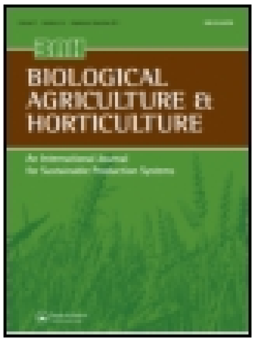

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Aggregate stability and visual evaluation of soil structure in biodynamic cultivation of Burgundy vineyard soils

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ABSTRACT

An on-vineyard approach was used to investigate effects of the biodynamic preparations horn manure and horn silica (BD) on the soil structure in five vineyards on different bedrocks and that had been under organic management for different time periods. The underlying hypothesis was that the effects of the biodynamic preparations increase aggregate stability and improve soil structure. The results showed that soil aggregate stability during wet sieving was not different in the treatment with biodynamic preparations (BD+), compared with that without preparations (BD-). Based on visual evaluation (VESS), improvements in soil structure in the BD+ treatment, compared with BD-, were not significant for macropores/biopores, drop test topsoil or subsoil colour, but significant improvements were observed in drop test subsoil (p = 0.009), topsoil colour (p < 0.000), root penetration (p = 0. 017), structure of surface (stable aggregates, little encrustation, p = 0.006), structure of topsoil (p = 0.030), structure of subsoil (p < 0.000) and the colour change from topsoil to subsoil was at a greater depth (p = 0.049). Based on previously reported results showing significant changes in the microbial activity in soil from the BD+ treatment, using the same soil samples, it was thought possible that the observed differences in soil structure between BD+ and BD- were linked to the differences in the microbial activity.

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Aggregate stability; horn manure; horn silica; VESS; viticulture

Introduction

In wine production, the soil, together with the climate, plays a key role for the terroir (Van Leeuwen et al. 2004) and can influence the quality of the wine (Van Leeuwen and Seguin 2006; Canfora et al. 2018). Physical soil properties, such as high bulk density and high penetration resistance, have been reported to have negative effects on the grape quality and the yields in viticulture (Quezada et al. 2014). Many vineyard soils have been strongly damaged by erosion, due to the low aggregate stability of soils with low organic matter contents (Le Bissonnais et al. 2007; Martínez-Casasnovas and Concepción Ramos 2009). One of the main objectives of organic farming is to increase biological interactions in the soil to improve the physical, chemical and biological properties (Coll et al. 2011; Radić et al. 2014; Burns et al. 2016; Canfora et al. 2018).

The soil, and in particular the microbial community of the soil, has been reported as an important component of the terroir (Van Leeuwen et al. 2004; Zarraonaindia et al. 2015; Canfora et al. 2018) and to contribute significantly to site-specific wine quality (Van Leeuwen and Seguin 2006). Questions regarding food quality and the environmental impact of cultivation methods have

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increased the interest in organic viticulture (Tassoni et al. 2013; Villanueva-Rey et al. 2014; Parpinello et al. 2015, 2019; Picone et al. 2016) and thus also the interest in converting from conventional to organic viticulture systems (Coll et al. 2011; Döring et al. 2015a; Picone et al. 2016; Hendgen et al. 2018; Soustre-Gacougnolle et al. 2018).

Ten % of the organic vineyard area, worldwide, is currently farmed biodynamically (Hendgen et al. 2018; Soustre-Gacougnolle et al. 2018). Biodynamic agriculture is the oldest form of organic farming and is based on Rudolf Steiner's anthroposophical concepts (Koepf et al. 1990). One of the characteristics of biodynamic farming systems is the use of specific preparations as compost additives and for field spraying (Reganold et al. 1993; Carpenter-Boggs et al. 2000; Turinek et al. 2009). The two most important preparations are the field spray preparations horn manure (P500) and horn silica (P501). In practice, the application rates of these preparations are extremely low and only 100 g ha^{-1} fermented cow manure and 4 g ha^{-1} quartz flour are applied per treatment for horn manure and horn silica, respectively. The preparations are stirred in water for 1 h before use. For more details on the production of horn manure and horn silica see Masson (2014) and for details on the ingredients of the preparations see Botelho et al. (2015). Auxin like effects on plants were found in assessments of horn manure by Radha and Rao (2014) and by Giannattasio et al. (2013). The use and effectiveness of these preparations is a topic of controversy, as discussed by Faust et al. (2017) and Juknevičienė et al. (2019).

In a long-term viticulture field trial in Geisenheim, Germany, the three cultivation methods of integrated, organic and biodynamic vineyard management were clearly distinguished in a main component analysis of growth parameters, plant health and yield (Meissner et al. 2019), and in this study the biodynamic and the organic treatments differed only in terms of the application of the biodynamic preparations. Using image-forming methods, grape juice samples from the integrated, organic and biodynamic cultivation methods from this long-term field trial in Geisenheim could be differentiated and classified in all five cultivation years investigated (Fritz et al. 2017, 2020). Kokornaczyk et al. (2014) were able to distinguish between organic and biodynamic wines using the droplet evaporation method. In terms of grape yield and disease indices, Botelho et al. (2015) found increases in leaf enzymatic activities, which are typically correlated with plant biotic and abiotic stress and associated with induced plant resistance. According to Guzzon et al. (2016), biodynamic production systems of grapes had a positive influence on the development of the microbiota on the grapes in years with difficult climatic conditions, compared with conventional production systems. The yeast microbiota were found not vary between organic and biodynamic Sangiovese red wines (Patrignani et al. 2017). Vineyard soil bacterial diversity and composition were found to be different when comparing biodynamic and organic systems (Burns et al. 2016).

In studies on the chemical characteristics of wine, Parpinello et al. (2015), Laghi et al. (2014) and Picone et al. (2016) were able to distinguish between wine from organic and biodynamic cultivation with regard to the chemical substances. With the sensory evaluation of wine, Ross et al. (2009) were also able to distinguish between organic and biodynamic wines, whereas Meissner (2015) could only partially distinguish between the wines from the different systems and Parpinello et al. (2015) reported that the wines could not be distinguished.

It is more difficult to apply the randomised block design in vineyards that are on slopes compared with when they are on flat surfaces (Reeve et al. 2005) and for this reason on-farm (on-vineyard) approaches are widely used in vineyard research (Coll et al. 2011; Radić et al. 2014; Salomé et al. 2014, 2016; Villanueva-Rey et al. 2014; Likar et al. 2017). In the current study an on-farm approach was used for vineyards on slopes to investigate the effects of the application of the biodynamic preparations horn manure and horn silica on the structure of the soil under organic management. In this study, visual evaluation of the soil structure (VESS) was carried out using the spade diagnosis method (Beste 1999, 2003; Ball et al. 2007; Guimarães et al. 2011). In the last two decades, important basic work to develop a standardisable visual assessment of soil structure with spade diagnosis has been undertaken (Beste 1999, 2003; Ball et al. 2007, 2013, 2015, 2017; Shepherd et al. 2008; Guimarães et al. 2011, 2017; Giannattasio et al. 2013; Sonneveld et al. 2014; Van Leeuwen et al. 2018; Emmet-Booth et al. 2019; Valani et al. 2020). As VESS is a qualitative or a semi-quantitative method, assessment of aggregate stability by wet sieving was also performed as an additional test (Yoder 1936; Karamia et al. 2012; Deviren Saygına et al. 2012; Besalatpour et al. 2013; Lourdes et al. 2016) and the mean weight diameter (MWD) stability index was calculated according to Angers et al (2006). Due to the high content of stones in the soils, it was not possible, as originally planned, to take undisturbed soil samples to measure the water retention function, hydraulic conductivity, and air conductivity. On the same soil samples as were used in the present investigation, the microbial biomass and different microbial activity indices were also examined, with full details outlined in Fritz et al. (2020), and the results suggested that the biodynamic preparations had significant effects on the microbial community in the soil, as reported in Fritz et al. (2020).

The hypothesis underlying the present experiments was that the application of horn silica and horn manure will improve the soil structure in the vineyard and increase aggregate stability.

Material and methods

Study sites and sampling

Soils were sampled from five vineyards located at three sites in Burgundy, France, between the Mâcon (Saône et Loire region) and Beaune (Côte d'Or region) areas (Table 1). Each of the five vineyards had previously been divided into two halves and from the time when the management of the vineyard was changed to this regime (see 'BD preparations used since' in Table 1), one half in each vineyard had been treated annually with the biodynamic preparations (BD+ treatment) 500P and 501 (BioDynamie Services sarl Pierre et Vincent Masson, France), whereas the other half had received no BD preparations (BD- treatment) throughout the respective time periods (Table 1) (Fritz et al. 2020).

Vineyards A1 (N 46°16`3.65″; E 4°45`55.35″) and A2 (N 46°16′4.05″; E 4°45′55.40″), each approximately 4000 m^2 and located next to each other, belong to a vineyard in the Vinzelles locality. At this site, the bedrock consists of bioclastic enclosures, ferruginous oolites, calcareous and siliceous marl belonging to the Jurassic Aalénien-Bajocien inferior formation. Vineyard B (N 46°31′0.10″; E 4°43′26.15″), with an area of 2600 m^2, is located near the Bray region and at this site the bedrock consists of limestone and calcareous marl belonging to the Jurassic Sinémurien

Table 1. Information on the sites and the land-use and management factors of the three wine-growing sites (further details in Fritz et al. 2020).

	A: Vinzelles	B: Bray	C: Bouzeron
ASL (m)	257	265	299
Slope	11%	11%	11–19%
Facing	East	West	East to south-east
MAP (mm)	773	786	805
MAT (°C)	10.7	10.6	10.4
Clay (%)	34	34	24
Silt (%)	52	52	52
Sand (%)	14	14	24
Bedrock	Limestone and marl (Aalénien-Bajocien)	Limestone and marl (Sinémurium)	Limestone and marl (Oxfordien)
Soil type (FAO-WRB 2014)	Cambic Leptosol	Cambic Leptosol	Calcaric Leptosol
Vineyard since	A1: 1976 A2: 1951	2013	C1: 1977 C2: 1999
Grape variety	Vitis riparia	Chardonnay	Aligoté doré
Stock	3309	3309	161–49
BD preparations used since	2001	2013	2015
BD preparations (year^{-1})	2 × 500P + 501	2 × 500P + 3–5 × 501	2 × 500P + 501
BD application rate (year^{-1})	40 l ha^{-1}	30–35 l ha^{-1}	35 l ha^{-1}
Additional preparations	*Equisetum arvense* L.	No	*Valeriana officinalis* L.
Tillage in the row (year^{-1})	1 × hoeing, 4 × discing	Hoeing	2 × undercutting
Plant cover between rows	> 70% (under-sowing)	< 10%	< 10%

Notes: MAP = mean annual precipitation; MAT = mean annual temperature.

formation. Vineyards C1 (N 46°53′59.20″; E 4°43′ 51.15″) and C2 (N 46°53′ 56.20″; E 4° 43′48.75″), each approximately 3800 m^2, are located near Bouzeron, where the bedrock consists of oolitic limestone and calcareous marl belonging to the Jurassic Oxfordian superior formation (Fritz et al. 2020). More information on the vineyards, including mean annual precipitation and temperature, ASL, slope, facing, soil type, grape variety, BD preparation use, tillage, plant cover, clay, silt and sand, is described in Table 1. All sites were managed according to organic farming standards.

Soil samples were taken in July 2016 from each BD+ and BD- treatment, at distances of 5.1 m between each treatment pair. Sampling points were evenly distributed within the grapevine plant rows across each vineyard, with exact repetition in the corresponding treatments. From the five sites, six replicate soil samples in each half of the vineyard (0–10 cm depth) were taken, using a steel ring (diameter 9.7 cm, height 10 cm). The soil samples were stored in polyethylene bags at 4°C for up to one month until analysis.

Aggregate stability

The same soil samples, as collected by methods described above, were also used for assessment of microbial activity (as previously reported in Fritz et al. (2020)) and for assessment of aggregate stability as reported in the study described here. To measure aggregate stability, the aggregates were separated carefully and without shaking, by using a sieve with a mesh size of 12.5 mm. Wet sieving was carried out, based on the method of Yoder (1936) and Hartge and Horn (2009), in a sieve tower with sieves of 25 cm diameter and mesh sizes of 8, 4, 2, 1, 0.5 and 0.25 mm. 20 g (± 1 g) air-dried aggregates were carefully placed on the 8 mm sieve. The aggregates were saturated with water for 30 minutes while just touching the water surface. The sieves were then moved up and down at 30 rotations per minute and a lifting height of 4 cm for 10 minutes. The contents of each sieve were dried for 24 hours at 105 °C. The mean weight diameter (MWD) was calculated according to Angers et al (2006).

Visual evaluation of soil structure (VESS) – spade method

Visual evaluation of soil structure was done on the basis of the VESS method (Beste 2003; Ball et al. 2007, 2013, 2017; Shepherd et al. 2008; Guimarães et al. 2011, 2017; Giannattasio et al. 2013; Sonneveld et al. 2014; Van Leeuwen et al. 2018; Emmet-Booth et al. 2019; Valani et al. 2020), using 5 categories for the scores, where 1 = best condition and 5 = worst condition. In contrast to the usual VESS method, the soil condition was not described with a single value, but evaluations were done individually for 10 different parameters: 1) structure, surface; 2) root penetration; 3) macro-pores /biopores; 4) colour of topsoil; 5) colour of subsoil; 6) structure, topsoil; 7) structure, subsoil; 8) drop test, top-soil; 9) drop test, subsoil and 10) depth at which the colour changed between top-soil and sub-soil (cm). Details of the parameters 1, 2, 3, 4 and 5 are described in see Table 2 and of the parameters 6, 7, 8 and 9 in Table 3.

The visual soil assessment was performed in the vineyards A1, A2, B and C2 (Table 1). Three pairs of soil samples per vineyard were collected and visually examined in July 2016, examining one BD+ and one BD- variant for each pair. Before sampling the soil blocks on the spade, each 40 cm deep into the ground x 25 cm wide and 15 cm thick, for visual soil evaluation, the structure of the surface was first assessed at the sampling point (spade diagnosis see Beste 1999, 2003; Ball et al. 2007; Guimarães et al. 2011). The evaluation of the spade sample was carried out in accordance with the evaluation sheets shown in Tables 2 and 3. The criteria for the assessment of soil rooting and colour were carried out according to Brunotte et al. (2011). The assessment of the structure of the surface was carried out according to Brunotte et al. (2011) and (Shepherd et al. 2008). The evaluation of the microstructure was carried out according to Diez et al. (2017). The evaluation of the macropores/biopores, as well as the degree of consolidation of the soil block after the drop test was based on Sponagel (2005). For the evaluation of the root penetration of the soil, the lateral

Table 2. The score key for the visual soil evaluation of the parameters: 1) Structure of surface; 2) Root penetration; 3) Macropores/biopores; 4) Colour topsoil; and 5) Colour subsoil, with the spade method, modified according to Brunotte et al. (2011), Shepherd et al. (2008) and Diez et al. (2017).

Parameter	1 2	3	4 5
	Favourable / Desirable		Unfavourable / Undesirable
1) Structure of surface	• Intact stable aggregates • Worm excrement • Very little or no encrustation or surface cover ≥ 70 %	• Encrustation 2–3 mm thick • Encrustation broken with significant cracks or surface cover > 30% and < 70%.	• Silting • Encrustation > 5 mm thick • Encrustation almost everywhere with small cracks or surface cover ≤ 30%
2) Root penetration	• Continuous over all horizons • Root distribution even • No root blockage	• Roots predominantly in earthworm tunnels and splits	• Kinked root, no roots • Root blockage • Roots felt on compacted layers or on aggregate surfaces • Uneven
3) Macropores/biopores	• Many earthworm tunnels in the profile wall and profile floor (> 5) • Newly created earthworm tunnels in the processing horizon • Old earthworm tunnels in the subsoil filled with earthworm excrement and humus material	• Some earthworm tunnels visible	• No open biopores on the soil surface • In crumbs, few vertical earthworm tunnels • Few biopores in the subsoil
4) Colour, topsoil **5) Colour, subsoil**	• Uniform colour within the horizons (dark) • Smooth change between the horizons		• Blue and grey coloured areas in the horizons (reduction zones) • Rust stains (lack of air) • Abrupt change between the horizons

break edge of the soil cuboid was evaluated. The side to be examined was carefully roughened with a fork, so that the break edge was as natural as possible. In the spade diagnosis, macropores (visible to the human eye) and biopores were evaluated together. The pores on the underside of the soil cuboid were mainly examined, as this is where the edge broke open most naturally and where the pores were most clearly visible.

The depth at which the colour changed between the topsoil and the subsoil was recorded as the parameter 'depth of colour change between topsoil and subsoil', which was recorded as cm soil depth (see Tables 2 and 5) and the reference to the sample as topsoil or subsoil always referred to the depth at which the colour changed in the soil.

For the drop test, the soil cuboid was dropped from a spade onto a solid surface from a height of approximately 1 m. According to Sponagel (2005), the degree of compaction of the soil was determined from the two soil layers, topsoil and subsoil (in accordance with the depth where the colour changed). During the subsequent careful division of the soil by hand, the aggregate structure, such as granular, crumbly, polyhedron structure, was determined (see Table 3). Separate evaluations were carried out for the topsoil and the subsoil.

Statistical evaluation

The results presented in the tables are arithmetic means. For aggregate stability, all test series were checked for normal distribution using the Shapiro test. As the data were not normally distributed, the Wilcox test was used to compare the mean values. Outliers were removed if their value exceeded the standard deviation twice. Statistical evaluation was performed with

Table 3. The score key for the visual soil evaluation of the parameters: 6) Structure, topsoil; 7) Structure, subsoil; 8) Drop test, topsoil; 9) Drop test, subsoil, with the spade method modified, according to Sponagel (2005).

Parameter	1	2	3	4	5
	Favourable / Desirable			Unfavourable / Undesirable	
6) Structure, topsoil **7) Structure, subsoil**			**Single particle structure** Loose	Compressed/solid	
Non-aggregated structure		**Coherent structure** • Loose coherent • Porous • Decomposing under pressure		• Solidly compacted • Tightly stored • Few (no) macropores	
Aggregated structure	**Granular** • Porous • Loose • Finely aggregated	**Crumbly**, crumbs < 50 mm • Unsharply defined, porous aggregates • Decomposing at light/strong pressure			
		Sharp-edged structure (polyhedron structure) • Subpolyhedralstructure (round)		• Polyhedral structure (sharp-edged)	
8) Drop test, topsoil **9) Drop test, subsoil**					
Degree of compaction	Very loose	Loose	Medium	Solid	Very solid
Reaction of the block during the drop test	Falling apart already at the time of collection	Falls apart on collision into numerous fragments or into its individual parts	Falls apart on collision into a few fragments, which can be further divided by hand	Falls apart on collision into a few fragments that cannot or can only with difficulty be broken up by hand	Does not fall apart on collision

R 1.0.136 (R Core Team 2014). The visual soil evaluation data were tested for normality of distribution using Kolmogorov-Smirnov test with a Lilliefors significance correction (Lilliefors 1967). In normally distributed data the significance of BD-treatment effects was analysed using the paired sample T-test for the treatment pairs. The parameters of root penetration, the evaluation of the colour of the topsoil and subsoil were not normally distributed. The significance of BD-treatment effects from the non-normally distributed data was calculated using the Wilcoxon sign rank test on paired samples (Siegel 1956). Statistical evaluation was carried out with SPSS statistical software (SPSS 24).

Results

Measurement of aggregate stability by wet sieving revealed significantly lower MWD for sites A1, A2 and B compared to sites C1 and C2 (Table 4). There were no differences in MWD between the BD- and BD+ variants.

In the visual assessment of the soil structure, the colour change between the topsoil and the subsoil was significantly deeper in the soil for BD+ than for BD-, considering the mean values of all sites (Table 5). In the ranking evaluation there were no significant differences between BD- and BD + for macropores/biopores, drop test of topsoil or in the subsoil colour. However, for the drop test of subsoil and for the topsoil colour, the scores for BD+ were lower (low score indicate better conditions) than with BD- at all sites and for the mean values of all sites for these parameters the differences were highly significant (Table 5 and Figure 1).

At three out of four locations, root penetration was better (lower scores) in the BD+ treatment than in the BD- (Figure 2) and for the overall mean values (of all locations) of this parameter the difference between the treatments was significant ($p = 0.017$). The structure of the surface, topsoil and subsoil, had

Table 4. Aggregate mean weight diameter (MWD) (mm) after water bath according to screen sizes 8, 4, 2, 1, 0.5, 0.25 mm. Sites A1, A2, B, C1 and C2.

	A1	A2	B	C1	C2	Overall mean (all sites)
BD-	6.52	7.72	6.16	9.16	8.02	7.52
BD+	7.16	6.50	6.02	9.04	8.74	7.47
Mean BD	6.84 a	7.11 a	6.09 a	9.10 b	8.40 b	

Note: Different letters within the row indicate significant differences ($p < 0.05$) between the sites in accordance with the Wilcox test. There were no significant differences between BD- and BD+.

Table 5. Visual soil assessment of mean depth of the colour change between topsoil and subsoil, macroproes/biopores, drop test topsoil and subsoil, colour topsoil and subsoil, at 4 study sites, without (BD-) and with (BD+) application of biodynamic preparations; score ratings of 1–5, with 1 = best structure and 5 = worst structure. Study sites: A1, A2, B and C2.

Study site	Colour change between topsoil and subsoil (cm)	Macropores/ biopores (score rating 1–5)	Drop test, topsoil (score rating 1–5)	Drop test, subsoil (score rating 1–5)	Colour, topsoil (score rating 1–5)	Colour, subsoil (score rating 1–5)
A1						
BD-	13.33	3.78	1.00	3.33	3.00	3.00
BD+	18.00	2.89	1.00	2.50	1.50	3.00
A2						
BD-	11.00	3.12	1.00	2.67	3.00	3.67
BD+	12.67	2.45	1.17	2.33	1.83	3.00
B						
BD-	20.00	2.89	1.67	2.00	2.50	3.00
BD+	20.67	3.11	1.00	1.33	1.83	3.00
C2						
BD-	10.00	3.34	1.50	2.50	2.83	3.00
BD+	12.33	3.34	1.17	1.83	2.00	3.00
Mean						
BD-	13.58 *	3.28	1.29	2.62 **	2.83 ***	3.17
BD+	15.92 *	2.95	1.08	2.00 **	1.79 ***	3.00

Notes: Asterisks indicate significant BD treatment-specific differences (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). For description of the parameters, see Tables 2 and 3.

lower scores (low score indicate better conditions) at all sites for BD+ than for BD- and for the overall means of the sites for these parameters the differences were significant to highly significant (Figure 2).

Discussion

General site effects

The aggregate stability, measured by the MWD, was higher at sites C1 and C2 compared to sites A1, A2 and B, while according to the visual soil assessments, site C2 was within the same ranges as sites A1, A2 and B for all parameters. The soil at sites C1 and C2 had a 10% lower clay content (Table 1), but higher soil organic carbon concentrations, as reported by Fritz et al. (2020), than the soil at sites A1, A2 and B.

Biodynamic preparations

The application of the BD preparations P500 (horn manure) and P501 (horn silica) did not result in significant differences in aggregate stability (Table 4). However, according to VESS, there were significant improvements of the soil with application of the BD preparations (BD+), compared with BD-, for 7 out of the 10 parameters. Statistically, the differences were most obvious between BD+ and BD- for topsoil colour and for the structure of the surface and the subsoil (Table 5, Figure 1b and 1d).

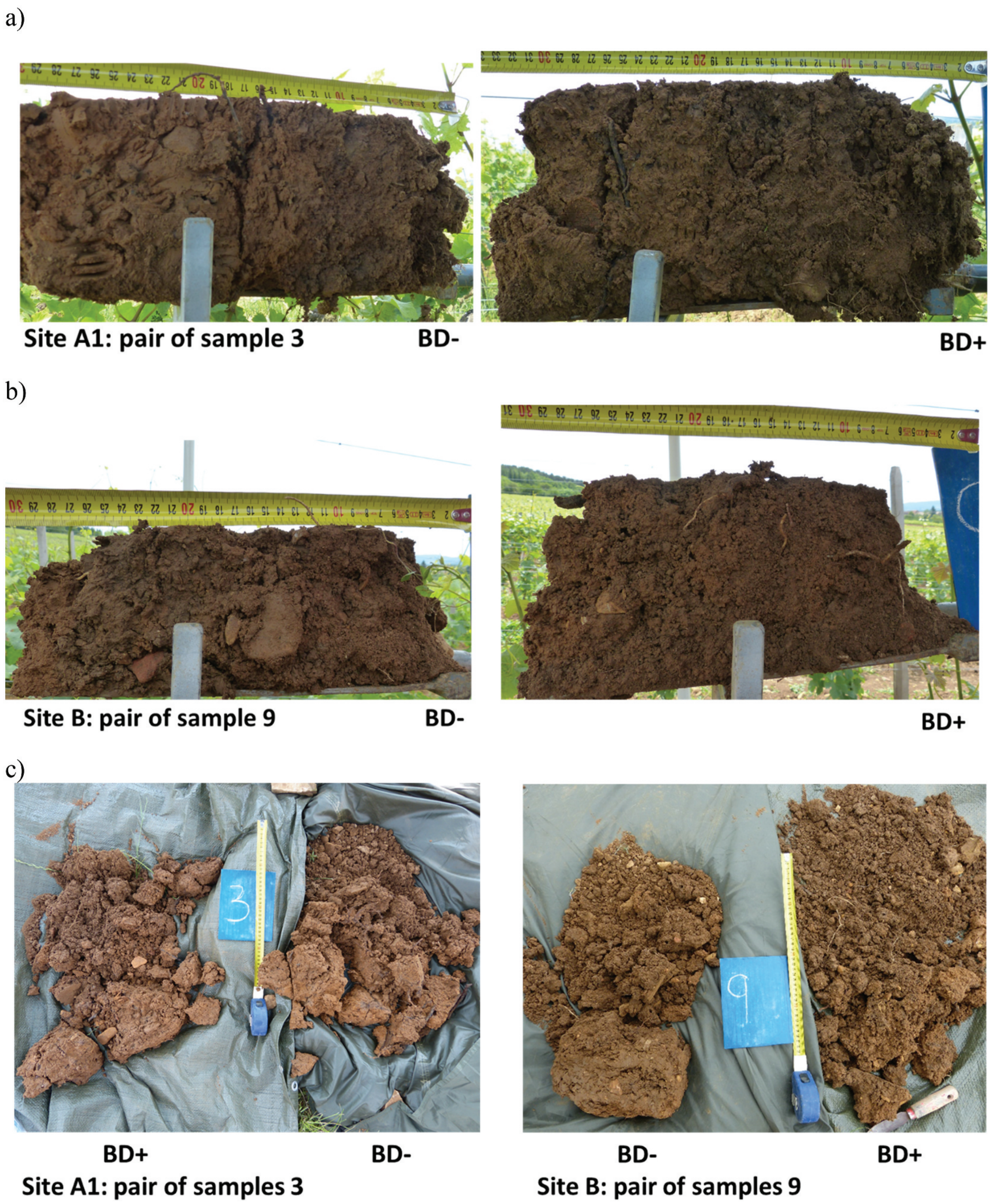

Figure 1. Pictures from spade diagnosis (a and b) and drop test (c). Examples are from site A1, pair of sample 3 (a and c (left)) and from site B, pair of sample 9 (b and c (right), both without (BD-) and with application of biodynamic preparations (BD+).

Results of the drop test of the topsoil and subsoil showed similar trends to the results of the structure of the topsoil and subsoil in the investigation (Table 5, Figure 1c and d). Guimarães et al. (2011) compared normal scoring of soil structure with scoring after breaking up the slice by dropping (drop shatter) and they reported that normal scoring of soil structure or scoring soil

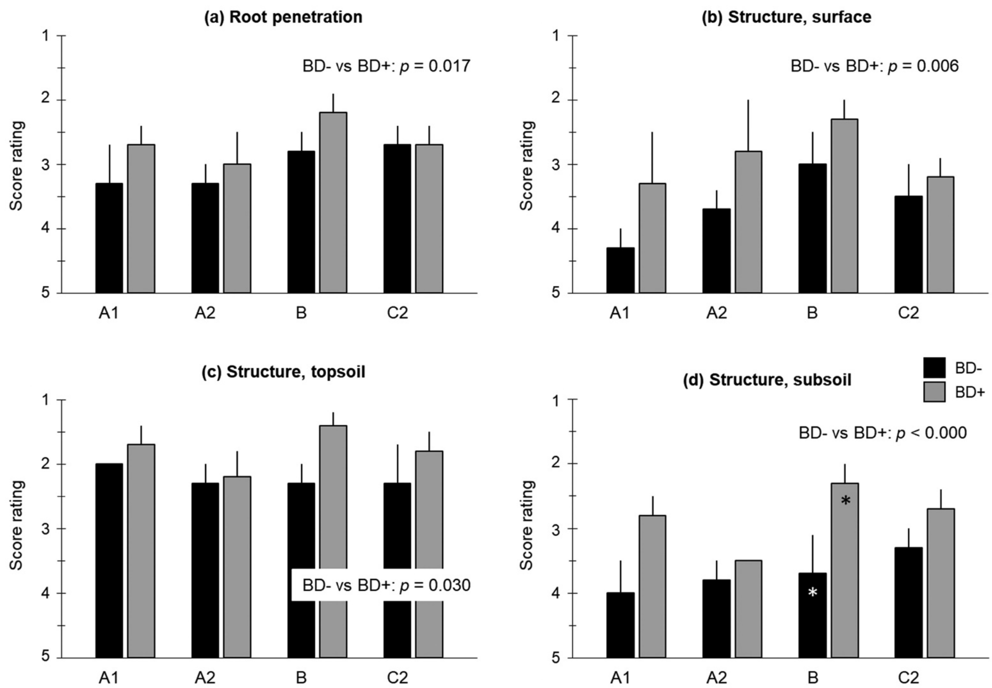

Figure 2. (a) Root penetration; (b) Structure, surface; (c) Structure, topsoil; and (d) Structure, subsoil, at four vineyard sites, without (BD-) and with application of biodynamic preparations (BD+); Statistical significance of the differences between the overall mean values (all locations) are marked with BD- vs BD+ (t-test) in the Figures. An asterisk indicates a significant difference between the BD treatments at one site (t-test, $p < 0.05$); Score ratings are 1–5, with 1 = best structure; 5 = worst structure. Error bars indicate standard deviation. For details of study sites A1, A2, B, C2, see .Table 1

after dropping also resulted in the same score. In the study reported here, the normal scoring of soil structure led to a statistically clearer distinction between the treatments BD- and BD+.

Using the same soil samples as used in the present study, further parameters were also investigated and the results were previously reported by Fritz et al. (2020). Those results showed that for the parameters bulk density, carbonate, soil organic carbon (SOC), total N, soil N:C, microbial carbon (MBC), microbial nitrogen (MBN), ergosterol, CO_2 C (basal respiration rate) and qCO_2 (mg CO_2-C g^{-1} MBC d^{-1}) there were no significant differences between BD- and BD+. However, significant differences between the treatments BD+ and BD- were found for soil pH, MB-C:N, MBC:SOC and for 16 of 18 substrates in the assessment of multi-substrate-induced respiration rates, i.e. in parameters that indicated sensitively variable microbiological soil processes. Based on the results reported in Fritz et al. (2020) it was suggested that the use of the biodynamic preparations had significant effects on the microbial community in the soil. A long-term experiment in Darmstadt, Germany, showed that biodynamic cultivation, compared with organic cultivation (the only difference was the use of biodynamic preparations), led to a more efficient use of soil organic carbon by the microbes (Sradnick et al. 2013). In the long-term DOC-experiment in Switzerland, a biodynamic management system (compared with a non-biodynamic system i.e. a systems comparison, where the differences between the systems were not only the use of biodynamic preparations) led to improved use of carbon by the microbial biomass, higher biological activity, higher proportions of more stable organic matter and higher SOC and MBC contents (Mäder et al. 2002; Fließbach et al. 2007; Birkhofer et al. 2008). In the same DOC-experiment in Switzerland, the biodynamic system was also shown to have an impact on the microbial community in the soil (Hartmann et al. 2015). Higher biological activity in soil in response to the application of horn manure and horn silica was also reported by Juknevičienė et al. (2019) and Vaitkevičienė et al. (2019) in three-year trials with pumpkin and potatoes. Burns et al. (2016) also reported that the bacterial diversity and composition in vineyard soil were different comparing biodynamic and organic systems (also in trials where the differences between the systems were not only the use of biodynamic preparations).

Investigations have indicated that the use of biodynamic preparations had a compensatory effect against unfavourable growth conditions (Raupp and König 1996), affected the activity of the microorganisms in the soil (Fritz et al. 2020), affected the yield (Spiess 1978; Sharma et al. 2012; Vaitkevičienė et al. 2019; Juknevičienė et al. 2019), affected the contents of secondary plant substances (Juknevičienė 2015) and affected the germination of the seed in the following generation (Fritz and Köpke 2005). These effects were explained to correspond to better self-regulation of the plants in the form of increased resilience (Schneider and Ullrich 1994; Döring et al. 2015b).

As the application rates of the preparations were extremely low (100 g ha^{-1} fermented cow manure and 4 g ha^{-1} quartz flour per treatment for horn manure and horn silica, respectively), it was thought unlikely that the effect on the soil structure observed in this study were due directly to the additions of C, N, K or P made by the application of the preparations. In order to better understand the mode of action of the biodynamic preparations, there are different explanatory models. One explanation is that the preparations influence the microbial community and have a regulating effect. For example, bacteria may recognise and react to extremely low concentrations of signal molecules such as carbohydrates and peptides, which are produced in the microbially mediated slow maturation under oxygen-deficient conditions during the production of the preparations (Spaccini et al. 2012). This could result in greater microbial activity in the rhizosphere (Reeve et al. 2010; Giannattasio et al. 2013) or the stimulation of natural defences (Schneider and Ullrich 1994; Botelho et al. 2015). In studies by Ortiz-Álvarez et al. (2020), it was reported that the fungal networks of vineyard soils under biodynamic management were higher clustered communities (more closely connected in the cooperation), lower modular (less self-contained groups, such as islands in the cooperation) with less co-exclusion proportion compared to that in organic and conventional vineyard soils. These characteristics of biodynamic soils were considered as favourable for a high suppressive effect of the soil against pathogens and for a high resilience potential of the soil, summarised by Ortiz-Álvarez et al. (2020) as 'based on that, we can hypothesise that fungal communities that give rise to small-world and collaborative networks, as it is found in biodynamically managed soils, can be more resistant to the continuously changing environment imposed by climate change and land use' .

A further complementary explanation of bacterial regulation is that biodynamic preparations act via hormonal effects. For example, in the horn manure preparation, strains of bacteria have been detected that produce indoleacetic acid (Radha and Rao 2014), and this preparation has also been reported to contain nondegraded lignin residues that have an indoleacetic acid-like activity (Spaccini et al. 2012). Giannattasio et al. (2013) found strong auxin-like effects in horn manure and Fritz (2000) reported gibberellic acid-like effects of horn silica. The significant differences between BD+ and BD- treatments for the results of the multi-substrate-induced respiration method (Fritz et al. 2020) supported the hypothesis that the microbial community may have a role in the effects of the horn manure and horn silica preparations. The changes in the activity of the microbial community may have been the cause of the changes in the soil structure in BD+ treatment, compared with that in BD-.

Conclusions

In the present investigation, the soils in the BD+ and the BD- treatments showed no differences in terms of aggregate stability, but for the visual evaluation of soil structure there were significant improvements in the soil structure in the BD+ treatment. In order to gain a better understanding of the possible relationships between soil structure and the activity of the microbial community, it was recommended that for further investigations of the BD+ treatment, it would be interesting to measure substances that are specifically known to have important roles in the formation of the soil structure, for example, extracellular polymer substances (EPS) and glomalin. In addition to the visual assessment of the soil structure, further investigations should also include assessment of core scale physical properties of the soils, such as water retention functions, saturated hydraulic conductivity, and air permeability.

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No potential conflict of interest was reported by the author(s).

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