Microbial functional diversity indicators in vineyard soils under organic and biodynamic land management

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Research Article

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Abstract

Background: An on-vineyard approach was used to investigate the effects of biodynamic (BD) preparations on microbial functional diversity and extracellular polymeric substances (EPS) in four vineyards on different bedrocks under organic management.

Methods: Soil organic carbon (SOC), total N, microbial biomass carbon (MBC), multi substrate-induced respiration (MSIR), with 17 substrates and H_2O, EPS, and glomalin-related soil protein (GRSP) were measured in soils taken from vineyards without (BD-) and with (BD+) biodynamic preparations.

Results: The vineyards with BD preparations had improved all soil chemical and biological properties. The MSIR approach was again able to separate clearly BD+ and BD-, confirming previous studies. The glomalin-related soil protein (GRSP) was negatively related to the microbial respiratory response of all substrates added.

Conclusions: Lower ratios of EPS-carbohydrates and particularly EPS-protein indicate that soil microorganisms have to divert less substrate to the formation of EPS, so that more of a substrate can be used for the production of microbial biomass.

Background

Soil microbial functions play an essential role in nutrient cycling and decomposition, and the understanding of these ecosystem processes is central to the efficient management of specific agricultural regimes for soil quality maintenance [1, 2]. The quality of organic matter inputs is one factor that influences the present microbial community`s ability to metabolize these inputs, and to produce “binding agents” such as extracellular polymeric substances (EPS) [3, 4] and glomalin-related soil protein (GRSP), responsible for improving soil aggregate stability [5, 6].

In viticulture, the improvement of soil physical and chemical properties, i.e., aggregate stability, is of particular importance due to the susceptibility of vineyards to erosion [7, 8]. Therefore, farming management practices that prioritize soil quality enhancement through sustainable practices, e.g., humus build-up are essential, such as organic and biodynamic agricultural approaches. Both alternative farming methods share similar practices, such as the use of organic fertilizers, compost, and often reduced tillage, among others [9].

Biodynamic agriculture additionally considers the use of natural preparations (known as biodynamic (BD) preparations) applied to the soil, crops, and compost. The BD preparations consist of cow manure (horn manure), finely ground silica (horn silica) applied in the field, and plant-based ferments applied to the compost [10]. Research studies in peer-reviewed journals showed that BD preparations are able to promote soil aggregate formation [11], higher nutrient storage [12, 13], and N status of the microbial community [14]. It is well-documented that the application of BD preparations have a balancing effect on

the microbial functional diversity [15]. More recently, it was shown that BD preparations can act as biofertilizers, increasing the abundance of plant growth promoting microorganisms [16].

The functional capacity of microorganisms, i.e., their ability to decompose and to convert substrates, is often measured through multi-substrate induced respiration (MSIR). In this method, a selection of substrates, mimicking root exudates or microbial decomposition products, is added to soil and the respiratory response is registered [17, 18]. MSIR has been thoroughly used to analyze the effect of land use management [19, 20, 21] and fertilization systems [18] on microbial functional diversity.

One of the mechanisms involved in resource usage by microorganisms is their ability to produce EPS. This polymer is excreted to produce a safe interface between microorganisms and the soil matrix [22, 23]. Microorganisms can be found embedded in that hydrated substance [24] that serves as self-protection against environmental stressors [25, 26] and binds whole microbial communities to soil aggregates, which is known to be structurally important in soil aggregate formation [27, 28, 29].

The role that GRSP plays in soil structure maintenance is similar to EPS, however with a different origin. Glomalin was originally reported by Wright and Upadhyaya [30] to be produced by arbuscular mycorrhizal fungi (AMF), but it was later described as a soil glycoprotein [31, 32, 33, 34] therefore, named GRSP. The aggregation capabilities of GRSP are suggested to be indirectly connected to carbon stabilization in soil [35, 36, 37]. Therefore, its presence can be used as an indicator of soil quality.

Considering the already mentioned BP effects on soil, quantifying EPS and GRSP could provide insights into microorganisms functioning under such conditions. We postulate that the presence of EPS and GRSP can be the underlying cause for the advantages of BD preparations. Therefore, the aim of the present work is to analyze the effects of biodynamic farming on the functional diversity of microorganisms and the production of EPS and GRSP in the soils of four vineyards of the Burgundian region in France. The underlying hypotheses are: (1) The application of BD preparations positively affects the metabolic activity of microorganisms. (2) Soils under biodynamic farming show higher EPS and GRSP production because of the application of BD preparations.

Materials and methods

Site description and sampling

This study was conducted with soils from the Burgundian region, France, where grape cultivation dominates many landscapes. Four farmers who owned vineyards of about 0.5 ha were randomly selected for this study. The oldest vineyard was established in 1988, and prior to the establishment into two halves (approximately 2500 m^2 each). One half was annually sprayed with BD preparations (BD+), whereas the other half received no BD preparations (BD-). The BD preparations spayed were 500P (horn manure) and 501 (horn silica), obtained from BioDynamie Services Pierre et Vincent Masson, France. Vineyard soils were sampled in October 2020.

Topsoil samples were collected from 0 to 10 cm depth using stainless steel cylinders (v = 739 cm^3; h = 10 cm, inner diameter = 9.7 cm). Six replicates were taken from each plot, distributing the sampling points across the central vineyard rows and within grapevine plants. Samples were carried to the laboratory, sieved (< 2 mm), and stored in polyethylene bags at 4°C prior to analysis.

Multi-substrate induced respiration

Carbon substrate utilization patterns were analyzed by the multi-substrate induced respiration (MSIR) approach using the MicroResp™ method [17, 38]. Water content was adjusted to 35% of the soil WHC. Samples with higher WHC were air-dried at room temperature (18–20°C) until the required water content was reached. 300 mg soil was added into 1.1 mL wells of a deep-well microtiter plate (Nunc, Thermo Electron, Langenselbold, Germany) and pre-incubated for 7 days at 25°C in the dark prior to multi-SIR analysis.

Substrate utilization patterns of carbon compounds were determined by measuring the respiration rates after the addition of 17 low organic molecular weight substrates to the soil samples. The substrates added are considered as essential rhizosphere carbon sources [39, 40, 41]. A spectrum of 17 substrates plus water was selected according to previous studies [15, 18, 42, 43] including six amino acids: γ-aminobutyric acid (GABA), L-serine (Ser), L-alanine (Ala), L-cysteine (Cys), L-glutamine (GluN), and L-leucine (Leu), three amino sugars: N-acetyl-glucosamine (NAG), D-glucosamine (GlcN), and D-galactosamine (GalN)), four neutral sugars: L-arabinose (Ara), D-galactose (Gal), D-glucose (Glc), and D-fructose (Fru); one sugar alcohol: sorbitol (Sor), one phenolic organic acid: protocatechuic acid (ProCa); and two carboxylic acids: malic acid (MA) and citric acid (CA). Substrate solutions were prepared in distilled water at a concentration of 8 mg g^{-1} dry soil and 20 µl aliquots were dispensed in each well. Due to the lower solubility of Cys, GluN, Leu, and ProCa, solutions were prepared at 4, 2, 1.3, and 0.8 mg g^{-1} soil, respectively.

The colorimetric CO_2 trap consists of a deep-microplate containing gel with pH indicator dye [17] stored in the dark with wet towels and soda lime to avoid desiccation or CO_2 reaction prior to analysis [42]. Soil was added to the well plate and left open for 30 min after substrate addition to allow CO_2 release of any carbonate- acid based substrate reaction [38]. Afterwards, both deep microplates (96 wells) were placed face to face and immediately sealed. The color of the gel plates was measured directly before sealing (T0) and after 4 h (T1) of incubation at 25°C and CO_2-trap absorbance of 572 nm (FLUOstar, BMG, Offenburg, Germany) [18]. Respiration rates (µl CO_2 g^{-1} soil h^{-1}) are calculated from absorption data as µl $CO_2 = 51 \times (0.2 + ABS)^3$ [44], where ABS (absorbance) is the difference between T1 and T0 addition to the substrate utilization patterns. Values for basal respiration (BR) and soil microbial biomass C (MBC) were calculated from MSIR respiratory response. BR (µg CO_2C g^{-1} soil d^{-1}) was calculated based on H_2O-induced respiration: µl CO_2 g^{-1} soil $h^{-1} \times 24 \times 0.515 \times 0.2727$ [45], using conversion factors from h

in d, µl in µg, CO_2 in CO_2C, respectively. MBC (µg g^{-1} soil) was calculated based on Glc-induced respiration: µl CO_2 g^{-1} soil h^{-1} × 30 [46].

EPS extraction

EPS extraction
EPS was extracted from all 6 soils of this experiment, following the procedure originally proposed by Frølund et al. [47], and modified by Redmile-Gordon et al. [3] using a cation exchange resin (CER), with the omission of the pre-extraction step, as proposed by Bublitz et al. [48].

The process consisted of washing CER (Dowex 'Marathon C' Na form, strongly acidic, 20–50 mesh) with phosphate buffered saline (PBS) for 1h in the dark, at 4°C. PBS was prepared with 2 mM Na_3PO_4× 12 H_2O [0.760 g L^{-1}], 4 mM NaH_2PO_4× H_2O, [0.552 g L^{-1}], 9 mM NaCl [0.526 g L^{-1}], and 1 mM KCl [0.0746 g L^{-1}]. Field moist soil was weighed, and 2.5 g dry-weight equivalent was placed into a centrifuge tube. Washed CER was added to the soil in an amount based on the soil organic carbon (SOC) content. According to Redmile-Gordon et al. [3], 177.8 g CER should be used for each g $^{-1}$ SOC. 25 mL cold PBS were added together with the CER and tubes were shaken in the dark for 2 h at 120 rev min^{-1}. Tubes were then centrifuged at 4200 *g* for 20 min, the supernatant was extracted, and extracts were stored at -20°C until protein and carbohydrate quantification took place.

GRSP extraction

The glomalin-related soil protein (GRSP) was extracted following the easily extractable glomalin (EE-GRSP) protocol from Wright and Upadhyaya [30]. A 1 g soil sample was weighed into 50 mL centrifuge tubes and 8 mL of 20 M sodium citrate was added. Samples were then autoclaved at 121°C for 30 min and placed in ice immediately after to avoid extracted GRSP to recombine with soil particles. Cooled down extracts were centrifuged at 3500 *g* for 20 min. The supernatant was decanted and stored at -20°C.

Total protein quantification

Total GRSP and EPS proteins were quantified with a modified Lowry assay [49] corrected for soil extracts potentially containing confounding fractions of polyphenols [4, 40]. Standards of Bovin Serum Albumin (BSA, Sigma A7906) were compared with the samples, whose absorbance was read in a microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany). Confounding compounds were excluded from quantified proteins by measuring the absorbance of samples i) with and without reagents containing $CuSO_4$, and ii) by applying a mathematical correction as by Frølund et al. [47]: $ABS_{protein}$ = 1.25 (ABS_A -ABS_B), where ABS_A is the absorbance of samples using reagents with $CuSO_4$, ABS_B is the absorbance of samples without $CuSO_4$, and $ABS_{protein}$ is the theoretical absorbance of proteins.

Total carbohydrate quantification

EPS carbohydrate content was determined with the method proposed by Mopper and Gindler [51], and modified by Joergensen et al. [52], in which Cu^{2+} in the ends of mono- and disaccharides is reduced to Cu^{+}. A step of hydrolysis prior to quantification was performed, as proposed by Bublitz et al. [45], in which 1.5 M H_2SO_4 was added to EPS extracts in a proportion 1/1 (v/v) for a final acid concentration of 0.75 M. The extracts were then hydrolysed for 10 min at 100°C in an autoclave. A reagent consisting of 25 mL of an aqueous buffer (4% Na_2CO_3, 4% $[(NaPO_3)_6]$ and 0.2% aspartic acid solution) mixed with 3 mL bicinchoninic acid (Sigma D8284–5G) (40 g L^{-1}) and 0.45 mL of a $CuSO_4 \times 5\ H_2O$ solution (63 g L^{-1}) was added to the hydrolysed extracts, which were then heated for 120 min at 60°C. Total carbohydrates in hydrolysed extracts were photometrically detected at a wavelength of 562 nm [52].

Statistical analysis

Data are presented as arithmetic means of six independent replicates on an oven dry weight basis (about 24 h at 105°C). The normality of data was evaluated using Shapiro-Wilk test and the homogeneity of variance assessed using Levene´s test. If necessary, data was ln-transformed to meet the assumptions of analysis of variance (ANOVA). Significant differences between treatments were tested by two-way ANOVA, using vineyard and BD preparations as factors, followed by the Holm-Sidak test. ANOVA and correlation analyses were carried out using SigmaPlot 13.0 (Systat, San José, USA). Discriminant function analysis was conducted with SPSS 16.0 statistical software (SPSS, IBM, Ehningen, Germany) to evaluate the differences between the vineyard locations, treatments, and specific soil conditions.

Results

Vineyard effects on soil chemical and microbial properties

The soil properties exhibited a high variation between the four vineyards (Table 1). The soil pH was acidic at the sandy vineyard Fleurie and alkaline at the other three loamy and clayey vineyards. This led to lowest contents of SOC and total N at Fleurie (Table 2), whereas Lavernette and Prés Culey formed a pair varying around 23.6 mg SOC and 2.31 mg total N g^{-1} soil, averaging the BD- and BD + treatments.

Table 1
Site characteristics, soil properties, soil types [53], biodynamic practices of the four French vineyards.

	Prissé	Fleurie	Lavernette	Prés Culey
ASL (m)	207	298	177	280
Slope (%)	14	14	10	13
Facing	North to north-east	South to west	East	East
Azimuth	+ 16°	+ 222°	+ 95°	+ 97°
MAP (mm)	650	632	663	671
MAT (°C)	13.1	11.2	11.6	11.7
Clay (%)	25	5	39	43
Silt (%)	64	16	46	51
Sand (%)	11	79	15	6
pH-H_2O	7.7	5.0	7.9	8.4
Bedrock	Limestone and marl	Porphyritic granite	Tuff, ignimbrite, and dacite	Limestone
Dominating soil type	Rendzic Leptosols (60%)	Colluvisols (100%)	Alocrisol (60%)	Rendosols (35%)
Vineyard since	2000	1989	1988	2016
Grape variety	Chardonnay	Gamay	Chardonnay 277	Savagnin + Pineau gris
Stock	3309	3309	SO4	161 − 49
BD preparations since	2018	2016	2007	2016
BD preparations (y^{-1})	2 × 500P + 1 × 501	2 × 500P + 3 × 501	2 × 500P + 1 × 501	2 × 500P + 3 × 501
BD application rate (y^{-1})	40 l ha^{-1}	40 l ha^{-1}	40 l ha^{-1}	40 l ha^{-1}
Plant cover between rows	1 over 2 rows	Naturally sparse	No	No
Latitude (north)	46° 19' 7"	46° 11' 29"	46° 15' 34"	46° 31' 3"
Longitude (east)	4° 43' 36"	4° 40' 29"	4° 45' 13"	4° 44' 19"

ASL: above sea level; MAP: mean annual precipitation; MAT: mean annual temperature.

Based on the respiratory response to adding 17 substrates and H_2O, DF1 separated acidic and sandy Fleurie from the other 3 vineyards and Prés Culey from Prissé and Lavernette. DF2 separated Prissé and Fleurie from Lavernette and Prés Culey (Fig. 1).

Despite differences in SOC content, Prissé and Lavernette formed a pair of vineyards according to a mean MBC around 600 µg g^{-1} soil, averaging the BD- and BD + treatments, followed by Prés Culey and Fleurie (Table 2). Consequently, the largest difference was observed for the MBC/SOC ratio, which was on average 3.1% at Prissé and Lavernette and 1.5% at Fleurie and Prés Culey. The significantly largest basal respiration was measured at Prissé, followed by a distinct decline in the order Lavernette > Prés Culey > Fleurie. The substrate-induced activities exhibited similar response patterns as MBC and basal respiration, with interchangeable maxima at Prissé and Lavernette, followed by Prés Culey and a minimum at Fleurie (Fig. 2a, b, c; Supplementary Tables 1a and 1b). An exception was the S-containing amino acid cysteine (Fig. 2d).

In contrast to respiration, the metabolic quotient qCO_2 showed more variable site-specific pattern and varied around 12 mg CO_2 g^{-1} MBC d^{-1} (Table 2).

EPS-carbohydrates and EPS-proteins widely varied around 258 and 114 µg g^{-1} soil, respectively (Table 3), and showed strong interrelationships with MBC (r = 0.90 and r = 0.76) and basal respiration (r = 0.80 and r = 0.69). GRSP varied around 1475 µg g^{-1} soil and formed also two site pairs, with maximum contents at Fleurie and Prés Culey and minimum contents at Prissé and Lavernette. GRSP exhibited significant negative relationships with MBC (r = -0.71), EPS-carbohydrates (r = -0.61) and EPS-protein (r = -0.53).

Table 2
Mean contents of SOC, total N, and MBC, the MBC/SOC ratio, the basal respiration rate (CO_2C) and the metabolic quotient qCO_2 in vineyard soils without (BD-) and with (BD+) application of biodynamic preparations.

	SOC	**Total N**	**MBC**	**MBC/SOC**	**CO_2C**	**qCO_2**
	(mg g^{-1} soil)		(µg g^{-1} soil)	(%)	(µg g^{-1} soil d^{-1})	(mg g^{-1} d^{-1})
BD-						
Prissé	16.4 b	1.65	523 b	3.2 a	7.1 a	13.7 ab
Fleurie	12.8 c	1.17	148 d	1.2 b	2.3 d	15.8 a*
Lavernette	21.0 a	1.99	651 a*	3.1 a*	5.0 b	7.6 c
Prés Culey	23.9 a	2.41	279 c	1.2 b	3.3 c	12.1 b*
Mean	18.6	1.81	412	2.2	4.5	**12.3**
BD+						
Prissé	18.2 b	1.74 b	640 a*	3.5 a*	8.6 a	13.5 a
Fleurie	14.8 c	1.31 c	267 d*	1.8 c*	2.9 d	11.1 ab
Lavernette	25.0 a	2.38 a	595 b	2.4 b	5.7 b	9.6 b
Boisseau	24.4 a	2.47 a	428 c*	1.8 c*	3.8 c	8.9 b*
Mean	**20.6**	**1.98**	**469**	**2.4**	**5.3**	10.8
Probability values						
BD	0.01	< 0.01	< 0.01	0.02	0.01	0.01
Vineyard	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Vineyard × BD	NS	NS	< 0.01	< 0.01	NS	< 0.01
CV (± %)	14	8.7	9.4	12	15	15

CV = mean coefficient of variation between replicate samples (n = 6); different letters within a column indicate a vineyard-specific difference for each treatment (Holm-Sidak test, $P < 0.05$); an asterisk indicates a significant higher value between the BD treatments, showing vineyard × BD interactions; bold numbers indicate a significant higher mean between the BD treatments.

Table 3
Mean contents of EPS-carbohydrates (EPS-carb), EPS-proteins (EPS-prot), and glomalin-related soil protein (GRSP) as well as the ratios EPS-carbohydrates / EPS-protein, EPS-carbohydrates / MBC, EPS-protein / MBC, and GSRP / EPS-protein in vineyard soils without (BD-) and with application of biodynamic (BD+) preparations.

	EPS-carb	**EPS-prot**	**GRSP**	**EPS-carb/**	**EPS-carb/**	**EPS-prot/**	**GRSP/**
	(μg g^{-1} soil)			EPS-prot	MBC	MBC	EPS-prot
BD-							
Prissé	301 b	157 b	980 b	2.2 a	0.64 a	0.31 b	7 c
Fleurie	31 d	24 d	2330 a	1.3 b	0.21 c	0.16 c	98 a*
Lavernette	509 a*	209 a	1060 b	2.5 a	0.79 a	0.33 ab	5 c
Prés Culey	145 c	113 c	1770 a	1.2 b	0.53 b	0.45 a	15 b
Mean	247	**126**	1530	1.8	**0.55**	**0.31**	32
BD+							
Prissé	412 a*	129 b	1040 b	3.3 a	0.65 a	0.19 b	9 c
Fleurie	39 c*	25 d	1550 a	1.5 b	0.15 c	0.10 c	64 a*
Lavernette	409 a	143 a	1200 b	2.9 a	0.69 a	0.24 ab	8 c*
Prés Culey	211 b*	109 c	1880 a	2.0 b	0.49 b	0.26 a	18 b
Mean	**268**	102	1420	**2.4**	0.48	0.20	25
Probability values							
BD	0.01	0.01	NS	< 0.01	0.02	< 0.01	NS
Vineyard	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Vineyard × BD	< 0.01	NS	NS	NS	NS	NS	0.01
CV (± %)	18	19	24	18	18	22	29

CV = mean coefficient of variation between replicate samples (n = 6); different letters within a column indicate a vineyard-specific difference for each treatment (Holm-Sidak test, $P < 0.05$); an asterisk indicates a significant higher value between the BD treatments, showing vineyard × BD interactions; bold numbers indicate a significant higher mean between the BD treatments.

BD preparation effects on soil chemical and microbial properties

The application of BD preparations generally increased SOC, total N, MBC, basal respiration, and the MBC/SOC ratio (Table 2) as well as the EPS-carbohydrate content and the EPS-carbohydrates/EPS-

protein ratio (Table 3). The application of BD preparations did not affect the GRSP content and the GRSP/EPS-protein ratio but had generally significant negative effects on EPS-protein and the EPS-protein/MBC and EPS-carbohydrates/MBC ratios. DF1 separated BD- and BD + at Prés Culey and Lavernette, whereas DF2 separated BD- and BD + at Prissé, Fleurie, and Prés Culey.

The soil properties exhibited a high variation between the four vineyards (Table 1), leading to numerous significant interactions of all MSIR substrates with BD preparations. These interactions were particularly caused by the different microbial response to BD preparations at Lavernette, where the MBC (= Glc) (Table 2) and the respiratory response to the application of Ara, Gal, Fru, and CA (Supplementary-Table 1) responded significantly stronger without BD preparation than with preparation contrasting the other three sites. The strongest effect showed the BD preparations at sandy and acidic Fleurie with significant effects of all chemical and soil biological properties (Table 2, 3, Fig. 2; Supplementary-Tables 1, 2, 3, and 4).

Discussion

General soil properties

The soil properties exhibited always significant difference between the four vineyards. SOC and MBC were lowest at the sandy and acidic vineyard Fleurie, which is typical for these soil properties [54, 55]. The number of soils is small to verify systematic effects of other soil properties, for example clay. In contrast to Fleurie, the MBC/SOC was exceptionally high at Prissé and Lavernette [55, 56], indicating a recent strong increase in the annual C input by organic fertilizers and intercropping. The MBC/SOC ratio indicates substrate availably to the soil microbial community [55, 56]. The reason for the relatively low MBC/SOC ratio at Prés Culey cannot be explained by the current dataset. One reason could be high Cu concentrations in soil due to past applications of $CuSO_4$ as a fungicide [9, 57].

No data exist for comparing the contents of EPS carbohydrates and EPS proteins in vineyard soils. However, the EPS carbohydrates and EPS proteins contents are in the range of arable and grassland soils exhibiting similar SOC and MBC contents [58, 59]. The low ratio of EPS-carbohydrates to EPS-protein indicates a microbial origin of the EPS [60], as plants excrete mainly carbohydrates as EPS [61, 62]. The plant-derived mucilage must be rapidly transformed into microbial EPS [63, 64]. This view is supported by the close correlations between MBC and EPS-carbohydrates and EPS-proteins.

In contrast to EPS, some information exists on the GRSP content of vineyard soils [65, 66, 67, 68]. Sharifi et al. [68] measured around 1400 µg g^{-1} soil easily extractable GRSP in different aggregate fractions of vineyard soils, which is similar to the current data. Ferreira et al. [66] measured between 2200 and 3400 µg g^{-1} soil easily extractable GRSP in vineyard soils with *Crotalaria juncea* as plant cover within the spacing between two rows of grape plants, which is somewhat above the current data. In addition, Ferreira et al. [66] observed significant positive correlations of GRSP with AMF colonization and AMF spores. A strong but negative relationship of GSRP with EPS was obtained in the current dataset,

indicating some connections between these two SOC fractions, which are important components of microbial residues [69]. However, the GRSP contributes approximately 4% to soil organic matter (SOC × 2) but nearly 25% total N (GRSP / 3.125, which is the C/N ratio of protein). This suggests that not all GRSP extracted by citric acid is AMF derived. In addition, AMF-derived glomalin should be a fraction of all fungal and bacterial EPS and not a six-times multitude of EPS.

BD preparations

The positive effect of biodynamic preparations on SOC and total N was not found in previous research in vineyards by Fritz et al. [15]. Therefore, we are reserved in interpreting these results. However, the respiratory MSIR confirms again positive effects of BD preparations observed in long-term field experiments in Darmstadt [18] and Bonn [42] but also in Burgundian vineyards [15]. The possible reasons for BD effects have been repeatedly explained in detail [15, 16, 42, 70].

EPS responded more quickly to environmental soil conditions than SOC, total N, and MBC [60], i.e., BD preparation effects are particularly valid for this type of microbial indices. The reduced contents of EPS-proteins after application of BD preparations suggest that less extracellular enzymes and less scaffoldings are necessary for microbial performance in soil. This means that more of a substrate could be directed directly into the microbial biomass and less into microbial residues during growth. This energy saving mechanism is even more obvious in the higher EPS carbohydrate to EPS protein ratio and in the lower ratios of EPS-carbohydrates to MBC and EPS-protein to MBC in the BD + treatments. The same change of these ratios has been observed by Bublitz et al. [60] in the biodynamic FYM treatment of the famous DOK experiment [71, 72].

The Lavernette soil showed the strongest respiratory response to the neutral sugars Ara, Gal, Glc (= MBC), and Fru (Fig. 2a) in comparison with the other three vineyards. However, the application of BD preparations reduced the respiratory response only in this vineyard. This means that less neutral sugars are catabolized to CO_2 and more substrate is diverted into microbial anabolism [43, 73], first into microbial biomass and then into microbial necromass [74], which led to the strongest increase in SOC content after the application of BD preparations. They seem to have again a harmonizing and balancing contrary effect as repeatedly observed by others not only on soil microbial indices [15, 42, 75], but also on plant development indices [76]. The harmonizing effect of the BD preparations was less expressed on the respiratory response particularly to the addition of most amino acids, for example Ala (Fig. 2c), indicating that the anabolic demand for N remained high even at Lavernette.

The most distinct respiratory response between the vineyard soils was observed after application of cysteine, a sulphur-containing amino (Fig. 2d). The order declined as follows: Prissé > Fleurie > Lavernette > Prés Culey. The suggest a strong S-deficiency at Prés Culey and a rather good S- availability at Fleurie in comparison with the other substrate added. However, this view needs to be confirmed by other measurements, e.g. S content in grape plants, total soil S, $CaCl_2$ extractable SO_4^{2-}, or MBS [77]. In line with the current view, Cys has the strongest positive linear relationship with DF2 (Supplementary-

Table 1b), pointing to the importance of S nutrition not only in arable [75, 78] but also in vineyard systems.

Conclusions

The vineyards with BD preparations had improved soil chemical and soil biological properties. The MSIR approach was again able to clearly separate BD + and BD-, confirming previous studies. The glomalin-related soil protein (GRSP) was negatively related to the microbial respiratory response of all substrates added. This suggests a close link due to an unknown mechanism. The respiratory response to most of the substrates was similar except that to the S-containing amino acid cysteine, indication a close relationship to S nutrition of soil microorganisms. The most striking feature were the lower ratios of EPS-carbohydrates and particularly EPS-protein to the soil microbial biomass. This indicates that soil microorganisms have to divert less substrate to the formation of EPS, so that more of a substrate can be used for the production of microbial biomass, which is an important source of microbial necromass and finally soil organic matter.

Abbreviations

BD: biodynamic; EPS: extracellular polymeric substances; GRSP: glomalin-related soil protein; Substrates: GABA: γ-aminobutyric acid; Ser: L-serine; Ala: L-alanine; Cys: L-cysteine; GluN: L-glutamine; Leu: L-leucine; NAG: N-acetyl-glucosamine; GlcN: D-glucosamine; GalN: D-galactosamine; Ara: L-arabinose; Gal: D-galactose; Glc: D-glucose; Fru: D-fructose; Sor: sorbitol; ProCa: protocatechuic acid; MA malic acid; CA: citric acid.

Declarations

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Authors' contributions

TAB carried out the laboratory analyses, interpreted the results, and wrote the first draft. VM performed the experiments. HRG, JF, and RJ interpreted the results, finalized and reviewed the manuscript with TAB. All authors read and approved the final manuscript.

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Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

VM is founder and currently employed at BioDynamie Services. The remaining authors declare no competing interests.

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Figures

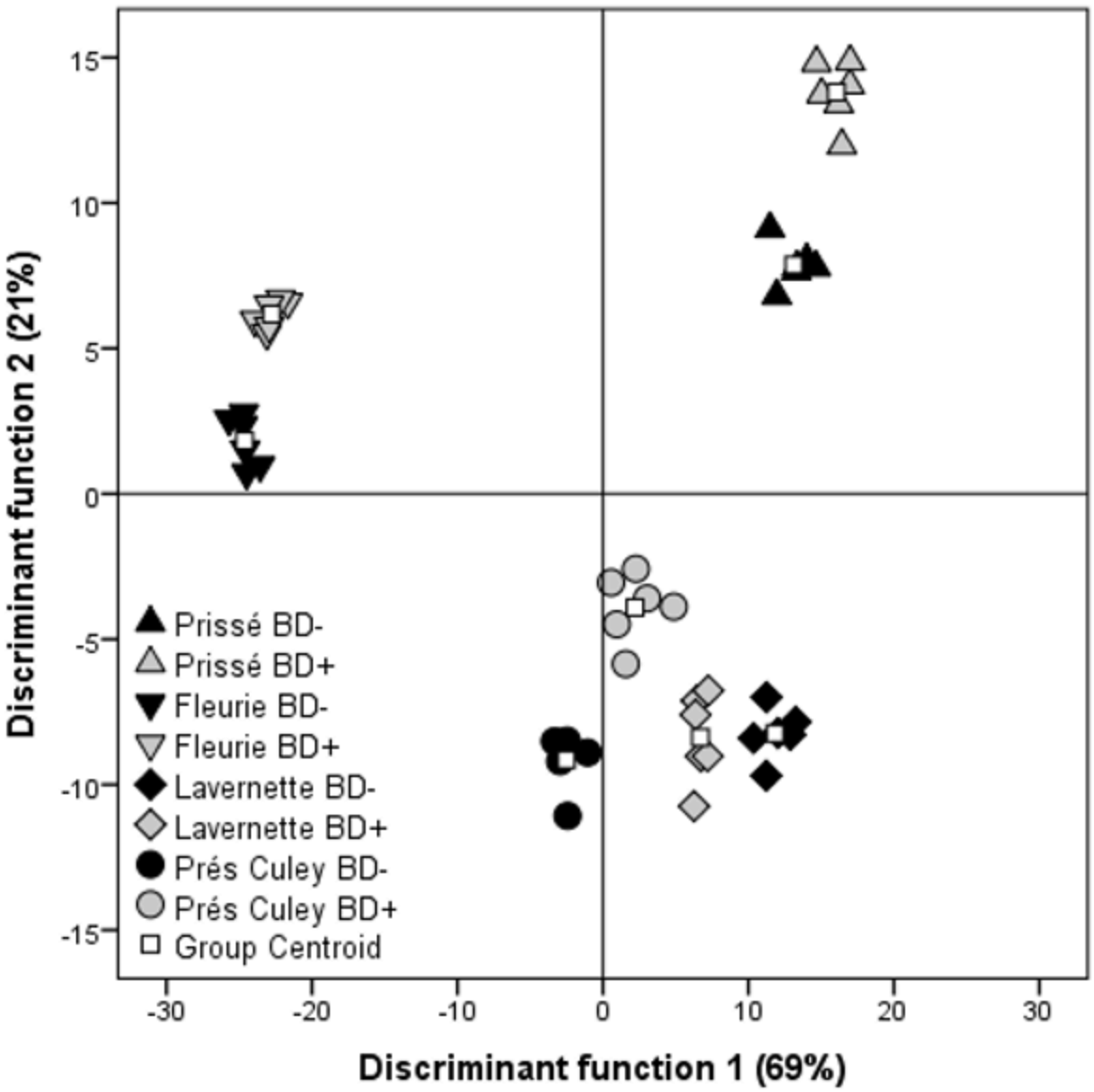

Figure 1

Discriminant function analysis based on the multi-substrate induced respiration rates of 17 substrates plus distilled water for the four vineyard locations and treatments without (BD-) and with (BD+) biodynamic preparations.

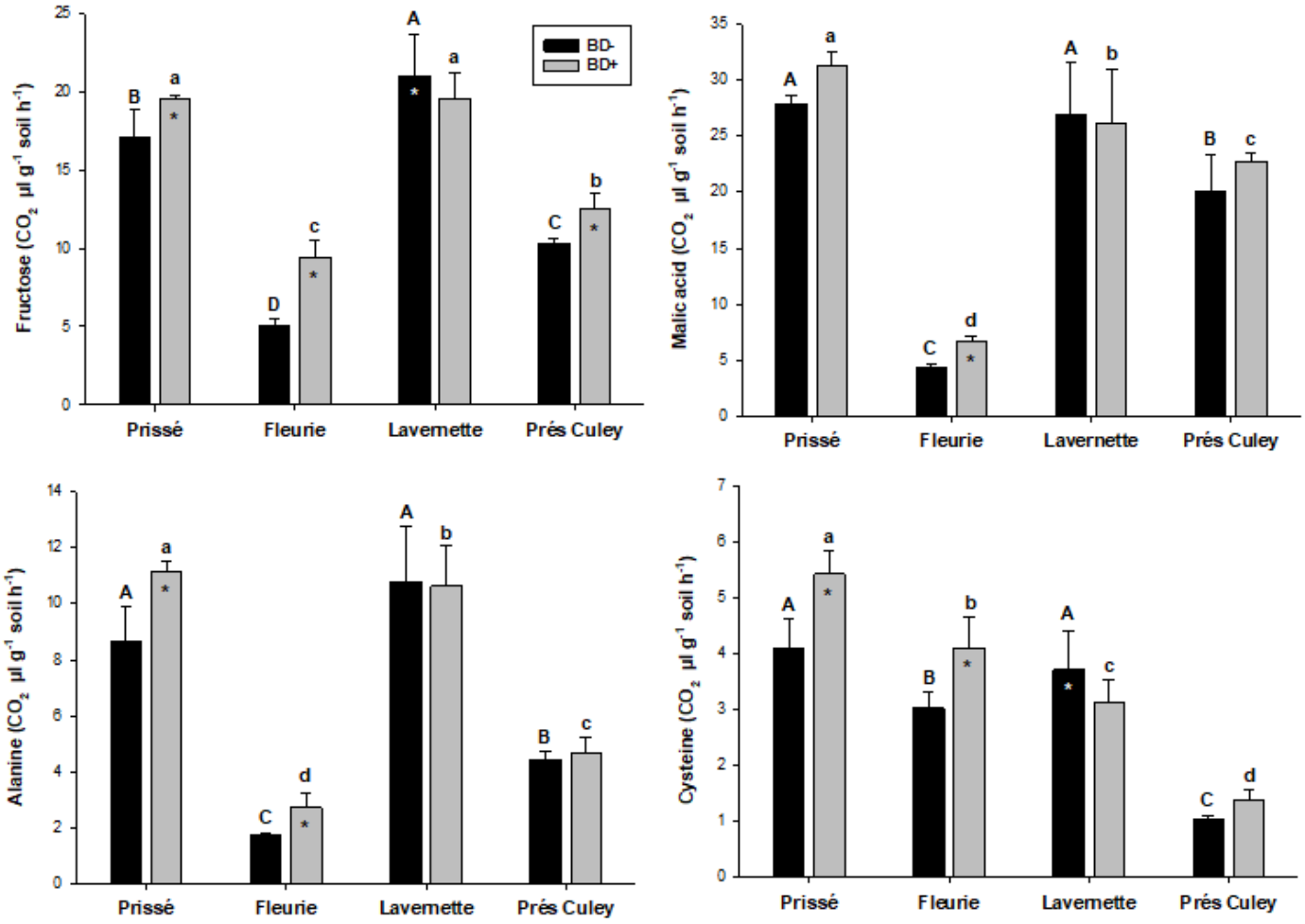

Figure 2

Mean induced respiration rates for (a) D-Fructose, (b) Malic acid, (c) L-Alanine, and (d) L-Cysteine at the four vineyard locations without (BD-) and with (BD+) biodynamic preparations.; different letters on top of the bars represent a vineyard-specific difference for each biodynamic treatment (Holm-Sidak test, $p < 0.05$); asterisks represent a significant difference between the BD treatments for each vineyard location.

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