USA. Values are the means with standard errors shown by the vertical bars (n = 5). Bars with different letters are significantly different using one-way analysis of variance and Tukey's honestly significant difference mean separation at $P \leq 0.05$. S + NT, S + T, and UV = alley with a sown cover crop and no-tillage, alley with a sown cover crop and tillage, and under-vine zone with no-grass cover and tillage, respectively.

DISCUSSION

In the present study, different interrow management practices did not strongly influence shoot biomass. However, it significantly affected root development in S + NT. This suggested that the absence of tillage for 22 years created a soil environment that supported exploration and establishment by roots, conversely to the conditions that occur with tillage during spring and autumn in S + T. Vegetation composition, at least during the period of this study, also differed in tillage and no-tillage treatments: S + NT was colonized during the 22-year period by grasses and weeds that differed from S + T, where only the annually sown cover crop species were observed, resulting in an increase in plant diversity with potential positive effect on root biomass (Zhu et al., 2014). However, the similar C and N contents and C/N ratios of shoot and root of these two treatments indicated a comparable biochemical decomposability. This suggests that functional type of plants rather than species composition drives the C and N composition of the plant biomass. Both the treatments supported Graminaceae and N-fixing species emerging from the seedbank. In contrast, the effective vegetation control carried out in UV drastically eliminated the vegetation cover, reducing both shoot and root biomass.

Soil characteristics only partially reflect the differences in C and N in the vegetation biomass, suggesting that microbial activity and stabilization mechanisms contribute differently to SOM accumulation depending on the type of disturbance as suggested by Raiesi and Kabiri (2017). In S + NT, higher soil TOC and TN contents were likely due to the higher contribution of root biomass with respect to above-ground biomass (Post and Kwon, 2000). The use of tillage in S + T together with apparent lower root biomass led instead to a reduced content of TOC and TN, supporting the previous study that showed the relatively greater importance of intact (i.e., no tillage) root biomass to soil organic matter pools than that of the incorporated shoot biomass (Gale et al., 2000). Further, TOC and TN contents in S + T were comparable to those of UV, although the OC-input derived from vegetation biomass in UV was 20 times lower than S + T.

The highest vegetation biomass input in S + NT led to the highest DOC pool, in line with the results obtained in the surface soil layers of annual (Jacobs et al., 2009, 2010; Wang et al., 2014) and perennial (Steenwerth and Belina, 2008b; Agnelli et al., 2014) agroecosystems with cover crops and/or crop residue as a mulch. As reported by Chantigny (2003), the dissolved organic fraction can be derived from vegetation residue decomposition and rhizodeposition and feed the nutrient pools. The inorganic N content was low, most likely due to plant uptake and competition between the plants and microorganisms for N sources, especially during spring (Jackson et al., 1989; Kuzyakov and Xu, 2013). Corresponding increases in soil water content and temperature also promote microbial activity, consequently promoting a rapid consumption of

inorganic N and immobilization of microbial biomass (Burger and Jackson, 2003; Steenwerth and Belina, 2008a). The higher MBC and MBN and higher rate of potential N mineralization and potential microbial respiration in S + NT demonstrate a greater biological activity supported by soil conservation practices, similar to that observed in other annual (Wright et al., 2005; Jacobs et al., 2009) and perennial (Steenwerth and Belina, 2008a, b) agroecosystems with vegetation cover and less frequent tillage disturbance. The higher MBC/TOC ratio in S + NT compared to the other treatments indicated a higher microbial efficiency in utilizing C sources and a greater substrate availability provided by leaf and root turnover (Anderson and Domsch, 1989; Stockfisch et al., 1999). This may highlight that even with relatively low disturbance over 20 years (shallow tillage), as performed in S + T, microorganisms might become less efficient in incorporating C in their biomass. This was also indicated by large reductions in MBN and potential N mineralization (Jacobs et al., 2009).

In UV, the scarce presence of herbaceous plant species limited nutrient uptake leading to higher concentrations of NO3--N, available P, and exchangeable cations in soil compared with those of the other treatments. The lower microbial efficiency in UV, expressed by both low MBC/TOC ratio and potential microbial respiration, possibly reflects limited competition with grasses for nutrient forms as a consequence of glyphosate application. A similar reduction in microbial respiration after glyphosate application was indeed observed in a meta-analysis study on agricultural soil by Nguyen et al. (2016), and in vineyard inter-row after tillage and glyphosate application by Karl et al. (2016).

Based on the potential microbial respiration and the derived percentage of TOC evolved as CO2-C, a strong impact of soil management on C cycling dynamics mediated by soil microbial activity was observed. Decomposition of the labile C pool in S + NT was faster than in S + T and UV (data not shown), similar to that in no-tillage permanent cover treatments in other agroecosystems (Werner, 1997; Jacobs et al., 2010). As indicated earlier, the combination of vegetation cover and no-disturbance increased SOM and subsequent substrate availability, supporting greater microbial biomass and respiration. The metabolic quotient (qCO2), i.e., the maintenance energy requirement of soil microbes, among treatments was 20.0 ± 4.0 µg CO2-C mg MBC-1 h-1 (n = 15), indicating a reduced substrate use efficiency, in which large amounts of substrate are diverted toward catabolic processes at the expense of anabolic processes (Anderson and Domsch, 1990; Dinesh et al., 2012). In all treatments therefore, mineralization prevailed over accumulation, as suggested also by the relatively low and similar TOC/TN ratios. On the other hand, when substrate availability was low, as in UV, microbial respiration was limited as deduced from the lower percentage of TOC evolved as CO2-C.

The coarse and fine POM fractions are known to be sensitive to tillage (Cambardella and Elliott, 1992; Six et al., 1999; Peregrina et al., 2010). Thus, it is not surprising that S + NT had higher percent of coarse and fine POM-C than the other two treatments (Fig. 4), most likely due to lower aggregate disturbance coupled with a greater vegetation biomass input (Tisdall and Oades, 1982; Golchin et al., 1994, 1995; Six et al., 2000). In S + T, the disturbance by tillage and the lower aggregate resistance to breakdown may have led to both lower OM protection and faster mineralization processes. This is in agreement with the results found by Laudicina et al. (2017) in a vineyard from Sicily. The coarser fractions contained a lower amount of POM with a much higher C/N ratio, indicating that only fresh material feeds this pool. However, even the < 53 µm OM fraction was unexpectedly affected, the lower C/N ratio was an index of intense decomposition, similar to the findings reported in a wine grape vineyard under permanent cover crop and autumn tillage established on an Entisol in Italy (Belmonte et al., 2016).

The results of the present study elucidate why aggregate stability is not necessarily connected to vegetation cover and plant residue input, at least during the study period. The greater stability of aggregates in S + NT might be attributed to the mutual interactions among long-term biomass input, microbial activity, and protection offered by the mineral phases (Degens, 1997; Six et al., 2004). The enhanced microbial activity in the absence of tillage favoured the interaction and stabilization of SOM into

microaggregates (Six et al., 2000), consequently improving the aggregate resistance to water breakdown (Belmonte et al., 2016). This interaction was further confirmed by the negative correlation between aggregate loss and TOC ($r = --0.865$, $P \leq 0.01$), DOC ($r = --0.909$, $P \leq 0.01$), and MBC ($r = --0.836$, $P \leq 0.01$). In S + T, the decrease in organic C input, largely due to the tillage disturbance and lower root biomass, limited the input of temporary aggregating agents. The consequent reduction of microaggregates led to a topsoil that was more vulnerable to aggregate disruption. In contrast, despite scarce vegetation biomass input in UV, the aggregate loss was unexpectedly lower than that in S + T. This stronger aggregation might have led to the development of an efficient system of SOM protection, limiting mineralization by microorganisms, as demonstrated by the intermediate values of DOC, fine and coarse POM, and potentially mineralizable N. Furthermore, this might have led to the similarity in TOC contents in S + T and UV despite drastic distinctions in biomass inputs.

CONCLUSIONS

Vineyard management significantly affected soil functionality through several and interconnected actions on microorganisms, SOM dynamics, and aggregate stability. The no-tillage practice was associated with protective mechanisms against microbial mineralization through the presence of relatively more stable soil aggregates and greater vegetation biomass, resulting in the higher SOM accumulation. Just three annual tillage passes in the tilled soils led to a reduction in vegetation biomass input, especially from roots, followed by a faster turnover of both SOM and aggregates. Not surprisingly, diminished biomass input under the vine-limited microbial activity, slowed SOM decomposition and aggregate turnover. These findings suggest that tillage, even when performed infrequently, negatively affects soil functions in vineyards, and that maintaining an annual vegetation cover in the alleys cannot fully mitigate its impacts. Thus, the combination of tillage with maintaining cover crops or resident vegetation becomes a comparatively poorer option than no-tillage when the goal is to improve SOM content and aggregate stability. To this end, the use of the no-tillage permanent vegetation cover is instead recommended. Furthermore, when technically feasible, widening permanent vegetation cover into the under-vine area covering 20%--30% of the vineyard floor would contribute to improving the overall vineyard soil conservation.

ACKNOWLEDGEMENTS

We thank Mr Charles Schembre from the Napa Valley Resource Conservation District of USA for providing information and the access to the vineyard property. This study was supported by the USDA-ARS Sustainable Viticulture Production Systems, USA (No. 2032-21220- 006-00, NP305 Crop Production).

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