Received: 28 Avril 2019 | Accepted: 2 September 2019 | Published: 18 October 2019
DOI:10.20870/oeno-one.2019.53.4.2403

OENO One VINE AND WINE OPEN ACCESS JOURNAL

Conversion to organic and biodynamic viticultural practices: impact on soil, grapevine development and grape quality

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ABSTRACT

Aim: The effects of integrated, organic and biodynamic management on soil quality and the growth and morphological development of Riesling grapevines were assessed during the first 4 years of a long-term field trial in Geisenheim, Germany. The overall aim was to understand the effects of these different viticultural practices on soil quality and plant morphology as the basis for product quality.
Methods and results: As indicators of soil quality, earthworm abundance and the activity of selected enzymes were assessed. The vegetative and reproductive development of the grapevines, as well as their susceptibility to fungal diseases in the field, wood and grape composition, and grape yield, were investigated. Individual variables were subjected to analysis of variance. Additionally, all variables were subjected to multivariate principal component analysis. Compared with plots under integrated management, plots under the two biological treatments were characterized by higher soil quality and lower vegetative growth and grape yield, and therefore higher exposure of grapes and lower grape cluster compactness, and, probably as a result of these morphological differences, lower incidence of acetic acid rot. Principal component analysis clearly differentiated the three treatments, and showed that biodynamic management had more pronounced effects than organic management in terms of enhanced soil fertility and reduction of vegetative growth.
Conclusions: In the present study, organic and especially biodynamic management resulted in a morphology favouring production of high-quality grapes. The treatments differed in terms of fertilization and plant protection methods as well as choice of cover crops. Therefore, further research is necessary with respect to root growth and the nitrogen and water uptake dynamics of vines and cover crops. The differences between grapes produced under organic and biodynamic management emphasize the need for more research on the mode of action of biodynamic preparations.
Significance and impact of the study: In recent years, both winegrowers and consumers have expressed steadily growing interest in organic and especially biodynamic wine production. The present study contributes to a better understanding of the effects on grapevine growth and morphological development of shifting to these methods as a way to increase product quality.

KEYWORDS

farming systems, plant morphology, vegetative growth, reproductive development, biodynamic management, integrated management, organic management, soil fertility, must quality, sustainability, viticultural practice

INTRODUCTION

Plant production methods, along with site and climatic conditions, have a major impact on the growth and development of crops, including grapevines (Baeumer, 1992). Fertilization methods influence growth and differentiation processes in plants, in terms of both morphology and chemical composition (Herms and Mattson, 1992; Brandt and Mølgaard, 2001). Compared with wild plants, crops are exposed to a more nutrient-rich growth environment, with positive effects on yield and basic nutrient content (Dixon, 2001). However, if nutrients are applied in excess, photosynthetic assimilation may be insufficient to enable incorporation of all absorbed nutrients as higher molecular compounds such as secondary metabolites (Bell and Henschke, 2005). High amounts of low-molecular nitrogen-containing compounds, such as nitrate and free amino acids, create a plant substrate that fosters development of pests and diseases (Chaboussou, 1996).

Compared with annual crops, grapevines have a rather low nutrient demand (Nendel and Kersebaum, 2004). However, although recommended levels of fertilizer have been extremely reduced and cover crops have been used frequently in viticulture since the 1980s (Linsenmeier *et al.*, 2007), nutrient losses by erosion, leaching and denitrification remain a major problem (Nendel and Kersebaum, 2004; Novara *et al.*, 2018).

In organic viticulture, nitrogen availability may be limited, because no mineral fertilizers are applied (regulation EU 834/2007). When an intensively fertilized vineyard is converted to the organic system, growth reduction, with concurrent yield decrease, and a less nitrogen-dominated tissue composition (wood, leaves and grapes) are to be expected, as described by Brandt and Mølgaard (2001). In production of high-quality wine, these changes are partially desired because of their positive effects on quality-determining variables such as acidity and phenolic and anthocyanin content (Acevedo-Opazo *et al.*, 2010; Santesteban *et al.*, 2011).

In recent years, both winegrowers and consumers have shown steadily growing interest in organic wine production (Mann *et al.,* 2012; Willer *et al.*, 2013). In the course of this development, biodynamic wine production especially is receiving increasing attention (Castellini *et al.*, 2017), because winegrowers have reported remarkable positive influences of biodynamic practices on soil quality, grapevine development, plant health and wine quality (Preston, 2008; Masson, 2009; Meissner, 2015). In biodynamic agriculture, in addition to compost fertilization and cover crops, preparations of specific fermented plant materials are used to enhance soil fertility and microbial diversity (e.g. Reganold *et al.*, 1993; Mäder *et al.*, 2002), improve the composting process (e.g. Reeve *et al.*, 2010) and enable more balanced crop growth and development (e.g. Fritz and Köpke, 2005; Reeve *et al.*, 2005). In particular, horn silica (BD 501), which consists of fermented ground silica derived from quartz of feldspar, is used to support inner maturation, that is, formation of higher molecular compounds, with concurrent reduction of vegetative growth (Koepf *et al.*, 1990; Athmann, 2011).

The mode of action of biodynamic preparations is still not well understood. Because the preparations are applied in very small quantities, direct nutrient effects are unlikely. One possible mode of action is effects on bacterial regulation, because bacteria detect and react to extremely low levels of signal molecules in their environment, and biodynamic preparations may contain such molecules (Reeve *et al.*, 2010). According to Giannatasio *et al.* (2013), concentrations in the micromolar range at which the preparations are applied are sufficient to induce biological activity in soil bacteria or plants, potentially fostering microbial proliferation and thus greater rhizospheric activity via the large amounts of carbohydrate and peptide that arise from microbially mediated slow maturation under conditions of low oxygen during production of the preparation (Spaccini *et al.*, 2012). A recent study identified stimulation of natural defence compounds as a possible mode of action of biodynamic preparations: Botelho *et al.* (2016) found that biodynamically as compared with organically managed grapevines showed increased activity of several enzymes typically correlated with plant biotic and abiotic stresses and associated with induced plant resistance.

In the field of viticulture, different aspects of the quality of organic, biodynamic and conventionally produced grapes and wines have been compared in several studies, with inconsistent results. Tinttunen and Lehtonen (2001), using combined analysis of ultraviolet visible spectra and measurement of phenolic compounds, were unable to differentiate wines produced organically from those produced conventionally; however, they did find a higher resveratrol content in the organic wines. Reeve *et al.* (2005) compared organic and biodynamic vines and grapes produced in a long-term field trial and found no differences in terms of yield but a higher ratio of yield to pruning weight and higher Brix values in the biodynamic vines and grapes. Tassoni *et al.* (2013) found no differences in biogenic amine, anthocyanin or polyphenol content or antioxidant activity between wines from organic, biodynamic and conventional production. Kokornaczyk *et al.* (2014), using the droplet evaporation method, successfully distinguished organic wines from biodynamic wines. Laghi *et al.* (2014) compared organic and biodynamic wines with a metabonomics approach and found differences in terms of some amino acids, alcohols and polyphenols. Granato *et al.* (2015) were able to differentiate organic and biodynamic grape juice samples from conventional samples based on instrumental taste profile, antioxidant activity and chemical composition; however, they were unable to distinguish organic from biodynamic grape juice.

All these authors studied the effects of different viticultural practices (organic, biodynamic and conventional) on the harvested product. However, to date, there has been no comprehensive study assessing the growth and morphological development of grapevines produced by the different methods as a basis for improving product quality. Therefore, the aim of the present study was to assess the effects of converting to organic or biodynamic grapevine production on soil variables; vegetative and reproductive development of the grapevines; wood, leaf, grape and must composition; and grape yield.

Our hypotheses were as follows: first, that, compared with integrated management, organic management results in improved soil fertility (indicated by increased earthworm abundance and higher enzyme activity); second, that reduction in vegetative growth, with more open canopy and less compact grape cluster structure, results in lower incidence of fungal diseases; and third, that application of biodynamic field and compost preparations results in more pronounced effects in terms of enhanced soil fertility, disease resistance and reduction of vegetative growth.

MATERIALS AND METHODS

1. Experimental site

This study was carried out as part of the Geisenheim system comparison trial INBIODYN. The experimental site is a 0.8-ha Riesling (clone Gm 198-30) vineyard on sandy loam at Geisenheim University, Germany (49°59′N, 7°56′E), with a mean annual temperature of 10.7°C, mean annual rainfall of 535 mm, and mean rainfall of 360 mm from April to October during the vegetation period (DWD, German weather service, Geisenheim station). The soil is a deeply ploughed anthrosol, with sandy clayey loam in the topsoil and gravelly sandy loam in the subsoil. The soil pH is 7.1.

The site is characterized by a high content of plant available nutrients (calcium acetate lactate-extractable P and K) throughout the soil profile (P_2O_5 and K_2O per 100 g soil at different soil depths, determined according to Schaller, 2000: 73 mg and 44 mg, respectively, from 0 to 30 cm; 74 mg and 38 mg, respectively, from 30 to 60 cm; and 71 mg and 32 mg, respectively, from 60 to 90 cm). In 2010, the plots were checked for uniformity in terms of particle size distribution, soil moisture, pH, humus content, carbon-to-nitrogen ratio, and phosphorus, potassium, and magnesium content. The plots under the different treatments did not differ significantly in terms of any of these variables (data published in Döring *et al.*, 2015).

2. Experimental set-up

The grapes are grafted on two different rootstocks: *Vitis berlandieri* Planch ´ *Vitis riparia* Michx cv. SO4 and *Vitis riparia* Michx ´ *Vitis cinerea* Engelm. cv. Börner. Grape samples from the harvest years 2006–2009 were analysed. The vines were planted in 1991 at a spacing of 1.2 m within rows and 2 m between rows. The pruning system is a single guyot with about 6–8 buds per m^2. The vineyard was managed according to good agricultural practice (GAP) until 2005.

In January 2006, the vineyard was divided into replicate plots under integrated (GAP), organic or biodynamic management (the last two subject to regulation EU 834/2007) in a complete block design with four replicates (see Figure S1). Each plot consists of four rows with 64 vines in each row and is divided into two subplots with one rootstock each. Only the inner two rows were used for sampling. For each variable, an equal number of samples were taken from the two rootstocks.

TABLE 1. Comparison of the different viticultural practices used in the present study.

Character	Integrated management	Organic management	Biodynamic management
Perennial cover crop	Grass mixture	Diverse cover crop mixture (Wolff mixture)	Diverse cover crop mixture (Wolff mixture)
Annual cover crop	Rye plus vetch	Diverse cover crop mixtures	Diverse cover crop mixtures
Under-vine management	Herbicides	Mechanical	Mechanical
Fertilization method	Compost plus mineral plus legumes from annual cover crops	Compost plus legumes from perennial and annual cover crops	Compost plus legumes from perennial and annual cover crops
Plant protection	Organic fungicides	Copper (maximum 3 kg ha^{-1} $year^{-1}$), sulphur, plant strengtheners	Copper (maximum 3 kg ha^{-1} $year^{-1}$), sulphur, plant strengtheners
Use of biodynamic preparations	None	None	Horn manure, horn silica, compost preparations

In 2007, weather conditions, particularly precipitation, were close to their long-term annual mean values during ripening and grape harvest. In contrast, 2006 was characterized by very high precipitation from August to October (220 mm compared with a long-term annual mean of about 130 mm) and very heavy rainfall events close to grape harvest. In 2008 and 2009, heavy rainfall events occurred shortly before harvest (see Figures S2–S5).

3. Management

Management was according to GAP for integrated management, EU 834/2007 and the Ecovin Standard for organic management, and EU 834/2007 and the Demeter Standard for biodynamic management. The different treatments are summarized in Table 1.

Compost fertilization was used in each of the treatments. For integrated management, the compost was made from communal green waste with 1.4 % and 1.5 % nitrogen on a dry matter basis in 2006 and 2007, respectively, and a carbon-to-nitrogen ratio of 14.7 in 2007 (data on carbon for 2006 is missing due to technical problems). For organic and biodynamic management, the compost was made from organic cow manure with 1.9 % and 1.0 % nitrogen on a dry matter basis in 2006 and 2007, respectively, and a carbon-to-nitrogen ratio of 13.1 in 2007 (supplemented with biodynamic preparations under biodynamic management only).

The compost for the two biological treatments was prepared from the same batch of manure under the same conditions except for the addition of biodynamic preparations 502–507 for biodynamic management. These preparations consist of specific fermented plant materials (from 502 to 507: *Achillea millefolium* L. flowers, *Matricaria recutita* L. flowers, *Urtica dioica* L. shoots, *Quercus robur* L. bark, *Taraxacum officinalis* L. flowers and *Valeriana officinalis* L. extract) and are applied with the aim of improving the composting process by stimulating nutrient cycling (Koepf *et al.*, 1990). For details of their content, production and application, see Table S1.

For all the treatments, 50 kg of nitrogen ha^{-1} was applied in 2006 and 2007, equivalent to, respectively, 6.4 and 5.9 t compost ha^{-1} on a fresh matter basis to plots under integrated management and 4.7 and 8.4 t compost ha^{-1} on a fresh matter basis to plots under the biological treatments. In 2008 and 2009, no compost was used for any of the treatments following GAP, because mean values for biomass production and leaf and wood nutrient content were in the optimum range (Tables S2 and S3). In 2006, calcium nitrate (15 % nitrogen) and, as foliar fertilizer after flowering, urea (46% nitrogen) were additionally applied at 25 kg ha^{-1} and 5 kg ha^{-1}, respectively, to plots under integrated management. For all the treatments, nitrogen was also available via incorporation of the biomass of legume-containing cover crop mixtures.

Cover crops were used for all the treatments, with permanent and winter mixtures alternating between rows. The permanent mixture for integrated management was chosen according to GAP, with the aims of preventing erosion, enabling easy care, and minimizing nutrient and water competition for the vines. Therefore, the mixture was composed of grassland species (Table S4). For organic and biodynamic management, the very diverse Wolff mixture® was chosen; it is widely used in organic and biodynamic viticulture throughout Germany. The mixture contains a great variety of species and was developed to provide nutrients via leguminous crops, above-ground biodiversity, and optimized utilization of both top- and subsoil resources and the supply of organic material via combining different root systems. The mixture is

TABLE 2. Timeline of management operations in the field trial.

Operation	2006				2007				2008				2009			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Calcium nitrate (3.75 kg of N/ha) and urea (2.3 kg of N/ha)			Red													
Compost: 50 kg of N/ha[a]			Grey					Grey								
Sowing winter cover crops			Grey					Grey			Grey					
Sowing summer cover crops		Grey				Grey				Grey				Grey		
Mulching summer cover crops[b]						Grey					Grey			Dark green	Grey	
Mulching winter cover crops[b]						Grey	Red			Grey						
Pruning			Grey				Grey				Grey				Grey	
Plant protection		Grey	Grey			Grey	Grey			Grey	Grey			Grey	Grey	
Application of biodynamic field preparations		Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	Light green	

[a] 25 kg of N/ha in 2007. [b] Integrated management, mulching; organic and biodynamic management, rolling or rolling and mulching after about 4 weeks. Red, integrated management only; dark green, organic and biodynamic management; light green, biodynamic management only; grey, all treatments.

composed of material from 30 species, 80% of which are legumes (see Table S4).

Owing to continuous germination of previously established former grasses in the first 3 years after conversion to organic and biodynamic management, the cover crops, which had been intended to be permanent, had to be re-established every year, in spring, in every second row. Winter mixtures of 80% *Secale cereale* and 20% *Vicia villosa* were used for plots under integrated management, and again, very diverse mixtures were used for plots under organic and biodynamic management (45 % legumes in 2006, 7 % legumes in 2007 and 63 % legumes in 2008; Table S5). Winter cover crops were sown into the rows of the permanent cover crops of the previous year in September 2006, October 2007, and August (organic and biodynamic management) and September 2008 (integrated management). Under integrated management, the permanent cover crops were mulched two or three times during their growth. Under organic and biodynamic management, the permanent cover crops were rolled, with the aims of maintaining high biodiversity by stimulating flowering and conserving soil moisture by creating a mulch cover. Winter cover crops were mulched (integrated management) or rolled or mulched (organic and biodynamic management) and, in all three treatments, ploughed under in spring. For detailed information on cover crop management, see Table S6.

Canopy management was the same for all the treatments. Except for the first pruning at the start of the vegetation period, no further shoot thinning or defoliation was carried out during the vegetation period, to avoid inhomogeneity due to management. Tillage was mostly carried out on the same date for all treatments. The area underneath the vines was kept free from weeds by application of herbicide to plots under integrated management, and mechanically for plots under the biological treatments (see Table S6).

Plant protection was based on control of symptoms of fungal diseases and pests and monitoring of climate data. Under integrated management, different fungicides and magnesium sulphate products (to prevent bunch stem necrosis) were used, following the recommendations for plant protection under that treatment. For both organic and biodynamic management, plant protection was the same: before flowering, the plant-strengthening product MycosinVin® was applied; during and after flowering, copper products and wettable sulphur and/or potassium hydrogen carbonate were used. Grapevine berry moths (*Lobesia botrana*) were controlled using pheromones (Table S7).

In biodynamic management, the field preparations horn manure (BD 500) and horn silica (BD 501), were used. The former consists of fermented cow manure and is applied with the aim of stimulating soil processes and root growth, and the latter consists of fermented ground silica from quartz of feldspar and is applied with the aim of stimulating plant physiological processes and improving crop quality (Koepf *et al.*, 1990). BD 500 was applied twice, in March or April and May (May and June in 2006), and BD 501 was applied three times, in May or June, August and September of each year (Table S8).

A timeline of management operations in the field trial is given in Table 2.

4. Soil variables

Soil mineral nitrogen content (N_{min}) was measured five or six times between June and November in each of the years 2006–2008. Eight samples per row were taken with a soil core sampler at two depths (0–30 and 30–60 cm). Rows with summer and winter cover crops were sampled and analysed separately. After homogenization (Schäfer BHM II, Euskirchen, Germany), samples were analysed according to Schaller (2000) by flow injection analysis at 540 nm (FOSS FIAstar 5000, Hillerød, Denmark).

Earthworms were extracted from the soil in December 2007, November 2008 and April 2009, from an area of 0.25 m^2, using a modified mustard extraction method (Gunn, 1992) combined with hand sorting to a soil depth of 25 cm over an area of 0.0625 m^2.

Enzyme activity was determined in August 2007 and July 2008 from mixed samples of summer and winter cover crops to a soil depth of 10 cm, according to Schaller (2000), with the modification that no carbon tetrachloride was added. Samples were ground (Schäfer BHM II) and stored at 4 °C until measurement.

Dehydrogenase activity was measured directly after sampling. First, 5 mL of triphenyltetrazoliumchloride (TTC) was added to 5 g of soil, enabling dehydrogenases in the soil to react with TTC to form triphenylformazane (TPF). Next, the TPF was extracted with 25 mL of acetone and its rate of production determined colorimetrically (546 nm). The dry matter content of the sample was determined in parallel, and values were converted to an hourly rate based on weight of dry matter.

The activity of β-glucosidase, neutral phosphatase and urease was measured after drying the ground soil samples at 25 °C for 2 weeks and then mincing them (Retsch Backenbrecher, Haan, Germany). For determination of β-glucosidase activity, 3.5 mL of a substrate–buffer mixture comprising 0.452 g of *p*-nitrophenyl-β-D-glocopyranoside in 75 mL of sterilized water and phosphate-citrate buffer with pH 6.2 (33.9 portions of 0.1 M citric acid and about 66.1 portions of 0.2 M disodium chloride, yielding 100 mL of buffer solution, the pH of which was adjusted with hydrogen chloride) was added to 1 g of soil moistened with 0.5 mL of toluene. For determination of neutral phosphatase activity, 5 mL of a substrate–buffer mixture comprising 1.067 g *p*-nitrophenylphosphate-disodium chloride in 50 mL of sterilized water and phosphate-citrate buffer with pH 6.5 (58 portions of 0.1 M citric acid and about 142 portions of 0.2 M disodium phosphate, yielding 200 mL buffer solution, the pH of which was adjusted with hydrogen chloride) was added to 1 g of soil moistened with 0.5 mL of toluene. Soil and substrate were incubated for 1.5 h. The rate of production of the reaction product, nitrophenols, was measured photometrically using a flame photometer at 400 nm (ELEX 6361, Eppendorf, Germany). For determination of urease, 1 mL of urea solution (5.435 g of urea in 1 L of sterilized water) was added to 2 g of soil moistened with 0.5 mL of toluene. After an incubation time of 5 h, 2 mL of saturated potassium sulphate solution was added for exchange with the adsorbed ammonium. The concentration of ammonium was then measured by flow injection (FIAstar 5000).

5. Crop variables

The number of leaf layers, proportion of inner leaves, number of exposed grapes and gaps in the canopy were assessed using the point quadrat method (Wilson, 1960; Smart and Robinson, 1991) on 27 August 2008 and 9 September 2009. A stick was inserted orthogonally to the leaf wall 10 times per plot to a distance of 20 cm, and the number of leaf layers and number of grapes touching the stick were counted (if none, 'gap' was recorded).

The nutrient content of the leaves was determined four times in each experimental year. The third or fourth basal leaf on the shoot of 13 vines per plot was sampled. First, its petioles were removed. Leaves were then washed, rinsed with distilled water, dried at 60 °C for 3 days and ground (Cyclotec, Foss, Hillerød, Denmark). Nitrogen and phosphorus content was determined using flow injection (FIAstar 5000); calcium and potassium content using a flame photometer (ELEX 6361); and copper, iron, magnesium, manganese and zinc content using atomic absorption spectroscopy (Perkin Elmer 4100, Waltham, MA). Leaf chlorophyll content was measured for the third, fourth or fifth leaf of the uprising shoot and the third, fourth or fifth leaf of the middle shoot for every plot, using a handheld photometer (Minolta SPAD-502, Osaka, Japan). Six measurements were obtained at defined places in the centre of each leaf. Values were calibrated in the laboratory by extracting the chlorophyll of leaf slices representing differentclasses of the photometric values on each sampling date and by measuring chlorophyll a and b with a photometer (Specord 200, Analytik Jena, Jena, Germany).

Pruning weight was measured shortly after pruning on four vines per plot. After drying, the nutrient content of wood was determined as described for leaf analysis but with the addition of measurement of starch, glucose, fructose and sucrose content. The fresh weight of the wood samples was determined before they were dried in the oven at 60 °C for 4 days. After determination of their dry weight, the samples were ground (Retsch-Mill ZM2000) and passed through sieves (mesh < 0.1 mm).

To determine starch content, a subsample of 0.5 g of powder was extracted by exposing the sample to a mixture of DMSO and 8N hydrochloric acid (ratio, 4:1) for 60 min at 60 °C. After cooling, the pH of the mixture was adjusted to 4.5 and the volume adjusted to 100 mL with distilled water. Starch was hydrolysed using β-amyloglucosidase (Merck, Darmstadt, Germany) in a citrate buffer and kept in a water bath at 60 °C for 15 min. The amount of D-glucose released was determined using a commercial D-glucose assay kit (R-Biopharm, Darmstadt, Germany), following the instructions of the manufacturer. Samples were mixed with a TEA [Tris-acetate-EDTA] ATP–NADP buffer (R-Biopharm) and absorbance was recorded at 340 nm (Specord 500, Analytik Jena). The hexokinase enzyme mixture (HK-G6PDH) was added and absorbance read after 15 min. The D-glucose content of the samples before digestion was determined. Values for starch content were converted to percentage of dry weight by reference to a standard curve.

To determine soluble sugar content, a similar but separate extraction procedure was conducted using 0.5 g of wood powder in 25 mL of distilled water for 60 min at 60 °C. Glucose, fructose and sucrose content were determined using a commercial enzyme assay kit with absorbance read at 340 nm. The values obtained were converted to percentage of dry weight by reference to a standard curve.

Total soluble carbohydrate content as a percentage of dry weight equaled the sum of values for glucose, fructose and sucrose content as a percentage of dry weight. The values for total non-structural carbohydrates equaled the sum of values for soluble carbohydrates, including starch.

The mean shoot length of 18 main shoots per plot was determined in May of each of the years 2007–2009. In 2008, these 18 shoots were labelled, and in July the number of internodes, number of secondary and tertiary lateral shoots, number of leaves on the main shoot and lateral shoots and number of bunches were determined for one main shoot per plot whose length corresponded to the mean length of the 18 shoots measured.

Grape cluster compactness was assessed for 50 clusters per plot in August 2007, July 2008 and September 2009. A rating scheme with five classes was used (Table 3).

Degree and frequency of *Botrytis cinerea infection* was determined on two to four dates between August and October in all four experimental years. Additionally, in October 2008 and 2009, acetic acid rot was assessed for 100 clusters per plot. For both diseases, a rating scale with seven classes was used, in which class 1 corresponded to zero infection and class 7 corresponded to >75% infection.

Grapes were harvested on 9 October 2006, 25 September 2007, 16 October 2008 and 14 October 2009. For determination of grape yield, missing vines were considered. Single-berry weight was determined for 70–80 grapes per plot on four to six dates during ripening. Total acidity, pH and yeast-available nitrogen (nitrogen by the orthophthaldialdehyde method, NOPA) in the must were determined 12 h after pressing and cooling. For analysis of total titratable acidity and pH, a titrator (Titrino 719S, Metrohm,

TABLE 3. Rating scale for grape cluster compactness (Source: Geisenheim University).

Class	Description
1	Grapes very loose: berries do not touch each other, and main axis of shoot can be bent > 90°
2	Grapes loose: berries touch each other, and main axis of shoot can be bent 45–90°
3	Strong grape cluster structure: berries still movable, and main axis of shoot can be bent 10–45°
4	Compact grape cluster structure: berries not movable, but not deformed, and main axis of shoot can be bent ≤ 10°
5	Very compact grape cluster structure: berries deformed by pressure of neighbouring berries, and main axis of shoot cannot be bent

Switzerland) was used. The NOPA content was determined photometrically (Specord 200) using the method described by Dukes and Butzke (1998). Must density in degrees Oechsle (°Oe) was determined using a refractometer (Type 2556/93, Leo Kübler, Germany).

6. Statistical analysis

Data were tested for significance using ANOVA, with treatment and, if applicable, rootstock or soil management (cover crop or cultivated soil) as fix factors and field repetition as a random factor. This was followed by subjecting the data to Tukey's test at $\alpha = 0.05$. All variables were submitted to principal component analysis; for variables that were measured several times per year (i.e. single-berry weight, leaf and wood nutrient content, and the degree and frequency of *Botrytis cinerea* infection); only values for the last date of measurement in each experimental year were entered. Only components with eigenvalues > 1 were included in the analysis. All tests were carried out with PASW Statistics 18 (SPSS, IBM, Armonk, NY, USA).

RESULTS

The mineral nitrogen content of the soil (N_{min}) was similar in plots under organic and biodynamic management. In comparison with the biological treatments, integrated management resulted in significantly higher N_{min} at several sampling dates in each of the years 2006–2008 (Figure 1).

Earthworm abundance tended to be higher in plots under the two biological treatments (Figure 2a). Based on the mean values for the years 2007–2009 combined, there were 45 % and 94 % more individuals in plots under organic and biodynamic management, respectively, than in those under integrated management. However, these differences were not significant. Regarding

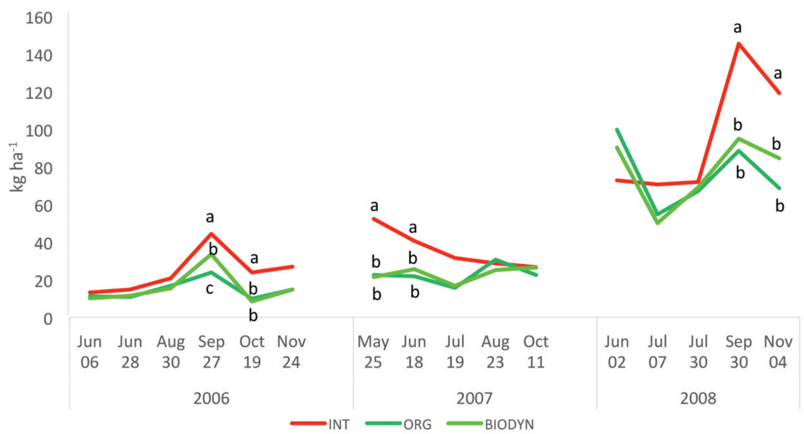

FIGURE 1. Mean concentration of soil mineral nitrogen (NH_4^+–N and NO_3^-–N) at a soil depth of 0–60 cm in plots under integrated (INT), organic (ORG) and biodynamic (BIODYN) management, 2006–2008 ($n = 4$).

Nitrogen content was measured in soil between rows with permanent and annual cover crops. Different letters indicate significant differences between treatments on the given date (Tukey's test, $\alpha = 0.05$).

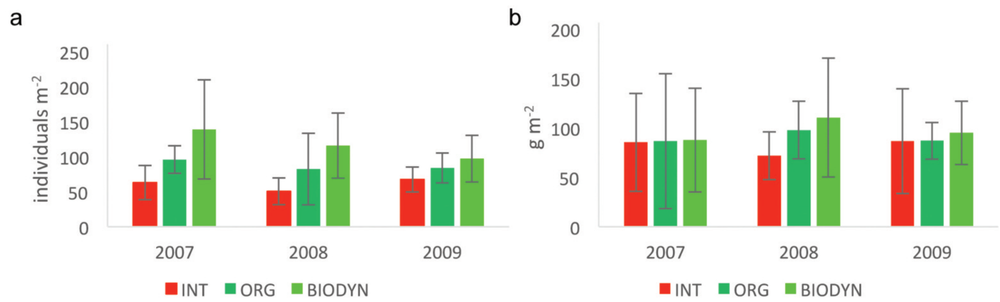

FIGURE 2. Earthworm abundance (a) and fresh weight biomass (b) in plots under integrated (INT), organic (ORG) and biodynamic (BIODYN) management (means ± standard deviation, n = 4).

TABLE 4. Mean activity of selected enzymes in plots under integrated, organic and biodynamic management (n = 4).

Variable	Year	Management		
		integrated	organic	biodynamic
β-glucosidase activity (µmol nitrophenols·g^{-1}·h^{-1})	2007	2.60	2.80	2.73
	2008	1.73	1.89	1.86
Dehydrogenase activity (µg triphenylformazane·g^{-1}·h^{-1})	2007	6.23 a	8.32 b	7.20 ab
	2008	7.38	8.00	8.16
Neutral phosphatase activity (µmol nitrophenols·g^{-1}·h^{-1})	2007	0.91a	1.00ab	1.14b
	2008	1.16	1.26	1.27
Urease activity (µmol CH_4N_2O·g^{-1}·h^{-1})	2007	35.09	35.90	32.76
	2008	0.71	0.78	0.76

Different letters indicate significant differences between treatments on the given date (Tukey's test, α = 0.05).

TABLE 5. Mean values of crop variables indicating vegetative growth for vines under integrated, organic and biodynamic management (*n* = 4).

Variable	Year	Management		
		integrated	organic	biodynamic
Shoot length (cm)	2007	67.63	62.72	62.16
	2008	76.69 a	73.97 ab	70.01 b
	2009	83.63 a	78.14 b	79.03 b
Pruning weight (t/ha)	2006	2.57 a	2.24 b	2.16 b
	2007	3.30 a	2.46 b	2.45 b
	2008	3.20 a	2.77 b	2.67 b
	2009	3.96 a	3.22 b	3.03 b
No. of leaf layers[a]	2008	1.42	1.20	1.13
	2009	2.69 a	2.39 b	2.30 b
Proportion of inner leaves (%)[b]	2008	15.50 a	10.25 b	9.50 b
	2009	32.60 a	28.28 b	26.95 b
Leaf chlorophyll content (mg/g fresh weight)	2007	0.72 a	0.70 b	0.69 b
	2008	1.46 a	1.42 ab	1.41 b

[a] Optimum range, 1.0–1.5 (Smart and Robinson, 1991). [b] Optimum range, < 10% (Smart and Robinson, 1991).
The pruning weight data for 2006 have been reported previously (Fritz et al., 2017). Different letters indicate significant differences between treatments in the given year (Tukey's test, α = 0.05).

earthworm biomass, the different treatments had no clear effects (Figure 2b).

Dehydrogenase and neutral phosphatase activity were tendentially or significantly higher under the two biological treatments, whereas there were no treatment effects on β-glucosidase or urease activity (Table 4).

Values for crop variables indicating vegetative growth, that is, shoot length, pruning weight, number of leaf layers, proportion of inner leaves and leaf chlorophyll content, were tendentially or significantly higher for vines under integrated management than for those under the two biological treatments (Table 5).

Consequently, the proportion of the canopy representing gaps and the proportion of grapes that were exposed, both morphological features promoting reproductive development of the grapes, were tendentially or significantly higher under the two biological treatments, and grape cluster compactness was lower (Table 6).

Accordingly, single-berry weight was tendentially or significantly higher during ripening for vines under integrated management in all four experimental years (Table 7).

The morphological development of vines under the different treatments was reflected by the appearance of exemplary shoots with leaf series and grapes. The number of grapes was not

TABLE 6. Mean values of crop variables affecting reproductive development for vines under integrated, organic and biodynamic management ($n = 4$).

Variable	Year	Management		
		integrated	organic	biodynamic
Gaps in the canopy (%)	2008	17.00 b	25.00 a	22.50 ab
	2009	3.25 b	3.00 b	5.50 a
Exposed grapes (%)	2008	36.88	35.50	36.75
	2009	25.58 b	37.20 ab	41.03 a
Grape cluster compactness (cluster flexibility index)	2007	3.91 a	3.46 b	3.13 c
	2008	2.25 a	1.89 b	1.75 b
	2009	3.35 a	2.99 b	2.83 b

Different letters indicate significant differences between treatments in the given year (Tukey's test, α = 0.05).

TABLE 7. Mean values of the crop variable single-berry weight for vines under integrated, organic and biodynamic management (n = 4).

Date	Management		
	integrated	organic	biodynamic
2006			
8 August	0.63	0.62	0.62
17 August	0.77	0.75	0.76
23 August	0.93	0.86	0.87
6 September	1.15	1.09	1.11
13 September	1.18 a	1.10 b	1.07 b
20 September	1.26 a	1.21 ab	1.19 b
2007			
15 August	1.49	1.44	1.43
21 August	1.76	1.69	1.70
4 September	1.84	1.74	1.68
12 September	1.92	1.87	1.82
19 September	1.87	1.80	1.79
2008			
13 August	1.19	1.14	1.19
27 August	1.51	1.42	1.37
10 September	1.72 a	1.60 ab	1.57 b
24 September	1.79	1.65	1.65
1 October	1.74 a	1.60 b	1.53 b
8 October	1.76	1.72	1.68
2009			
13 August	1.06	1.01	1.00
27 August	1.39	1.26	1.28
10 September	1.51	1.48	1.45
1 October	1.75	1.63	1.63

Different letters indicate significant differences between treatments in the given year (Tukey's test, α = 0.05).

significantly affected by the different treatments, but tendentially lower under biodynamic management (Figure 3c). Although the main shoot produced an equal number of leaves, regardless of the kind of treatment, integrated management resulted in a significantly higher number of lateral shoots and a significantly higher number of leaves on lateral shoots (Figure 3a,b). Tertiary lateral shoots were found only in vines under integrated management. Compared with integrated management, biodynamic management resulted in shorter shoots, as indicated by a significantly lower main shoot length and lower internode length (Figure 3d,e) despite an equal number of internodes (see Figure 3a). Together, the results for these variables reflect the stronger

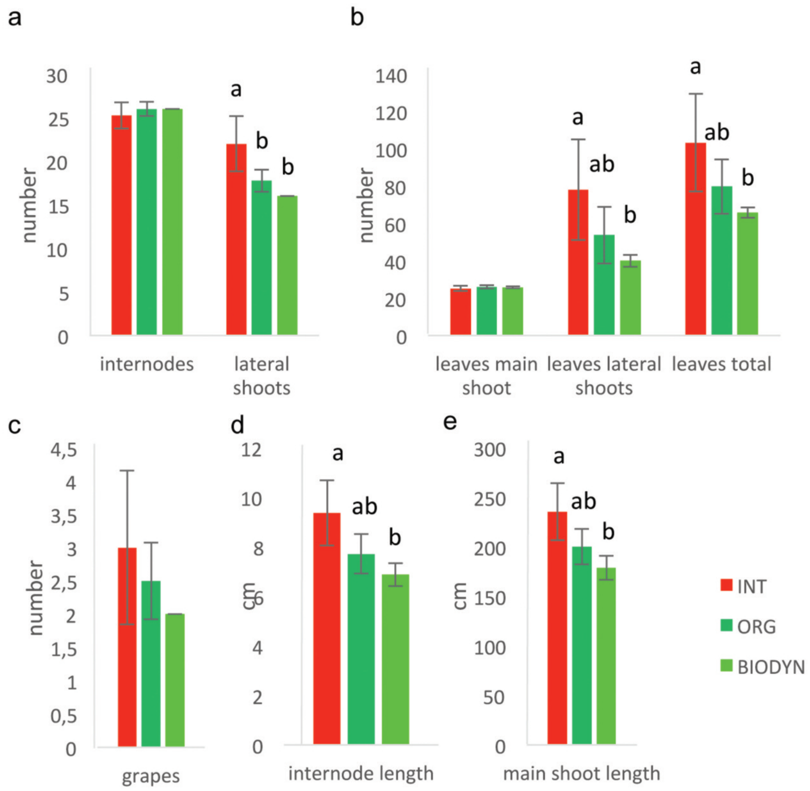

FIGURE 3. Variables for which measurements were taken in 2008 from exemplary shoots of vines under integrated (INT), organic (ORG) and biodynamic (BIODYN) management: number of internodes and number of lateral shoots (a), number of leaves on the main shoot and lateral shoots, and total number of leaves (b), number of grapes (c), internode length (d) and main shoot length (e) (means ± standard deviation, $n = 4$).
Different letters indicate significant differences between treatments on the given date (Tukey's test, $\alpha = 0.05$).

vegetative growth of the vines under integrated management.

Values for the nutrient content of leaves and wood were generally in the normal range for each nutrient, with only very few significant differences between treatments (see Tables S2 and S3). The only clear treatment effect was a significantly higher copper content in both leaves and wood in vines under the two biological treatments; in the leaves, this was accompanied by a significantly lower zinc content. In 2006, the nitrogen content of wood was higher in vines under integrated management.

Grape yield was consistently higher in vines under integrated management. These differences were significant in all four experimental years except 2008 (Figure 4).

Must composition was minimally affected by the different treatments (Table 8). Other than higher must density in 2006 and higher total acidity in 2007 and 2009, the results for integrated management did not differ significantly from those for organic and biodynamic management.

In vines under integrated management, the degree and frequency of *Botrytis cinerea* infection was significantly lower in 2007 and significantly higher in 2009 (Table 9).

The degree and frequency of acetic acid rot were significantly higher in vines under integrated management in both 2008 and 2009 (Figure 5).

As shown in the graphical evaluation of the principal components (Figure 6), the three treatments were clearly differentiated by the first component, which explained 40.6% of total variance. Samples from vines under integrated management featured strong shoot and leaf growth as well as high grape yield, but also compact grape clusters and high susceptibility to acetic acid rot. Organic management and especially biodynamic management were characterized by lower vigour, higher earthworm abundance and higher amounts of carbon-based wood components. The degree and frequency of *Botrytis cinerea* infection had both high positive and high negative factor loads in different years. The full list of variables submitted to principal component analysis, and the factor loads, is given in Table S9.

DISCUSSION

1. Soil fertility: earthworm abundance and enzyme activity

The establishment and maintenance of soil fertility are important objectives for the sustainable use of vineyards. Soil biology is more sensitive to changes in soil quality than to changes in physical or chemical soil variables (Crecchio *et al.,* 2001). Therefore, as part of the present study (the Geisenheim field trial), earthworm abundance and biomass and the activity of key soil enzymes were measured as indicators of soil fertility.

Earthworm abundance and the activity of enzymes of the P-cycle (e.g. neutral phosphatase) and dehydrogenase as an oxireductase enzyme participating in all biogeochemical cycles were promoted in vines under organic and biodynamic management compared with those under integrated management. These results indicate the improvement of soil fertility and thus support our first hypothesis. Compared with conventional management, organic management has been shown to increase earthworm abundance (Mäder *et al.,* 2002) and overall enzyme activity (several studies, e.g. Mäder *et al.,* 2002, García-Ruiz *et al.*, 2008).

It is well established that organic amendments can positively affect soil faunal and microbial biomass

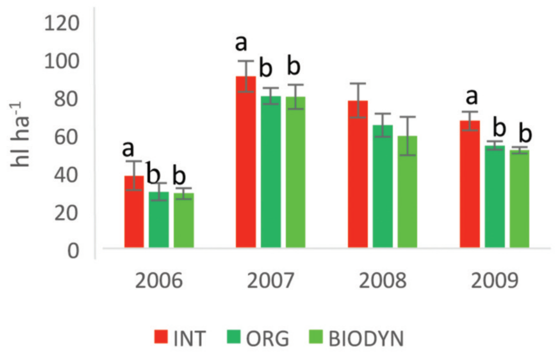

FIGURE 4. Grape yield in vines under integrated (INT), organic (ORG) and biodynamic (BIODYN) management (means ± standard deviation, $n = 4$). Different letters indicate significant differences between treatments for the given year (Tukey's test, α = 0.05).The data for 2006 have been reported previously (Fritz *et al*., 2017).

TABLE 8. Mean values for must composition of grapes after harvest from vines under integrated, organic and biodynamic management ($n = 4$).

Variable	Year	Management		
		integrated	organic	biodynamic
Must density (°Oe)	2006	97.13 b	100.38 a	100.38 a
	2007	89.25	89.06	90.00
	2008	82.31	85.00	85.00
	2009	99.63	98.31	98.81
pH	2006	3.05	3.02	3.06
	2007	2.98	2.97	2.96
	2008	3.16	3.16	3.14
	2009	3.19	3.21	3.21
Total acidity (g/L)	2006	8.46	8.07	7.94
	2007	11.17 a	11.01 ab	10.68 b
	2008	13.95	13.55	13.50
	2009	6.72 a	6.57 ab	6.45 b
NOPA (mg/L)	2006	101.00	94.50	94.25
	2007	150.75	128.50	123.00
	2008	161.00	155.75	165.50
	2009	111.75	115.50	124.75

NOPA, nitrogen by the orthophthaldialdehyde method.
Different letters indicate significant differences between treatments for the given year (Tukey's test, α = 0.05). The data for 2006 have been reported previously (Fritz *et al.*, 2017).

TABLE 9. Mean degree and frequency of *Botrytis cinerea* infection in vines under integrated, organic and biodynamic management ($n = 4$).

Variable	Date	Management		
		integrated	organic	biodynamic
	2006			
	9 September	1.53	2.98	3.38
	26 September	31.10	33.14	36.53
	5 October	59.43	56.45	57.91
	2007			
	23 August	1.20	1.08	0.54
	13 September	9.91 b	11.51 ab	14.84 a
Degree of infection (%)	24 September	14.89 b	16.35 ab	18.54 a
	2008			
	10 September	3.83	2.79	2.49
	24 September	24.36 a	20.43 ab	16.33 b
	13 October	50.83	52.24	44.01
	2009			
	9 September	1.40 a	0.34 b	0.54 b
	6 October	18.48 a	10.75 b	9.28 b
	2006			
	9 September	20.00	36.75	41.75
	26 September	86.50	86.75	86.75
	5 October	99.75	99.75	100.00
	2007			
	23 August	8.75	7.50	5.50
	13 September	46.00 b	52.25 ab	56.50 a
Frequency of infection (%)	24 September	60.75 b	70.00 ab	72.00 a
	2008			
	10 September	21.00	22.00	20.50
	24 September	79.50	81.75	77.25
	13 October	99.50	99.75	100.00
	2009			
	9 September	13.50 a	5.25 b	6.50 b
	6 October	71.50 a	48.25 b	50.50 b

Different letters indicate significant differences between treatments for the given date (Tukey's test, $\alpha = 0.05$). The data for 5 October 2006 have been reported previously (Fritz *et al*., 2017).

and activity. Because the total amount of compost used was about equal under the different treatments (the total for both years being 12.3 t/ha under integrated management and 13.1 t/ha under organic and biodynamic management), and compost was applied in 2006 and 2007 only, the main difference related to input of organic carbon was presumably in the use of cover crops. Further research is needed to determine if the use of more diverse cover crop mixtures, including deep-rooting species, provides, via rhizodeposition, more below-ground organic carbon in the form of both root exudates and lysates. This organic carbon could provide both a food source for earthworms and a substrate for enzymes, as possibly indicated by the correlation of dehydrogenase and β-glucosidase activity with soil organic carbon (Crecchio *et al.*, 2001) and soil microbial biomass carbon (García-Gil *et al.*, 2000).

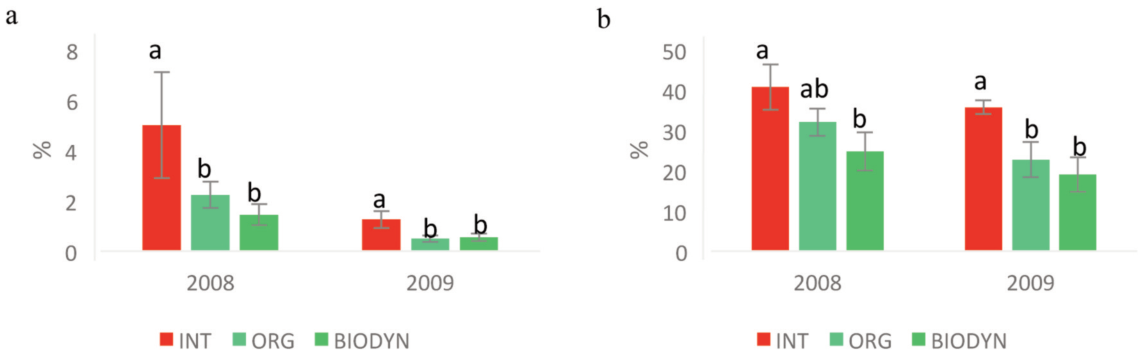

FIGURE 5. Degree (a) and frequency (b) of acetic acid rot in vines under integrated (INT), organic (ORG) and biodynamic (BIODYN) treatment (means ± standard deviation, n = 4).
Different letters indicate significant differences between treatments in the given year (Tukey's test, α = 0.05).

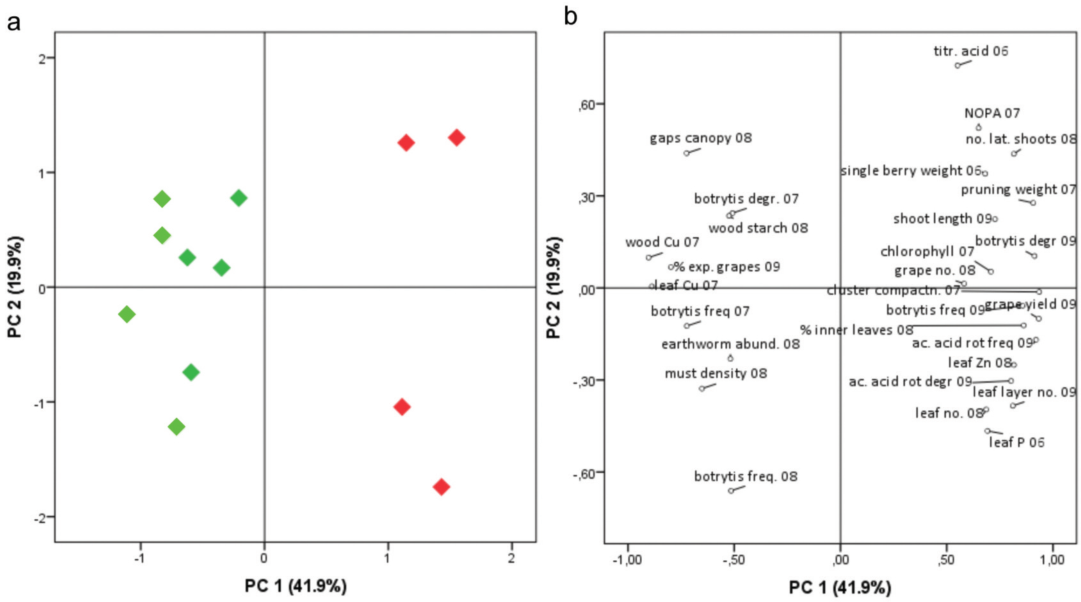

FIGURE 6. Biplots showing the location of the single-treatment objects (a) for integrated management (¨), organic management (¨) and biodynamic management (¨) and trait vectors with factor loads for the first principal component (PC 1) > ± 0.8 (b) based on principal component analysis of variables from field

An increase in β-glucosidase activity after the introduction of cover crops has been reported by Dick (1994). This may have been due to roots from grapevines as well as cover crops stimulating enzyme activity by either releasing enzymes or sustaining higher microbial activity in the rhizosphere, as has been shown for trees (Kotroczó *et al.*, 2014). Furthermore, earthworms may have profited from improved habitats as a consequence of increased soil porosity resulting from the use of diverse cover crop mixtures, as suggested by Riley *et al.* (2008). The same authors also reported a positive influence on earthworm abundance of the use of leys in crop rotation.

Enzyme activity is suspected to be impaired by high heavy metal content in soil (García-Gil *et al.,* 2000). However, although dehydrogenase activity correlated inversely with copper content in soil at our experimental site (Schmieg, 2008), the higher soil copper content resulting from plant protection under organic and biodynamic management apparently had only a minor impact on enzyme activity in the present study. The heavy metal content of the municipal waste compost used in integrated management and the cow manure compost used in organic and biodynamic management was not analysed. It is possible that the cumulative amount of heavy metal in plots under integrated management was higher than in those under the biological treatments. Also, it is likely that application of herbicide to the plots under integrated management decreased earthworm abundance and enzyme activity. Negative effects of pesticides on earthworms are well documented (e.g. Lee, 1985; Edwards and Bohlen, 1996; Zang *et al.*, 2000; Pelosi *et al.,* 2013). However, in terms of enzyme activity, negative effects have been found for only some pesticides and some enzymes (i.e. phosphatase), and other enzymes (i.e. urease) even show higher activity after pesticide application (Sannino and Gianfreda, 2001). In the present study, the activity of urease (an enzyme of the N-cycle) was not promoted by organic and biodynamic management. Previously, urease activity has been shown to decrease with increasing application of ammonia-based nitrogen fertilizer, presumably because the addition of the end product of the enzymatic reaction suppresses enzyme synthesis (Dick *et al.,* 1988; Bandick and Dick, 1999).

According to Olander and Vitousek (2000), phosphatase activity is strongly regulated by the supply of phosphorus. Because most organic phosphorus is bound in phosphate esters, it is mineralized independently of carbon via phosphatase, and biotic demand for phosphorus drives phosphatase production and phosphorus mineralization. Bowles *et al.* (2014) found a strong relation between microbial biomass and phosphodiesterase activity and pointed out the importance of soil microbial biomass for regulating investments in phosphatases. The dynamics of phosphorus availability and microbial biomass were not measured in the present study. Therefore, it can only be hypothesized that besides reduced exposure to pesticides, lower phosphorus availability and higher microbial biomass in the soil of plots under the biological treatments may also have contributed to higher phosphatase activity.

2. Vegetative growth, canopy structure and susceptibility to fungal diseases

Supporting our second hypothesis, vegetative growth was reduced in vines under the two biological treatments compared with those under integrated management, with shorter shoots with shorter internodes, fewer lateral shoots, and lower pruning weight, number of leaf layers, proportion of inner leaves, leaf chlorophyll content and grape yield. Grape yield from vines under organic and biodynamic management was 10–25% lower than from those under integrated management. In 2007, the only year without losses due to *Botrytis cinerea* infection, yield was generally higher for all treatments: 90 hL/ha and about 80 hL/ha from vines under integrated management and the biological treatments, respectively.

The fact that wood and leaf nutrient content and must composition were minimally affected indicated that none of the treatments were prone to result in nutrient deficiency. The higher leaf and wood copper content in vines under organic and biodynamic management are due to the application of copper-containing agents to protect the plants against *Plasmopara viticola*. Application of these agents probably also explains the lower zinc content of the leaves of vines under the two biological treatments, because copper can inhibit zinc uptake (Scheffer and Schachtschabel, 2001).

A notable result is that the lower number of leaves in vines under organic and biodynamic management was exclusively due to the lower number of lateral shoots. This resulted in a morphology that improved the grapes' exposure to light, through more gaps in the canopy, a higher proportion of exposed grapes and lower grape cluster compactness. Consequently, the microclimate for vines under the two biological treatments was less favorable towards infection with fungal diseases and their spread from one berry to another. This probably explains the lower incidence of acetic acid rot in 2008 and 2009 and, in the year in which canopies were denser (i.e. 2009), the lower incidence of *Botrytis cinerea* infection despite the application of fungicide to vines under integrated management.

In a study of vine responses to pathogens at the level of gene expression and at the biochemical level, Soustre-Gacougnolle *et al.* (2018) found higher levels of anti-oxidative and antifungal secondary metabolites and higher expression of silencing and immunity genes in vines under biodynamic management than with conventional viticulture. Similarly, Schneider and Ullrich (1994) showed that, among other inducers of systemic resistance, BD 501 effectively inhibited disease development in tobacco and cucumber plants, presumably by increasing the activity of different enzymes. Further research is needed to determine whether, besides differences in plant morphology, such mechanisms also reduce susceptibility to pathogens in plants under organic and biodynamic management.

The plant-available phosphorus and potassium content of soil was high, regardless of treatment. However, soil mineral nitrogen content was higher in plots under integrated management on several dates in 2006, 2007 and 2008: in 2006 and 2008, mainly in September and October (i.e. the weeks before harvest), and in 2007, in May and June. Because equal amounts of nitrogen were applied in the form of compost to all plots, regardless of treatment, and mineral fertilizer was applied to those under integrated management only in 2006, these differences are probably not a consequence of the application of nitrogen fertilizer. Rather, the following processes could have resulted in the higher mineral nitrogen content in the soil of plots under integrated management.

- Slower mineralization of nitrogen from the compost used in organic and biodynamic management, due to a larger carbon-to-nitrogen ratio. However, the carbon-to-nitrogen ratio was similar for all treatments in 2007, and although data are missing for 2006, it can be assumed that compost from green waste has a carbon-to-nitrogen ratio that is equal to or larger than that of compost from manure.

- Higher nitrogen and water uptake, especially from the subsoil, by cover crops in plots under organic and biodynamic management. In a study at our experimental site, Döring *et al.* (2015) measured lower transpiration rates and stomatal conductance as well as higher water stress in the vines under the two biological treatments from 2010 to 2012, and attributed this finding to differences in soil management and fertilization. In the first 4 years after conversion discussed here, fertilization differed minimally between the treatments, therefore cover crops and soil management are probably the factors with the most influence.

Neither cover crop biomass nor nitrogen uptake by cover crops was measured. However, the cover-cropping strategies followed under integrated versus organic and biodynamic management differed. For integrated management, grassland cover crops with low nutrient demand and shallow root systems were chosen. Permanent cover crops were frequently mulched, enabling rapid mineralization of the nutrients stored in the cover crop biomass. In contrast, for organic and biodynamic management, permanent cover crops were rolled, allowing them to flower. Thus, they provided habitats for above-ground biodiversity and presumably resulted in slower nutrient mineralization, because a smaller amount of organic matter was affiliated.

The choice of cover crop mixture used for organic and biodynamic management followed a strategy of optimum resource utilization in the top- and subsoil, with a combination of shallow- and deep-rooting species; the latter extended deeper than the space with the maximum root-length density of the vines, well below a soil depth of 40 cm (Lehnart *et al.,* 2008, Linsenmeier *et al.,* 2011). There is evidence that plants with different root systems affect physical, chemical and biological soil characteristics. For example, taprooted alfalfa (*Medicaga sativa* L.) has been shown to increase nitrogen and carbon input (Fischer *et al.,* 2013, Hafner and Kuzyakov, 2016) and soil aeration, especially in deeper soil layers (Uteau *et al.,* 2013), compared with taprooted chicory, thus creating favorable conditions for microbial growth (Hafner and Kuzyakov, 2016). Root channels are hotspots for the acquisition of nutrients from subsoil, with high levels of oxygen, microbial activity, and plant-available nutrients, especially if colonized by anecic earthworms (Athmann *et al.,* 2017).

In the present study, the diverse crops with different root systems in the Wolff mixture may have improved the structure of the soil and consequently its physical, chemical and biological characteristics. Simultaneously, the root growth of the vines may have been directed to deeper soil layers, enabling nutrient uptake from these areas. This was a finding of Morlat and Jacquet (2003) and suggests competition for water and nutrients in the upper soil layers and the reduced penetration resistance of the deeper soil layers. Through these mechanisms, the cover crops would have enabled more complete exploration of nutrient and water reserves in the subsoil by the vines. At the same time, they would have removed water and nutrients present in excess from a quality perspective – an effect that is also attempted by deficit irrigation (e.g. Acevedo-Opazo *et al.*, 2010, Santesteban *et al.*, 2011). Monteiro and Lopes (2007), Tesic *et al.* (2007), Giese *et al.* (2014) and Muscas *et al.* (2017) have shown that cover crops can be equally successful in reducing excessive grapevine vigour. Also, the effectiveness of cover crops in reducing pests and diseases by modifying nutrient status and grapevine vigour is receiving increasing attention (Muscas *et al.*, 2017). It has to be pointed out, however, that the effects of cover crops depend on the interaction of climate aridity, edaphic conditions and agronomic management (see the results of the field study by Uliarte *et al.*, 2013, on the performance of different cover crop species under temperate and arid conditions, and the review by Pardini *et al.,* 2002). Moreover, cover crops need skillful management. This includes precise timing of sowing, rolling and mulching events to prevent them from being overly competitive and to synchronize nitrogen supply from leguminous crops with periods of high nitrogen demand by the vines.

More research is necessary, including studies to determine the effects of different management operations and their timing, patterns of root growth, and nutrient and water uptake by cover crops, as well as consequences of cover crop use for soil structure and, successively, for grapevine root-length density and the distribution of grapevine roots in the heterogenized subsoil. The implications of the use of diverse cover crop mixtures for above- and below-ground biodiversity, and the ecological services provided by this diversity (e.g. pollination and pest control), also require future research. It can be hypothesized that the more diverse cover crop mixtures used in organic and biodynamic vineyards will have an impact not only on above-ground biodiversity (via providing habitats for insects; Hartwig and Ammon, 2002) but also on below-ground biodiversity (via rhizodeposition and associated microbes, as found by Burns *et al.,* 2016) and consequently on soil functionality.

3. Biodynamic versus organic management

Most of the variables assessed in terms of the vegetative and reproductive development of the vines showed large differences between the results for those under integrated management, which had the most vigorous growth, and those under the two biological treatments. In many cases, the differences were significant only between vines under integrated management and vines under biodynamic management. Furthermore, in the comprehensive evaluation of mean values by principal component analysis, compared with the four organic plots, all four biodynamic plots had more negative values for the first principal component. This indicates more pronounced effects in terms of enhanced soil fertility and reduction of vegetative growth in vines under biodynamic management, thus supporting our third hypothesis.

Notably, the increase in earthworm abundance, as compared with the soil of plots under integrated management, was considerably higher in the soil of plots under biodynamic management than in that of plots under organic management in 2007–2009. This is consistent with the findings of Mäder *et al.* (2002) and Reeve *et al.* (2005). However, in all three studies, the differences were not significant.

In all 4 years for which results are available, vines under biodynamic management had the lowest pruning weight, and in 2 of the 3 years for which results are available, the shortest shoot length. On most dates of measurement, berry weight and consequently grape cluster compactness and the degree and frequency of infection with *Botrytis cinerea* were lower than in vines under organic management. The only difference between organic and biodynamic management was the application of the biodynamic preparations in the latter.

A plausible hypothesis, potentially complementing the theory that bacterial regulation effects underlie the mode of action of the biodynamic preparations (Spaccini *et al.*, 2012; Giannatasio *et al.*, 2013) by stimulating natural defence compounds (Botelho *et al.*, 2016), is that the biodynamic preparations act via hormonal effects. Radha and Rao (2014) analysed the composition of the microbial community in biodynamic preparations, including the horn manure preparation BD 500, and found that all bacterial strains analysed produced indole acetic acid. This finding is supported by the results of studies by Giannatasio *et al.* (2013), who found that BD 500 shows strong auxin-like effects, and Spaccini *et al.* (2012), who detected large amounts of undegraded lignin residues, which are known to exhibit indole acetic acid–like activity and may account for biostimulation of microbes and plants. In a study by Fritz (2000), 44 of 47 variables that showed significant reactions in response to application of horn silica to beans and lettuce were variables known to be influenced by gibberellic acid (see also Fritz and Köpke, 2005). Gibberellic acid is used in integrated grape production to reduce grape cluster compactness and thereby the risk of infection with *Botrytis cinerea*. Further research is necessary to investigate whether application of biodynamic preparations stimulates production of gibberellic acid.

CONCLUSIONS

In the first 4 years after conversion to organic and biodynamic management as part of the Geisenheim system comparison trial INBIODYN, soil mineral nitrogen content and vegetative growth of grapevines were reduced significantly by the two biological treatments, resulting in a morphology that increased the grapes' exposure to light and consequently reduced the susceptibility of the vines to acetic acid rot and also, in a year in which the plants had very dense canopies overall, to *Botrytis cinerea* infection. In many cases, the only significant differences were between integrated and biodynamic management.

Differences in nitrogen supply between plots under integrated management and those under the two biological treatments in these first 4 years of the trial were possibly not primarily due to fertilizer application but to cover crop management. Further research is needed to determine how choice and management of cover crop species affect soil structure, root growth, and consequently the uptake of water and nutrients by the vines.

Principal component analysis clearly differentiated integrated, organic and biodynamic management. Overall differences between plots under integrated and biodynamic management were larger than differences between plots under integrated and organic management, emphasizing the need to elucidate the exact mode of action of the biodynamic preparations.

Acknowledgements: The authors gratefully acknowledge financial support from Software AG Foundation, Darmstadt, Germany, and Damus Foundation, Mannheim, Germany. Helpful comments on the manuscript from Prof. Dr Timo Kautz, Humboldt University Berlin, Germany, are greatly appreciated.

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