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Vineyard management system affects soil microbiological properties

Sofia Di Giacinto[1†], Matthias Friedel[2†*], Christian Poll[3], Johanna Döring[2], Robert Kunz[1] and Randolf Kauer[2]

[1]Department of Soil Science and Plant Nutrition and [2]Department of General and Organic Viticulture, Hochschule Geisenheim University, Von-Lade-Str. 1, 65366 Geisenheim, Germany
[3]Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, Emil-Wolff-Str. 27, 70599 Stuttgart, Germany

[†] These authors contributed equally to this work

*Corresponding author: matthias.friedel@hs-gm.de

ABSTRACT

Aim: The aim of this study was to investigate the effects of integrated (INT), organic (ORG) and biodynamic (BD) management systems with similar C- and N-inputs on soil microbiology in a viticultural long-term field trial.
Methods and results: Within the systems comparison, soil samples were taken 10 years after conversion, throughout the growing season. To gather information about microbial community structure, the activity of five soil enzymes was measured, and phospholipid fatty acids (PLFA) and neutral lipids fatty acids (NLFA) profiles were analysed accompanied by comprehensive soil analysis. pH associated with BD was significantly higher compared to INT soil. Copper and N-min values in INT were significantly lower compared to the organic systems. BD and ORG were characterised by a higher β-D-glucosidase and urease activity and a higher abundance of fungi and bacteria. INT had larger quantities of mycorrhizae indicator NLFAs.
Significance and impact: Results from this study contribute to a better understanding of the microbial community structure and nutrient cycling under organic and biodynamic viticulture.

KEYWORDS

PLFA, organic viticulture, soil microbiology, biodynamic viticulture, management systems, mycorrhizae, cover crop

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/2458

INTRODUCTION

The loss of agricultural soil due to erosion and loss of soil fertility is one of the most drastic challenges for global agriculture in the 21st century (Brundtland *et al.*, 1987; Forster *et al.*, 2013). The sustainable management of soil in agriculture is strongly promoted by European policies (García-Ruiz *et al.*, 2008) and aims at preserving and/or improving soil quality (Widmer *et al.*, 2006) Microorganisms are involved in soil nutrient cycling and soil aggregate formation, as well as plant pathology and plant growth promotion (Widmer *et al.*, 2006). Consequently, soil maintains its functionalities and productive efficiency only through conserving a diverse and active soil microbiome. Weed and pest control strategies, tillage and fertiliser applications associated with agricultural management systems can affect composition, abundance and activity of the soil microbial community, e.g. (Lundquist *et al.,* 1999; Mäder *et al.,* 2002) and thus either degrade or amend soil quality.

Integrated (INT), organic (ORG) and biodynamic (BD) agricultural management systems are the main systems used in Europe. The integrated system aims at integrating natural regulation processes with anthropic interventions in order to partially reduce external inputs, but it allows the use of mineral fertilisers, synthetic fungicides and herbicides. Integrated production follows integrated crop management rules and regional guidelines, when present (Bradley *et al.*, 2002). Organic agriculture is a holistic approach that supports agro-ecosystem health, including soil biological activity. Agronomic, biological and mechanical methods are promoted over synthetic materials and off-farm inputs (FAO, 1999). Organic management in Europe follows European Union Regulation (EEC) No. 834/2007, which excludes synthetic pesticides and inorganic fertilisers and highlights the preservation of soil fertility and biodiversity. Organic vineyard management often includes the application of multi-species cover crops, organic fertilisers, green manure and mechanical weed control rather than herbicide use. Biodynamic agriculture must follow the same regulations as organic agriculture; it is based on Rudolf Steiner's Agriculture Course held at Koberwitz (now Kobierzyce, Poland) in 1924 and is recognised as one of the first organic agricultural movements (Vogt, 2007). Biodynamic standards are similar to organic agriculture norms, but differ in the application of biodynamic preparations on plants (horn silica, preparation 501), soil (horn manure, preparation 500) and compost (herbal preparations 502–507; Freyer, 2016). The preservation of soil fertility by supporting the soil microorganisms has been at the core of organic and biodynamic agriculture movements since they began, and has thus been a focus of many studies comparing agricultural management systems.

The response of soil microorganisms to management practices has been intensively studied in a system comparison trial based on a ley rotation (Fließbach *et al*., 2007; Mäder *et al.*, 2002), but less is known about the soil in perennial crops, such as grapevines. Studies conducted in olive orchards (Benitez *et al.*, 2006; García-Ruiz *et al*., 2008), apple orchards (Bougnom *et al.*, 2012; *Meyer et al.*, 2014) and vineyards (Okur *et al*., 2009) compared enzymatic activities, soil respiration parameters and arbuscular mycorrhizal fungi in different agricultural management systems. In all these studies the soil microbiome was strongly affected by the management system.

Vineyard soil is often highly degraded because of the intense use of phytosanitary products and frequent tillage (Miguéns *et al.*, 2007). The historical and present use of copper-based fungicides, still the only effective method against *Plasmopara viticola* allowed in organic viticulture, has made viticulture one of the land use forms with the highest copper accumulation in European agriculture (Ballabio *et al.*, 2018). Copper concentrations found in vineyards can inhibit enzymatic activities (Mackie *et al.*, 2013; Wightwick et al., 2013).

Despite the restriction to copper-based fungicides in organic farming, significantly elevated soil enzymatic activities were found in ORG or BD as compared to INT crop rotations (Mäder *et al*., 2002) and olive orchards (Benitez *et al*., 2006). Protease, urease, alkaline phosphatase and dehydrogenase activities were significantly higher in organic than in conventional vineyard soils (Okur *et al*., 2009). Long-term organic fertilisation (García-Ruiz *et al*., 2008) and the use of cover crops (Virto *et al.*, 2012; Peregrina *et al.*, 2014) can enhance the activity of soil enzymes and might explain these differences. Meyer *et al*. (2014) observed that the alteration index three (AI3), an enzymatic soil degradation index, was higher in conventional

soils compared to organic apple orchard soils, indicating a more degraded soil.

Basal soil respiration (R_BCO_2) in apple orchards (Bougnom *et al*., 2012) and microbial biomass carbon (MBC) in crop rotations (Fließbach *et al*., 2007) seem to be higher under BD and ORG management, while the metabolic quotient for CO_2 (qCO_2) is lower in these systems under crop rotation (Mäder *et al*., 2002). Similar results have been reported for vineyards: MBC (Okur *et al*., 2009; Freitas *et al*., 2011) and R_BCO_2 (Freitas *et al*., 2011) were higher in ORG or BD systems compared to conventional vineyards while qCO_2 was lower (Probst *et al*., 2008; Freitas *et al*., 2011). In a long-term study (Zaller and Köpke, 2004), qCO_2 was significantly lower in soils treated with biodynamic farmyard manure compared to traditional farmyard manure. While the quantity and carbon turnover of soil microbiota seem to be enhanced under organic and biodynamic farming, results concerning the composition of the soil microbial community are not always in accordance.

Phospholipid-derived fatty acids (PLFAs) are commonly used as chemotaxonomic markers in microbial ecology. PLFA analysis can be used to determine biomass and composition of microbial communities (Frostegård *et al.,* 2011) and have been used to compare the effects of different management practices on soil microbiota. PLFA profiles from organic and conventional soil show clearly discernible patterns, but also depend strongly on management-independent factors such as soil type, time of the year, and adaptation to environmental conditions (Bossio *et al.*, 1998; Lundquist *et al.,* 1999; Birkhofer *et al.,* 2008).

PLFA analysis also allows for quantification of arbuscular mycorrhizal fungi (AMF). AMF are symbionts of the fine roots of about 80 % of all plant species (Gianinazzi and Schüepp, 1994), accounting for 5–50 % of the biomass of soil microbes in agricultural soil (Olsson *et al.*, 1999). They favour plant growth by improving root nutrition capacity and tolerance to pathogens, drought and heavy metals (Muchovej, 2001). Arbuscular mycorrhizal fungi colonisation in vineyard soil can be limited by soil fumigation (Menge *et al.*, 1983; Cheng and Baumgartner, 2004), season, rainfall (Schreiner, 2005) and available soil phosphorous (Karagiannidis and Nikolaou, 1999; Karagiannidis *et al.*, 2015).

Mäder *et al.* (2000) observed that percentage of root length colonised by AMF was 30–60 % higher for plants grown in soil from low-input farming systems (ORG and BD) than in those grown in INT (with and without farmyard manure) farmed soils. Around 50 % of AMF variance was attributed to soil chemical parameters such as soluble soil phosphorous. In vineyards, organically farmed plots were characterised by a higher AMF sporulation, a three times higher root colonisation, and a greater number of AMF species compared to conventional vineyards (Freitas *et al*., 2011). In contrast, a recent study has shown a higher diversity of AMF in INT as compared to organic and biodynamic viticultural management systems (Hendgen *et al.*, 2018).

ORG as compared to INT agriculture has a more active, more diverse and more stable microbial community according to most of the systems comparisons presented to date. As multiple parameters are varied in a systems comparison, it is virtually impossible to trace these effects back to a single source of variance. It is likely, however, that these effects are caused by organic fertilisers, which have been shown to enhance microbial activity. In previous systems comparisons (e.g. Okur *et al*., 2009) with a few noteworthy exceptions (Mäder *et al*., 2000; Mäder *et al*., 2002; Fließbach *et al.*, 2007), organic fertilisers were often added in large amounts to organic systems, while integrated systems only received mineral fertilisers, thus introducing massive differences in organic C supply. The detrimental effect of the use of copper-based fungicides seems to be overridden by this additional carbon supply.

The aim of this study was to investigate the effects of different management systems with similar C- and N-inputs on soil microbiology in a viticultural long-term field trial by gathering enzyme activity and PLFA data accompanied by comprehensive soil analysis throughout the growing season.

MATERIALS AND METHODS

1. Experimental site

The experimental vineyard was located in the *Rheingau* region, Germany (49°59'; 7°56'). The vineyard, 0.8 hectare in size, was planted in 1991. The vineyard faces south (slope < 5%) and rows are oriented north–south. Vines (*Vitis vinifera* L. cv. Riesling clone Gm 198–30,

grafted to *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. SO4 and *Vitis riparia* Michx. × *Vitis cinerea* Engelm. cv. Börner rootstock, respectively) are planted at a spacing of 1.2 m within rows and 2 m between rows, using a vertical shoot positioning system. The soil of the experimental site is classified as a Luvisol from loess according to the official soil map (BodenViewer Hessen, viewed on 2 April 2020), but shows characteristics of a hortic anthrosol due to long viticultural use. The sand, silt and clay ratio is typical of loam (40/40/20) and relatively homogenous across soil layers and blocks (Table S1). Until the end of 2005, the vineyard was managed uniformly according to standards for integrated agriculture (code of good agricultural practice; Martinez, 2013). Conversion of individual plots to ORG and BD viticulture started in 2006. The experiment was set up as a complete block design, where the three factor levels of the main effect management system were replicated in four blocks. Each plot consisted of four rows with 32 vines each.

2. Management

The INT treatment was managed according to the code of good practice. ORG and BD plots were managed according to Regulation (EC) No 834/2007 and Regulation (EC) No 889/2008 and according to ECOVIN- and Demeter-Standards, respectively (Table 1).

Plant protection in BD and ORG plots was based on copper, sulfur and plant strengtheners while systemic fungicides were used in the INT management system (Table S2). Mulch mixture and Wolff-mixture (Table S3) were used as permanent cover crops, established in every second row, in INT and in ORG or BD plots, respectively. Rows with winter cover crops were tilled shortly before bloom (Table S4). Undervine weed was controlled mechanically in ORG and BD plots while in INT plots herbicides were used. ORG and BD plots received the same soil and vine management except for the use of BD preparations in the BD treatment. The biodynamic preparation horn manure (500; Masson and Masson, 2013) was applied twice at the beginning of the growing season and once after harvest. Horn silica (501; Masson and Masson, 2013) was applied three times a year during the growing season. In the previous years, but not in the experimental year, compost from farmyard manure was used for both ORG and BD plots. In BD plots compost preparations (502-507; Masson and Masson, 2013) were added to compost. Green waste compost was used for INT plots. Mineral fertilisers were applied in the INT treatment when soil analyses indicated a considerably elevated level of nitrogen in the ORG and BD treatments due to cover crop N fixation, but not in the experimental season.

The plots were checked for uniformity prior to data collection using a mixed linear model with treatment as a fixed factor and block and sampling depth (0–30, 30–60 cm) as a random factor with respect to soil moisture, pH, C/N ratio, and phosphor, magnesium and potassium content (Table S5, modified according to Döring *et al.,* 2015). In case of humus content, a mixed linear model with treatment as a fixed factor and block as a random factor was applied (Table S5, modified according to Döring *et al.* 2015). The treatments did not differ significantly in any of these parameters.

3. Soil sampling

Soil samples were collected once a month over a period of four months (May–August 2016). Sampling dates were 23 May, 13 June, 18 July and 18 August. Samples were taken from the middle of untilled rows under cover crop in the central row of each experimental block, leaving the outer rows as a buffer. Six samples were extracted with an iron drill at depths of 0–20 cm and pooled to get a representative sample for each of the 12 vineyard plots. On the first sampling date, all plots were additionally sampled for general soil parameters in depth intervals of 0–30, 30–60 and 60–90 cm for pH, P_2O_5, K_2O and MgO, and at 0–30 cm for the remaining general parameters. Soil samples were put into plastic bags, transported in a cool box and finally stored at 4 °C until further processing. The analysis of soil composition and enzymatic activities was conducted within 24 h after soil sampling. Samples for PLFA were sieved (2 mm) and frozen at -20 °C immediately after sampling.

4. Soil composition

General soil analysis was conducted to outline the main characteristics of the vineyard soil and to identify possible variables that could interfere with research results. The general soil analyses (pH, P, K, Mg, carbon content, soil type, heavy metals, water holding capacity) were carried out

only once, on soil samples from the first collection day. pH, potassium, phosphorus and magnesium concentrations were measured according to VDLUFA (Hoffmann, 1991). Total carbon (TC) was determined by Dumas combustion on an Elementar vario MAX cube (Elementar Analysensysteme, Langenselbold, Germany). Total inorganic carbon (TIC) was determined by Scheibler analysis (Schaller, 2000). Organic carbon (TOC) was determined as the difference between TC and TIC. The soil type was analysed by sedimentation and sieving method according to Schaller (2000). The heavy metal content in the soil was analysed by aqua regia dissolution and ICP-OES (inductively coupled plasma optical emission spectroscopy), according to VDLUFA (Hoffmann, 1991). Soil water holding capacity (WHC) expressed as w/w was measured according to Schaller *et al*. (2000). As WHC can be influenced by cover crop, soil organic matter content and microbial activity, it was treated as a dependent variable. The water content of the samples was expressed as relative water content (RWC in %), calculated as actual water content (w/w)/WHC. Mineralised nitrogen (N-min) and water content were analysed for all dates. Flow injection analysis (FIA star 5000 Analyzer, FOSS, DK) was used for the measurement of mineralised nitrogen (N-min) in soil (Schaller, 2000).

5. Enzymatic activities

Enzyme essays followed the method of Alef (1991). β-glucosidase (GLU) is a common and predominant enzyme in soil (Eivazi & Tabatabai, 1988) indicating the ability of soil to degrade cellulose. Dehydrogenase (DHA) is part of the respiration pathways of soil microorganisms (Das & Varma, 2010), and plays a significant role in the biological oxidation of soil organic matter. Urease (UR) is involved in urea hydrolysis and thus in the soil nitrogen cycle (Lloyd & Sheaffe, 1973) and catalase (CA) is an intracellular enzyme involved in the oxidoreductase metabolism of microorganisms (García-Gil *et al.*, 2000). Phosphatases (PHOs) are a broad group of enzymes involved in the phosphorus cycle and are strongly connected to soil fertility (Das & Varma, 2010). The biological fertility index (BIF) was calculated according to Stefanic *et al.* (1984).

$$BIF \equiv \frac{(1{,}5 \cdot DHA + k \cdot 100 \cdot CA)}{2}$$

where k is a proportionality factor of 0.01. The alteration index three (AI3) is an index for soil disturbance and was calculated according to Puglisi *et al.* (2006).

$$AI3 \equiv (7{,}87 \cdot GLU) - (8{,}22 \cdot PHO) + (0{,}49 \cdot UR)$$

6. PLFA and NLFA profiles

Phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) were analysed according to Frostegård *et al*. (1993). PLFAs were extracted from 4 g of frozen soil with a single-phase mixture of chloroform:methanol: citrate buffer (18.4 mL at 1:2:0.8 volume ratio). Lipid extraction was carried out by normal phase SPE with Si cartridges (Bond Elut Si, Aglient, Santa Clara, U.S.A.) conditioned with 2 mL chloroform. The extracted fatty acids were successively eluted with 2 × 2.5 mL chloroform (NFLAs), 8 × 2.5 mL acetone (glycolipids) and 2 × 2.5 mL methanol (PLFAs). The recovered polar lipids were transesterified to the fatty acid methyl esters (FAMES) by a mild alkaline methanolysis. FAMES were quantified by GC-FID. Chromatographic separation was achieved on a 50 m × 0.2 mm ID × 0.33 µm film thickness Agilent HP-5 column. 100 µL of the sample was injected at 260 °C in split mode. The column oven was held at 70°C for 2 min, ramped to 160°C at 30°C/min, then from 160°C to 280°C at 3°C min^{-1} and held for 15 min. The carrier gas was helium with a flow of 250 kPa (about 20 cm s^{-1}). The detector temperature was 280°C and the detector gases were air at a flow rate of 450 mL min^{-1} and hydrogen at a flow rate of 45 mL min^{-1}. The concentrations of PLFAs were expressed in units of nmol g^{-1}.

7. Weather data

Data for the 2016 growing season were provided by the German meteorological service (DWD) from a station close to (400 m) the experimental vineyard.

8. Statistical analysis

All statistical analyses were computed using the R software package (R Development Core Team, 2006) and principal component analysis was computed using the FactomineR package (Le *et al.*, 2008). A mixed linear model with treatment as a fixed factor and block and date as random factors was applied. A likelihood ratio test was performed to test the significance of the factor treatment. If the treatment effect was significant

($p < 0.05$), a general linear hypothesis test with Bonferroni-Holm adjustment was carried out to compare the factor levels. For general soil analysis, sampling depth (0–30, 30–60 and 60–90 cm) was included in the model as a random factor. Prior to implementation of the mixed linear model residues of the respective datasets were graphically checked for normal distribution (histogram and Q-Q plot).

RESULTS

1. Experimental conditions

The experimental season of 2016 was characterised by high precipitation in spring, with 81 mm in May and 100 mm in June, and a comparatively dry summer (Figure S1). Consequently, the soils were water-saturated on the second sampling date (June 18), and dried up afterwards.

Average soil pH of the experimental vineyard was 7.4 (Table 2). pH values increased in the order INT < ORG < BD and a significant difference in pH values was detected between INT and BD plots.

Nutrient analysis showed the vineyard soil to be extremely rich in P_2O_5, K_2O, with a sufficient MgO concentration. MgO levels were influenced by management system ($p < 0.01$), showing higher concentrations in the ORG system compared to INT and BD. The INT system showed a slightly lower amount of P_2O_5. Average total lime ($CaCO_3$) concentration was 4.87. Nitrogen concentration in the analysed soils was significantly lower in the INT plots. Average concentrations were 0.11 % for the INT treatment and 0.12 % for the ORG and the BD treatment, respectively. Average organic matter levels were 1 %, and the C/N ratio was slightly higher than 11. Levels of trace elements within the field trial did not differ significantly among treatments, except copper (Cu) and cadmium (Cd). Copper concentration was significantly lower in INT compared to ORG and BD plots, whereas Cd levels were significantly lower in INT compared to BD plots. WHC did not differ among treatments.

2. Enzymatic activities

The experimental vineyard soil was characterised by low values of mineralised

TABLE 2. Results of the soil chemical analysis of integrated, organic and biodynamic management systems.

Parameter	INT (mean ± sd)		ORG (mean ± sd)		BD (mean ± sd)		Treat
pH	7.35 ± 0.19	b	7.40 ± 0.19	ab	7.47 ± 0.10	a	*
P_2O_5 (mg 100 g^{-1} soil)	68.20 ± 14.93	-	75.80 ± –11.01	-	75.86 ± 12.80	-	ns
K_2O (mg 100 g^{-1} soil)	30.17 ± 8.01	-	30.83 ± 10.04	-	31.00 ± 9.02	-	ns
MgO (mg 100 g^{-1} soil)	10.67 ± 2.46	b	12.58 ± 2.19	a	10.83 ± 1.47	b	**
$CaCO_3$ (%)	5.12 ± 3.21	-	4.49 ± 1.59	-	4.99 ± 1.16	-	ns
N (%)	0.11 ± 0.01	b	0.12 ± 0.01	a	0.12 ± 0.01	a	*
total-C (%)	1.97 ± 0.55	-	1.90 ± 0.36	-	2.05 ± 0.16	-	ns
Inorganic C (%)	0.61 ± 0.39	-	0.54 ± 0.19	-	0.60 ± 0.14	-	ns
Organic C (%)	1.36 ± 0.20	-	1.36 ± 0.22	-	1.45 ± 0.13	-	ns
C/N ratio	12.5 ± 3.11	-	11.0 ± 1.63	-	12.0 ± 1.83	-	ns
Mn (ppm)	533.21 ± 9.42	-	547.79 ± 14.50	-	553.75 ± 22.24	-	ns
Fe (ppm)	18073.75 ± 2617.56	-	18432.50 ± 2788.03	-	18195.00 ± 3349.25	-	ns
Cu (ppm)	82.40 ± 20.11	b	98.68 ± 24.54	a	98.19 ± 21.29	a	**
Zn (ppm)	95.13 ± 11.92	-	102.23 ± 19.38	-	103.00 ± 12.87	-	ns
Ni (ppm)	23.13 ± 3.70	-	24.59 ± 5.46	-	25.03 ± 5.01	-	ns
Cd (ppm)	0.84 ± 0.60	b	1.17 ± 0.95	ab	1.26 ± 0.87	a	*
Pb (ppm)	30.22 ± 6.66	-	32.94 ± 6.49	-	32.79 ± 5.01	-	ns
WHC	30.75 ± 1.54	-	30.69 ± 1.78	-	31.14 ± 1.47	-	ns

Statistical significance, *p<0.05 and **p<0.01, of the treatment effect determined by likelihood ratio test (ns, not significant). a,b indicate statistical significance (p<0.05) for the fixed factor treatment determined by general linear hypothesis test with Bonferroni-Holm adjustment.

TABLE 3. Soil enzymatic activities and microbial community analysis (PLFA) under integrated, organic and biodynamic vineyard management.

Parameter	INT (mean ± sd)		ORG (mean ± sd)		BD (mean ± sd)		Treat
Soil analysis							
N-min (NO_3-N kg ha^{-1})	4.42 ± 2.43	b	12.27 ± 11.78	a	11.79 ± 6.81	a	**
RWC (%)	47.74 ± 11.15	-	47.01 ± 13.20	-	48.52 ± 12.73	-	ns
Enzymatic activity							
GLU (µg ρNG g^{-1} h^{-1})	564.52 ± 163.10	b	730.96 ± 176.79	a	753.96 ± 192.63	a	***
CAT (% of O_2 released)	8.76 ± 3.35	b	13.38 ± 6.78	a	12.61 ± 6.98	ab	*
UR (µg NH_4-N g^{-1} h^{-1})	24.07 ± 5.44	b	27.31 ± 5.35	ab	29.44 ± 4.92	a	*
DHA (µg TPF g^{-1} h^{-1})	0.656 ± 0.137	b	0.714 ± 0.152	ab	0.756 ± 0.132	a	*
PHO (µg ρNP g^{-1} h^{-1})	217.81 ± 82.03	-	222.09 ± 73.95	-	227.8 ± 82.7	-	ns
Biological fertility index (BIF)	4.87 ± 1.74	b	7.22 ± 3.47	a	6.87 ± 3.47	ab	*
Alteration index 3 (AI3)	-6.18 ± 11.52	-	-0.44 ±12.43	-	-1.74 ± 14.02	-	ns
Fatty acid – indicator							
PLFA 16:1n7 - Bacteria (nmol g^{-1} soil)	5.90 ± 1.01	b	7.09 ± 0.91	a	7.43 ± 0.72	a	***
PLFA 18:2n6 - Fungi (nmol g^{-1} soil)	2.44 ± 0.49	b	3.06 ± 0.66	a	3.07 ± 0.63	a	**
PLFA 20:4n6 - Protozoa (nmol g^{-1} soil)	0.63 ± 0.20	-	0.63 ± 0.30	-	0.58 ± 0.14	-	ns
NLFA 16:1n5 - AMF (nmol g^{-1} soil)	31.13 ± 12.18	a	15.82 ± 8.27	b	14.31 ± 5.44	b	***

Data presented are means across all sampling dates ± standard deviation. Statistical significance, *p<0.05, **p<0.01 and ***p<0.001, of the treatment effect determined by likelihood ratio test (ns = not significant). a,b indicate statistical significance (p<0.05) for the fixed factor treatment determined by general linear hypothesis test with Bonferroni-Holm adjustment.

nitrogen (N-min) in the first 20 cm of soil, which is normal under cover crops. N-min was significantly affected by the management system ($p < 0.01$), with higher values in the ORG and BD compared to the INT system (Table 3).

Relative water content (RWC) did not differ significantly among treatments (Table 3).

GLU, CAT, UR and DHA activities showed higher values in both organic systems compared to INT (Table 3). GLU, UR and DHA were significantly higher in BD plots compared to INT plots, whereas in ORG plots only GLU and CAT were significantly higher in comparison to the INT treatment. The enzymatic index BIF determined here was significantly affected by the management system, whereas Alteration Index Three (AI3) did not differ among treatments. BIF was significantly elevated in ORG compared to INT plots.

3. PLFA and NLFA profiles

Four main PLFAs and NLFAs were analysed as chemotaxonomic markers to describe the composition of the soil microbial community (Table 3). Bacteria, fungi and AMF populations were strongly influenced by the management system while protozoa marker (PLFA 20:4ω6) did not differ among treatments. PLFA 16:1ω7 and PLFA 18:2ω6, bacteria and fungi population indicators, were significantly higher in both ORG and BD systems compared to INT. NLFA 16:1ω5, the AMF marker, was significantly higher in INT soil as compared to ORG and BD systems.

4. Principal component (PCA) analysis

A scores plot of a PCA containing all microbial analyses (Figure 1A) showed that all samples from specific dates were clustered together. This confirmed the importance of seasonal factors. In all sampling date associated clusters, INT samples deviated in a similar manner to ORG and BD samples, among which no systematic difference was visible. This shows that the differences between INT and ORG or BD samples were stable over the experiment.

Variable loadings (Figure 1B) showed that mycorrhizae development was negatively related to other microbiological parameters, especially fungi, protozoa and bacteria, urease activity and N-min, which were tightly correlated among themselves (see also Figure S2). However, only

N-min and Urease activity showed a significant negative correlation with mycorrhiza presence (Figure S2). While variables from this group were separating the ORG and BD from the INT treatment, soil water content, GLU and PHO were the main variables separating the respective sampling dates. As no treatment:date interactions were observed in the experiment, a correlation analysis was conducted on the seasonal means of the microbiological parameters and the soil chemical data (Figure S3). This analysis revealed that fungi and bacteria communities were associated with total soil N, and fungi benefited from a lower C:N ratio. Protozoa were negatively correlated with soil pH. None of the groups seemed to be affected by copper concentration in the soil.

DISCUSSION

Most of the general soil parameters were not significantly influenced by the management system. Similar to a previous study (Fließbach *et al.,* 2007), organic systems showed higher pH values compared to INT soil, possibly related to the use of acidifying mineral fertilisers in the INT treatment. Independently from the treatment, pH was higher in 2016 compared to the beginning of the trial (Table S5).

Copper concentration of the experimental vineyard was higher than the European average for vineyards (Ballabio *et al*., 2018), with the ORG and BD showing a 20 % higher level than INT. This might be due to the regular use of Cu products in the organic plant protection strategy. Contrary to other studies, the increasing Cu level in ORG and BD systems did not have a negative effect on either β-glucosidase or dehydrogenase activities (Fernández-Calviño *et al.*, 2010; Mackie *et al*., 2013) nor on bacterial or fungal abundance (Ge & Zhang, 2011). This might be explained by the fact that the difference in Cu concentration in the soil between ORG/BD and INT treatments was only 16 mg kg^{-1} of soil. Both the difference among treatments and average Cu concentrations are well below levels that were shown to have significant effects on the microbial community (Díaz-Raviña *et al.*, 2007) or toxic effects on plants (Kabata-Pendias, 2010).

Higher MgO levels were associated with the ORG system in the current study. Mäder *et al.* (2002) also observed higher magnesium levels in ORG compared to conventional and BD systems in the long-term field trial on annual crops in Switzerland. As soil management, cover crops and fungicides applied were identical under ORG and BD treatments in the current trial, it can be hypothesised that biodynamic preparations applied in the BD treatment might have led to the observed changes. Reeve *et al.* (2005) found content of magnesium in the soil of organically managed vineyard plots to be higher compared to biodynamically managed soils in the first year after conversion. In a different study, Reeve *et al.* (2010) analysed composted grape pomace of ORG and BD origin and found ORG composts to have higher amounts of magnesium compared to their BD counterparts. This might be one reason why MgO contents under ORG management were significantly higher in the current trial, but the mechanisms behind it remain unclear. MgO levels in 2016 were lower compared to the beginning of the trial (Table S5), probably due to MgO uptake by the vines.

A combination of factors may be responsible for the constantly elevated N-min values in the ORG and BD treatments. The use of a legume-rich cover crop in ORG and BD systems has great potential to enhance short-term soil N availability (Kuo *et al.*, 1997; Utomo *et al.*, 1990). N fixation in singe-legume cover crops can reach several hundred kg of N ha^{-1} a^{-1} (Karpenstein-Machan & Stuelpnagel, 2000). In addition, compost made exclusively from crop residues or municipal yard waste as used in the INT treatment differs from manure compost by C/N ratio (Hartz *et al.*, 2000) and may be less readily mineralisable. Furthermore, agricultural practices performed in ORG and BD (rolling on 24 May 2016 and mulching on 8 June 2016, Table S3) may have accelerated nitrogen release from cover crop residues. These practices also seem to have stimulated the growth of a microbial community characteristic for the turnover of organic matter. This was reflected in the analysis of soil enzymes and PLFAs: UR, CA, DHA and GLU activities as well as bacteria and fungi markers were elevated in ORG and BD treatments and correlated positively with N-min as well as with markers for bacteria, fungi and protozoa. Increased activities of these enzymes have been reported after establishment of cover cropping and organic fertilisation in vineyards (Okur *et al*., 2009; Virto *et al*., 2012; Peregrina *et al*., 2014). However, in the first years of this systems comparison, only DHA and PHO activities were elevated under ORG and BD

management (Meißner, 2015). The biological fertility index (BIF), based on DHA and CA activities, was within the range provided by Riffaldi *et al.* (2002). ORG was characterised by higher BIF compared to INT, thus indicating a more fertile soil (Stefanic *et al*., 1984). BIF under BD management showed the same tendency ($p < 0.1$) as ORG. The ratio of fungi/bacteria was similar among treatments and constant among sampling dates, indicating rather stable microbial communities. In addition, the high abundance and low seasonal variation of protozoa indicators found in all treatments shows that the microbial ecosystem seems to be stable. PHO activity and AI3 were not affected by the management system, as were phosphate levels in the soils.

Mycorrhizae, which are sensitive to soil disturbance (Oehl *et al.*, 2003), were found in elevated concentrations in the INT treatment. This stands in contrast to data published by Mäder *et al*. (2000; 2002), who showed higher AMF presence in ORG or BD soils based on crop rotations, but is in accordance with findings of Hendgen *et al*., (2018), who reported a shift in the fungal community towards an increased Glomeromycota presence under INT management. Phosphorus and nitrogen are the most important regulators of arbuscular mycorrhizal symbiosis (Nouri *et al.*, 2015). As there were no differences in phosphate concentration among treatments and N-min was the only soil parameter that was significantly correlated to AMF presence (Figure S3), it is likely that higher N-min values in ORG and BD soils could partially explain lower AMF marker concentration in the respective treatments. As reported for other crops (Graham *et al.*, 1986; Griffioen *et al.*, 1994), the higher copper level in both organic systems may also have inhibited AMF. However, differences in copper concentration between INT and ORG or BD treatments in our study were much lower compared to these studies, and other fungi or bacteria did not seem to be negatively affected by Cu concentration in the ORG or BD treatments. AMF could substantially contribute to plant nutrition, thus understanding dynamics of grapevine mycorrhizal colonisation is of major importance, especially in farming systems with low external input.

Consistent differences among treatments in the microbial community analysis as well as in soil enzymatic activities were found in the current study. INT and ORG or BD systems were shown to have unique and stable microbial communities. This may be related to the type of cover crop selected, but also to the differences in the management regimes of ORG or BD and INT. These include a different selection of fungicides, differences in soil pH, a higher frequency of machine overpasses as well as the use of mulching/rolling of the cover crop in ORG and BD, and differences in compost composition. Döring *et al.* (2015) and Meißner (2015) observed growth, yield and fruit quality of grapevines in the experimental vineyard in which this study was conducted. ORG and BD systems in their studies consistently showed significantly lower growth and yield compared to the INT treatment, despite elevated N-min values and an intact microbial community. Comparable results were obtained in the 2016 growing season (Table S6). Consequently, competition for water and nutrients by the deep-rooting cover crops and thus phytohormonal regulation may be responsible for the reduction of growth in ORG and BD systems. This is supported by the fact that ORG and BD systems often had lower water potential compared to INT (Döring *et al.,* 2015).

Preparation 500, used as a field spray preparation in biodynamic agriculture, is characterised by a rich microbial population and shows elevated values of β-glucosidase, alkaline phosphatase, chitinase and esterase enzymatic activities (Giannattasio *et al.*, 2013). Nevertheless, BD plots did not show significant differences compared to the ORG system (except for MgO levels). Consequently, the application of biodynamic preparations did not affect the microbiological parameters considered in the current study.

CONCLUSIONS

The results of the current study indicate that organically managed soil has a more active (higher soil enzymatic activities) and richer bacterial and fungal community, most likely related to the cover crop and its management. Higher AMF colonisation associated with integrated vineyard management could be attributed to lower N-min availability, lower soil pH and to a lower soil disturbance. PLFA and enzymatic activities showed unique and stable communities for INT and ORG or BD treatments. The application of biodynamic

preparations did not affect the microbiological parameters considered in our study.

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