

Research Article

Assessing the Short-Term Effects of No-Till on Crop Yield, Greenhouse Gas Emissions, and Soil C and N Pools in a Cover-Cropped, Biodynamic Mediterranean Vineyard

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Background and Aims. No-till is considered a core practice of conservation and climate-smart agriculture. Nevertheless, recent evidence suggests that the benefits of this practice for climate change mitigation might be overestimated, particularly in the short term. **Methods and Results.** In a three-year field experiment, we investigated the environmental and agronomic performance of this practice by looking at changes in soil physical properties, C and N pools, as well as vine yield and grape quality. No-till increased stratification in the distribution of active soil C (POXC), further accentuating the already existing difference between top and subsoil. No-till also slightly reduced the daily efflux of CO₂ from the soil during the rainy season, showing that these plots were less prone to lose C than tilled plots. Nonetheless, no-till did not increase total soil C stocks. This, together with the lack of differences in cumulative N₂O emissions, resulted in similar global warming potential in till and no-till plots. Vine yield and grape quality remained unchanged in the no-till compared to the tilled plots. **Conclusions.** Even though no-till did not result in short-term climate change mitigation, results of this study suggest changes in the ecological processes leading to C accumulation and mineralization and that may result in future C sequestration. There were no deleterious effects of no-till on grape yield and quality. **Significance of the Study.** This study shows that reducing tillage intensity in vineyards is a feasible strategy from an agronomic standpoint.

1. Introduction

Soil organic matter (SOM) provides a basal resource for the soil food web and is therefore considered to be the foundation of a healthy soil ecosystem. SOM dynamics including processes of mineralization, and stabilization, strongly regulate the release of greenhouse gases such as CO₂ and N₂O, and the sequestration of carbon (C) with consequences for climate change mitigation [1, 2]. The use of cover crops is

a well-recognized strategy to diversify cropping systems while increasing SOM and sequestering C [3–5]. Both above and belowground biomass of cover crops contributes to litter production and to the increase in soil C stocks in Mediterranean vineyards [3, 6]. The production of root exudates can also contribute to substantial amounts of C sequestration (Sokol et al., 2019). Nonetheless, cover crop management has large implications for the climate change mitigation potential of this practice.

Tillage is commonly used to incorporate cover crop residues into the soil and it is estimated that more than 90% of cultivated land worldwide is subjected to some degree of tillage [7]. In winegrape production, tillage is also commonly used to increase soil porosity and to manage weeds under the vines during the growing season. This practice is particularly critical for biodynamic vineyards where the use of synthetic herbicides is precluded [8]. There is a large amount of conflicting evidence on the effects of tillage and tillage intensity on SOM and C sequestration, and therefore, its potential to mitigate climate change [9–11]. Tillage promotes the rapid incorporation of plant residues and oxygenation of the soil, increasing microbial biomass and activity and therefore accelerating nutrient cycling. A large body of scientific evidence shows that this boost in decomposition rates triggers large emissions of CO₂ and therefore reduces the net amount of plant C sequestered in soils [12–14]. The disruption of soil aggregates leads to the exposure of previously protected C to microbial attack, further increasing soil C losses [15–17]. Destruction of soil structure has other associated negative effects on soil health, such as soil compaction and consequently reducing water infiltration and plant-available water [15].

In light of all the concurrent evidence described above, no-till is considered a core practice of conservation and climate-smart agriculture [18]. Nevertheless, the benefits of this practice for climate change mitigation are not yet clear. It has been suggested that no-till causes the redistribution and stratification of C within the soil profile, rather than a net increase, leading to the overestimation of C increases when only the topsoil (<10 cm depth) is analyzed [15, 19, 20]. While CO₂ emissions typically decrease under no-till [21], several studies suggest potential tradeoffs through the short-term increase in denitrification rates and release of N₂O, a greenhouse gas with a global warming potential 298 times higher than CO₂ [22–24]. This increase in N₂O emissions is associated with higher bulk density and anaerobic microsites under no-till [25], which have been suggested to be particularly relevant shortly after the transition to no-till but disappear in the long term as soil C accumulation leads to decreases in bulk density [26]. This means that the capacity of no-till to mitigate climate change may be overstated, at least in the short-term [27]. Some studies show high rates of SOM accumulation, higher C stabilization, and lower GHG emissions in highly disturbed tilled soils, due to a more efficient transformation of plant residues into microbial biomass and mineral-associated organic matter [28, 29].

Under certain crops and conditions, transition to no-till could decrease yields [30]. Data regarding the potential effects of no-till for winegrape production is still scarce but some studies have shown changes in fruit quality related to increases in grape anthocyanin contents [31], decrease in phenolic content [32] or no changes in fruit yield and quality [33]. This uncertainty regarding the benefits of no-till for climate change mitigation, crop yield, and quality increases farmer reluctance to incorporate this conservation practice. The lack of data is particularly critical in arid and semi-arid regions where issues of soil degradation and climate change

are most acute and where soil organic matter accumulates more slowly [34, 35]. As a perennial crop of large relevance in many semiarid regions, wine grapes could be critical in the conservation of soil resources when managed properly [36].

In this study, we evaluated the short-term effects of transitioning to no-till on the yield and quality of Syrah grapes, soil C and N pools, soil biophysical properties, and greenhouse gas emissions in a biodynamically-managed vineyard. This study builds on the study by Lazcano et al. [37]; which evaluated the combined effects of sheep grazing and tillage on soil C and GHG emissions during a 2-year period. We hypothesized that, after three years, no-tilled soils would show higher soil organic matter, active C aggregate stability, and infiltration rates. We also expected that no-tilled soils would also show a strong stratification in soil C, with shallow soil depth (0–15 cm) having significantly more C than the soil at 15–30 cm, whereas tilled soils would show a homogeneous distribution of soil C throughout the 30 cm depth.

2. Material and Methods

2.1. Experimental Design. A three-year field trial was conducted between April 2018 and February 2021 in a *Vitis vinifera* L. cv. Syrah (Dureza × Mondeuse Blanche) biodynamic commercial vineyard located in the Paso Robles American Viticulture Area (AVA), San Luis Obispo County (California, USA). The study was conducted in parallel to a two-year grazing and tillage trial as described in Lazcano et al. [37]. The climate in the area is Mediterranean with an annual average temperature of 15.3°C, average annual rainfall of 364 mm, and two well-differentiated seasons, the dry (April–October) and the wet season (November–March) (Figure 1). The soil at the experimental site is a linne-calodo complex classified as 12% Linne (Fine-loamy, mixed, thermic Calcic Pachic Haploxerolls) and 10–11% Calodo (Loamy, mixed, thermic, shallow Calcic Haploxerolls), with 30% clay. This soil is characterized by 6% CaCO₃ in the <2 mm fraction and a pH of 8.2.

The rootstock (140 RU, *Vitis* parentage *berlandieri* × *rupestris*) was planted in 1992 and grafted to Syrah in 2004 (Beaucastel clone “C”). Since vine establishment, soil management included annual seeding of a cover crop mix in the fall (15% *Avena sativa*, 30% *Vicia faba*, 20% *Vicia americana*, 10% *Vicia sativa*, and 25% *Pisum sativum* subsp. *Dundale*). Fertilizer management consists of broadcasting grape pomace compost (C:N ratio of 16.5) at a rate of 11 t·ha⁻¹ once a year in the fall. The vineyard has been tilled and subjected to compost application and sheep grazing during the dormant season (November–March) for around 6 to 8 years. Vines are watered using drip irrigation once after harvest to replenish the soil profile and, when necessary, through the growing season using visual cues of plant water stress.

We assessed the short-term effects of transitioning to no-till on soil C, N, and greenhouse gas emissions. The experimental design consisted of alternating till and no-till blocks, each block with four rows of vines and three tractor rows. Till and no-till blocks were not randomized but

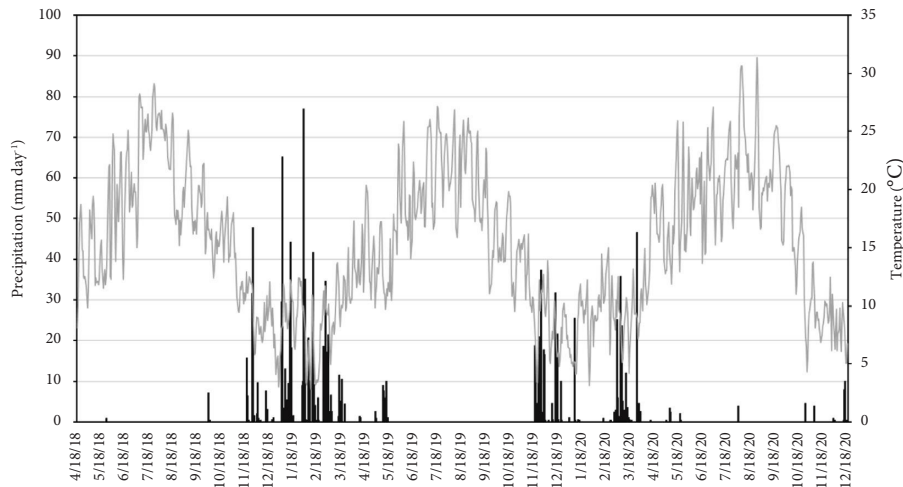


FIGURE 1: Precipitation and temperature were registered at the experimental site over the course of the study.

alternated side by side. Each treatment (till or no-till) was replicated four times, resulting in a total of 8 experimental plots. Within each plot, two functional locations, receiving different soil management, were considered: (1) the soil under the vine canopy (vine row), which receives water and is not tilled, and (2) the soil in the alleys (tractor row), which does not receive water through irrigation and may be tilled, depending on the treatment.

Management of the experimental plots included compost and cover crop seed broadcasting in Autumn each year during the wet seasons; mowing of the cover crop in the Autumn and Spring during the wet season when the cover crop reached sufficient height (30 cm approx.) (as described in [37]). Till plots were tilled a total of four times (November 2018, May 2019, November 2019, and May 2020) using a three-point offset disk to 10–25 cm depth, with two tractor passes in each event. No-till plots were left undisturbed and only mowed for the duration of the experiment (3 years).

2.2. Soil and Plant Sampling and Analysis. Aboveground cover crop biomass was measured three times during the study (April 2018, April 2019, and February 2020) by using a 1 m² quadrant as described in Lazcano et al. [37]. Total C and N were determined in dried and ground cover crop samples via dry combustion using a Vario Max CNS elemental analyzer (Elementar, Langensfeld, Hesse, Germany).

In March 2021 after three years of treatment implementation, we collected soil samples at the two functional locations within the central vine and tractor rows of each plot. Soil samples were collected to 30 cm depth using a Giddings manual bulk soil core sampler with a diameter of 5 cm (Windsor, CO, USA) and immediately split into two depths (0–15 and 15–30 cm). Additional soil samples were collected to a depth of 15 cm at the time of gas sample collection, directly after the tillage treatments and after harvest. Soils were stored in plastic bags at 4°C and transported to the lab prior to analysis. Grape yields were determined in mid-September in 2018, 2019, and 2020, when

berries reached approximately 23°C Brix. To determine yields we randomly selected 10 vines from the two central rows of each plot and measured the total number and fresh weight of all grape bunches on each vine [37]. A subsample of 200 berries was collected in each plot for further chemical analysis. Berries were homogenized to measure berry anthocyanins and total phenolics following a previously published protocol [37, 38].

Infiltration rates were determined in March 2021 at the time of soil sampling using mini-disk infiltrometers (METER Group Inc., USA). Soil moisture was determined gravimetrically by oven drying at 105°C for 24 hours. Soil bulk density (g cm⁻³) was determined based on the known volume of the soil core, the fresh weight of the soil core, and the gravimetric moisture content of the fresh soil sample. No significant amount of gravel was detected in the samples. Soil moisture was determined gravimetrically by oven drying at 105°C for 24 hours. Soil water-filled pore space (%WFPS) was calculated using the soil gravimetric water content (w) as follows:

$$\%WFPS = \frac{(w * \text{bulk density})}{[1 - (\text{bulk density}/2.65)]} * 100\%. \quad (1)$$

The water holding capacity of the soil samples was determined gravimetrically by determining the difference between the soil weight at field capacity and permanent wilting point. Aggregate stability was determined as the percentage of soil remaining on a 250 μm sieve after wet sieving. Ten grams of air-dried soil was placed on a 250 μm sieve and submerged in deionized water for 5 minutes to allow for rewetting. Subsequently, wet sieving took place by oscillating the sieve at a constant speed for 2 minutes. The soil remaining on the sieve was recovered and oven dried for the determination of the percent aggregate stability.

Total C and N were determined in dried and ground soil samples via dry combustion using a Vario Max CNS elemental analyzer. Combustion at 650°C was used to account for the maximum recovery of organic C and minimal inorganic C recovery [37, 39, 40]. Nitrate (NO₃⁻-N) and

ammonium ($\text{NH}_4^+\text{-N}$) in soil samples were determined colorimetrically in 2 M potassium chloride (KCl) soil extracts [41] using a Thermo Scientific Evolution 201 UV-visible spectrophotometer (Madison, Wisconsin, USA). Active C, also known as permanganate oxidizable carbon (POXC), was determined in 2.5 g air-dried soil samples based on a reaction with 2 M potassium permanganate (KMnO_4), after which the color change was determined on a spectrophotometer (Milton Roy, Houston, TX) at 550 nm [42].

Microbial biomass C (MBC) was determined through the fumigation-extraction method. Briefly, a soil subsample (6 g fresh weight) was subjected to fumigation with chloroform for 24 h prior to extraction with 0.5 M K_2SO_4 . In parallel, a second subsample was directly extracted with 0.5 M K_2SO_4 without fumigation. The concentration of dissolved organic C (DOC) was analyzed in fumigated and nonfumigated samples on a Dohrmann Phoenix 8000 UV-persulfate oxidation analyzer (Tekmar-Dohrmann, Cincinnati, OH). Microbial biomass C was calculated as the DOC in the fumigated minus the nonfumigated soil samples with a K_e factor of 0.35 (Horwath and Paul, 1994; Vance et al., 1987).

2.3. Analysis of Soil N_2O and CO_2 Emissions. Gas samples from the experimental plots were collected one day before and for 4-5 days after each of the main management events including mowing, tillage, irrigation, and harvest as well as after the first precipitation event in fall, to measure baseline and event-related fluxes. Sampling was done once a month between these management events as described in Lazcano et al. [37]. Fluxes of N_2O and CO_2 were measured using static flux chambers [43] made of two PVC rings (20 cm diameter and 12 cm height): a bottom ring or collar and a cap covered with insulating reflective material to reduce heating within the chamber. The chambers were vented and equipped with a thermocouple to track changes in chamber temperature during chamber closure. The collars were inserted into the soil to a depth of approximately 10 cm in the central tractor and vine row in each plot [37]. During flux measurements, the collars were capped, and gas samples were taken with an air-tight polypropylene syringe by slowly withdrawing 20 mL of gas through sampling ports capped with rubber septa. Gas samples were immediately transferred from the syringe into pre-evacuated 12-mL Exetainer glass vials (Labco Ltd., Buckinghamshire, UK). Gas samples were collected at regular time intervals of 0, 15, 30, and 45 min after chamber closure [37].

Gas samples were transported to the laboratory and analyzed on a Shimadzu GC-2014 gas chromatograph (Shimadzu Scientific, Kyoto, Japan). N_2O and CO_2 concentrations in the samples were calculated using a calibration curve based on a set of analytical grade standards. Chamber gas concentrations were converted to mass per volume units assuming ideal gas relations using chamber air temperature values [44]. Fluxes were calculated from the rate of change in chamber N_2O and CO_2 concentration over the sampling intervals, taking into account chamber

volume and soil surface area [37, 43, 45]. When the data had a nonlinear trend, the slope to the first derivative of the second-order polynomial was used as the flux, rather than the linear model, using the Microsoft Excel LINEST function [46]. Fluxes were not considered when the fit to the linear or LINEST function was poor ($R^2 < 0.80$). Cumulative emissions were determined by trapezoidal integration of daily fluxes measured in each chamber over a specific period (management events or baseline measurements between events). Cumulative emissions from each chamber location were calculated under the assumption that the measured fluxes represent mean daily fluxes, and that means daily fluxes change linearly between measurements [37]. Global warming potential (GWP) was calculated as follows:

$$\text{GWP} = \Delta\text{SOC}(\text{kg CO}_2\text{eq ha}^{-1}) + \text{N}_2\text{O}(\text{kg CO}_2\text{eq ha}^{-1}), \quad (2)$$

where ΔSOC is the difference between soil C stocks, in $\text{kg}\cdot\text{ha}^{-1}$, between 2018 and 2021. The change in soil C stock was then transformed to CO_2 equivalents by multiplying by 44/12. N_2O ($\text{kg CO}_2\text{eq}\cdot\text{ha}^{-1}$) is the cumulative emissions over the period of the study multiplied by 273.

2.4. Data Analysis. General linear models were used to evaluate the effects of tillage and functional location on the average daily emissions, cumulative emissions of N_2O and CO_2 , and ancillary soil variables measured at the time of gas sampling (%WFPS, NH_4^+ , and NO_3^-). Differences in average daily emissions at the different locations due to the tillage treatments were assessed throughout April 2018 through December 2020, including four tillage events. Daily emissions, cumulative emissions of N_2O , CO_2 , and ancillary soil variables, were also clustered into seasons (wet and dry) for further data analysis. Wet seasons (November through March) included the months with precipitation and coincided with vine dormancy whereas dry seasons included months without precipitation and with active grape vines (April through October) (Figure 2). A total of five seasons' worth of data were collected, including three dry seasons (2018, 2019, and 2020) and two wet seasons (2018-2019 and 2019-2020). The effect of tillage was assessed within the two functional locations (tractor and vine row) and within seasons (wet or dry). Tukey HSD tests were used for pairwise comparisons among the different treatment levels. When residuals were not normally distributed, response variables were transformed prior to the analysis by using $\log_{10} + 10$ (N_2O , CO_2) or square root (NO_3^-). Correlations between daily fluxes and ancillary variables measured at gas sampling were run using nonparametric Spearman's ρ .

General linear models were used to evaluate the effects of tillage treatments on soil N and C, at the two functional locations (vine, tractor row) and soil depths (0-15 cm, 15-30 cm), in 2021, three years after practice implementation. We also used general linear models to evaluate differences in crop yield with tillage, and year as fixed factors. All statistical analyses were conducted with JMP Pro v.15.1.0. (2019, SAS Institute Inc.).

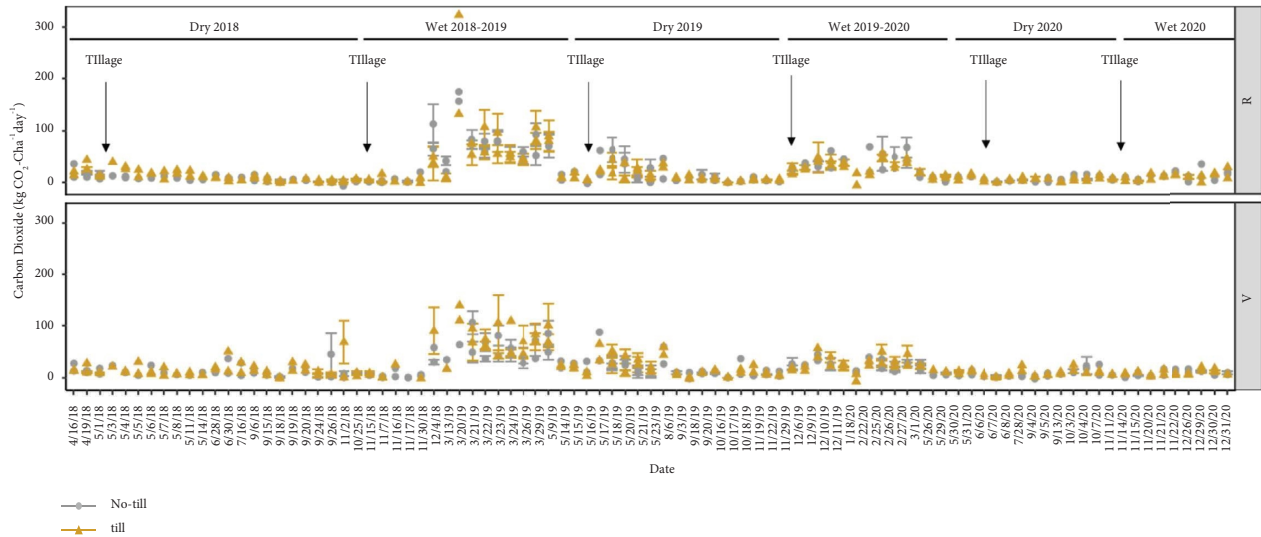


FIGURE 2: CO₂ daily fluxes were measured during the study in the soil in the tractor row (R) and under the vines (V) in either till (orange) or no-till (gray) plots. Values are means ± standard error of 4 replicates.

3. Results

3.1. Effects of the Tillage Treatments on Soil C and N Pools. Soil organic C was significantly higher in the topsoil (0–15 cm depth) as compared to the subsoil (15–30 cm depth) (depth: $F = 52.9$; $p < 0.001$) (Table 1). No statistically significant differences in soil organic C were observed between tilled and nontilled plots after three years of practice implementation. Similar trends were observed for total soil N, which was significantly higher in the topsoil (0–15 cm) as compared to the subsoil (depth: $F = 68.9$; $p < 0.001$), without significant differences between tilled and nontilled plots at either depth (Table 1).

The concentration of active C or POXC was also significantly higher in the topsoil as compared to the subsoil (depth: $F = 32.8$; $p < 0.001$) (Table 1). We observed a significant effect of tillage three years after the start of practice implementation, which depended on the soil depth interval. No-till plots showed statistically significant differences between the two soil depths, with topsoil having more POXC than subsoil, whereas tilled plots had similar POXC concentration at the two depths (depth \times tillage: $F = 4.20$; $p = 0.05$). Microbial biomass C (MBC) was similar between tractor and vine rows but higher in the 0–15 cm than in the 15–30 cm soil layer (depth: $F = 16.9$; $p < 0.001$) of both tractor and vine rows. Nonetheless, MBC remained unaffected by the tillage treatments. The concentration of plant-available N (NO_3^- -N) in soil samples was similar across depths locations and tillage treatments (Table 1). No significant differences were found in ammonium concentrations in soil samples collected at the different locations, depths, and tillage treatments (Table 1).

3.2. Effects of the Tillage Treatments on Soil Physical Properties. Analysis of soil physical properties in 2021, three years after the start of the experiment revealed that bulk density was

similar across locations and depths and remained unaffected by the tillage treatments (Table 2). Aggregate stability was significantly higher in the topsoil (0–15 cm) than in the subsoil (15–30 cm) (Table 2) (depth: $F = 4.34$; $p = 0.049$), although no differences were found between tillage treatments at either location or depth. No significant differences were found in water infiltration rates in the tractor row of tilled versus nontilled plots (Table 2). Soil water holding capacity (WHC) was unaffected by tillage, although we observed a trend for slightly higher WHC in the topsoil of no-till plots ($F = 3.54$; $p = 0.073$) (Table 2) that would be worth investigating in long-term experiments.

3.3. Effects of the Tillage Treatments on GHG Emissions and GWP. Daily fluxes of CO₂ and N₂O measured from April 2018 to December 2020 were analyzed by season for a total of five seasons including 3 dry seasons and 2 wet seasons. The daily fluxes of CO₂ ranged from 0 to 399 kg CO₂-C ha⁻¹·day⁻¹ being generally higher in wet as compared to dry seasons throughout the study (Figure 1). Emissions of CO₂ were significantly higher in tilled vs. nontilled plots during the 2018 dry season (tillage: $F = 6.92$; $p = 0.009$) and 2018–2019 wet season (tillage: $F = 5.71$; $p = 0.017$), irrespectively of the location (vine or tractor row) (Figure 1). This trend was reversed in the 2019–2020 wet season where no-till plots had significantly higher emissions as compared to tilled plots (tillage: $F = 4.19$; $p = 0.0423$). No significant differences in CO₂ emissions were found between the tractor and the vine row. Despite the differences observed in daily fluxes, cumulative emissions of CO₂ were not different between treatments or locations at any of the seasons included in this study (Table 3).

Daily fluxes of N₂O ranged between –13 and 70 g N₂O-N ha⁻¹·day⁻¹ throughout the study (Figure 3). Fluxes of N₂O were not significantly different between tilled and nontilled plots at either of the locations studied (vine or tractor row) in

TABLE 1: Different C and N pools were analyzed in the soil samples collected at two depths (0–15 and 15–30 cm) from the tractor and vine rows of the experimental plots in 2021, three years after practice implementation. Values are means \pm standard errors. Letters within the same column indicate significant differences between treatments, locations, and depths at $p < 0.05$.

		Total C (%)	Total N (%)	SOM (%)	POXC ($\text{mg}\cdot\text{kg}^{-1}$)	MBC ($\text{mg}\cdot\text{kg}\text{ soil}^{-1}$)	$\text{NH}_4^+\text{-N}$ ($\mu\text{g}\text{g soil}^{-1}$)	$\text{NO}_3^-\text{-N}$ ($\mu\text{g}\cdot\text{g soil}^{-1}$)	
Tractor <i>r</i>	0–15	No-till	3.0 ± 0.2^a	0.3 ± 0.02^a	5.17 ± 0.31^a	827.3 ± 32^a	453.24 ± 113^a	1.28 ± 0.07	11.9 ± 0.9
		Till	3.0 ± 0.1^a	0.3 ± 0.0^a	5.14 ± 0.16^a	774.7 ± 15^{ab}	378.46 ± 62^a	1.41 ± 0.09	9.0 ± 0.6
	15–30	No-till	2.1 ± 0.0^b	0.2 ± 0.0^b	3.69 ± 0.06^b	631.6 ± 38^c	221.58 ± 25^b	1.37 ± 0.23	12.9 ± 2.3
		Till	2.5 ± 0.1^b	0.3 ± 0.01^b	4.25 ± 0.14^b	652.8 ± 52^{bc}	162.42 ± 19^b	1.33 ± 0.16	10.8 ± 2.1
Vine <i>r</i>	0–15	No-till	3.0 ± 0.2^a	0.3 ± 0.02^a	5.22 ± 0.30^a	846.8 ± 62^a	369.24 ± 40^a	1.15 ± 0.15	9.9 ± 0.7
		Till	2.8 ± 0.1^a	0.3 ± 0.01^a	4.77 ± 0.21^a	783.7 ± 25^{ab}	381.27 ± 51^a	1.28 ± 0.11	8.5 ± 1.2
	15–30	No-till	2.2 ± 0.1^b	0.2 ± 0.01^b	3.80 ± 0.25^b	639.1 ± 21^c	186.37 ± 46^b	1.29 ± 0.08	10.8 ± 1.4
		Till	2.2 ± 0.2^b	0.2 ± 0.02^b	3.79 ± 0.30^b	714.7 ± 17^{bc}	270.46 ± 83^b	1.66 ± 0.22	11.4 ± 2.1

TABLE 2: Physical properties of the soil samples collected from the tractor and vine rows of the experimental plots in 2021, three years after practice implementation. Values are means ± standard errors. Letters within the same column indicate significant differences between treatments, locations, and depths at $p < 0.05$.

			Bulk density ($\text{g}\cdot\text{cm}^{-3}$)	Stable aggregates (%)	WHC ($\text{g}\cdot\text{g soil}^{-1}$)
Tractor <i>r</i>	0–15 cm	No-till	0.844 ± 0.05	66.5 ± 4.57 ^a	1.11 ± 0.03
		Till	0.897 ± 0.05	69.2 ± 1.83 ^a	1.03 ± 0.06
	15–30 cm	No-till	0.791 ± 0.09	64.9 ± 5.04 ^b	1.12 ± 0.03
		Till	0.862 ± 0.06	60.6 ± 2.53 ^b	1.11 ± 0.02
Vine <i>r</i>	0–15 cm	No-till	0.805 ± 0.06	65.7 ± 4.48 ^a	1.10 ± 0.02
		Till	0.859 ± 0.04	69.7 ± 1.90 ^a	1.04 ± 0.01
	15–30 cm	No-till	0.769 ± 0.04	60.7 ± 2.57 ^b	1.09 ± 0.02
		Till	0.798 ± 0.12	65.1 ± 1.74 ^b	1.05 ± 0.02

TABLE 3: Cumulative CO₂ and N₂O emissions by season were measured at the tractor row and soil under the vines in tilled and nontilled plots. Letters within the same row (season) indicate significant differences between tillage treatments at $p < 0.05$.

	Tractor row		Vine row	
	No-till	Till	No-till	Till
kg CO₂-C ha⁻¹ season⁻¹				
Dry 2018	428 ± 170	1029 ± 467	656 ± 266	125 ± 934
Dry 2019	1610 ± 810	4305 ± 1787	1839 ± 1109	3514 ± 989
Dry 2020	789 ± 129	970 ± 245	1183 ± 568	1556 ± 564
Wet 2018-2019	28853 ± 19789	80296 ± 45596	9893 ± 3730	44724 ± 24205
Wet 2019-2020	10320 ± 5376	6612 ± 3199	12071 ± 3991	9143 ± 9562
Wet 2020	221 ± 95	366 ± 113	376 ± 136	162 ± 46
g N₂O-N ha⁻¹ season⁻¹				
Dry 2018	48.2 ± 31	114.3 ± 104	89.1 ± 82	48.7 ± 30
Dry 2019	363 ± 303	177 ± 103	383 ± 186	194 ± 113
Dry 2020	119.3 ± 148	145.5 ± 55	62.9 ± 26	141.9 ± 52
Wet 2018-2019	441 ± 385	790 ± 750	78.6 ± 34	3102 ± 2031
Wet 2019-2020	1246 ± 412	1598 ± 556	260 ± 106	1521 ± 1303
Wet 2020	-13.3 ± 15	1.6 ± 3	-48.7 ± 49	2.9 ± 2

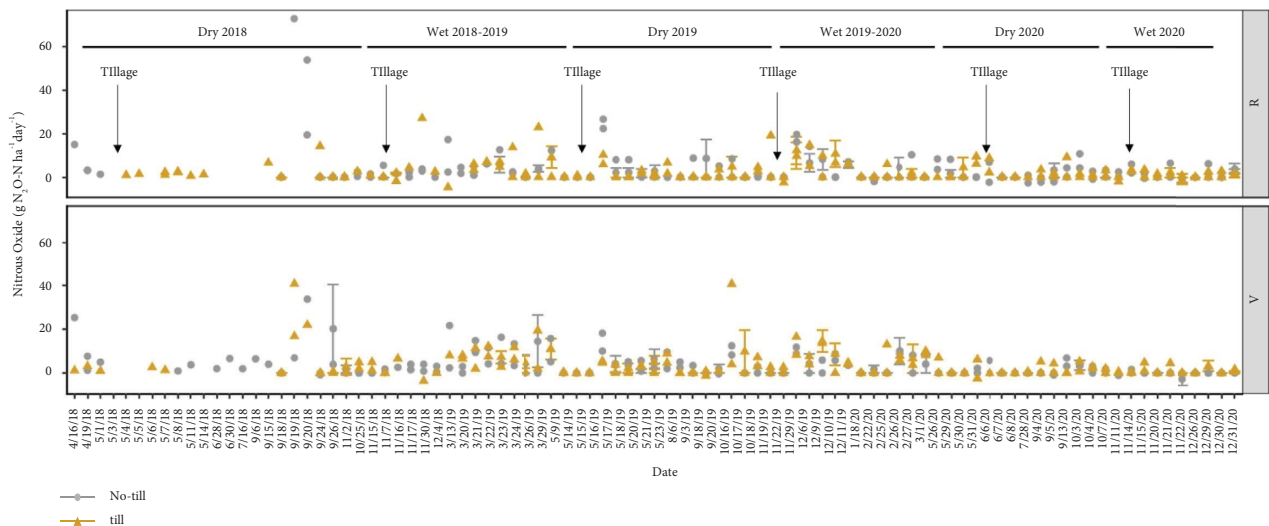


FIGURE 3: N₂O daily fluxes were measured during the study in the soil in the tractor row (R) and under the vines (V) in either till (orange) or no-till (gray) plots. Values are means ± standard error of 4 replicates.

the 2018 dry season (tillage: $F = 0.79$; $p = 0.391$), 2018-2019 wet season (tillage: $F = 2.09$; $p = 0.153$), 2019 dry season (tillage: $F = 3.74$; $p = 0.061$), 2019-2020 wet season (tillage:

$F = 0.17$; $p = 0.678$), and 2020 dry season (tillage: $F = 1.02$; $p = 0.323$) (Figure 3). We did not have sufficient data and degrees of freedom to perform statistical analysis for the

TABLE 4: Change in soil C stocks, CO₂ equivalents and global warming potential of the till and no-till plots during the three years of this study. Values are averages of 4 replicates ± standard errors. Negative values indicate C drawdowns or inputs into the system while positive C indicates C outputs or losses. Letters within the same column indicate significant differences between treatments and years at $p < 0.05$.

		Δ soil C (kg CO ₂ eq·ha ⁻¹)	GWP N ₂ O (kg CO ₂ eq·ha ⁻¹)	GWP (kg CO ₂ eq·ha ⁻¹)
Tractor <i>r</i>	No-till	39.17 ± 82	315.3 ± 78	354.5 ± 56
	Till	-248.1 ± 221	404.2 ± 175	156.1 ± 356
Vine <i>r</i>	No-till	187.4 ± 67	117.9 ± 35	305.29 ± 68
	Till	23.1 ± 128	718.4 ± 476	741.6 ± 525

2020 wet season. During the 2019-2020 wet season, the tractor rows had slightly higher N₂O emissions than the vine rows (location: $F = 5.03$; $p = 0.032$), but no other differences between the locations were found throughout the study. Despite the differences observed in daily fluxes, cumulative emissions of N₂O were not different between treatments or locations at any of the seasons included in this study (Table 3).

The CO₂ and N₂O daily fluxes throughout the study were significantly and positively correlated ($p < 0.0001$). N₂O fluxes were positively correlated to soil water-filled pore space ($p = 0.031$) and negatively correlated to the soil content of ammonium (NH₄⁺-N) ($p < 0.001$). The CO₂ fluxes were negatively correlated to the soil content of ammonium (NH₄⁺-N) ($p < 0.001$).

We used the cumulative N₂O emissions of the till and no-till treatments as well as the change in C stocks to calculate the global warming potential (GWP) of the practices over the three years of the study (Table 4). No significant differences were found among tillage treatments in the change of C stocks ($F = 2.64$; $p = 0.13$), or GWP ($F = 0.21$; $p = 0.65$) at either of the locations sampled in this study (tractor and vine row).

3.4. Effects of the Tillage Treatments on Cover Crop and Vine Yield. Large interannual variability was observed in cover crop growth and therefore potential C and N inputs to the soil. Cover crop biomass was significantly higher in 2019 compared to 2018 and 2020 (Table 5) ($F = 18.76$; $p < 0.001$). The N input from the cover crop was higher in 2019 ($F = 19.48$; $p < 0.001$) although C inputs were higher in 2018 compared to 2020 ($F = 8.31$; $p = 0.0028$). No differences were observed in cover crop biomass C and N inputs between till or no-till plots.

No significant differences were observed either in crop yield between tilled or no-tilled plots, irrespectively of the year (Table 5) (tillage: $F = 0.05$; $p = 0.815$), although we did observe strong interannual variability in this parameter (year: $F = 7.05$; $p = 0.005$). We did not detect any significant differences between tillage treatments in crop quality as assessed through the content of anthocyanins (tillage: $F = 0.008$; $p = 0.929$) and phenolics (tillage: $F = 0.30$; $p = 0.588$), although both quality parameters showed strong interannual variability (anthocyanin: $F = 121.6$; $p < 0.001$; phenolics $F = 48.74$; $p < 0.001$) as it would be expected.

4. Discussion

Soils under no-till are subjected to a lower degree of disturbance than tilled soils, having generally higher structural stability and C sequestration [47, 48]. These changes have

been shown to have direct impacts on C and N cycling, potentially reducing C turnover and CO₂ emission but triggering the release of N₂O through denitrification in anaerobic microsites [23, 49].

In this study, three years after the transition to no-till in this biodynamically-managed vineyard, we observed little change in soil physical properties, C and N pools. No-till increased stratification in the distribution of active soil C (POXC), further accentuating the already existing difference between top and subsoil. Similar increases in topsoil POXC with no-till were observed by Bongiorno et al., [50] in 10 long-term experiments evaluating the effects of tillage across an edaphoclimatic gradient in Europe. It is well known that the transition to no-till systems causes a redistribution of C within the soil profile rather than a net increase [51]. Thus, no-till soils accumulate more C and have a higher bulk density on the surface than tilled soils where soil C is incorporated at depth [47, 52–54]. Our results suggest a trend for higher C accumulation in the topsoil of no-till plot and potential for future C sequestration [55], although no significant differences were observed between till and no-till plots in SOM, total C and N at either depth.

Transition to no-till slightly reduced the daily efflux of CO₂ from the soil during the rainy season, showing that these plots were less prone to lose C than tilled plots. Nonetheless, these differences in daily fluxes were not translated to cumulative seasonal emissions. Similar increases in CO₂ efflux from tilled vineyard soils during precipitation events were reported by Steenwerth et al., [56]; who suggested that these fluxes were driven by changes in soil C content, WFPS, and temperature.

Emissions of N₂O from vineyard soils are generally lower than other crops grown in Mediterranean regions [6]; yet, the lack of tillage disturbance can trigger emissions of this greenhouse gas, especially in fine-textured soils, as the one in this study [23]. In our study, daily fluxes of N₂O were positively correlated to soil WFPS and negatively to ammonium concentration suggesting higher emissions from nitrification and denitrification processes. However, opposite to what we had expected, no significant increases in N₂O daily fluxes and cumulative emissions were observed in the no-till plots through the course of this study, which suggests that seasonal changes in soil moisture and available N were stronger drivers than soil management. These results are in line with Garland et al. [57]; who reported no significant differences in N₂O emissions between tilled and no-till plots in a Mediterranean vineyard one year after the start of the treatments, and show that there are no associated environmental tradeoffs to conversion to no-till.

TABLE 5: Effects of tillage on cover crop (CC) biomass and associated C and N inputs, vine yield, and grape chemistry in 2018, 2019, and 2020. Letters within the same column indicate significant differences between treatments and years at $p < 0.05$.

		CC biomass (g·m ⁻²)	CC C input (g·m ⁻²)	CC N input (g·m ⁻²)	Yield (kg·vine ⁻¹)	Anthocyanins (mg·g ⁻¹)	Phenolics (mg·g ⁻¹)
2018	No-till	123 ± 25 ^b	52.3 ± 10.6 ^a	1.6 ± 0.3 ^b	2.63 ± 0.4 ^b	0.26 ± 0.02 ^b	0.09 ± 0.04 ^b
	Till	158 ± 25 ^b	67.4 ± 11.3 ^a	2.4 ± 0.3 ^b	2.72 ± 0.5 ^b	0.30 ± 0.02 ^b	0.05 ± 0.03 ^b
2019	No-till	222 ± 27 ^a	38.2 ± 4.9 ^{ab}	4.8 ± 0.9 ^a	3.86 ± 0.3 ^a	0.23 ± 0.03 ^b	0.03 ± 0.02 ^b
	Till	154 ± 18 ^a	49.1 ± 11.2 ^{ab}	3.4 ± 0.4 ^a	3.94 ± 0.5 ^a	0.20 ± 0.03 ^b	0.04 ± 0.02 ^b
2020	No-till	59 ± 17 ^c	24.4 ± 7 ^b	1.3 ± 0.3 ^b	2.99 ± 0.1 ^b	0.89 ± 0.07 ^a	0.14 ± 0.07 ^a
	Till	62 ± 5 ^c	25.4 ± 2 ^b	1.5 ± 0.1 ^b	2.60 ± 0.2 ^b	0.88 ± 0.08 ^a	0.16 ± 0.08 ^a

Altogether, the lack of differences in soil C stocks and cumulative GHG emissions led to similar GWP between till and no-till plots. This lack of effects could be due to the short duration of the study relative to the SOM buildup in semiarid, Mediterranean regions. The slower buildup of SOM is expected given that the rate of carbon accumulation for a given practice is strongly dependent on environmental conditions (i.e., temperature and precipitation) that regulate microbial activity [58, 59]. This suggests that the building of organic matter and soil C stocks in no-till vineyards may take more than 5 years [51, 60–62]. For instance, Wolff et al., [63] reported significant increases in SOC seven years after the transition to no-till in a California vineyard, which caused significant decreases in the GWP of the practice as compared to conventional tillage with a disk to 10 cm depth.

It is also possible that the lack of significant differences between tilled and nontilled plots SOM, C, and N is due to the tillage implement used in this vineyard (disk) compared to other systems, such as moldboard plow or chisel plow, which are known to produce a higher disturbance intensity [21]. In this region of California, growers employ different implements and apply different tillage intensities and frequencies depending on the type of soil and production goals. Laudicina et al. [48] reported a significant short-term (5 years) reduction in bulk density together with increases in aggregate stability, and total soil C of a Mediterranean vineyard soil but only when no-till was compared to rotary tiller (higher intensity), whereas there were almost no differences with a spading machine (lower intensity). A moderate degree of tillage intensity may not have negative effects on soil health and C sequestration, particularly when stacked with other conservation practices (cover crops, compost). In a long-term field experiment comparing different agricultural systems Autret et al., [64] estimated that, under conservation practices (i.e., no-till), the lack of tillage explained only 20% of the C accumulation while the majority of the C inputs were attributed to crop residues and cover crops.

The vineyard studied here has consistently received large inputs of cover crops and, despite being tilled for the last 6–8 years, it also shows some of the highest levels of SOM as compared to the typical values reported for vineyards in California [65]. These high levels of SOM could not only be partially explained by the fine texture of the soil which allows for the formation of stable, mineral-associated organic matter (MAOM) [66, 67] but also by long-term management as it has been observed previously for biodynamic systems

[68–71]. High levels of SOM in organically managed cropping systems have also been associated with tightly coupled N mineralization and immobilization [72] which explains the lack of differences between till and no-till plots in inorganic N observed in our study. Most likely, this is also associated with the lack of significant differences in crop yield and grape quality.

In summary, even though implementation of no-till for three years did not lead to increases in soil organic matter and soil C stocks, we observed a trend towards higher C stratification and reduced CO₂ emissions in no-till plots that suggest changes in the ecological processes leading to C accumulation and mineralization. Adopting no till-practices has associated environmental benefits through the reduction of the use of machinery and fuel consumption. This suggests the environmental benefits of this practice, although longer studies and life cycle analysis would be needed to verify this trend. There were no deleterious effects of no-till on grape yield and quality, proving that reducing tillage intensity is a feasible strategy from an agronomic standpoint.

5. Conclusions

Even though no-till did not result in short-term climate change mitigation, results of this study suggest changes in the ecological processes leading to C accumulation and mineralization and that may result in future C sequestration. There were no deleterious effects of no-till on grape yield and quality.

Data Availability

Data will be made available upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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