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Assessment of the Impact of Distinct Vineyard Management Practices on Soil Physico-Chemical Properties

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ABSTRACT: Vines are one of the most ancient crops, with great relevance worldwide but especially in wine-growing areas in Southern Europe. In the Bairrada wine region of north-central Portugal, vineyards have long been managed intensively, with frequent tillage and application of fertilizers and phytochemical products. During the last decade, however, these conventional practices are increasingly becoming substituted by more sustainable management practices, in particular integrated production (IP) and, to a lesser degree, no-tillage (NT) and biodynamic (BD). This study investigated differences in soil quality of 4 vineyards managed with each of these practices for at least 6 years. Twelve topsoil (0-15 cm) samples were collected in vineyard rows and inter-rows, during one sampling campaign, and analyzed for selected physical and chemical properties. These physical properties were texture, bulk density and penetration resistance, while the chemical properties included pH, electrical conductivity, and the contents of organic matter, nutrients, cations, and metals. Nearby forest soils were also sampled as a reference, since this was the prior land-use in the study sites. The obtained results demonstrated that conventional practices were associated with diminished soil quality, as indicated by lower contents of organic matter and nutrients, such as total nitrogen (TN) and phosphorus (TP), and exchangeable cations, as well as by a higher concentration of Cu and, in some samples, of Ni and Pb. Cu concentrations were also relatively high under NT, so that overall soil quality, particularly associated with fertility, was best under IP.

KEYWORDS: Vineyards, management practices, long-term impacts, soil properties

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Introduction

Vineyards are an important type of perennial croplands that are established practically on all continents and cover a total area of 7.4 million ha.[1] In 2018, 45% of this area was located in Europe, with Portugal having the fourth largest vineyard area (190 kha) and contributing 2.1% to the total wine production, and ranking ninth on the list of wine exporting countries.[1] The wine sector is an important economic sector in Portugal, not only because wine amount to 52% of total sales of the drink industry[2] but also due to increasing enotourism.[3] Wine growing in Portugal is still largely based on intensive management practices that focus on maximizing grape yields. Traditional wine-growing practices in Portugal involve frequent tillage to minimize weed cover and soil compaction, post-harvest removal of crop residues, and high application rates of mineral fertilizers and phytopharmaceuticals for weed and pest control.[4,5] Such conventional management practices, however, can have significant side effects in terms of soil quality as well as surface water quality.[6,7]

Vineyards in Mediterranean regions are among the most degraded agricultural crop systems.[5,8] This is at least in part due to the intensive management practices, while the poor soils,[9] time of plantation and parent material,[10] steep slopes and intense rainfalls[11] also play a key role. Land degradation in Mediterranean vineyards is associated with loss of soil organic matter (SOM) due to accelerated mineralization,[12] decrease of nutrient contents,[13] topsoil compaction[14] and reduced water infiltration capacity,[15] enhanced soil erosion rates,[11] accumulation of metals and organic pollutants,[5] and associated loss of soil biodiversity due to habitat deterioration, and, overall, a reduced provisioning of ecosystem services.[16] Due to enhanced runoff and erosion, and the associated transport of nutrients, pesticides and their degradation products accumulated in soils, Mediterranean vineyards have been identified as relevant sources of diffuse contamination and, thereby, an important threat to—typically scarce—water resources.[3,17] Aside from its off-site impacts, soil erosion can also decrease in-situ soil fertility and, thereby, reduce crop yields and economic revenues of farmers,[4,9] which, in turn, can promote land use changes, including land abandonment.[13]

The environmental problems caused by agricultural intensification have been raising growing concerns regarding traditional farming systems and, arguably, have motivated a gradual shift toward more sustainable practices.[18] In Europe, this shift is mostly triggered by governmental subsidies to farmers, mainly through the Common Agriculture Policy[11] and, to a lesser extent, by changing consumer preferences for sustainable and healthy products.[19] Several wine producers have adopted sustainable practices as a strategy to provide product quality differentiation,[20] for example, through specific certifications or membership in prestigious professional associations.[19] Organic vineyards in Europe increased from 87 577 ha in 2004 to 315 579 ha in 2014,[21] with European southern countries showing a particularly rapid expansion.[22] Vineyards under biodynamic (BD) practices amounted to 147 000 ha across the world in 2012, and these practices have been receiving growing attention in the past 10 years.[23]

The growing use of sustainable practices in viticulture has become an object of frequent research interest, with a special focus on their implications for wine quality[24] and soil quality, including soil biodiversity.[23,25] Three practices have received special attention in terms of soil quality impacts: (1) application of organic amendments (eg, manure and compost) for increasing SOM and available nitrogen contents;[15,26] (2) reduced tillage for increasing SOM content,[12] decreasing soil compaction, and increasing hydraulic conductivity,[15] and for decreasing runoff and erosion;[13] (3) permanent grass cover (spontaneous or seeded) for increasing SOM content, improving soil structure, enhancing water infiltration, and decreasing soil erosion.[9,13] Ferreira et al[11] found that alternating tillage practices (performed only in every second inter-row, while the inter-row besides the tilled one is covered by spontaneous vegetation) reduced runoff, erosion and nutrient losses, but not sufficiently to halt soil degradation. Borsato et al[20] reported that organic viticulture, without application of mineral fertilizers and chemical pesticides and with less-frequent tillage, increased soil carbon stocks, and reduced soil compaction as well as erosion. Villanueva-Rey et al[26] and Picone et al[27] claimed that BD viticulture, with organic management practices, based on minimum soil disturbance, application of organic matter and fermented compost, and the use of natural products for pest control, stimulate soil nutrient cycling and improves soil fertility.

Overall, however, there is still a limited knowledge of the impacts of sustainable management practices on soil quality of vineyards.[29] This is especially true for the long-term impacts, as many key soil quality indicators are known to respond slowly to changes in management (eg, soil organic carbon and cation exchange capacity).[5] This study aims to assess such long-term soil quality impacts by comparing conventional viticulture (CV) with 3 different sustainable management practices that have been implemented for more than 6 years. They are no-tillage (NT), integrated production (IP), and BD management. Assessing the impacts of different management practices on soil properties can guide winegrowers and decision makers to minimize land degradation and adopt more sustainable agriculture.

Material and Methods

Study area

The study was carried out in the Denomination of Controlled Origin of Bairrada wine region (Figure 1), which produces 3.6% of the Portuguese wines.[2] Vineyards in this region are mostly subject to conventional practices, but an increasing number is now being managed under IP practices, supported by governmental subsidies. A reduced number of vineyards is being managed with NT or BD practices. For this study, one representative vineyard was selected for each of these 4 management practices (Figure 1), all of them for at least 6 years. Conventional management (CV) involves intensive tillage (3-5 times per year) with heavy machinery (wheeled tractor with chisel), to maintain the soil surface bare and break soil crusting, and large inputs of fertilizers and phytochemical products. Integrated production is regulated by the principles of soil conservation and fertility improvement, imposing reduced tillage to maintain a partial, spontaneous soil cover (eg, *Poaceae, Sonchus oleraceus*, and *Calendula arvensis*), regulated fertilization rates, and selective application of phytochemical products (eg, preventive and curative fungicides) in accordance with compulsory instructions issued by regional authorities. Under both CV and IP, management practices are highly mechanized, with tillage being performed with a chisel, and foliar fertilizers and phytochemical products being applied through dispersers. The selected NT vineyard was relatively old (~60 years) and had an irregular scheme of vines (Figure 2), reflecting the fact that its planting was done without machinery. The NT vineyard was first managed with conventional practices, except tillage, for about 40 years, then was abandoned for about 14 years and was finally rehabilitated, due to vines extreme resistance to drought periods and the high quality of the wine produced, despite the very low crop yield. Rehabilitation of old, abandoned vineyards has regularly occurred in the Bairrada region over the last years. The selected BD vineyard is being managed under the principles formulated by Rudolf Steiner (1861-1925), based on maintaining soils fertile, keeping plants healthy to better resist diseases and pests, and producing high-quality food.[24] These principles are mostly implemented through manual practices, and various Steiner preparations (liquid sprays and compost made from medicinal herbs and/or organic residues) are applied on the vines and the soil to enhance the vines' vegetative-reproductive balance. Table 1 presents the main characteristics and management practices of the 4 vineyards studied here. All 4 vineyards are rain fed, and their pruning and harvesting are done manually.

The climate of the Bairrada region is Mediterranean but has a strong influence of the Atlantic Ocean.[11] Its lithology is heterogeneous, with materials ranging from Paleozoic sedimentary rocks to Cenozoic unconsolidated sediments.[6] However, the

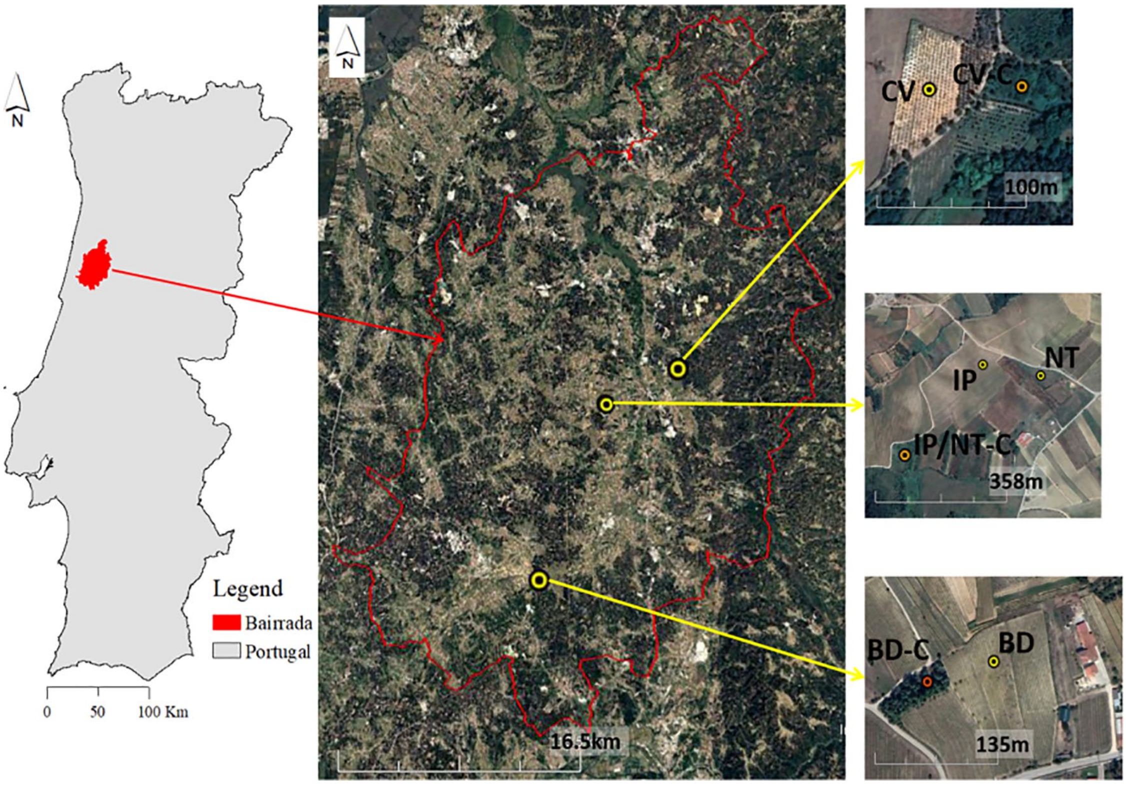

Figure 1. Location of Bairrada wine region in Portugal (left) and the study sites inside the Bairrada (right): vineyards managed under conventional (CV), integrated production (IP), no-tillage (NT) and biodynamic (BD), and associated controls (-C).

Figure 2. No-tillage vineyard, with irregular vine distribution (A), opposing to aligned vine plantation, used in the integrated production (B), conventional and biodynamic vineyards investigated.

vineyards are generally cultivated on limestones and marls from the Jurassic, and the soils have high amounts of carbonates.[31] Vineyard soils predominantly correspond to Cambisols, with a heavy texture that is susceptible to the formation of soil crusts under dry conditions. CV overlays Humic Cambisol, IP and NT Chromic Cambisol, whereas BD has a calcic Cambisol.[6,32]

Besides the 4 vineyards, 3 neighboring forest stands with the same lithology, and soil type were selected to represent reference conditions (CV-C and BD-C are the forest sites nearby CV and BD vineyards, respectively). In the case of the nearby IP and NT vineyards, a single forest site (IP/NT-C) was selected (Figure 1).

Table 1. Main characteristics of the investigated vineyards, regarding year of plantation and start of the current practice, and differences in the main management practices, including tillage, fertilization and use of phytochemicals. Specific information about the type and application rate of pesticides was not provided by farmers.

TYPE OF VINEYARD	YEAR OF PLANTATION (START OF CURRENT MANAGEMENT)	MEAN SLOPE (°)	TILLAGE OPERATIONS	FERTILIZATION	HERBICIDE APPLICATION	FUNGICIDE AND INSECTICIDE APPLICATION
Conventional (CV)	2005 (2007)	2	3-5 per year (20 cm depth)	Mineral fertilizers applied on soil every 2 years; 2 foliar fertilizations per year	At least twice per year in vine row	Preventive and curative; >10 times per year
Integrated production (IP)	2005 (2012)	12	2 per year (15 cm depth)*	Manure applied 1-2 times per year (25 ton/ha); 2 foliar fertilizations per year	1-2 times per year in vine row	Preventive and curative; ~8 times per year
No-tillage (NT)	1970 (1970)	5	None over the last years	Not applied over the last years	1-2 times per year in vine row	Preventive and curative; ~8 times per year
Biodynamic (BD)	2010 (2014)	8	Once every 3-4 years	Manure applied once per year (20 ton/ha), complemented with specific fermented preparations*	None (seeded ground cover)	None (preventive homeopathic treatments based on infusions or plant extracts, and Bordeaux mixture)

*Tillage is performed in alternate rows, so that each area is tilled only once per year.
*Preparations 500 and 501, based on cow manure and silica, respectively.[30]

Soil sampling and physico-chemical analyses

For each vineyard, one plot of approximately 380 m² was selected, comprising 5 rows and 5 inter-rows, and 30 vines per row in CV, IP, and BD. At each plot, 12 composite samples were randomly collected, 6 in the row of the vine and 6 in the inter-row (area between vine rows). In NT, an unaligned vineyard, row samples were taken ~50 cm from the vine and the inter-row samples at higher distance. In the 3 control sites (forest), a smaller plot of ~200 m² was selected (due to high shrub biomass and difficulties to move inside) and 6 composite samples were collected. Each composite sample comprises 3 individual disturbed samples (~200 g), collected manually with a shovel at 0-15 cm depth. In each site, including vineyards and control sites, 3 undisturbed soil surface samples were also collected (in the inter-row vineyard zones, between wheels' trails) with metal core rings (250 cm³), for bulk density analyses. All samples were collected in April and early July 2018, and transported to the laboratory in a dark chilled cooler (~4°C).

In the field, soil penetration resistance was assessed in each vineyard and control site, using a digital cone penetrometer (FIELDSCOUT SC 900, from Spectrum Technologies, Inc). Three random places within each plot, including 3 in vine rows and 3 in vine inter-rows for vineyards, were selected for penetrometer measurements. The penetrometer was operated by placing the cone on the soil surface with the shaft oriented vertically, and then pressed into the soil until it reached different depths. Resistance measurements were performed every 2.5 cm of soil, until maximum possible penetration depth was reached. In each measurement place, several repetitions were performed until similar results were found (minimum of 3 measurements). All the measurements were performed by the same person, on the same day for all the study sites (18/04/2018). All the sites are under the same meteorological conditions, and thus similar soil moisture content.

Undisturbed core soil samples were oven-dried at 105°C and weighted, for bulk density analyses through the gravimetric method. Disturbed soil samples were oven-dried at 38°C and sieved (mesh size of 2 mm), and then used to determine particle size and all the chemical parameters. Duplicate samples were used for quality control purposes and mean concentration values (repeated analysis of each sample) were used for data analysis.

Particle size distribution was performed using the Robinson pipette method.[33] Soil texture was then classified according to USDA particle-size classification. Soil pH and electrical conductivity were measured in a soil/water suspension of 1:5 v/v, using the potentiometric method.[34,35] Soil organic content was analyzed by oxidation at 600°C and quantified through infrared analyzer (LecoSC-144 DR, Strohlein Instruments), and converted to SOM content using a conversion factor of 1.72. Total nitrogen (TN) was determined using the Kjeldahl method,[36] which quantifies organic and some inorganic (NH_3/NH_4^+) forms. Nitrogen under ammonia (NH_4) and nitrate (NO_3) forms were quantified with an automated segmented flow analyzer (SAN++, Skalar), after extraction from 5 g of soil samples.[37,38] Total phosphorous (P) was analyzed after extraction with HCl 37% and HNO_3 65% in 3:1v/v (ISO 111466), and quantified with Vanadate molybdate acid solution using a visible spectrophotometer (Spectrometer T80+, PG Instruments, Ltd).[39] Plant-available soil phosphorus (P_2O_5) and potassium (K_2O) were quantified after extraction with the Egnér-Riehm method.[40]

Ascorbic acid method was used to quantify P_2O_5 concentration using a molecular spectrophotometer (Philips PYE Unicam SP6-350), and K_2O concentration using an atomic absorption spectrometer (AAS; Perkin Elmer).[41] The exchangeable cations (Ca, Mg, K, and Na) were determined after extraction with NH_4COOCH_3 1N, buffered at pH = 7, at 1:10 (w/v) by shaking for 2 h, followed by centrifugation at 3000 r/min for 10 min and filtration. Exchangeable cations were then quantified in extract solutions using AAS (Perkin Elmer).[42]

Boron content (B) was determined after digestion of 12.5 g of soil with 25 mL of $ClCa_2$ 0.01 M solution, buffered at pH = 7, for 10 min at 100°C (DigiPREPMINI, SCP science). Digested solutions were centrifuged at 3000 r/min for 7 min, and the supernatants were analyzed in a continuous flow Analyzer (San^{++}, Skalar).[43] Additional micronutrients, including Fe and Mn, and heavy metals (Cu, Zn, Ni, Cd, Cr, Cd and Pb) were analyzed through extraction of 3 g of each soil sample according with ISO 111466:1995(E),[44] and quantification through AAS.[45]

Data analyses

Statistically significant differences in the soil parameters between (1) row and inter-row samples collected in each vineyard, and (2) between each vineyard and associated control site, were investigated using the nonparametric Mann–Whitney test. Significant differences between the 4 vineyards managed with different practices were investigated separately for (1) row, and (2) inter-row zones, as well as (3) considering all the samples together (collected in both row and inter-row zones), using Kruskal-Wallis test. This test was also used to assess significant differences between the 3 control sites. Average and standard deviation values for each soil chemical property within the vineyard sites included all row and inter-row measurements. Whenever significant differences between vineyards were identified, the post hoc least significant difference (LSD) test was used to identify distinctive sites. The non-parametric tests were used, given the non-normal distribution of the parameters, investigated through Shapiro-Wilk and Levene tests. All tests were performed with a 0.05 significance level, using IBM SPSS Statistics 25 software. Due to non-normal distribution of soil properties, median and interquartile range (difference between third and first quartile) are presented instead of mean and standard deviation.

Results and Discussion

Impact of management practices on physical soil properties

All vineyards have a clay loam texture, except CV which has a sandy loam one, whereas the control sites CV-C and BD-C present a loam, and IP/NT-C a silt loam texture. Bulk density in inter-row zones was similar between all 4 vineyards (median values of 1.4-1.6 g/cm^3; $P > 0.05$) and the 3 control sites (1.2-1.3 g/cm^3; $P > 0.05$) (Table 3, presented in section below), with the latter displaying slightly lower values than the associated vineyards ($P > 0.05$). Results from the vineyards are in accordance with bulk density findings from other studies, such as those from Coll et al,[46] reporting similarities between conventional (1.2 g/cm^3) and organic (1.3-1.4 g/cm^3) vineyards. Bogunovic et al[47] also reported similar soil surface bulk density between conventional, NT and tillage managed practices (1.6-1.7 g/cm^3), due to the impact of wheel traffic. The increase of bulk density with machinery traffic intensity[48] can also explain the similarities found in the 4 vineyards investigated in this study, since soil sampling was performed in the traffic affected area. The results from forest areas, are also within the range of bulk density values reported in central Portugal, including limestone soils (0.8-1.3 g/cm^3).[49]

Management practices, however, display a significant impact on soil penetration resistance ($P < 0.05$), with increasing values over soil depth (Figure 3). In CV, soil penetration resistance in vine row was higher than in inter-row and control site ($P < 0.05$), with the 2 latter displaying similar results ($P > 0.05$), due to the influence of tillage operations in the inter-row zone. In NT, penetration resistance was similar in both vineyard zones and the control site ($P > 0.05$), due to the lack of machinery traffic. IP showed highest penetration resistance in the inter-row zone, and lowest in the control ($P > 0.05$), with the difference between the latter and the row zone being possibly driven by soil texture (clay loam vs silt loam). In BD, the control site displayed highest penetration resistance, possibly because it is a small forest area surrounded by agricultural soils (Figure 1), used by farmers during work breaks to rest in the shadow, which enhances soil compaction due to vehicular traffic. Both row and inter-row zones have similar penetration resistance in the surface layer (0-15 cm), due to limited vehicular traffic applied in the inter-row (Table 1); but the inter-row zone has higher values in the deeper layer, possibly due to past conventional vineyard practices, using heavy machinery. Van Dijck and van Asch[48] also reported greater penetration resistance bellow than above the depth of harrowing in vineyards, indicating the presence of subsoil compaction.

In general, inter-row in IP presents highest penetration resistance ($P < 0.05$) than NT and BD, with no and minimum tillage and similar texture, and even than CV with more machinery traffic but with lighter texture. Increasing soil penetration resistance reduces water and air fluxes (eg, infiltration rate and aeration), with negative consequences for root development and soil biology, which enhances soil degradation and loss of soil quality.[14,51] Tillage provides changes in the inter-row and row morphology,[10] and has been widely reported to destroy natural porosity of the soil,[13] whereas sustainable management practices, such as NT and BD, are typically associated with higher root density in inter-row and soil biodiversity, which enhances macroporosity,[15] thus decreasing penetration resistance.

Differences in soil chemical properties between rows and inter-rows

Distinct management practices between row and inter-row zones of a vineyard affected only part of the chemical soil properties investigated (Table 2). Within all the 4 vineyards, no significant differences between both zones were recorded for pH, EC, NH_4, TP, P_2O_5, K_2O, Fe, Mn, Cu, Cd, and Cr ($P > 0.05$). For the other chemical properties, some vineyards recorded significant differences in few parameters (Table 2; $P < 0.05$). No-tillage, however, given the unaligned disposition of vines and abandonment over several years did not record any significant spatial variability in the chemical properties ($P > 0.05$). In NT, the parameters P_2O_5, B, Mn, Cu and Pb showed the lowest concentrations in both zones ($P < 0.05$), comparing with the other vineyards, as well as lowest Mg in the row zone ($P < 0.05$), and highest Fe in the inter-row ($P < 0.05$) (Table 2).

Conventional vineyard recorded significant differences in the concentrations of exchangeable cations and Pb ($P < 0.05$). Inter-rows showed higher concentrations of (1) Ca and Na than row zone (median values of 39.5 vs 9.0 cmol/kg and 1.4 vs 0.3 cmol/kg, respectively), with Na recording the lowest row concentrations in CV ($P < 0.05$), possibly due to the chemical composition of the mineral fertilizer applied on the leaves and then dripping into the soil (enriched in these nutrients), and (2) highest Pb (25 vs 11 mg/kg), perhaps driven by the widespread spray of large amounts of pesticides, which due to wind effect may also settle on inter-row soil. Nevertheless, concentrations of Pb, as well as P_2O_5, Fe, Zn, Cd, and Ni in both row and inter-row zones of CV recorded the lowest values between all vineyards ($P < 0.05$). On the other hand, Mg and K recorded higher concentrations in rows than inter-rows (53.5 vs 4.1 cmol/kg and 6.7 vs 3.0 cmol/kg, correspondingly), and probably driven by the type of foliar fertilizer used, comprising not only K but also enriched with Mg. The lowest concentrations of K in the inter-row zone, were in fact the lowest within the 4 vineyards ($P < 0.05$). Previous studies, however, reported higher Cu in rows than inter-rows, due to the use of Cu phytopharmaceuticals on the vines, under which the soil is left largely undisturbed.[52] Statistical differences were found between the inter-row and row zones in IP vineyard ($P < 0.05$). Highest values of SOM were recorded in inter-row (median of 2.5% and interquartile range of 0.5%) which can be a consequence of manure application, as recorded in studies elsewhere.[13] Likewise, highest values of NO_3 were also recorded in inter-row (median of 18.7 mg/kg), possibly due to the type of mineral fertilizer applied on the soil inter-row, leading to susceptible losses through runoff and leachate[53] which may explain the high spatial variability in the inter-row results. Also, Ni was higher in the inter-row (49 mg/kg), perhaps driven by manure application.[52] Opposing, Ca was higher in the row than inter-row zone (251.7 vs 154.7 cmol/kg; $P < 0.05$), possibly due to the use of Bordeaux mixture. This product was responsible for higher Cu in vine rows under organic practices elsewhere.[54] Despite no significant difference in Fe between both vineyard zones, Fe concentrations in the row zone recorded the highest values within the 4 study sites ($P < 0.05$).

In BD, K was greatest in the inter-row (11.5 vs 4.4 cmol/kg), maybe driven by manure application, whereas B was highest in the row (0.30 vs 0.08 mg/kg), possibly due to vine supplementation. Nevertheless, highest B and electrical conductivity in both row and inter-row zones, were noticed in BD comparing with the other vineyards ($P < 0.05$). BD was also the vineyard with lowest concentration of SOM and K in the row ($P < 0.05$), but highest K in the inter-row zone ($P < 0.05$), due to organic matter incorporation. According to Mackie et al,[51] chemical differences between row and inter-row zone depend on the age of the vineyard and technical operations within the vineyard, such as incorporation of mineral fertilizers or organic matter.

Impact of management practices on chemical soil properties

CV did not affect soil pH, given the similarities with the control (median values of 5.5, Table 3) ($P > 0.05$), with both vineyard and control sites recording significantly lower values than the other vineyards and controls, respectively ($P > 0.05$). In the other vineyards, IP and NT management practices increased the pH from 8.1 (control) to 8.2 and 8.4, respectively ($P < 0.05$), whereas BD just led to a slight increase (8.3 vs 8.1 in the control site; $P > 0.05$).

BD and CV did not affect electrical conductivity, given the slight differences comparing with associated controls (220 vs 239 dS/m and 110 vs 115 dS/m, correspondingly; $P > 0.05$) (Table 3). IP and NT decreased electrical conductivity, based on control values (249 dS/m) ($P < 0.05$), with NT having a greater impact than IP (116 vs 150 dS/m, respectively; $P < 0.05$), possibly due to lower mineralization rate of SOM favored by NT operations.[55,56]

All vineyards present significantly lower SOM than associated control sites (1.1-2.3% vs 2.4-4.1%) ($P < 0.05$) (Figure 4A), as expected. IP recorded significantly higher SOM (2.3%) than the other vineyards ($P < 0.05$), with similar values between NT (1.3%) and BD (1.4%), and slightly lower in CV (1.1%) ($P > 0.05$). These results are thought to be a consequence of the management practices, given the similar values between the control sites ($P > 0.05$). These findings support previous studies reporting increases in SOC and SOM driven by the incorporation of organic residues in the soil,[55] and decreasing values due to lower mineralization rates of SOM resulting from minimum and NT practices, when compared with conventional tillage.[12,57] Considering the low amount of SOM in Mediterranean vineyards, shifting conventional to more sustainable management practices will mitigate fast mineralization

Table 2. Mean concentrations and standard deviation of the chemical parameters quantified in the row (R) and inter-row (IR) zones of the vineyards with 4 distinct management practices investigated (n = 6). Bold numbers highlight the statistical significant differences between both vineyard zones ($P < 0.05$). Different capital letters indicate significant differences ($P < 0.05$) between rows of the 4 vineyards, and different lower case letters indicate significant differences ($P < 0.05$) between the inter-rows.

	CONVENTIONAL		INTEGRATED PRODUCTION		NO-TILLAGE		BIODYNAMIC	
	R	IR	R	IR	R	IR	R	IR
pH	5.6 (0.7) A	5.5 (0.6) a	8.5 (0.1) BC	8.2 (0.1) b	8.3 (0.)1 B	8.4 (0.1) bc	8.4 (0.0) C	8.2 (0.1) bd
EC (dS/m)	101 (69) A	111 (21) a	136 (19) B	185 (68) b	119 (8) A	108 (16) a	207 (91) C	225 (21) c
SOM (%)	1.2 (0.1) A	1.1 (0.2) a	**1.6 (1.5)** B	**2.5 (0.5)** b	1.4 (0.1) AC	1.1 (0.5) ac	1.2 (0.4) C	1.4 (0.3) c
TN (mg/kg)	939 (81) A	875 (136) a	1066 (963) B	1474 (271) b	906 (133) A	807 (205) a	940 (270) A	912 (250) a
NH_4 (mg/kg)	12.0 (0.3) A	11.7 (0.2) a	10.2 (2.9) B	10.2 (0.7) b	10.0 (1.8) C	9.1 (0.6) c	11.1 (2.2) A	11.4 (0.4) a
NO_3 (mg/kg)	13.8 (12.8) A	9.6 (4.6) a	2.4 (1.0) A	18.7 (29.8) a	6.0 (3.8)1 A	4.9 (0.4) a	8.7 (7.5) A	12.9 (5.0) a
TP (mg/kg)	194 (115) A	239 (86) a	346 (60) B	395 (140) b	325 (29)5 A	292 (57) a	432 (191) A	321 (129) a
P_2O_5 (mg/kg)	33 (27) A	34 (19) a	17 (14) A	22 (17) a	11 (4) B	6 (3) b	33 (14) A	22 (41) a
K_2O (mg/kg)	160 (76) A	153 (74) a	193 (17) A	135 (59) a	283 (21) B	180 (156) b	132 (73) A	202 (56) a
Ca (cmol/kg)	**9.0 (22.6)** A	**39.5 (18.2)** a	**251.7 (19.3)** B	**154.7 (57.7)** b	209.9 (106.9) B	229.4 (51.9) b	155.2 (12.2) A	163.8 (18.5) ab
Mg (cmol/kg)	**53.5 (53.4)** A	**4.1 (1.4)** a	4.3 (2.4) A	10.3 (22.0) a	3.5 (1.8) B	3.5 (1.9) a	11.8 (1.6) A	13.2 (2.1) a
K (cmol/kg)	**6.7 (3.4)** A	**3.0 (1.0)** a	6.8 (2.4) B	8.1 (1.4) b	7.0 (1.4) B	6.9 (3.5) b	**4.4 (0.8)** C	**11.5 (1.0)** c
Na (cmol/kg)	**0.3 (0.7)** A	**1.4 (0.4)** a	0.7 (0.2) B	0.9 (0.4) b	0.9 (0.5) AB	1.0 (0.6) ab	0.8 (0.7) C	0.4 (0.4) c
B (mg/kg)	0.33 (0.39) A	0.34 (0.17) a	0.32 (0.34) A	0.28 (0.11) a	0.73 (0.27) B	0.58 (0.16) b	**0.30 (0.07)** C	**0.08 (0.04)** c
Fe (mg/kg)	8784 (1611) A	10 243 (1882) a	19 839 (4241) B	17 586 (2029) b	19 315 (2796) C	21 483 (5142) c	13 342 (3316) D	12 669 (1925) d
Mn (mg/kg)	257 (49) A	249 (49) a	337 (89) A	295 (122) a	428 (156) B	419 (237) b	231 (26) A	226 (15) a
Cu (mg/kg)	71 (13) A	74 (21) a	58 (23) A	65 (11) a	195 (52) B	197 (31) b	79 (5) A	65 (6) a
Zn (mg/kg)	20 (4) A	20 (3) a	**40 (12)** B	**36 (6)** b	37 (1) B	36 (8) b	29 (4) C	30 (8) c
Cd (mg/kg)	1 (1) A	1 (0) a	5 (1) B	5 (1) b	5 (1) B	4 (1) b	4 (2) B	4 (2) b
Ni (mg/kg)	11 (3) A	17 (3) a	**41 (6)** B	**49 (5)** b	37 (6) C	36 (3) c	41 (5) AC	28 (13) c
Cr (mg/kg)	13 (8) A	18 (3) a	31 (8) B	28 (4) b	28 (2) C	32 (2) c	30 (3) B	25 (9) ab
Pb (mg/kg)	**11 (15)** A	**25 (3)** a	36 (7) B	35 (3) b	39 (2) C	41 (3) c	33 (3) B	32 (11) b

Table 3. Median concentration and interquartile range (numbers within brackets) of bulk density (BD) and several chemical parameters quantified in the vineyards (including both row and inter-row) managed under conventional (CV), integrated production (IP), no-tillage (NT) and biodynamic (BD) practices, and associated control sites (-C) (n = 12). Note that IP and NT have the same control site due close proximity. Bold numbers highlight the statistical significant differences between vineyard and associated control ($P < 0.05$). Different capital letters indicate significant differences ($P < 0.05$) between the 4 vineyards, and different lower case letters indicate significant differences ($P < 0.05$) between the 3 control sites.

	CONVENTIONAL		INTEGRATED PRODUCTION/NO-TILLAGE			BIODYNAMIC	
	CV	CV-C	IP	IP-C/N-C	NT	BD	BD-C
BD (g/cm³)	1.4 (0.0) A	1.2 (0.1) a	1.4 (0.0) A	1.3 (0.1) a	1.6 (0.2) A	1.3 (0.2) A	1.2 (0.3) a
pH	5.5 (0.6) A	5.5 (0.5) a	**8.2 (0.1)** B	**8.1 (0.2)** b	**8.4 (0.1)** B	8.3 (0.2) B	8.1 (0.0) b
EC (dS/m)	110 (43) A	115 (94) a	**150 (58)** B	**249 (48)** b	**116 (17)** A	220 (20) C	239 (31) b
NH_4 (mg/kg)	**11.8 (0.3)** A	**8.9 (3.6)** a	**10.2 (1.1)** B	**13.0 (1.1)** b	**9.3 (126)** B	11.3 (0.6) AB	9.5 (4.6) a
NO_3 (mg/kg)	**9.6 (11.6)** A	**7.3 (4.1)** a	**3.3 (10.1)** AB	**2.0 (5.2)** a	5.0 (1.8) B	**11.6 (6.1)** A	**0.9 (1.1)** b
TP (mg/kg)	227 (115) A	325 (62) a	354 (103) A	363 (41) a	313 (35) A	350 (197) A	272 (74) a
P_2O_5 (mg/kg)	**34 (10)** A	**7 (7)** a	18 (17) B	**16 (7)** b	**9 (5)** B	**28 (29)** A	**6 (6)** a
K_2O (mg/kg)	156 (67) A	154 (72) a	182 (63) A	**202 (24)** a	**273 (114)** B	170 (79) A	160 (76) a
Ca (cmol/kg)	**35 (40)** A	**207 (7)** a	230 (97) B	240 (46) a	224 (62) BC	**157 (15)** AC	**334 (47)** a
Mg (cmol/kg)	4.5 (47.2) A	121 (6.6) a	5.1 (7.7) AB	**24.1 (10.4)** a	**3.5 (1.9)** B	12.5 (2.1) AB	10.1 (1.8) a
K (cmol/kg)	4.0 (3.6) A	3.1 (1.4) a	**7.7 (2.1)** B	**4.9 (1.1)** a	**6.9 (2.1)** B	**4.6 (7.4)** B	**4.2 (1.6)** a
Na (cmol/kg)	1.0 (1.1) A	1.2 (0.4) a	**0.7 (0.2)** AB	**1.2 (0.3)** a	0.9 (0.5) A	**0.7 (0.5)** B	**1.8 (0.4)** b
B (mg/kg)	**0.34 (0.25)** A	**0.14 (0.04)** a	**0.29 (0.14)** A	**0.16 (0.08)** a	**0.61 (0.25)** B	**0.17 (0.22)** C	**0.14 (0.09)** a
Fe (mg/kg)	**9147 (2444)** A	**6992 (2806)** a	**18765 (2965)** B	**11367 (2656)** b	**19640 (4367)** C	**12942 (3119)** D	**8987 (1406)** a
Mn (mg/kg)	253 (45) A	312 (51) a	307 (91) A	**322 (35)** a	**420 (181)** B	**227 (19)** A	**414 (37)** b

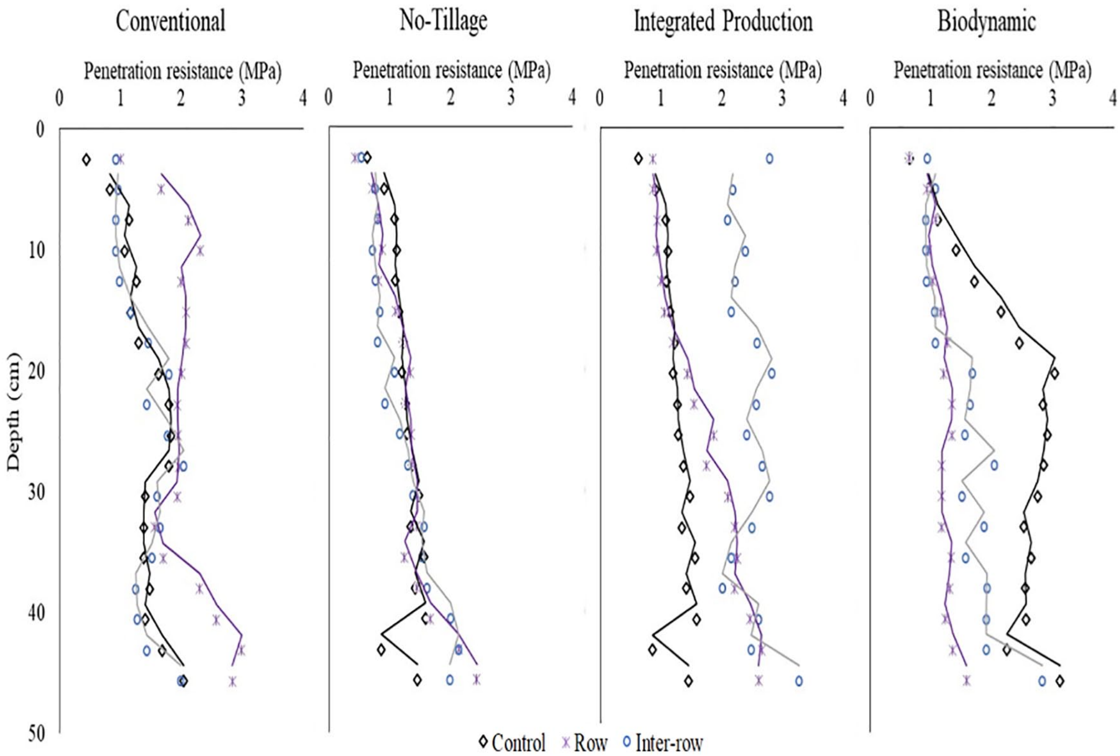

Figure 3. Median values of penetration resistance over soil depth in the 4 vineyards investigated, including in row and inter-row zone and comparison with the control site.

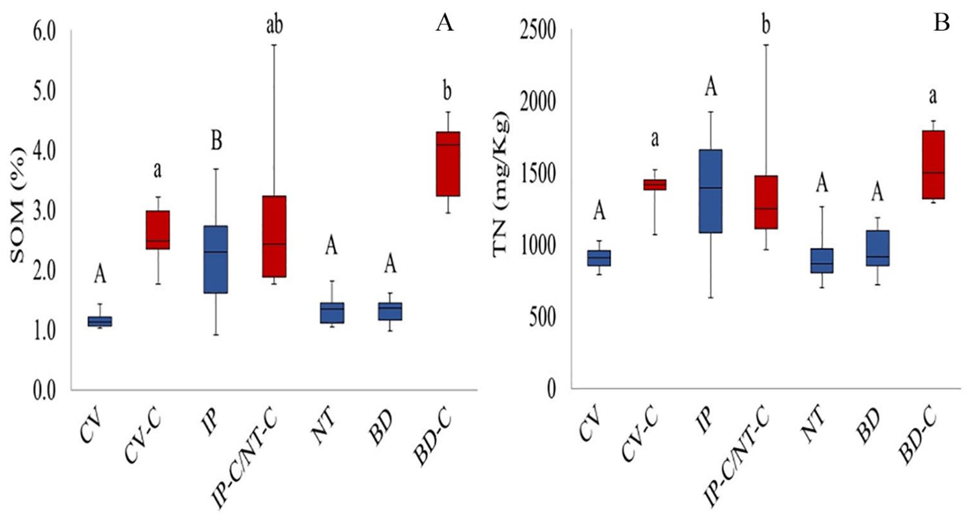

Figure 4. Impact of the 4 management practices investigated on (A) soil organic matter content (SOM), and (B) total nitrogen concentration (TN) on soil surface (0-15 cm), in comparison with the background levels (control sites). BD indicates biodynamic; CV, conventional practices; IP, integrated production; NT, no-tillage; -C, control site.

and increase or maintain SOM.[13] The use of ground cover management, as in IP, has been also attributed to the increase of SOM through plant residues input.[58] Furthermore, ground cover also plays an important role on soil erosion mitigation,[9,13] typically associated with higher slopes, such as those recorded in IP (12°, the highest from the 4 study vineyards (Table 1)) and responsible for soil organic carbon losses.[30] In NT, the lack of any amendment over the last years and the scarce ground cover led to similar SOM to CV. Similar SOM between BD, NT, and CV ($P > 0.05$) may be due to the relatively recent conversion of the vineyard to BD management practices. Reeve et al[58] reported that changes in soil properties are only noticed in farms with more than 6 years of BD practices.

Similar results were found for TN (Figure 4B), with control sites (1245-1497 mg/kg, $P > 0.05$) showing significantly higher concentrations than the vineyards ($P < 0.05$), and with IP (1392 mg/kg) recording 1.5 to 1.6 higher TN median concentrations than the other vineyards (866-912 mg/kg, $P > 0.05$), in both row and inter-row zones (Table 2). Higher SOM and TN in IP are a consequence of the greater manure application (Table 1) and minimum tillage. Previous studies have reported the effectiveness of soil amendment practices in recovering or enhancing soil fertility, namely SOM and available nitrogen.[26] Furthermore, conservation tillage has been demonstrated to enhance nitrogen fixation due to increased soil microbial biomass.[25] In Mediterranean areas, several studies show that minimum tillage led to TN increases on soil when compared with conventional tillage.[57] In our study, manure application in IP provides higher values of SOM and TN than NT management ($P > 0.05$), with no organic amendment. Several studies testify the impact of organic fertilization in increasing TN contents in the soil.[57,60] TN is dominated by organic forms, since NH_4 represents a very low fraction (0.7-1.0% in the control sites and 0.8-1.3% in the vineyards), and NO_3, despite not being quantified in TN (Kjeldahl method), is also rather small (0.1-0.6% of TN in control sites and 0.5-1.6% in vineyards) (Table 3). Higher concentrations of NO_3 were recorded in CV (9.6 mg/kg, $P < 0.05$) due to high rates of mineral fertilization, whereas low values were measured in NT (5.0 mg/kg, $P < 0.05$) due to decades of crop production and poor fertilization. These results are much higher than those reported in Italian vineyards (1.9-1.5 mgNO_3/kg),[61] and highlight the susceptibility to NO_3 loss through leachate and runoff, as reported in a nearby Portuguese vineyard managed under IP practices.[9]

The management practices also affected total phosphorus (TP) concentrations, given the similarities between the 3 control sites (272-363 mg/kg, $P > 0.05$) (Table 3). CV (227 mg/kg) led to lower content of TP than IP (354 mg/kg; $P < 0.05$) and BD (350 mg/kg; $P < 0.05$). Nevertheless, NT did not show significant TP (313 mg/kg) differences comparing with the other management practices ($P > 0.05$). Available phosphorus comprised a relatively low fraction of TP (Table 3), particularly in the control sites (2.1-4.4%), and a variable fraction between vineyards (CV: 15.0%, BD: 8.0%, IP: 5.1% and NT: 2.9%). Although generally low P_2O_5 concentrations, BD (28 mg/kg) and CV (34 mg/kg) led to significant increases in the soil, comparing with control sites (6 mg/kg and 7 mg/kg, respectively; $P < 0.05$), but also with the other vineyards ($P < 0.05$). In BD, higher concentrations of P_2O_5 are provided by the incorporation of manure and fermented preparations into the soil (Table 1), as well as inter-row ground cover, as recorded elsewhere.[24] In CV, however, high applications of mineral fertilizers (N-P-K), assure the higher levels of P_2O_5 in the soil, affecting both row and inter-row zones (Table 2). NT (9 mg/kg), without any kind of fertilization, led to the lowest P_2O_5 between vineyards, in both zones ($P < 0.05$), and a decrease in comparison with the control site (16 mg/kg), where litter decomposition provides phosphorus input into the soil. In IP (18 mg/kg), the incorporation of manure into the soil led to a slight increase in P_2O_5 than recorded in the control site (16 mg/kg) ($P > 0.05$). According with Morugán-Coronado et al,[55] tillage operations do not have a significant effect on soil phosphorus content.

Available potassium displayed significantly higher concentrations in NT (273 mg/kg) than on the control site (202 mg/kg; $P < 0.05$), and the other 3 vineyards (156-170 mg/kg, $P < 0.05$) (Table 3), possibly due to accumulation in top soil layer driven by the absence of tillage practices.[62] conventional viticulture, IP, and BD recorded similar K_2O between each other ($P > 0.05$) and among the control sites (154-202 mg/kg; $P > 0.05$). Exchangeable form of potassium in the soil, however, showed similar results between NT, IP, and BD (6.9-7.7 cmol/kg; $P > 0.05$), which are greater than the control sites (4.6-4.9 cmol/kg) ($P < 0.05$).

Concentrations of K were similar between the control sites ($P > 0.05$), but CV (4.0 cmol/kg) recorded the lowest concentrations between all vineyards ($P < 0.05$), possibly due to the lower pH recorded (Table 3), which is often associated with K deficiency.[63,64] Conventional viticulture also recorded lower concentrations of Ca (35 cmol/kg) than the other vineyards ($P < 0.05$, Table 3), and recorded significantly lower values than the control site (207 cmol/kg; $P < 0.05$). In BD, a significant decrease in Ca was likewise noticed in comparison with the control site (157 cmol/kg vs 334 cmol/kg; $P < 0.05$). Despite IP (230 cmol/kg) and NT (224 cmol/kg) displayed the higher Ca between the 4 vineyards, their concentrations are similar to those found in the control site (240 cmol/kg; $P < 0.05$). Generally, high concentrations of Ca in all the study sites are driven by Cambisol properties. Concentrations of Mg are similar between IP (5.1 cmol/kg), BD (12.5 cmol/kg) and CV (4.5 cmol/kg) ($P > 0.05$), and among each one of these vineyards and associated control sites ($P > 0.05$). NT recorded lower Mg (3.5 cmol/kg) than the other vineyards, and significantly lower concentrations than the control site (24.1 cmol/kg; $P < 0.05$). In contrast, NT recorded higher concentrations of Na (0.9 cmol/kg) than IP and BD vineyards (0.7 cmol/kg), but similar to the concentration in control site (1.2 cmol/kg; $P > 0.05$). Despite all the vineyards displayed lower Mg than the control sites, significant decreases were only noticed in BD and CV ($P > 0.05$).

Regarding micronutrients (Table 3), all vineyards recorded significantly higher B and Fe than the correspondent control sites ($P < 0.05$). No-tillage showed higher increases comparing with the control site (3.8- and 1.7-folds for B and Fe, respectively), and the higher values between the vineyards (B: 0.61 mg/kg and Fe: 19 640 mg/kg). Lower concentrations of B and Fe were noticed in BD (0.17 mg/kg) and CV (9147 mg/kg), respectively. On the other hand, all the vineyards recorded lower Mn than the control sites, except NT (420 vs 322 mg/kg) which also displayed the highest concentrations between vineyards ($P < 0.05$). Lower concentrations of Mn were noticed in BD (227 mg/kg), accounting for about half of the concentration recorded in the control site (414 mg/kg).

Despite Cu and Zn are important micronutrients for plants, they can be contaminants due to the high concentrations in the soil. Higher mean concentrations of Cu were recorded in the vineyards than in control sites ($P < 0.05$) (Figure 5A). Higher

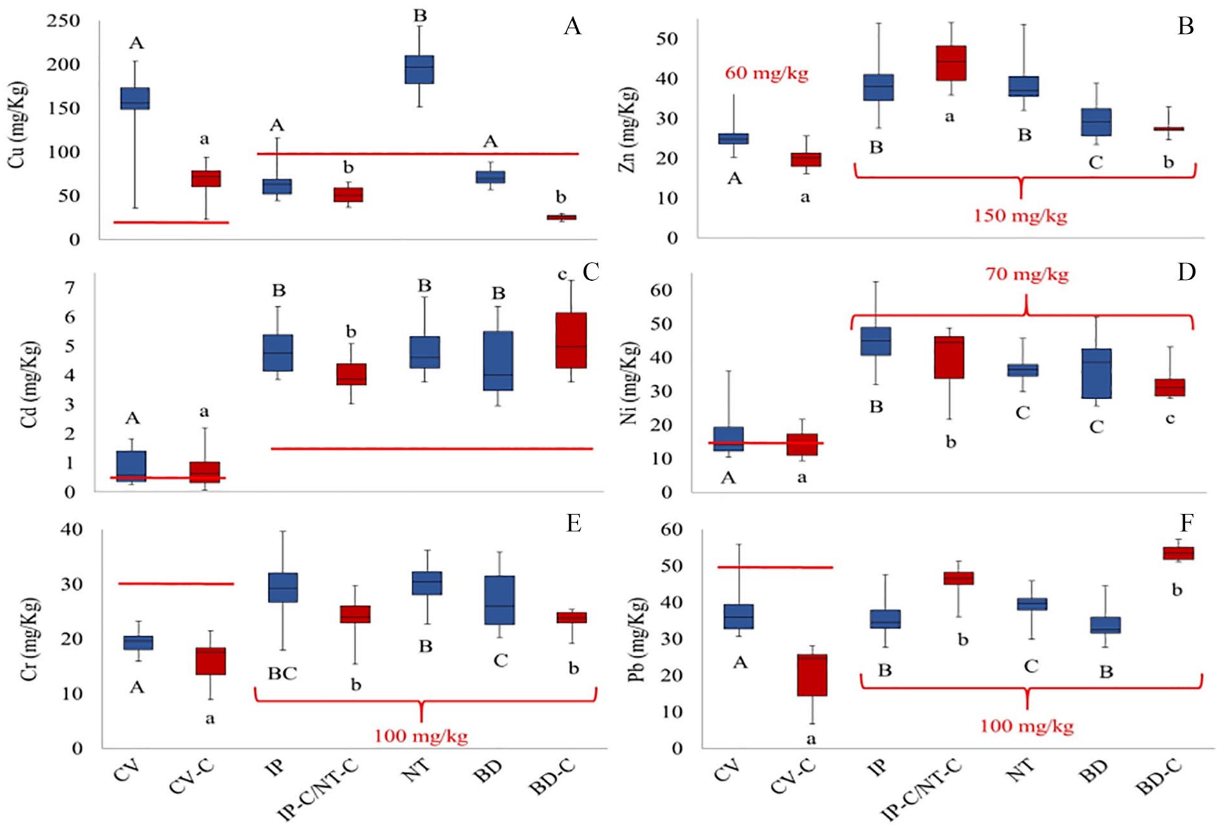

Figure 5. Impact of the 4 management practices investigated on total (A) copper, (B) zinc, (C) cadmium, (D) nickel, (E) chromium, and (F) lead concentrations measured on soil surface (0-15 cm), in comparison with the background levels (control sites). Horizontal red lines and red values indicate concentration thresholds for fertilization purposes, established by the Portuguese legislation for distinct ranges of soil pH (DL103/2015). BD indicates biodynamic; CV, conventional viticulture; IP, integrated production; NT, no-tillage; -C, control site.

Cu were measured in NT (197 mg/kg) and CV (156 mg/kg) than IP (63 mg/kg) and BD (68 mg/kg) ($P < 0.5$). Copper concentrations in NT and CV are higher than recorded in other old and abandoned vineyards within the Iberian Peninsula, but under more acidic soils.[65] In fact, the high concentrations measured in all the samples collected in NT and CV exceeded the maximum admissible contents in soils where fertilizers are intended to be applied (100 mg/kg and 20 mg/kg, respectively due to differences in soil pH; DL103/2015)[65]. Although Portuguese legislation does not establish any reference level of heavy metals in the soil, it presents thresholds for fertilization purposes for Cu, Zn, Cr, Ni, Cd, and Pb[66]. Based on this legislation, mineral fertilization practices in CV (Table 1) should not be performed. However, these fertilization thresholds were exceeded in all CV-C samples, which was a conventional vineyard during few decades before reconversion to forest land-use at least 20 years ago. Although BD recorded relatively low Cu, it represents twice higher concentrations than in the control site (36 mg/kg), which indicates its accumulation in the soil driven by the application of Bordeaux mixture to fight pests in the vineyard. Copper-based fungicides have been widely used in vineyards for more than a century,[54] which led to significant Cu accumulation in these agricultural soils worldwide.[3] Due to high Cu contamination of vineyard soils in Mediterranean areas and the phytotoxicity of this heavy metal, several researchers have suggested the need for remediation strategies.[17]

Zinc concentrations were similar between the vineyards and associated control sites ($P > 0.05$), except in CV (Figure 5B). Although CV recorded lower Zn between the vineyards (25 mg/kg), in both row and inter-row zones (Table 2), it enhanced the background concentrations (20 mg/kg; $P < 0.05$). This is a consequence of the wide use of fungicides and fertilizers in the conventional management practices,[17] and the prohibition of fungicides with Zn in IP and BD. Nevertheless, the concentrations of Zn in all the sites are far from the concentration thresholds in DL103/2015 (60 mg/kg for CV and CV-C, with $5 < pH < 6$, and 200 mg/kg for the other sites, with $pH > 7$), for the application of fertilizers.[66]

Spatial variations of Cd, Ni, and Cr were similar between the study sites (Figure 5 C to E), despite some differences between row and inter-row zones of some vineyards (Table 2), with only slight increases in the vineyards comparing with associated control sites ($P > 0.05$). The lowest concentrations of these metals were recorded in CV ($P < 0.05$), driven by the lower values measured in the control site ($P < 0.05$). However, 33% of the soil samples analyzed in CV exceeded the Ni thresholds provided by DL103/2015 (15 mg/kg for $5 < pH < 6$),[65] opposing to the other study sites where all the samples displayed lower concentrations, and thus can still receive fertilizing matter with Ni composition (Figure 5D). Regarding Cd, the DL103/2015 concentrations were exceeded in 50% of the soil samples in CV, but also in CV-C (0.5 mg/kg; DL103/2015[65]), and in all the samples collected in the other vineyards and control sites (1.5 mg/kg; DL103/2015[65]), indicating that fertilizers comprising Cd cannot be applied (Figure 5 C). Concentrations of Cr in all the study sites were in accordance with DL103/2015[65] (Figure 5E), thus, current fertilization practices involving the incorporation of Cr in the soil are acceptable. Despite similar concentrations of Pb between the vineyards (Figure 5F), CV led to a significant increase of background levels (36 vs 24 mg/kg, respectively; $P < 0.05$), whereas a decrease was noticed in the other vineyards, particularly in BD ($P < 0.05$). Based on DL103/2015,[65] Pb concentrations in the study sites do not represent a constrain for fertilization practices (Figure 5F). The application of phytopharmaceuticals, fertilizers, and organic amendments have been reported to enhance heavy metal contents in the soil.[3,54] The accumulation of heavy metals in the soil, comparing with concentrations in the control sites, enhances the risk of soil and water quality degradation, as reported by previous authors.[3,13]

Conclusions

The principal conclusions of this study comparing topsoil physicochemical properties of 4 vineyards representative of current management systems—that is, conventional, integrated, NT and BD—in the Bairrada wine region of north-central Portugal were as follows:

1. The 3 vineyards that had rows and inter-rows (ie, except NT) revealed statistically significant differences in topsoil chemical properties between them. However, these differences were not consistent across the 3 vineyards, probably reflecting different causes. For example, the use of mineral fertilizers could explain the significantly higher Ca concentration in the inter-rows of the conventional vineyard, where the use of Bordeaux mixture could be responsible for the significantly higher Ca concentrations in the rows of the vineyard under IP.
2. The conventional vineyard differed in a statistically significant manner from the other 3 more sustainably managed vineyards in terms of topsoil fertility, revealing the lowest concentrations of SOM, TN, total phosphorus, and exchangeable cations (Ca, Mg, and K). This reduced fertility probably reflected, at least in part, its more intensive tillage operations, favoring mineralization, but its coarser soil texture could also play a role (sandy loam soil vs clay loam).
3. The vineyard under IP revealed a higher soil fertility than the other 2 vineyards with sustainable management, with significantly higher contents of SOM, TN, and exchangeable potassium. This could reflect a combined effect of minimum tillage with manure application and additional foliar fertilization.
4. The vineyard under BD management differed least from the conventional vineyard, possibly due to the relatively recent adoption of these practices (6 vs 15 years).
5. All 4 vineyard soils revealed slightly but significantly higher metal concentrations than the neighboring forest sites representing the reference conditions, with the exception of Cd. This difference was most pronounced in the case of Cu, reflecting its long-standing and intensive use to mitigate pests, even under BD and IP systems. In the conventional and NT vineyards, the observed Cu concentrations may involve a risk of contamination. In the conventional vineyard, Pb and Ni exceeded concentration thresholds for the application of fertilizers in the soil, enhancing the susceptibility to land degradation and environmental pollution.

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Author Contributions

CF was involved in all stages of the research. AV and AK-B performed the laboratorial work. AC, OG-P and NA were involved in the design of the experimental setup and field work. All authors contributed for data analyses and manuscript preparation.

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