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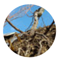

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ORIGINAL RESEARCH ARTICLE

Effects of biodynamic preparations 500 and 501 on vine and berry physiology, pedology and the soil microbiome

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ABSTRACT

In the pursuit of increasing sustainability, climate change resiliency and independence of synthetic pesticides in agriculture, the interest of consumers and producers in organic and biodynamic farming has been steadily increasing in recent decennia. This is, in particular, the case for the vitivinicultural industry in Europe, where more and more producers are converting from organic to biodynamic farming. However, clear scientific evidence showing that biodynamic farming improves vine physiology, vine stress resilience, soil quality related parameters and berry or wine quality is still lacking, despite the growing number of research studies on this issue. To investigate whether biodynamic farming methods have an impact on vine physiology, berry quality and the environment, a five-year experiment was set up in 2016 in a commercial vineyard in Switzerland. In this trial, the two main biodynamic preparations 500 and 501 were applied and compared to an organic control. Vine and berry physiology (net photosynthesis, vigour, sugar, organic acids, berry weight, yield) were assessed from 2016 to 2020. Soil physical properties (soil bulk density, water holding capacity, soil structural stability, macropore volume) were analysed from 2017-2020, and, soil fungal communities were analysed by DNA-sequencing in the last year of the experiment (2020).

None of the parameters related to vine and berry physiology showed significant differences throughout the duration of the experiments, except photosynthesis, which was higher when biodynamic preparations were applied at one time point. Similarly, the soil's physical properties were not influenced by the application of the two biodynamic preparations in all years. Regarding the soil microbiome, the preparations 500 and 501 neither led to significant differences in fungal diversity nor seemed to impact the soil fungal communities. The present study confirms previous findings of different research teams that did not observe significant differences between organic and biodynamic farming methods in terms of observed soil and vine parameters.

KEYWORDS: biodynamic viticulture, microbial diversity, organic viticulture, preparations 500 and 501, soil physical properties, vine physiology, berry quality

INTRODUCTION

In recent years, both winegrowers and consumers have shown a steadily growing interest in organic wine production, with an estimated winegrowing area of 454 000 ha, corresponding to 6.4 % of the world's viticulture surface certified organic production (OIV, 2021). In the course of this development, biodynamic grape and wine production is also receiving increasing attention with some of the most prestigious wineries converting to organic or biodynamic viticulture (Castellini *et al*., 2017; Reeve *et al*., 2005). The biggest international biodynamic association is Demeter. Demeter-certified agricultural farms have grown significantly in number (more than 5900 farms in 2019), with the certified surface area almost doubling to over 200,000 ha in 63 countries (Santoni *et al*., 2022). Regarding viticulture, in 2021, there were a total of 1012 wineries and 17 079 ha of Demeter-certified vineyards across the world (Sinpfendoerfer and Fischer, 2021)

Biodynamic winegrowers generally claim to produce lower environmental impact, higher vineyard biodiversity, better vine health and higher wine quality compared to organic farming. Nevertheless, this practice is still controversial in the viticultural industry and, in particular, within the scientific community. Despite an increasing number of scientific studies on the effects of organic, biodynamic and conventional viticulture in recent decennia, there is little evidence showing unambiguous differences between biodynamic and organic viticulture in terms of environmental impact, vine performance and berry quality (Döring *et al*., 2019). Several studies have compared the organoleptic quality of wines from biodynamically and organically grown vines, but no differences in wine sensory characteristics have been found (Collins *et al*., 2015; Parpinello *et al*., 2015; Patrignani *et al*., 2017). However, some studies have reported minor differences in the preferences for and in the sensory properties of biodynamic and organic Riesling (Meissner, 2015), Merlot (Ross *et al*., 2009) and Sangiovese (Parpinello *et al*., 2019) wines. In terms of vine physiology and berry quality, most studies have found differences between biodynamic and organic production when comparedto conventional viticulture, but not when comparing biodynamic with organic only. In general, organically and biodynamically managed vines show significantly lower growth and yield in comparison to integrated plots (Döring *et al*., 2015; Fritz *et al*., 2021; Meissner *et al*., 2019; Parpinello *et al*., 2019; Parpinello *et al*., 2015). Soil management and fertilisation strategies are supposedly responsible for these differences. As regards soils, Hendgen *et al*. (2018) found significant differences in the fungal community composition when comparing organic and biodynamic with conventional production, but again did not find any differences between organic and biodynamic management. Similarly, Longa *et al*. (2017) showed that the application of the preparations 500 and 501 did not affect microbial communities in the short term. In a meta-analysis, Christel *et al*. (2021) found that biodynamic farming displays higher soil ecological quality compared to organic farming. Soustre-Gacougnolle *et al*. (2018) report a higher expression of silencing and immunity related genes, and higher anti-oxidative and anti-fungal secondary metabolite levels in biodynamically managed vineyards, which suggests that the sustainability of biodynamic practices probably relies on fine molecular regulations. However, the latter study was not conducted using a controlled experimental design, but within a large network of commercial plots to which each producer applied his own definition of "biodynamic production"; this could have introduced biases in gene expression.

Despite the controversial scientific and empirical evidence of improved vine physiology, berry and wine quality and less environmental impact, demand for wines from biodynamically grown vines is growing and putting increasing pressure on both conventional and organic wine producers to apply biodynamic principals to vineyard management, thus increasing production costs (Castellini *et al*., 2017). We therefore tested the hypothesis that the application of the two main biodynamic preparations increases vine physiological performance and berry quality, leading to higher production costs.

The present study aimed to evaluate the long term effects of the biodynamic preparation 500 and 501 on vine physiology, berry and soil quality and the soil microbiome in a Swiss winegrowing region, planted with the most emblematic Swiss autochthonous grape variety, Chasselas (Rienth *et al*., 2020).

METHODS

The field experiment was conducted in a commercial vineyard in Mont-sur-Rolle, Switzerland (46°28'10.4"N 6°20'33.4"E). The experimental site was 0.76 hectare in size and planted in 2012 (*Vitis vinifera* L. cv. Chasselas clone RAC, grafted on 3309C).

The vines were planted with a spacing of 0.8 m within rows and 1.8 m between rows within a vertical shoot positioning system (VSP). Row orientation was north–south. Conversion to organic viticulture started in 2015 in accordance with Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008, prior to which the plot had been managed conventionally with spontaneous interrow grass cover and under-vine herbicide application.

The experiment was set up in a randomised complete block design with 18 homogenous blocks, each of which consisting of 4 rows with a total of 190 vines. Nine blocks were assigned as control blocks, to which pure water was applied instead of biodynamic preparation. In the remaining 9 treatment blocks, the two main biodynamic preparations, Horn Manure (500) and Horn Silica (501), were applied. Preparation 500 consists of fermented cow manure and is applied to the soil with the aim of stimulating soil processes and root growth. Preparation 501 consists of fermented ground silica from quartz of feldspar and is applied to leaves with the aim of stimulating plant physiological processes and improving crop quality (Koepf *et al.*, 1990). 500 was applied twice a year: in March or April and in May (May and June in 2016); meanwhile, 501 was applied three times a year, in May or June, August and September. All the measurements and analyses were carried out on the two middle rows, leaving at least 10 vines at the end of the blocks as buffer.

The organic and biodynamic blocks were both managed identically, except for the application of the two biodynamic preparations to the latter blocks. Downy and powdery mildew were controlled by organic fungicide treatments, depending on disease pressure, with 7 to 15 treatments being applied per season. Nitrogen supply of the vineyard was ensured by soil cultivation and the plowing-in of the cover crop mixture in every second row shortly before full bloom. Under-vine management was done mechanically without the use of herbicides.

1. Vine and berry physiology

Pruning weight was determined during the winter period by sampling 30 lignified shoots per block; these were obtained by cutting 1 m of the fruit cane after the second to last bud. They were then weighed using a standard scale (g per m of shoot). Leaf nitrogen content was assessed using an N-tester on 30 leaves per block in August of each season.

Leaf net photosynthesis was evaluated by gas exchange measurements on three well-exposed adult leaves per block at midday using a Ciras 3 Portable Photosynthesis System (PP Systems, USA). For the control of environmental parameters, photosynthetically active radiation (PAR) inside the leaf cuvette was adjusted to 1,500 mmol/m^2/s, temperature to 30 °C, relative humidity to 80 % and CO_2 concentration to 400 ppm

For berry quality, 50 berries per block (i.e., 450 per treatment) were sampled, 1 to 3 days prior to harvest. Berries were weighed to determine individual berry weight and subsequently pressed for further analysis. Organic acids and sugar were analysed by HPLC, a 1260 Infinity Agilent HPLC system consisting of a G4225A degasser, an isocratic G1310 pump system, a GT329B autosample injector, a G1316A column oven, and a G1314F UV-detector (Agilent Technologies, Santa Clara, CA, USA) connected to a Shodex RI-101 refractive index detector (Showa Denko, Kawasaki, Japan) maintained at 50C. The samples were pre-treated by solid phase extraction using Waters Oasis HLB and 6 cm^3 (200 mg) cartridges (Waters Corporation, Milford, MA, USA), then filtered through 0.2-mm nylon filters (Millipore, Burlington, MA, USA); 20 µL were directly injected into an Aminex HPX-87H HPLC column 300 × 7.8 mm, with a 9 µm particle size (Bio-Rad Laboratories, Hercules, CA, USA). Separations were carried out under isocratic conditions at 80C using a 0.65 mmol H_2SO_4 solution, with a mobile phase flow rate of 0.5 mL/min. Organic acids were detected at 210 nm.

To assess the vine water status photosynthetic carbon isotope composition, the $^{12}C/^{13}C$ ratio (also known as $\delta^{13}C$) was analysed in the sugars of must samples from berries in 2017 and 2018 according to Gaudillere *et al*. (2002).

Weather data was retrieved from the meteorological station in Mont-sur-Rolle (46°28’01.1”N 6°19’26.8”E; https://www.agrometeo.ch/).

2. Soil abiotic properties

Undisturbed soil samples of approximately 100 cm^3 were taken yearly at a depth of 5 to 10 cm from 2017 to 2020. Sampling took place every spring except in 2020 (autumn). The samples were taken in the middle of the inter-row in order to avoid disturbed environments (wheel passage, tillage under the vine). A total of 72 samples were analysed over the four years of the experiment (18 blocks*4 years).

To determine the volume of the samples at field capacity, the plastic bag method (Boivin *et al*., 1991) was used after equilibrium at a matric potential of -60 hPa. The samples were then oven-dried at 105 °C and the dry mass and the volume of the coarse fraction (> 2mm) were removed to calculate the following parameters: apparent density and porosity at -60hPa, water retention capacity at -60hPa (equivalent to pore volume smaller than 50 microns in diameter), and coarse pore volume greater than 50 µm. Structural stability was determined according to Le Bissonnais (2016). The mean weighted diameter (MWD) results from the average of the three structural stability tests. The organic matter content and the pH of the blocks were determined at the end of the experiment in 2020. The organic matter content of fine soil (< 2mm) was determined according to Walkley, A., Black (1934). The pH was measured in a 1:2.5 m/v water suspension.

3. Soil microbial analysis

Soil sampling was carried out at three sampling time points (24 June, 21 and 28 July) in 2020 (i.e., four years after the beginning of the experiment). Sampling was performed in two adjacent central rows. Within each block, eight subsamples (5x5x5 cm) were collected from the soil surface and then pooled in a plastic bag, resulting in a total of 18 samples per sampling date. Samples were stored in soft coolers containing ice packs and transported to the laboratory within a day. From each composite sample, a representative subsample of about 10 g was randomly taken and placed in a 50 ml Falcon tube and kept at -80 °C until DNA extraction.

3.1. DNA extraction and sequencing

DNA extraction was performed with 0.5 g of soil using the FastDNA SPIN Kit for soil (MP Biomedicals, Solon, OH) and following the recommendations of the manufacturer. DNA extracts were quantified using a Quawell q9000 spectrophotometer, adjusted to 20 ng µL-1 in ultra-pure water and stored at –20 °C. DNA samples (25 µL) were sent to the Centre for Comparative Genomics and Evolutionary Bioinformatics (Halifax, Canada) for PCR amplification and Illumina MiSeq sequencing. The internal transcribed spacer 2 region was amplified with the primer pair ITS86F and ITS4R to characterise the fungal communities. Further information regarding the PCR procedures and Illumina sequencing are provided in Fournier *et al*. (2020).

The amplicon data are available on EMBL European Nucleotide Archive under project number: PRJEB54862.

3.2. Sequence data processing and taxonomic assignment

The absence of sequencing primers in the dataset was verified using cutadapt (Martin, 2012). The reads analysis was carried out with the Divisive Amplicon Denoising Algorithm (DADA2) software (Callahan *et al*., 2016). The DADA2 pipeline infers exact amplicon sequence variants (ASVs) from sequencing data with filtering, dereplication, sample inference, chimera identification and merging of paired-end reads. The QIIME2 (Bolyen *et al*., 2019) was used for the taxonomy assignment of the ASVs with pre-trained Naive Bayes classifiers (Wang *et al*., 2007) and the UNITE database as a reference for fungi (Nilsson *et al*., 2019). Since our approach relies on extracted DNA, our data might include ASVs from extracellular DNA or encysted cells.

3.3. Statistical analyses

Microbial data was analysed by stepwise linear regression models computed to examine the effect of treatment, sampling day, pH and soil moisture content on microbial alpha diversity (Inverse Simpson). To visualise changes in fungi composition, nonmetric multidimensional scaling (NMDS) was conducted with the Bray–Curtis distance using the R function "metaMDS" (Oksanen *et al*., 2020). The drivers of the community compositional changes were then investigated using a permutational multivariate analysis of variance (PERMANOVA) applied to a Bray-Curtis dissimilarity matrix and using the R function "ADONIS" (Oksanen *et al*., 2020). All analyses were performed on a rarefied dataset (4950 sequences per sample).

Other soil, vine and berry physiology data was analysed using Excel stats and OriginPro, and using standard t-tests to test for significant differences between treatments.

RESULTS AND DISCUSSION

1. Vintage climatic characterisation

The monthly temperature and precipitation data for the five seasons of 2016 to 2020 is provided in Supplementary Figure S1. The long-term annual rainfall (1981-2010) in the region was 999 mm/m^2 and the growing season (1 April to 30 September) rainfall was 484 mm/m^2. Total annual rainfall in the five years of the study was 1256, 883, 1056, 1286 and 1225 mm respectively for 2016 to 2020. Growing season rainfall (1 April to 30 September) was 623 mm, 406 mm, 359 mm, 538 mm and 565 mm from 2016 to 2020. The annual average temperature of the region in the period 1981-2010 was 9.3 °C and 14.7 °C for the growing season from 1 April to 30 September. In the study plot, the mean annual temperature was 10.3, 11.3, 12.5, 12.0 and 12.5 °C and the mean growing season temperatures 15.9, 17.1, 18.7, 17.7 and 18.2 °C respectively for 2016 to 2020. Drawing from this climatic data, it becomes evident that the study region is undergoing global warming with temperatures increasing considerably in all the studied years, as compared to the reference period 1981 to 2010.

With a combination of low rainfall and high temperatures, the 2018 growing season was most affected by global warming, as shown in other studies in other European growing regions (Labbé *et al*., 2019; Rienth *et al*., 2020).

The main berry quality-determining compounds are illustrated in Figure 1 and the vine physiological parameters in Figure 2.

2. Vine physiology

When comparing the plant physiological parameters of the treated and non-treated vines - such as yield (Figure 1A), pruning weight (Figure 2B) and N-tester readings (Figure 2C) - as proxies for vine vigour and general physiological performance, no significant differences induced by biodynamic preparations were observed. This is in agreement with previous studies, which showed that the use of biodynamic preparations had little influence on vine vegetative growth (Döring *et al*., 2019).

Meissner *et al*. (2019) found limited significant impact on grapevine vegetative growth (reduced growth using biodynamic preparations). However, they used a broader combination of biodynamic preparations; therefore, their results are not strictly comparable to our findings.

No yield differences were observed in our study, which is in agreement with studies comparing organic and biodynamic treatments with cv. Merlot, cv. Sangiovese, cv. Cabernet-Sauvignon and cv. Riesling (Botelho *et al*., 2016; Collins *et al*., 2015; Döring *et al*., 2015; Meissner, 2015; Reeve *et al*., 2005). While Reeve *et al*. (2005) found that biodynamic treatments had no impact on pruning weights, they found the yield-pruning ratio to be significantly lower under biodynamic management; this difference was due to a slightly higher yield in the organic treatment, while pruning weights themselves did not differ between treatments. However, other studies have not found any differences in yield-pruning weight ratios between organic and biodynamic plots (Collins *et al*., 2015; Döring *et al*., 2015).

Differences in the yield of other crops have been observed; for example, in a study of ten biodynamic and organically managed greenhouses in Southern Germany (Zikeli *et al*., 2017) the biodynamic farms were found to produce significantly higher yields in tomatoes and cucumbers compared to the organic farms

Biodynamic preparations are claimed to stimulate soil nutrient cycling and to promote the photosynthetic activity of crops and compost transformation (Masson and Masson, 2013). In our study, net photosynthesis was significantly different, but to only one measurement point (8 May 2018, Figure 2D), whereas for all the other measurements we did not observe any differences between treatments similar to what was observed in other long terms studies on Riesling (Botelho *et al*., 2016; Döring *et al*., 2015).

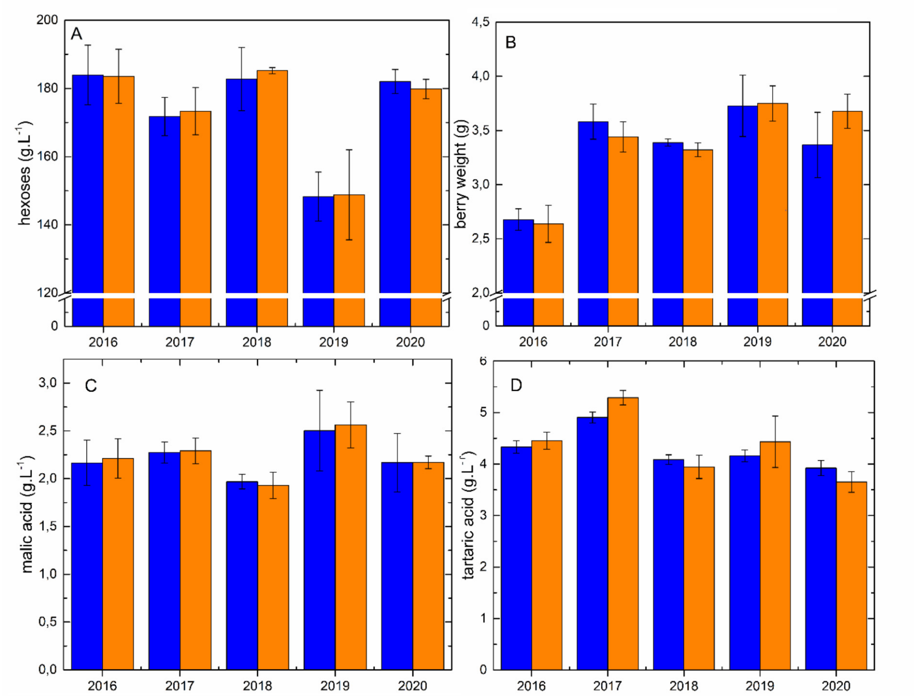

FIGURE 1. Berry quality characteristics.

A) Sugar concentration, B) Individual berry weight, C) Malic acid concentration, and D) Tartaric acid concentration. Orange bars: plots treated with biodynamic preparations 500 and 501. Blue bars: plots treated without biodynamic preparations.

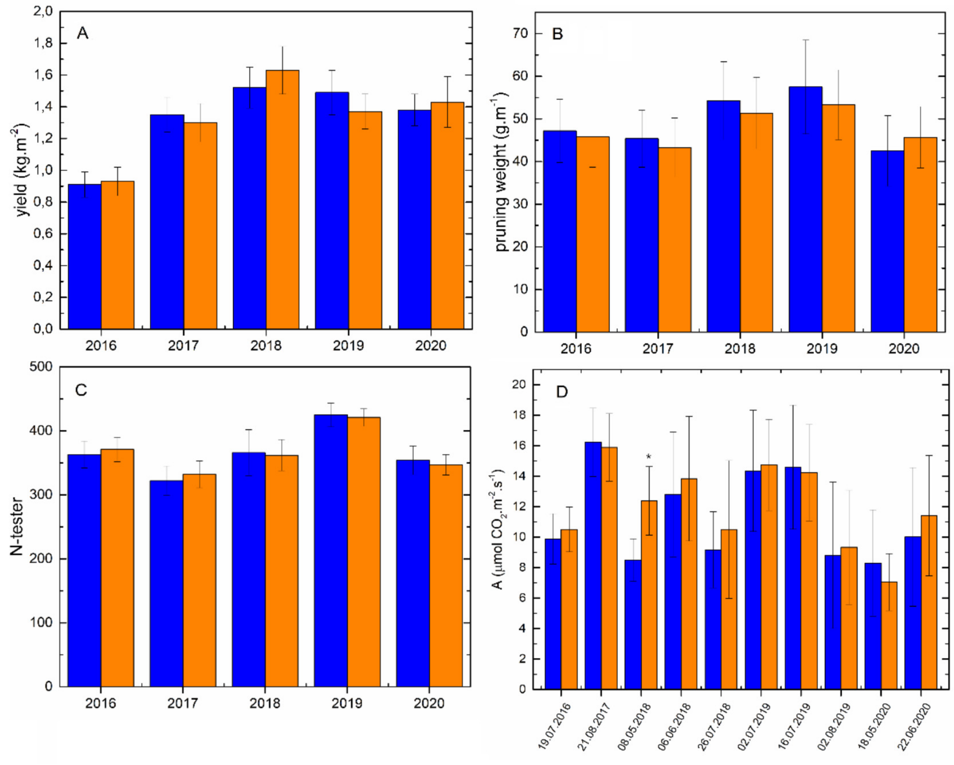

FIGURE 2. Yield and vine physiology.

A) Yield per square meter, B) Pruning weight per meter of shoot as a proxy for vigour, C) N-tester, and D) net photosynthesis. Orange bars: plots treated with biodynamic preparations 500 and 501. Blue bars: plots treated without biodynamic preparations. * indicates significant differences between treated and non-treated blocks in the respective year.

Interseasonal variations such as significantly lower berry weight, yield and higher sugar concentrations in 2016 can be explained by the vines still being in their juvenile phase in combination with a relatively cool year. In 2018, the slightly higher hexose concentrations are most likely the result of the particularly dry and hot conditions which lead to lower berry weights and thus increased sugar concentration. Lower malic acid concentrations in the same year can be explained by increased malic acid respiration due to high temperatures (Rienth *et al.*, 2016; Rienth *et al.*, 2021a). This is similar for 2020 which was relatively warm but wetter than 2018 and the opposite tendency for sugar and malic acid is observed in in 2019, which was cooler than 2020 and 2018 during the growing season.

No differences in sugar concentration between treatments was observed, which is in line with most of published biodynamic studies (Döring *et al.*, 2019). Only Reeve *et al.* (2005) found significantly higher Brix, total phenols and total anthocyanins in one out of four years in cv Merlot under biodynamic management when compared to organic management; however, biodynamic management consisted in applying additional products as well as 500 and 501, namely 502, 503, 504, 505, 506, 507 and Barrel compost.

The carbon 13 discrimination analysis of sugars in berries sampled in 2017 and 2018 gave values of -28.42 ± 0.50 (without biodynamic preparations) and -28.47 ± 0.43 (with biodynamic treatment) in 2017, and -28.62 ± 0.34 (without biodynamic preparations) and -28.45 ± 0.42 (with biodynamic treatment) in 2018. This suggests that no water deficit was experienced by the vines during berry ripening (Rienth and Scholasch, 2019) in both years and treatments.

The results of two recent studies on organic and biodynamic viticulture showed significantly lower pre-dawn water potentials in biodynamic plots for cv. Riesling in Germany (Döring *et al.*, 2015) and cv. Sangiovese in Italy (Botelho *et al*. 2015). However, the plots of the latter study were replicated but not randomised; therefore, the observed changes in physiological performance cannot be completely attributed to the treatment in this study due to possible soil heterogeneity.

In a recent metabolomic study conducted in two vineyards in the Veneto region in Italy on cv Garganega, Malagoli *et al.* (2022) applied 501 on leaves then carried out targeted and untargeted metabolite analyses of the leaves and berries. They observed changes in the chlorophyll content of the leaves and no variation in the free amino acid content of the berries; however, some individual amino acids were found to have increased in 501-treated vines, such as cysteine (+49.9 %), methionine (+100 %) and phenyl alanine (+24.9 %). Furthermore, the authors observed a higher concentration of epigallocatechin, and the pigment violaxanthin indicated a stimulation of the biosynthetic pathways of phenolics in the leaves and berries due to the application of 501; stimulation of the biosynthetic pathways has also been reported in a few studies on other types of crop. Jarienė *et al*. (2019) observed differences in total phenolic compound concentrations (TPCC) and total flavonoid concentrations (TFC) in the leaves of two mulberry (*Morus alba* L.) cultivars (Turchanka and Plodovaja 3) when 500 and 501 were applied: the

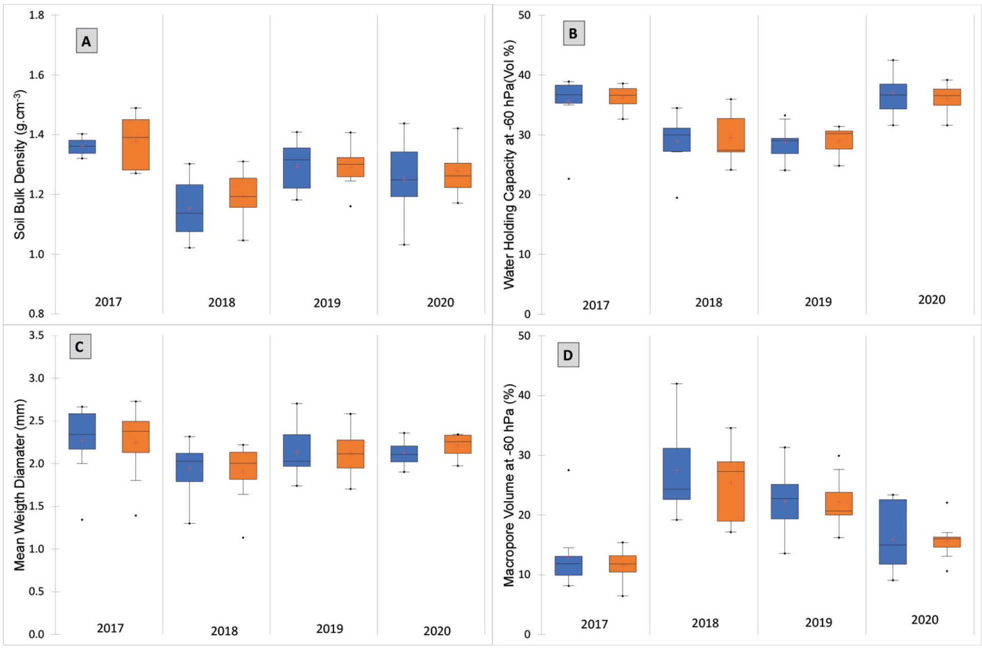

FIGURE 3. Soil physical properties.

A) Soil bulk density, B) Water holding capacity at -60hPa, C) soil structural stability assessed by Mean Weight Diameter, and D) Macropores volume at -60 hPa. Orange bars: plots treated with biodynamic preparations 500 and 501. Blue bars: plots treated without biodynamic preparations. * Indicates outliers.

TABLE 1. pH and SOC (Soil Organic Carbon) in 2020 from the plots treated with biodynamic preparations (500 and 501) and without biodynamic preparations.

Variable	Treatment	N	Mean	Std. Error	Minimum	Maximum
pH	With biodynamic preparations	9	7.68	0.0596	7.59	7.76
	Without biodynamic preparations	9	7.69	0.0636	7.60	7.76
SOC %	With biodynamic preparations	9	2.80	0.474	2.30	3.60
	Without biodynamic preparations	9	2.60	0.517	2.00	3.50

TABLE 2. Effects of selected variables on inverse Simpson diversity.

	Estimate	Std. Error	t value	Pr (> |t|)
(Intercept)	4.08804	0.04591	89.052	< 2e-16***
Sampling day	-0.09150	0.04878	-1.876	0.0641
pH	0.10050	0.04878	2.060	0.0424*

TABLE 3. Adonis analysis of the effect of sampling day, treatment, pH and soil moisture on fungal communities.

	Df	Sum of Sqs	Mean Sqs	F.Model	R2	Pr (> F)
Sampling day	1	10.1357	10.1357	68.102	0.42966	0.001***
Treatment	1	0.1270	0.1270	0.853	0.00538	0.402
pH	1	0.4444	0.4444	2.986	0.01884	0.018*
Soil moisture	1	0.3809	0.3809	2.559	0.01615	0.030*

Turchanka cultivar showed increased TPCC when only 500 was applied and Plodovaja 3 showed decreased TPCC and TFC when sprayed with 501. The combination of 500 and 501 had significant effects on quercetin-acetylhexoside and kaempferol-acetylhexoside accumulation in the mulberry leaves of both cultivars.

In our study, we did not analyse the phenolic compounds and can therefore not comment on the potential influences of biodynamic products on their synthesis. However, we did not observe any differences in the incidence of downy and powdery mildew or botrytis between treatments, which would potentially have been influenced by higher production of phenolic compounds, which serve as phytoalexins and phytotoxins against fungal diseases (Rienth *et al*., 2021a; Rienth *et al*., 2021b).

3. Soil physical properties and analysis

The vineyard parcel is situated on a homogenous colluvic cambisol soil (FAO/WRB, 2014) with a relatively high field capacity of 250 to 300 mm. This data was retrieved from a previous terroir study (Letessier and Fermond, 2004). All the analysed soil parameters are shown in Figure 3 A-D and Table 1. No significant differences between treated and control plots were observed from 2017 to 2020. The observed annual variations are due to soil tillage. Indeed, tillage was performed on every second inter-row alternately each year in August. More specifically, selected sampling inter-rows were plowed in August 2017 and 2019. This notably explains the strong bulk density differences between 2017 and 2018. Indeed, bulk density decreases after tillage, since the volume of the coarse pores increases and water retention (fine pores) decreases. Clearly, these variations were not influenced by the application of the biodynamic preparations. In a long-term field trial in Germany, Faust *et al*. (2017) did not find preparation 500 to have any additional positive effects on soil compared to those of composted farmyard manure fertilisation. However, Reeve *et al*. (2011) found that soil pH moderately increased when Pfeiffer field spray and other biodynamic preparations were applied.

4. Fungal diversity and community structure

A total of 1,553,659 high-quality soil fungal sequences were obtained from the nine blocks treated with water (without biodynamic preparations) and nine blocks reated with biodynamic preparations 500 and 501 at five sampling time points in 2020. The sequences belonged to 1648 fungal ASVs (Amplicon sequence variants).

No differences in diversity were detected between the samples treated with biodynamic preparations and those without biodynamic preparations. The pH was the only factor showing significant effects on fungal diversity (Table 2), while the fungal community composition was significantly affected by pH, the sampling date and the soil moisture (Table 3).

The results of our study indicate that the application of preparations 500 and 501 does not lead to significant differences in diversity and does not seem to impact the soil fungal communities.

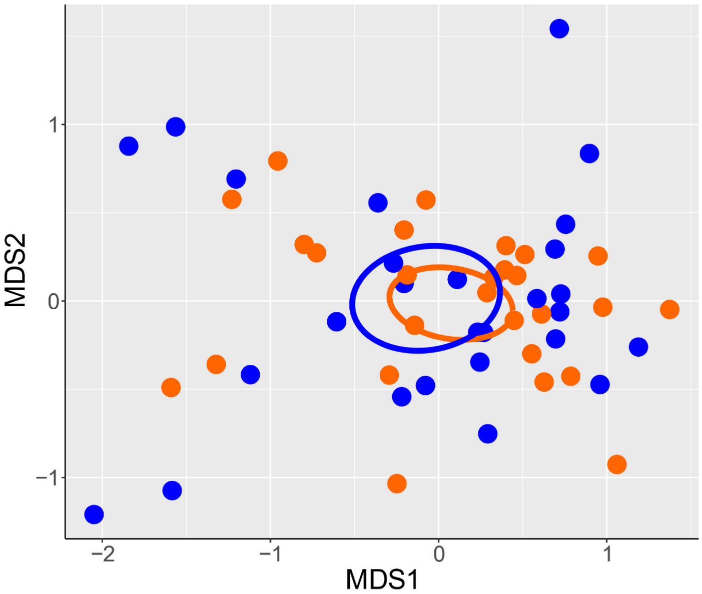

FIGURE 4. Non-metric multidimensional scaling (NMDS) plots of fungi obtained with the Bray-Curtis dissimilarity matrix.

Red and green dots represent samples collected from the plots treated with biodynamic preparations (500 and 501) and with water respectively. For each community cluster, ellipses represent 95 % confidence intervals around the centroid of each community cluster.

These results are in line with the study of Hendgen *et al.* (2018), in which the input of biodynamic preparations did not affect the fungal composition or richness compared to the organic treatment. However, the bacterial biodiversity increased in the topsoil under organic management compared to conventional viticulture, in which mineral fertilisers, herbicides and synthetic fungicides were applied (Hendgen *et al*., 2018).

Morrison-Whittle *et al*. (2017) quantified fungal communities in six conventional and six biodynamic vineyards by analysing samples from several different vineyard "habitats" (i.e., bark, fruit and soil) using metagenomic techniques; they found significantly higher species richness in biodnyamic fruit and bark communities, but not in the soil. In terms of types and abundance of fungal species, biodynamic management has been found to have a significant effect on soil and fruit (Morrison-Whittle *et al*., 2017 in Santoni *et al*., 2022). In a metaanalyis on the impact of farming systems on soil ecological quality, Christel *et al*. (2021) highlight that in the reviewed literature, microorganism abundance was enhanced in biodynamic farming compared to organic farming, with an increase of 71 % in the abundance measurements. Microorganism activity was also more stimulated in biodynamic farming than in inorganic farming: 54 % of the measurements showed a positive effect and 86 % of the soil fauna results showed similar effects of biodynamic farming and organic farming.

Spaccini *et al.* (2012) characterised the molecular composition of the biodynamic preparation 500 and found that it consists of a complex molecular structure, with lignin aromatic derivatives, polysaccharides and alkyl compounds as the predominant components. Biodynamic preparations appear to be enriched with biolabile components and, therefore, potentially conducive to plant growth stimulation. In the present study, however, the application of 500 to soil did influence fungal diversity or community structure.

CONCLUSIONS

The present study aimed to evaluate the effects of the application over four years of biodynamic preparations 500 and 501 on vine physiology, berry quality and soil physical properties and its microbiome. For all assessed parameters, no significant differences between the treated and control blocks were observed during the period of the experiment. The present study thus confirms the findings of several research groups, which showed that the differences between biodynamic and organic farming were almost never significant.

Thus, we could not confirm the empirical observations of the many biodynamic growers who frequently report that vines from biodynamic vineyards treated with preparations 500 and 501 are more stress resilient and healthier, and produce higher quality fruit and thus higher quality wine. Furthermore, the present study did not confirm the growers' empirical reports of higher soil quality in biodynamic vineyards.

However, we cannot rule out that longer trials combined with the analysis of additional parameters and/or a different methodological approach, such as epigenetics, might reveal some differences between organically- and biodynamically-managed vineyards. Furthermore, the formulation and manufacturing of the two applied preparations (500/501), which were commercial standard preparations, could have influenced the present results.

Due to experimental limitations, we were not able to conduct a full biodynamic holistic approach involving the application of not only the two main preparations 500 and 501, but also the different natural products often added by growers (e.g., different compost preparations and green manure) and following the lunar cycles; this may have affected results.

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