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ORIGINAL ARTICLE

Evaluation of the oenological suitability of grapes grown using biodynamic agriculture: the case of a bad vintage

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Abstract

Aims: We compare the evolution of the microbiota of grapes grown following conventional or biodynamic protocols during the final stage of ripening and wine fermentation in a year characterized by adverse climatic conditions.
Methods and Results: The observations were made in a vineyard subdivided into two parts, cultivated using a biodynamic and traditional approach in a year which saw a combination of adverse events in terms of weather, creating the conditions for extensive proliferation of vine pests. The biodynamic approach was severely tested, as agrochemicals were not used and vine pests were counteracted with moderate use of copper, sulphur and plant extracts and with intensive use of agronomical practices aimed at improving the health of the vines. Agronomic, microbiological and chemical testing showed that the response of the vineyard cultivated using a biodynamic approach was comparable or better to that of vines cultivated using the conventional method.
Conclusions: The work suggests that biodynamic cultivation of the grapevine may be sustainable even in difficult conditions, representing an interesting alternative to traditional vine-growing approaches.
Significance and Impact of the Study: This theme is topical and of interest to winemakers and consumers today, but is not easy to study due to the difficulties in finding vineyards with homogeneous characteristics, cultivated using different agronomical protocols. The particular climatic conditions observed in 2014 made this year a rare model, making it possible to verify the applicability of biodynamics to vine growing. The strict experimental plan gave results particularly useful for understanding the features of grape microbiota in a biodynamic context.

Introduction

Consumers today pay considerable attention to sustainability in the food industry and this can orientate the strategies of farmers and food producers. This trend stimulates the approaches designed to reduce the use of pesticides in agriculture and to improve the agricultural ecosystem. Of the different strategies, one of the oldest is biodynamics. Biodynamics is a spiritual-ethical-ecological approach to agriculture and food production developed in the early 1920s, on the basis of the teachings of Rudolf Steiner (1993), an Austrian educator and founder of the philosophy called 'Anthroposophy'. The biodynamic approach to agriculture has the primary objective of restoring the equilibrium of the farm ecosystem, by improving the health of plants and animals and thereby preventing the occurrence of pests and diseases (Demeter, 2012).

As regards viticulture and oenology, biodynamics has received increasing attention over the last few years. However, many doubts remain among winemakers in relation to its applicability on a large scale for the pro-

duction of wines. The main question regards the control of diseases of the grapevine such as *Plasmopara viticola, Uncinula necator* and *Botryotinia fuckeliana* (Fregoni 2006; Braccini 2010; Guerra and Steenwerth 2012) because in biodynamic agriculture this is based on the moderate use of copper and sulphur, assisted by plant extracts, and on intensive manual management of vineyards. The use of agrochemicals is strictly avoided (Demeter International 2012). While in years with favourable climatic conditions these methods are sufficient for dealing with diseases, there are doubts about the effectiveness of this strategy when adverse climatic conditions stimulate an increase in grapevine pests (Barata *et al.* 2008a, 2012b).

The microbiota of grapes plays a fundamental role in the development of fermentation and in the quality of the wines (Loureiro and Malfeito-Ferreira, 2003; Callejón *et al.* 2010; Verginer *et al.,* 2010; Fleet 2003). In biodynamic winemaking the use of selected starter cultures of yeast or lactic bacteria is avoided (Demeter International 2012) and therefore the fermentative microbiota is a consequence of microbial evolution on bunches during the last stages of the vegetative cycle of the grapevine (Renouf *et al.* 2005; Martins *et al.* 2012, 2014). The occurrence of vine diseases, and more generally crop protection strategies, could alter the composition of bunches and the availability of substrates, causing changes to the microbiota present within them (Lima *et al.* 1999; Barata *et al.* 2008b; Comitini and Ciani 2008; Cordero-Bueso *et al.* 2011). It has been shown that compromised integrity of the grapes favours the proliferation of yeasts, causing local alcoholic fermentation with the subsequent growth of acetic bacteria, stimulated by the presence of a small amount of ethanol (Nisiotou *et al.* 2011; Barata *et al.* 2012b). The main consequences are the presence of acetic acid in the grape must, even before alcoholic fermentation, the reduction of nutrients available to carry out regular fermentations and a generalized loss of the typical grapevine aromas, with the occurrence of some unpleasant smells such as ethyl acetate, diacetyl and volatile phenols (Loureiro and Malfeito-Ferreira 2003). Careful characterisation of the microbiota of grapes is therefore an effective way of evaluating the effectiveness of the biodynamic approach to vine-growing and wine production.

This work discusses the results of microbiological monitoring in an experimental vineyard in the northern Italy, which was divided into two homogeneous parts, to compare a conventional vine-growing protocol with biodynamic cultivation starting from 2011. We focused our attention particularly on the last stage of grape ripening during 2014, which was characterized by unusually adverse weather conditions in Italy, favouring the massive proliferation of vine pests. These are the most difficult conditions for the biodynamic approach to viticulture but, at the same time, it represents a good model for studying the effective applicability of biodynamics to large-scale vine growing. In this context the evolution of grape microbiota was taken into account as a marker of the suitability of the grapes for obtaining high-quality wine. Experimental winemaking provided additional information about oenological suitability of grapes produced using different agronomical approaches, contributing to furthering knowledge about biodynamics in oenology.

Material and methods

Vineyard and agricultural protocols, description and monitoring procedure

The 'Pozza' vineyard is located in San Michele all'Adige (46°11′ 45·852″ N 11°8′ 12·070″ E). The main characteristics of the vineyard are: Mean altitude: 260 m asl; area: 20 000 m^2; orientation: South–West; mean slope: 8·5%; year of vineyard planting: 2009; *Vitis vinifera* cultivars: Riesling (R) and Pinot Blanc (Pb); Riesling clones: 198–10 GM and 239–25 GM, Pinot Blanc clones: LB 16, LB 18; rootstock: SO4; vineyard training system: pergola trentina; planting system 2·80 × 0·5 m. Starting from 2011, the vineyard was subdivided into two homogeneous parts, the first cultivated using a biodynamic approach and the second by a traditional approach. We provided for a transition area between the two parts, consisting of at least 10 rows of vines. The main agricultural practices involved in both protocols are listed in Table 1. The dosage of agrochemicals used in the conventional protocol was adjusted according to the manufacturer's instructions. The sampling of grapes in the vineyard for agricultural and microbiological observation was performed on 72 vines for each cultivation method. The intensity and frequency of the attack of the main grapevine diseases was determined by observing 300 leaves (*Uncinula necator* and *Plasmopora viticola)* or 600 grapes (*Botryotinia fuckeliana*), according to OEPP/EPPO standards (2002). The frequency value was obtained using the ratio between positive samples (presence of the pest) and the total number of samples tested. The climate data were retrieved from the meteorological station of San Michele all'Adige (Italy), taking into account data for average temperature (°C) and total rainfall (mm) in the period between April and September.

Microbiological and chemical analysis of grapes and wine

Grapes (300 berries for each sample) were collected in the vineyard in sterile plastic bags, then analysed within 2 h. Bunches were homogenized using a stomacher blender (Seward, Worthing, West Sussex, UK) and diluted

Table 1 Main agricultural intervention and agrochemicals or biodynamic preparations involved in vine cultivation during 2014 in the 'Pozza' vineyard in San Michele All'Adige, Trento, Italy

Agricultural intervention	Traditional vine growing	Compounds adopted	Biodynamic vine growing
Fertilization	Chemical and organic fertilizers in pellet form		Green manure
Weeding	Chemical	Glyphosate	None
Soil management	None		Machining soil
Crop protection	Protection against *Uncinula necator*	Metraphenon, Penconazol, Quinoxifen, Spiroxamin, micronized sulphur	Sulphur powder
	Protection against *Plasmopara viticola*	Cyazofamid, Dimethomorph, Fluopicolid, Fosetyl aluminium, Copper sulphate, Trifloxystrobin	Copper sulphate
	Protection against other pests	Tiametoxan	None
Other agricultural practices	None		Distribution of biodynamic preparations 500 and 501 (Steiner 1993; Demeter International 2012)
Thinning and selection of grapes	Chemical and mechanical	Gibberellic acid	Manual
Lopping of branches	Mechanical		No looping, bending and tying
Harvest	Manual		Manual

with peptone water (1 g l^{-1} of Mycological Peptone; Oxoid, UK). Samples were analysed through a plate count (OIV, 2015) using three synthetic media: WL Agar (Oxoid) for yeast and acetic bacteria counts, Lysine Agar (Oxoid) to determine non-*Saccharomyces* yeast, MRS Agar (Oxoid) for lactic acid bacteria counts. All the plates were incubated at 25°C, in anaerobic conditions (Anaerogen; Oxoid) in the case of MRS, for a period of between 4 and 10 days, according to the characteristics of each microbial group. In the case of wines, samples were collected in a sterile glass bottle and immediately analysed, following the same method used for grapes. Chemical determination was performed using FT-IR (Winescan; Foss, Hillerød, Denmark).

Identification of microbial isolates from grapes or wine

Identification of microbial isolates at species level was performed starting from pure cultures obtained by successive subculture of isolates, collected by plate spreading of grapes or wine samples. In the case of yeasts, identification was performed using Fourier Transform Infrared spectroscopy (FT-IR) (Naumann *et al.* 1991; Kümmerle *et al.*, 1998; Ngo-Thi *et al.* 2003; Erukhimovitch *et al.* 2005; Wenning and Scherer 2013; Grangeteau *et al.* 2015) using a Tensor™ 27 spectrometer in combination with an HTS-XT Unit (Bruker, Billerica, MA). Yeast cells were dried to a transparent thin film on a 96-well zinc selenide (ZnSe) optical plate to perform FT-IR measurements using the middle infrared range between 4000 and 500 wave length cm^{-2} (25 000–2500 nm). The specific absorption patterns of the unknown yeast strains were compared to a reference database consisting of about 3000 strains representing 215 yeast species. As regards bacteria, extraction of DNA, micobiota was done by treating bacterial cells with lysis-buffer (0·1 mol l^{-1} Tris/HCl, 1% SDS, pH 9) and heating for 2 min at 99°C in a Thermomixer (Eppendorf, Hamburg, Germany), followed by phenol: chloroform: isoamyalcohol (25 : 24 : 1) extraction. DNA was precipitated using isopropanol and washed with 70% ethanol. After air drying, the DNA pellet was dissolved in 50 μl TE-buffer (10 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA, pH 8). A PCR fragment of 440 bp was amplified using Dream Taq Polymerase (Thermo Scientific, D) and the universal primers 91E (5′-GGAATTCAAAKGAATTGACGGGGGC-3′) and 13B (5′-CGGGATCCCAGGCCCGGGAACGTATTCAC-3′) (Mignard and Flandrois 2006). Cycling parameters saw initial denaturation for 4 min at 95°C and 35 cycles of 45 s at 95°C, 1 min at 55°C, 1 min at 72°C, followed by a final elongation step of 5 min at 72°C. Amplified PCR products were sequenced as single reads at SRD – Scientific Research and Development GmbH (Bad Homburg, Germany). Analysis of the 16s rDNA sequences was performed using the BLAST GenBank database of the National Center for Biotechnology Information (Bethesda, MD, http://www.ncbi.nlm.nih.gov).

Experimental winemaking

Grapes were manually harvested at the beginning of September (2 September Pinot Blanc; 10 September Riesling) and crushed using a 40 l Speidel Hydropress (D)

reaching a maximum pressure of 3×10^5 Pa. The grape must was cold clarified (3°C, 24 h) in stainless steel tanks (3 for each trial) with a nominal volume of 25 l, saturated with argon gas. After clarification the temperature of the grape must was restored to 20 ± 2°C to enable the starting of alcoholic fermentation, without the addition of selected yeast. The evolution of alcoholic fermentation was followed by daily measurement of sugar content using a PAL-1 (Atago, Japan) refractometer. At the end of alcoholic fermentation the wines were decanted and maintained at 20°C for 3 weeks to perform malolactic fermentation. The wines were stored at 3°C for 15 days, then stabilized with the addition of 0·1 g l^{-1} of sulphur dioxide (Dal Cin, I) and aged in closed tanks at 15°C for 3 months until analysis.

Statistical analysis

Statistical analysis of the data was carried out using STATISTICA 7.1 software (StatSoft Inc., Tulsa, OK).

Results

Effect of different agronomical protocols on the evolution of the vine cycle and grape maturation

In comparison to the seasonal trend for the last 10 years (Fig. 1), in the province of Trento the 2014 was characterized by climatic conditions very unsuitable for safe vine-growing. Mean temperature was slightly below average, but rainfall was higher and on 24 June, the regular vegetative cycle of the grapevine was altered by a strong hailstorm that affected about 40% of production, in terms of the quantity of grapes. Observations of the degree of infestation of the vineyard by *U. necator* and *P. viticola* were carried out during preblooming of the grapevine. *P. viticola* showed a significant degree of attack without differences between the different approaches considered. The cold, wet summer limited the vegetative cycle of *U. nectar* (Fregoni 2006), so there was no significant infestation in the vineyard. In contrast, the frequency and degree of attack by *B. fuckeliana* was very high after the hailstorm (Fig. 2). Pinot Blanc cultivated using the traditional system showed a level of attack by *B. fuckeliana* of about 76%, while Riesling cultivated in the same manner stood at about 71%. As regards vines cultivated using the biodynamic approach, the mean frequency of attack detected on Pinot Blanc was around 66%, while on Riesling the value remained about 57%. Comparison of the vineyards cultivated using biodynamic and traditional systems did not reveal significant differences in terms of the intensity of attacks, whereas in contrast Riesling samples appeared less sensitive to *B. fuckeliana* as compared to Pinot Blanc grapes. Considering that the agricultural protocol was the same for the two varieties of *V. vinifera*, the differences in the infestation of *B. fuckeliana* were due to intraspecific characteristics, such as the compactness of the bunch, the hardiness of the fruit cuticule or the evolution of maturation (Fregoni 2006; Lara *et al.* 2014).

Evolution of microbiota during the last stage of grape ripening

Microbial observation started in the month of August and took into account the main microbial groups of oenological significance (Loureiro and Malfeito-Ferreira 2003; Zott *et al.* 2008; Barata *et al.* 2012a). Comparing

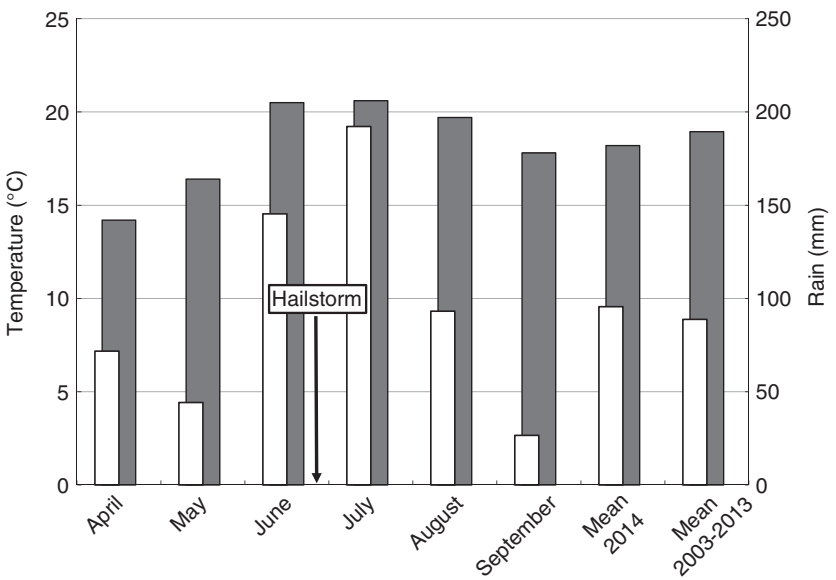

Figure 1 Main weather parameters in 2014 in the 'Pozza' vineyard in San Michele All'Adige (Trento, Italy) compared to those for the previous 10 years. The data refer to the period from April to September. ● Temperature (°C) ○ Rain (mm).

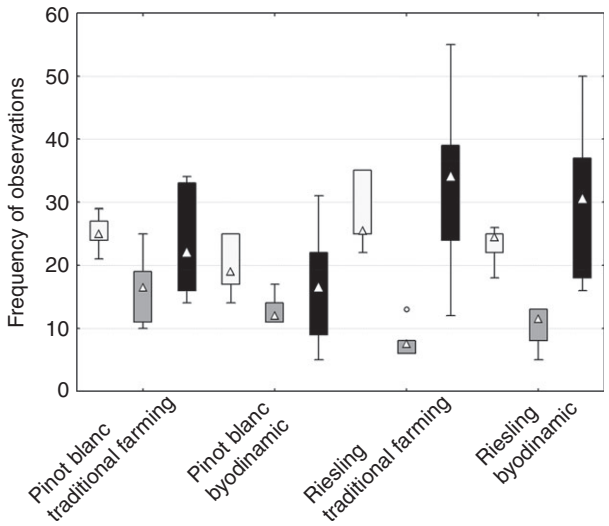

Figure 2 Box plot of the evolution of *Botryotinia fuckeliana* attack in the 'Pozza' vineyard in the last stage of grapevine ripening. White bars: 0–10% of frequency; grey bars: 11–25% of frequency; black bars: >26% of frequency. $n = 300$; Δ Median; [] 25–75%; ○ outlier.

data for the 2014 vintage with data for the two previous years, the different evolution of the microbiota during grape ripening was evident, linked to the different climate (Table 2). The 2012 and 2013 vintages were characterized by standard seasonal evolution and less pressure from vine pests, the microbiota on grapes remained below the three log units, while there was a negligible presence of acetic bacteria, under the plate count detection limits. In 2014 the microbiota profile differed in both quantitative and qualitative terms (Table 3). The evolution of the yeast population reflected the different features of Pinot blanc and Riesling, appearing to be linked to the timing of grape ripening and the sensitivity of the different cultivars to Botrytis attacks. Pinot blanc showed the maximum yeast concentration (over $3{\cdot}0 \times 10^4$ CFU g^{-1}) at the beginning of September, while in the case of Riesling, despite later harvesting, the yeast remained between $1{\cdot}2 \times 10^4$ CFU g^{-1} (biodynamic) and $1{\cdot}7 \times 10^4$ CFU g^{-1} (traditional). The proliferation of sour rot on grapes (Barata *et al.* 2012b) was reflected in the growth of acetic acid bacteria, over four log units. Lactic acid bacteria were not detected by any observations in the vineyard. From the qualitative point of view, comparison of the plate count on WL and Lysine Agar Medium (Table 3) indicates that the yeast population was mainly made up of non-*Saccharomyces* yeasts. FT-IR identification of yeast showed microbiota made up of nine species (Table 4): *Pichia kluyveri, Issatchenkia terricola, Metschnikowia fructicola, Rhodotorula laryngies, Hanseniaspora uvarum, Cryptococcus laurentii, Candida zemplinina.* According to the data furnished by the plate counts, the bacterial population was composed by acetic acid bacteria, especially *Gluconobacter cerinus*. Although other bacteria (*Curtobacterium* spp.), of which is unknown the activity in wine, were found. The complexity of the microbiota increased proportionally with the degree of ripening of the grapes, different profiles being observed in the two different grape varieties. The microbiota features belonging to different samples of grapes were not significantly different and appeared to be linked more to the characteristics of the cultivars of *V. vinifera* than the effect of the different agronomic practices. However, the lower concentration of micro-organisms recorded in 2014 in biodynamic grapes (Table 3) must be underlined and will be discussed in the next section of the paper.

Experimental winemaking

After crushing of the grapes, chemical characterization of both grape musts was performed, as reported in Table 5. We did not find differences between the main parameters of grapes, with the sole but relevant exception of yeast assimilable nitrogen (YAN) which was significantly higher in the case of the biodynamic agriculture as compared to traditionally cultivated grapes. The evolution of alcoholic fermentation took 7 ± 1 days in the case of Pinot Blanc (Fig. 3) and 14 ± 1 days in the case of Riesling (Fig. 3). In both fermentations *Saccharomyces cerevisiae* prevailed starting from the second observation, performed 72 h after crushing (Table 6), with the sole exception of Pinot Blanc Traditional sample where *H. uvarum* remained the main yeast strains for the first 4 days of fermentation. Besides *S. cerevisiae,* we identified *H. uvarum, C. zemplinina, P. kluyveri, I. orientalis* and *S. cariocanus* in the yeast group; of the bacteria, we confirmed the presence of *Gluconobacter* genus. All these micro-organisms tended to disappear after a few days' fermentation. Microflora composition appeared to be more related to the grape variety than to agricultural practices; the prevalence of *S. cerevisiae* ensured regular occurrence of fermentation, while no stuck fermentation or differences between the behaviour of the different approaches were observed, guaranteeing safe wines without anomalous composition (Table 7).

Discussion

The biodynamic approach considers the vineyard as a single organism and suggests some practices designed to improve the equilibrium and interaction between the different components in this ecosystem: soil, microbiota, vines, and other living organisms such as flora and invertebrates (Carpenter-Boggs *et al.* 2000; Reeve *et al.* 2005;

Table 2 Comparison of the incidence of the main vine pests and microbiota concentration in the 'Pozza' vineyard (San Michele all 'Adige, Trento, Italy) in three successive years with different weather conditions

	Physiological stage	*Cv.* of *V. vinifera*	Agricultural management	2012	2013	2014
Frequency of *Uncinula necator*	Harvest	Mean data	Mean data	<10%	<10%	<10%
Frequency of *Plasmopara viticola*		Mean data	Mean data	<10%	<10%	<10%
Frequency of *Botryotinia fuckeliana*		Mean data	Mean data	35%	<10%	70%
Yeast concentration (CFU g^{-1})	Complete veraison	Riesling	Conventional	27	400	910
		Riesling	Biodynamic	15	400	910
		Pinot Blanc	Conventional	60	250	450
		Pinot Blanc	Biodynamic	30	50	140
Acetic acid bacteria concentration (CFU g^{-1})		Riesling	Conventional	5	nd	230
		Riesling	Biodynamic	5	nd	4·50
		Pinot Blanc	Conventional	85	nd	nd
		Pinot Blanc	Biodynamic	50	nd	nd
Yeast concentration (CFU g^{-1})	Harvest	Riesling	Conventional	9100	4000	$1{\cdot}7 \times 10^4$
		Riesling	Biodynamic	7500	1300	$1{\cdot}2 \times 10^4$
		Pinot Blanc	Conventional	3500	2500	$3{\cdot}2 \times 10^4$
		Pinot Blanc	Biodynamic	1300	1800	$3{\cdot}8 \times 10^4$
Acetic acid bacteria concentration (CFU g^{-1})		Riesling	Conventional	3700	nd	$2{\cdot}5 \times 10^4$
		Riesling	Biodynamic	3600	nd	$2{\cdot}3 \times 10^4$
		Pinot Blanc	Conventional	450	nd	$2{\cdot}5 \times 10^4$
		Pinot Blanc	Biodynamic	450	nd	$1{\cdot}8 \times 10^4$

Data refer to samples made up of 300 berries (nd: not detected).

Table 3 Microbiological state of grapes during the last phases of ripening

Grape varieties	Agricultural protocol	Sampling date	Yeast (CFU g^{-1})	Non-Saccharomyces yeast (CFU g^{-1})	Acetic bacteria (CFU g^{-1})
Pinot Blanc	Traditional	11/08	450	450	n.d.
		22/08	3600	3600	450
		25/08	2800	2700	1200
		01/09	$3{\cdot}2 \times 10^4$	3200	$2{\cdot}5 \times 10^4$
Riesling		11/08	nd	nd	nd
		22/08	910	910	2300
		25/08	5500	5200	4100
		01/09	2300	2100	$1{\cdot}4 \times 10^4$
		08/09	$1{\cdot}7 \times 10^4$	$1{\cdot}6 \times 10^4$	$2{\cdot}0 \times 10^4$
Pinot Blanc	Biodynamic	11/08	140	140	nd
		22/08	2300	2300	910
		25/08	$2{\cdot}4 \times 10^4$	$2{\cdot}2 \times 10^4$	590
		01/09	$3{\cdot}8 \times 10^4$	$3{\cdot}5E \times 10^4$	$1{\cdot}8 \times 10^4$
Riesling		11/08	450	450	nd
		22/08	910	900	450
		25/08	3600	3200	1800
		01/09	1100	1000	6400
		08/09	$1{\cdot}2 \times 10^4$	$1{\cdot}1 \times 10^4$	$2{\cdot}3 \times 10^4$

Data refer to samples made up of 300 berries (nd: not detected).

Demeter International 2012). In this context, the use of some 'biodynamic preparations' is aimed at favouring the links between these different players in the vineyard ecosystem (Giannattasio *et al.* 2013) in order to obtain the ultimate scope of an increase in vine health, greater resistance to vine pests and high-quality grapevine production. One of the most interesting situations for testing the effectiveness of the biodynamic approach is when the vineyard ecosystem is perturbed by external and exceptional events. This is the case of a 'bad' vintage, when the sum of adverse climatic conditions leads to the insurgence of grapevine diseases. In the Trentino region 2014 was an exemplary case, as a result of bad weather conditions (Fig. 1) and an exceptional hailstorm causing

Table 4 Profile for microbiota found in grapes, according to maturation and agricultural practices. Data are expressed as the % of microbial isolation on the plate count

	Pinot blanc biodynamic				Pinot blanc traditional			
Sampling date	11/8	22/8	25/8	1/9	11/8	22/8	25/8	1/9
%								
R. laryngis	0·0	37·5	4·8	0·0	22·2	25·0	0·0	3·0
Hanseniaspora uvarum	100·0	12·5	54·7	30·1	22·2	12·5	55·6	24·0
Issatchenkia terricola	0·0	12·5	0·0	0·0	0·0	0·0	0·0	0·0
Pichia kluyveri	0·0	0·0	0·0	0·0	22·2	12·5	0·0	0·0
Candida zemplinina	0·0	0·0	0·0	22·4	0·0	0·0	8·6	4·5
Cryptococcus laurentii	0·0	0·0	9·5	0·0	22·2	12·5	0·0	1·0
Metschnikowia fructicola	0·0	0·0	0·0	0·0	0·0	0·0	3·7	0·0
Gluconobacter cerinus	0·0	25·0	28·6	25·2	11·1	25·0	19·8	25·5
Curtobacterium spp.	0·0	12·5	2·4	32·2	0·0	12·5	12·3	42·0
	Riesling biodynamic				Riesling traditional			
Sampling date	22/8	25/8	1/9	8/9	22/8	25/8	1/9	8/9
%								
R. laryngis	33·3	12·5	2·3	0·0	33·3	15·0	2·2	0·9
Hanseniaspora uvarum	33·3	12·5	20·9	20·5	0·0	0·0	2·2	25·7
Candida zemplinina	0·0	0·0	32·6	13·7	0·0	0·0	2·2	6·2
Cryptococcus laurentii	0·0	0·0	0·0	0·0	0·0	30·0	0·0	0·0
Metschnikowia fructicola	0·0	0·0	0·0	0·0	0·0	10·0	0·0	0·0
Gluconobacter cerinus	33·3	50·0	30·2	58·9	50·0	30·0	55·6	39·8
Curtobacterium spp.	0·0	25·0	13·9	6·8	16·7	15·0	35·5	27·4

Table 5 Mean chemical features of grape must obtained from grapes grown using different agricultural protocols (Mean data ± SD $n = 5$)

Grape varieties	Agricultural protocol	Sugar content (g l^{-1})	Tot. acidity (g l^{-1})	Tartaric acid (g l^{-1})	Malic acid (g l^{-1})	pH	Yeast assimilable nitrogen (mg l^{-1})
Pinot Blanc	Traditional	195 ± 5^a	7·5 ± 0·6^a	5·6 ± 0·3^a	4·2 ± 0·5^a	3·2 ± 0·1^a	57·7 ± 8·5^a
Pinot Blanc	Biodynamic	190 ± 1^a	7·6 ± 0·5^a	5·6 ± 0·2^a	4·4 ± 0·3^a	3·2 ± 0·1^a	101·0 ± 1·7^b
Riesling	Traditional	188 ± 3^a	8·9 ± 0·2^b	6·7 ± 0·3^b	4·3 ± 0·2^a	3·0 ± 0·1^b	37·7 ± 1·6^c
Riesling	Biodynamic	193 ± 6^a	9·5 ± 0·4^b	7·1 ± 0·1^b	4·6 ± 0·4^a	3·0 ± 0·1^b	57·7 ± 5^d

Statistical analysis: one-factor ANOVA and Tukey-B test. Different letters indicate statistically significant differences at $P < 0·05$.

damage in the vineyard, favouring the insurgence of grapevine pests (Fregoni 2006; Burruano and Granata 2008). This experiment was particularly remarkable, and not easily replicable, considering that the vineyard had already been cultivated using this kind of system for 3 years and it was therefore reasonably sure that the ecosystem had adapted well to the input provided by the different agricultural practices.

Of the different grapevine diseases, attention was focused on *B. fuckeliana* because the hailstorm in June favoured its proliferation by altering the integrity of bunches, and because the occurrence of this pest has dramatic effects in terms of the oenological suitability of grapes (Barbe *et al.* 2001; Nisiotou *et al.* 2011). As regards the effective damage due to this exceptional meteorological event, field observations showed a nonhomogeneous situation, with damage distributed in the vineyard on the basis of the different features of grapes. The production of less vigorous vines was almost completely impaired, while the more compact bunches recorded a lower level of damage. This may be due both to the proportionality of the damage in relation to the number of berries per bunch, and to the shielding capability of the more external berries, in the case of more compact bunches. We observed a generally high level of *B. fuckeliana* (Fig. 2), and we did not note statistically significant differences between traditional and biodynamic agricultural approaches. Despite these general results, some trends should be highlighted. In the Pinot Blanc trials the distribution of grape samples among the different classes

of intensity (Low, Medium, High, Fig. 2) was homogeneous, without significant differences between the three groups. The comparison between biodynamic and conventional management revealed a tendency to less sensitivity to Botrytis for biodynamic samples in all classes of intensity. In the case of Riesling the situation was more complex. In samples with a low level of attack (classes <25% of intensity) the occurrence of *B. fuckeliana* was higher in the part of the vineyard cultivated using the conventional protocol, probably because the intense manual activity carried out in the biodynamic vineyard countered minor outbreaks of mould in a more targeted and effective manner. In the samples with the highest frequency of attack (>25%, Fig. 2) the differences between the two agricultural management systems were minimal, but the use of agrochemicals was not decisive in the case of high pressure from vine pests.

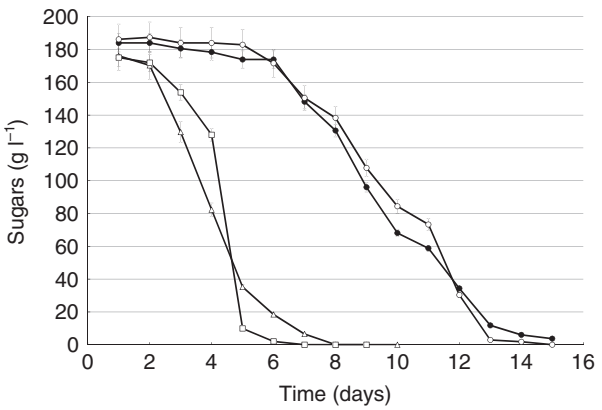

Figure 3 Evolution of alcoholic fermentation, expressed as sugar consumption. ● Riesling biodynamic; ○ Riesling traditional; Δ Pinot blanc biodynamic; □ Pinot blanc traditional.

The microbiota of grapes is a good indicator of the alterations occurring during grape ripening, because its composition is influenced by the presence of grapevine disease and weather conditions (Barata *et al.* 2008b, 2012b; Nisiotou *et al.* 2011). Furthermore, the microbiota of grapes is one of the main sources of fermenting micro-organisms (Martini 1993; Beltrán *et al.* 2002; Cappello *et al.* 2004), especially in winemaking protocols that do not provide for the use of selected yeast or bacteria cultures, such as the biodynamic approach. In the previous section we underlined the differences occurring between the microbiological counts performed in three successive years, demonstrating the particular features of 2014 and the relationship between vine disease and grape microbiota. The discussion here therefore focuses on the data relating to the 2014 observations. The evolution of oenological microbiota in grapes (Table 3) shows progressive acceleration of growth starting from veraison, according to the progressive availability of easily assayable substrates in bunches (Barata *et al.* 2012b). The high concentration of yeast observed in all samples at the end of ripening, more than four log units, is not surprising considering the exposure of the grapes to diseases favouring

Table 6 Microbiota profile following alcoholic fermentation. Data are expressed as the % of microbial isolation on the plate count

	Pinot blanc biodynamic					Pinot blanc traditional				
Sampling date	08/09	10/09	12/09	17/09	19/09	08/09	10/09	12/09	17/09	19/09
%										
Saccharomyces cerevisiae	6·2	60·7	98·2	100	100	16·3	21·8	63·2	55·6	97·3
S. cariocanus	0·0	0·0	0·0	0·0	0·0	0·0	0·0	15·8	37	2·7
Hanseniaspora uvarum	75·2	36·9	0·9	0·0	0·0	64·2	49·1	18·3	7·4	0·0
Candida zemplinina	14·7	2·4	0·9	0·0	0·0	15·5	28·6	2·7	0·0	0·0
Pichia kluyveri	1·6	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
I. orientalis	0·0	0·0	0·0	0·0	0·0	4·0	0·5	0·0	0·0	0·0
Gluconobacter cerinus	1·5	0·0	0·0	0·0	0·0	1·5	0·0	0·0	0·0	0·0
	Riesling biodynamic					Riesling traditional				
Sampling date	12/09	17/09	19/09	30/09	20/10	12/09	17/09	19/09	30/09	14/10
%										
Saccharomyces cerevisiae	40	100	100	100	100	15·9	97	100	100	100
Hanseniaspora uvarum	0·0	0·0	0·0	0·0	0·0	68·3	1·5	0·0	0·0	0·0
Candida zemplinina	0·0	0·0	0·0	0·0	0·0	7·9	1·1	0·0	0·0	0·0
Pichia kluyveri	20	0·0	0·0	0·0	0·0	7·9	0·0	0·0	0·0	0·0
I. orientalis	40	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Gluconobacter cerinus	0·0	0·0	0·0	0·0	0·0	1·5	0·0	0·0	0·0	0·0

Table 7 Chemical features of wines obtained from grapes grown using different agricultural protocols. (Mean data $n = 3$)

Grape varieties	Agricultural protocol	Ethanol (%)	Sugar (g l^{-1})	pH	Tot. acidity (g l^{-1})	Tartaric acid (g l^{-1})	Malic acid (g l^{-1})	Acetic acid (g l^{-1})
Pinot Blanc	Traditional	11·18	<1	3·31	5·7	3·33	2·04	0·28
Pinot Blanc	Biodynamic	11·12	<1	3·22	6·9	3·71	2·06	0·26
Riesling	Traditional	11·25	1·18	2·86	9·4	4·54	3·60	0·40
Riesling	Biodynamic	11·13	1·60	2·89	9·5	5·02	3·69	0·45

microbial proliferation (Nisiotou *et al.* 2011; Barata *et al.* 2012b). However, in agreement with observations of grapevine pests, the variables most influencing the qualitative and quantitative characteristics of the microbiota appear to be primarily related to morphological and physiological differences between Pinot Blanc and Riesling.

This tendency was confirmed by chemical characterisation of the grape must and by following experimental fermentation from both the chemical and microbiological point of view. Throughout the winemaking process, from the vineyard to wine fermentation, the selective pressure induced by the evolution of environmental conditions appeared to be the main reason for modifications to the microbiota, rather than the agricultural approaches adopted in the vineyard (Querol *et al.* 1994; Guerra *et al.* 1999; Renouf *et al.* 2005; Zott *et al.* 2008). This result is reasonably due to the particular environmental conditions of the 2014 vintage, which altered the usual evolution of microbiota. A significant exception can be found in the higher content of available nitrogen in biodynamic grapes. This parameter is crucial in determining the outcome of fermentation (Guzzon *et al.* 2011) and the aromatic expression of wines (Nicolini *et al.* 2004). While fertilization was carried out in both agronomic management systems, using different practices (Table 1), it is possible to assume that biodynamic practices favoured the equilibrium of the vines and optimized the composition of the grape must. The higher nitrogen content of biodynamic grape must probably favoured a prompt growth of *Saccharomyces,* resulting in an unexpected reduction in the biodiversity of biodynamic samples as compared to that observed in traditional grape must (Table 6).

In conclusion, the poor weather conditions in 2014 paved the way for intense attacks on vines by parasites. Despite this unfavourable situation, in terms of the healthiness of grapes and the microbiota present, biodynamic vine growing showed itself capable of ensuring comparable features to those obtained using a traditional agricultural approach, which is more invasive from an ecological point of view. Furthermore, the grapes produced in biodynamic vineyards were more suitable for winemaking, due to the high nitrogen content. These results, accompanied by analysis of fermentation behaviour and the wines obtained, could be considered promising indicators of a more balanced ecosystem in the vineyard, according to the principles of biodynamic practice. The results of this study suggest that biodynamic cultivation of the grapevine can be sustainable even in difficult years or winemaking conditions, representing an interesting alternative to the traditional vine-growing approach.

Conflict of interest

Raffaele Guzzon, Silvia Gugole, Roberto Zanzotti, Mario Malacarne, Roberto Larcher, Christian von Wallbrunn and Enzo Mescalchin state that there is no conflict of interest in relation to the content of this paper.

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