Supplemental Material

Ozonized Oleic Acid as a New Viticultural Treatment? Study of the Effect of LIQUENSO® Oxygenate on the Carpoplane Microbial Community and Wine Microorganisms Combining Metabarcoding and *In Vitro* Assays

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Table S1 : Schedule of phytosanitary treatments in the vineyard of the grape variety *Vitis vinifera* L. cv. Portugieser carried out in the season from 29 April 2019 to 10 September 2019. Application of fungicides followed the local recommendations of the plant protection service (DLR Rheinpfalz). Ozonide was applied at a concentration of 0,8% (v/v). The vineyard is located in Neustadt (Weinstraße), DE (49°22'28.2"N 8°11'28.3"E).

Date	NT	CT	OT
29.04.2019	-	-	ozonide (7)
06.05.2019	-	Netzschwefel Stulln (1), Folpan* 80 WDG (2), Polyram® WG (3)	ozonide (7)
13.05.2019	-	-	ozonide (7)
16.05.2019	-	Dynali (6), Folpan® 80 WDG (2)	-
20.05.2019	-	-	ozonide (7)
27.05.2019	-	Folpan® 80 WDG (2), Talendo® (4)	-
28.05.2019	-	-	ozonide (7)
03.06.2019	-	-	ozonide (7)
05.06.2019	-	Folpan® 80 WDG (2), Talendo® (4)	-
10.06.2019	-	-	ozonide (7)
18.06.2019	-	Folpan® 80 WDG (2), Luna® Experience(5)	ozonide (11)
24.06.2019	-	-	ozonide (7)

26.06.2019	-	Folpan® 80 WDG [2], Vivando® [3]	-
01.07.2019	-	-	ozonide [7]
08.07.2019	-	Folpan® 80 WDG [2], Sercardis® [3]	ozonide [7]
15.07.2019	-	-	ozonide [7]
17.07.2019	-	Folpan® 80 WDG [2], Dynali [6]	-
22.07.2019	-	-	ozonide [7]
29.07.2019	-	Folpan® 80 WDG [2], Systhane™ 20 EW [4]	ozonide [7]
05.08.2019	-	-	ozonide [7]
12.08.2019	-	Funguran®progress [1], Systhane™ 20 EW [4]	-
10.09.2019	HARVEST		

[1] Biofa GmbH, Münsingen, DE
[2] ADAMA Deutschland GmbH, Cologne, DE
[3] BASF SE, Ludwigshafen, DE
[4] Corteva Agriscience Germany GmbH, Munich, DE
[5] Bayer CropScience Deutschland GmbH, Langenfeld, DE
[6] Syngenta Agro GmbH, Maintal, DE
[7] Anseros Klaus-Nonnenmacher GmbH, Tübingen, DE

Table S2: Organisms used for 96-well microtiter-based efficacy assays and the incubation time to final photometric measurement of OD_{600}.

Species	Strain	Final measurement
Acetobacter aceti subsp. *aceti*	[1] DSM 3508	3 d.p.i.
Gluconobacter oxydans	[3] DLR-Isolate Bac31	8 d.p.i.
Levilactobacillus brevis (Basonym: *Lactobacillus brevis*)	[1] DSM 1268	1 d.p.i.
Lactiplantibacillus plantarum subsp. *plantarum* (*Basonym: Lactobacillus plantarum*)	[1] DSM 20205	1 d.p.i
Oenococcus oeni	[1] DSM 20255	6 d.p.i.
Pediococcus sp.	[1] DSM 1056	1 d.p.i.
Brettanomyces bruxellensis	[2] AWRI 1499	1 d.p.i.
Candida zeylanoides	[1] DSM 70185	1 d.p.i.
Hanseniaspora uvarum	[3] DLR-Isolate Y1	1 d.p.i.
Metschnikowia pulcherrima	[1] DSM 70321	1 d.p.i.
Pichia fermentans	[1] DSM 70090	1 d.p.i.
Saccharomyces cerevisiae	[4] Uvaferm 228	1 d.p.i
Schizosaccharomyces pombe var. *pombe*	[1] DSM 70572	1 d.p.i.
Zygosaccharomyces bailii	[1] DSM 70492	1 d.p.i.

[1]) Leibniz institute, DSMZ-German Collection of Microorganism and Cell Cultures GmbH, Braunschweig, DE
[2] AWRI-Australian Wine Reaserch Institute, Adelaide, AU
[3] Field isolates from German vineyards, reviewed and confirmed by sequencing
[4] Lallemand S.A.S.,Montreal, CA

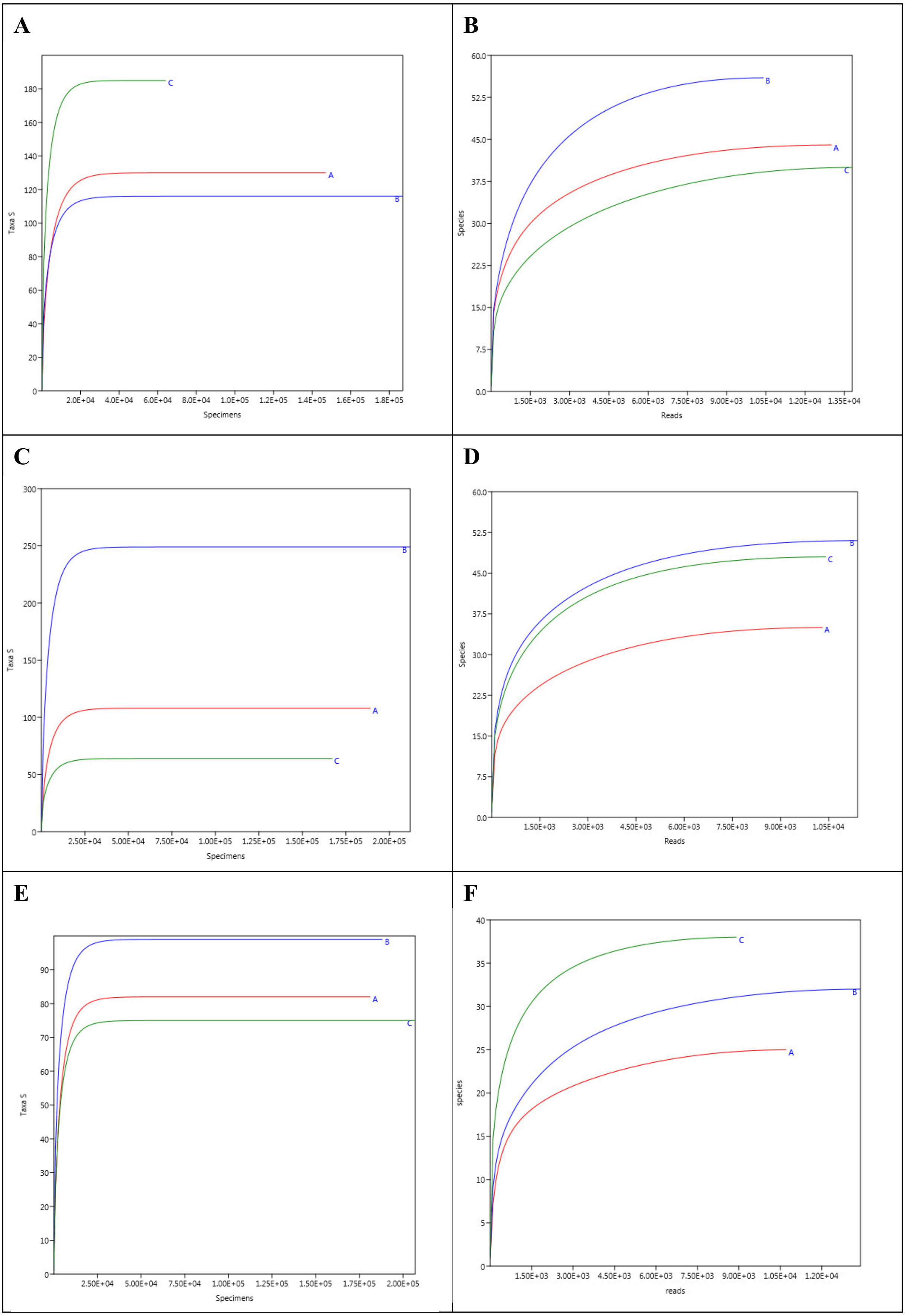
Table S3 : Species share of the individual samples in one triplicate of the same category expressed as percent of bacterial organisms (Proteobacteria, *Pantoea* spp., *Acetobacter* spp., *Lactobacillus* spp. and *Pseudomonas syringae*) or percent of fungal organisms (Ascomycota, Basidiomycota) respectively.

	Proteobacteria	*Pantoea* spp.	*Acetobacter* spp.	*Lactobacillus* spp.	*Pseudomonas syringae*	Ascomycota	Basidiomycota
Portugieser I (NT)	98	90	0	0	5	94	6
	81	70	0	0	2	94	5
	80	53	0	0,4	3	90	10
Portugieser I (CT)	99	90	0,2	0	7	92	8
	85	34	0	0,02	3	89	11
	99	93	0	0	0,6	84	16
Portugieser I (OT)	99	94	0,01	0	4	93	6
	69	66	0	0	1	89	10
	99	96	0,02	0	2	89	11
Portugieser II (NT)	42	18	0	0	0,8	90	8
	66	3	0	0	0,08	69	18
	75	64	0,01	0	0,06	81	13
Portugieser II (CT)	77	62	0	0	0,4	65	32
	50	8	0	0	0,4	13	87
	63	29	0	0	0,3	19	80
Portugieser II (OT)	82	79	0	0	0,2	79	21
	53	47	0	0	0,4	76	23
	64	38	0	0	0,1	84	15

Table S4: In vitro efficacy analysis of non-ozonized oleic acid. All values and standard deviations are expressed in % related to the corresponding negative controls (Table 3). Values significantly deviating from the corresponding C- ($p < 0,05$) are greyed.

	0,25% (v/v) oleic acid	0,4 % (v/v) oleic acid	0,8% (v/v) oleic acid	1,6 % (v/v) oleic acid	2,5 % (v/v) oleic acid	5 % (v/v) oleic acid	C-	C+
Acetobacter aceti	95.0 ± 4.6	99.3 ± 2.4	97.8 ± 3.5	96.4 ± 4.6	96.0 ± 9.3	92.0 ± 1.4	100.0 ± 3.2	0.0 ± 5.1
Gluconobacter oxydans	166.0 ± 20.6	140.0 ± 20.5	145.5 ± 10.4	149.0 ± 25.9	198.8 ± 64.8	140.7 ± 22.7	100.0 ± 17.0	0.0 ± 8.8
Levilactobacillus brevis	114.4 ± 6.4	107.0 ± 5.6	109.6 ± 4.7	112.5 ± 3.7	101.0 ± 5.5	77.2 ± 36.2	100.0 ± 9.1	0.0 ± 2.5
Lactiplantibacillus plantarum	109.2 ± 1.3	109.8 ± 5.9	109.7 ± 1.8	108.6 ± 1.6	104.7 ± 3.0	103.0 ± 2.4	100.0 ± 4.5	0.0 ± 1.7
Oenococcus oeni	147.0 ± 5.9	162.3 ± 43.5	139.6 ± 5.9	135.8 ± 1.1	131.1 ± 2.6	129.7 ± 6.5	100.0 ± 4.2	0.0 ± 3.5
Pediococcus sp.	93.1 ± 2.5	86.4 ± 8.9	84.4 ± 5.2	88.0 ± 0.5	85.4 ± 9.1	18.7 ± 2.7	100.0 ± 6.4	0.0 ± 3.6
Brettanomyces bruxellensis	105.6 ± 12.6	112.2 ± 17.2	113.4 ± 8.9	126.3 ± 37.2	122.2 ± 8.5	122.1 ± 11.1	100.0 ± 13.8	0.0 ± 2.3
Candida zeylanoides	105.2 ± 3.8	135.2 ± 15.0	116.1 ± 12.0	105.5 ± 7.4	101.1 ± 13.0	97.9 ± 18.6	100.0 ± 27.7	0.0 ± 3.5
Hanseniaspora uvarum	100.6 ± 5.8	94.9 ± 1.5	92.7 ± 6.3	97.8 ± 5.4	95.7 ± 3.8	93.8 ± 0.5	100.0 ± 7.6	0.0 ± 5.9
Metschnikowia pulcherrima	98.7 ± 2.9	103.2 ± 3.4	99.4 ± 1.7	96.3 ± 4.0	98.3 ± 4.4	96.9 ± 3.0	100.0 ± 3.3	0.0 ± 1.5
Pichia fermentans	103.6 ± 0.8	107.1 ± 2.1	104.4 ± 1.7	103.4 ± 2.4	105.0 ± 4.3	101.9 ± 8.8	100.0 ± 2.8	0.0 ± 0.6

Saccharomyces cerevisiae	97.4 ± 5.0	107.2 ± 4.7	105.3 ± 4.4	103.1 ± 4.8	108.0 ± 4.1	108.0 ± 3.9	100.0 ± 3.1	0.0 ± 1.0
Schizosaccharomyces pombe	95.0 ± 0.7	99.6 ± 6.7	101.3 ± 6.1	87.2 ± 6.6	104.3 ± 2.0	103.1 ± 2.9	100.0 ± 2.9	0.0 ± 3.6
Torulaspora delbruckii	98.1 ± 5.1	100.2 ± 4.6	91.2 ± 5.6	101.7 ± 3.9	102.1 ± 3.9	102.6 ± 5.4	100.0 ± 2.2	0.0 ± 1.1
Zygosaccharomyces bailii	110.5 ± 7.8	114.9 ± 10.1	112.8 ± 7.9	116.2 ± 14.8	115.7 ± 7.8	127.0 ± 4.8	100.0 ± 43.4	0.0 ± 1.2


A
Taxa S
0 20 40 60 80 100 120 140 160 180
Specimens
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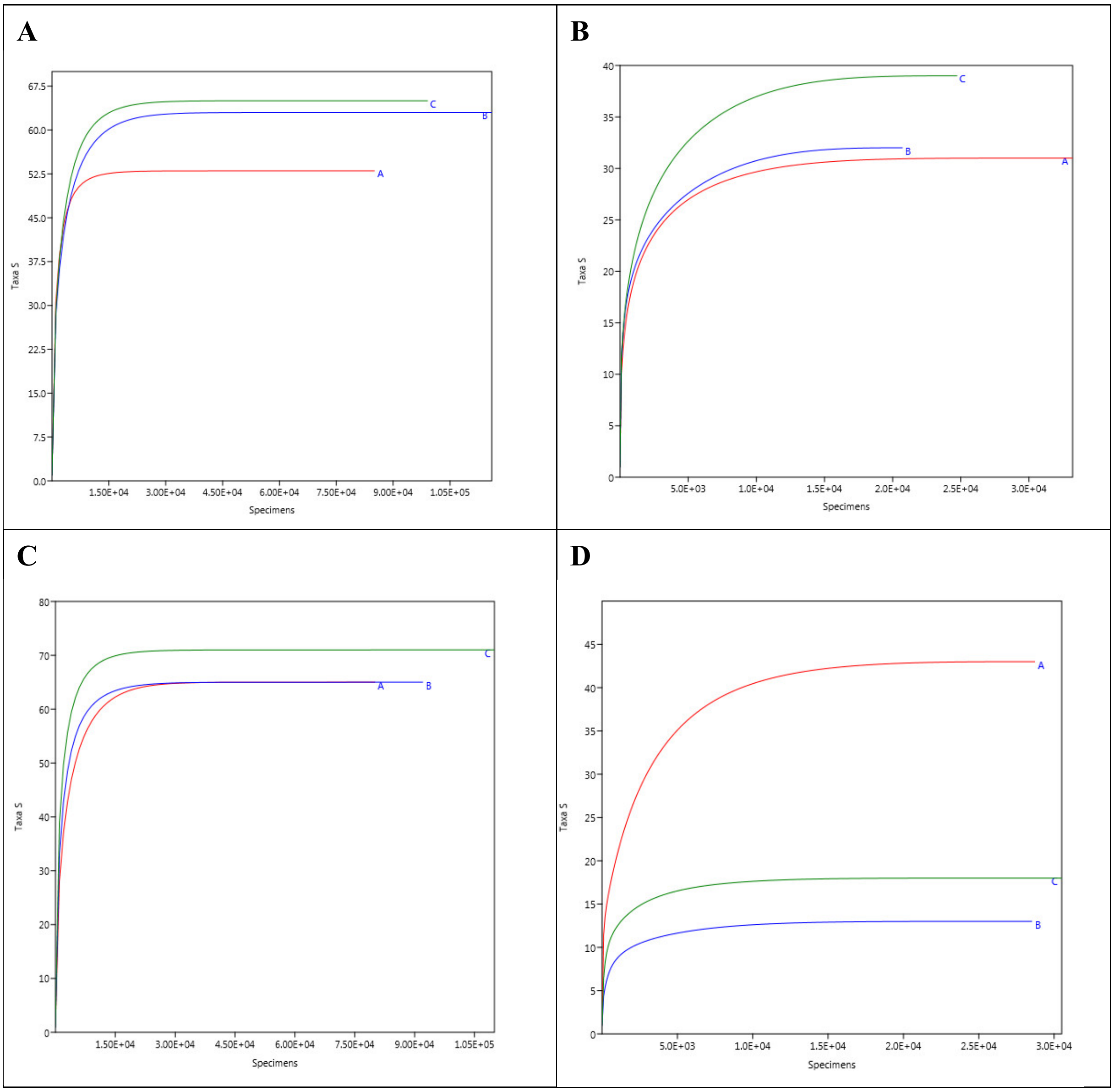
C
A
B
B
Species
0.0 7.5 15.0 22.5 30.0 37.5 45.0 52.5 60.0
Reads
1.50E+03 3.00E+03 4.50E+03 6.00E+03 7.50E+03 9.00E+03 1.05E+04 1.20E+04 1.35E+04
B
A
C
C
Taxa S
0 50 100 150 200 250 300
Specimens
2.50E+04 5.00E+04 7.50E+04 1.00E+05 1.25E+05 1.50E+05 1.75E+05 2.00E+05
B
A
C
D
Species
0.0 7.5 15.0 22.5 30.0 37.5 45.0 52.5 60.0
Reads
1.50E+03 3.00E+03 4.50E+03 6.00E+03 7.50E+03 9.00E+03 1.05E+04
B
C
A
E
Taxa S
0 10 20 30 40 50 60 70 80 90
Specimens
2.50E+04 5.00E+04 7.50E+04 1.00E+05 1.25E+05 1.50E+05 1.75E+05 2.00E+05
B
A
C
F
species
0 5 10 15 20 25 30 35 40
reads
1.50E+03 3.00E+03 4.50E+03 6.00E+03 7.50E+03 9.00E+03 1.05E+04 1.20E+04
C
B
A

Figure S1: Rarefaction curves obtained from 16S sequencing. Portugieser carpoplane sample triplicates of category I NT (A), CT (C) and OT (E) and category II NT (B), CT (D) and OT (F).



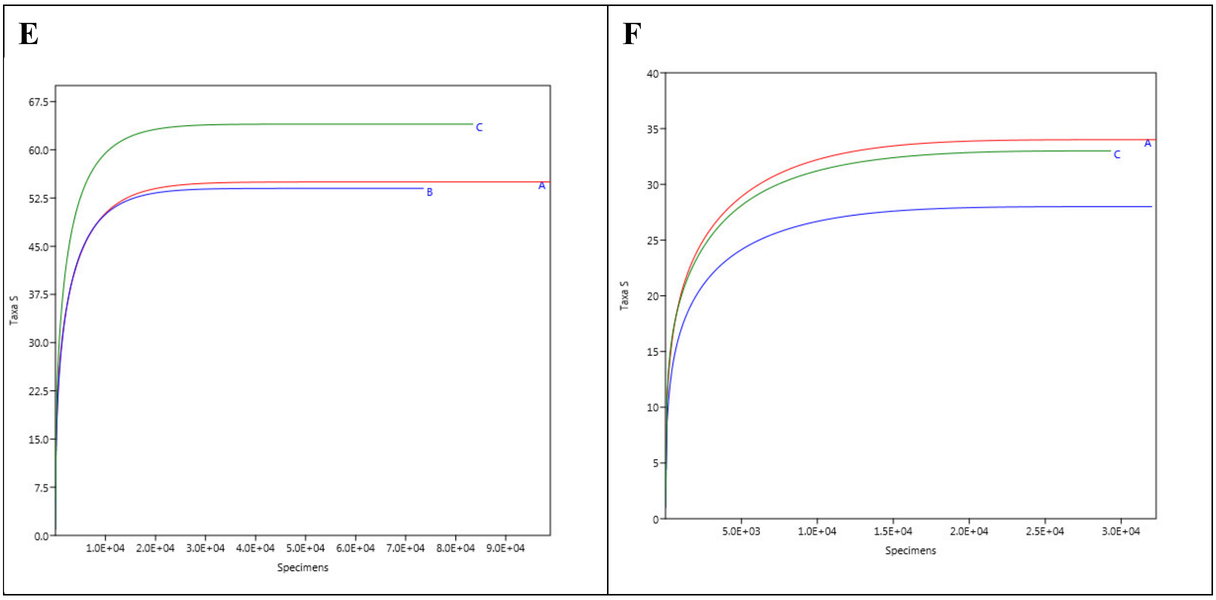
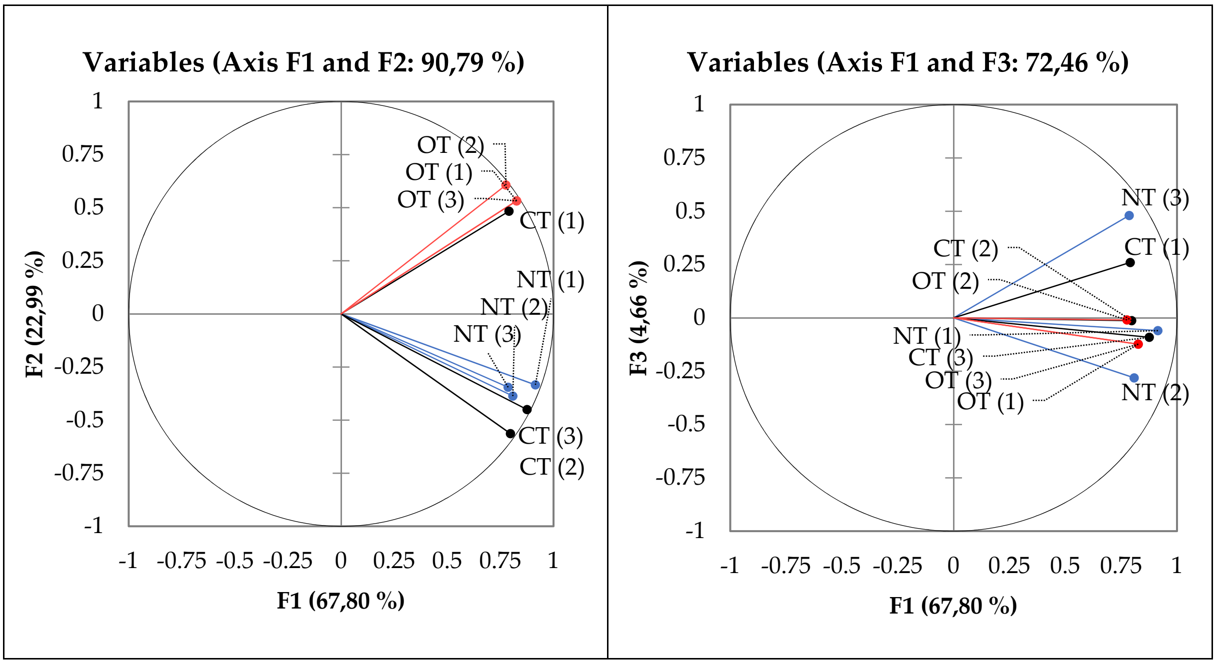


Figure S2 : Rarefaction curves obtained from ITS2 sequencing. Portugieser carpoplane sample triplicates of category I NT (A), CT (C) and OT (E) and category II NT (B), CT (D) and OT (F).



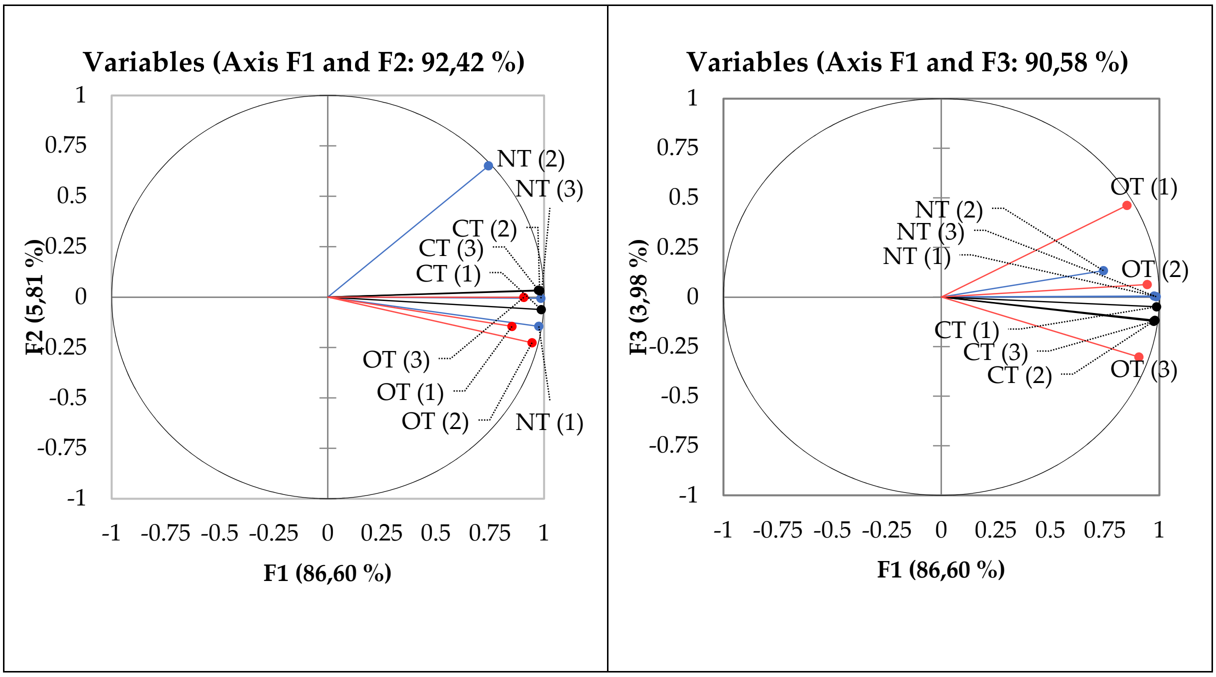
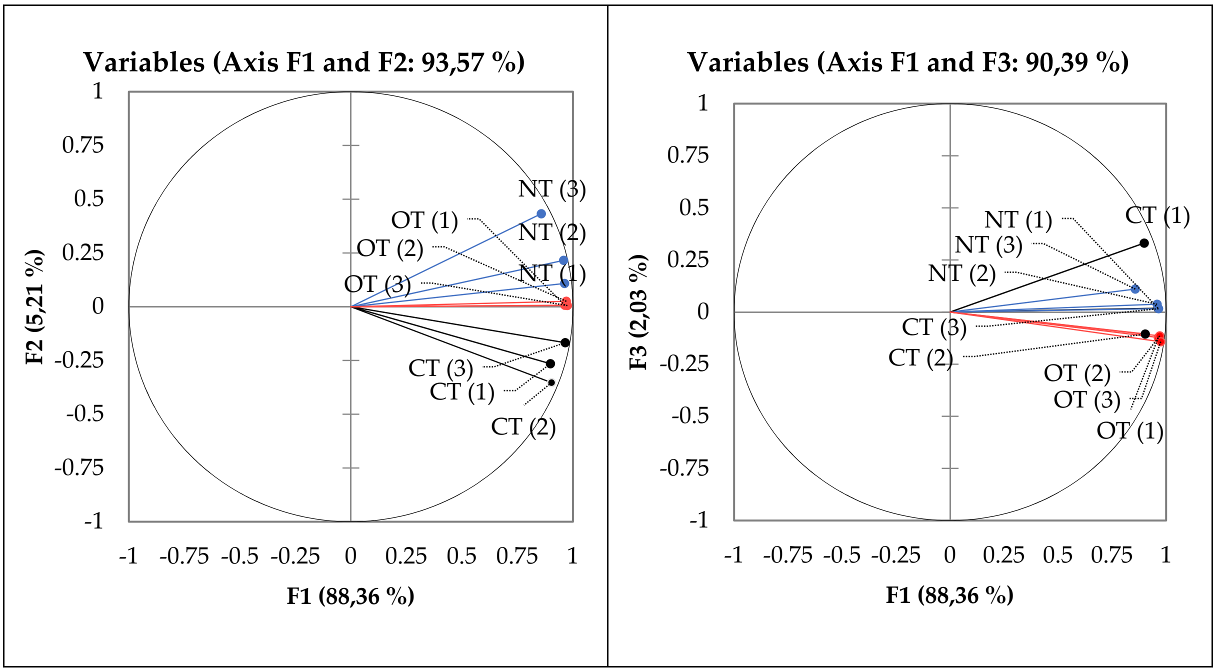


Figure S3: Principal Component Analysis of Portugieser I-16S (top) and Portugieser II-16S (bottom). The correlation circles represent Spearman's Rank correlations ($p < 0{,}05$) of individual samples. Sample types are color coded: NT=blue, OT=red, CT=black.



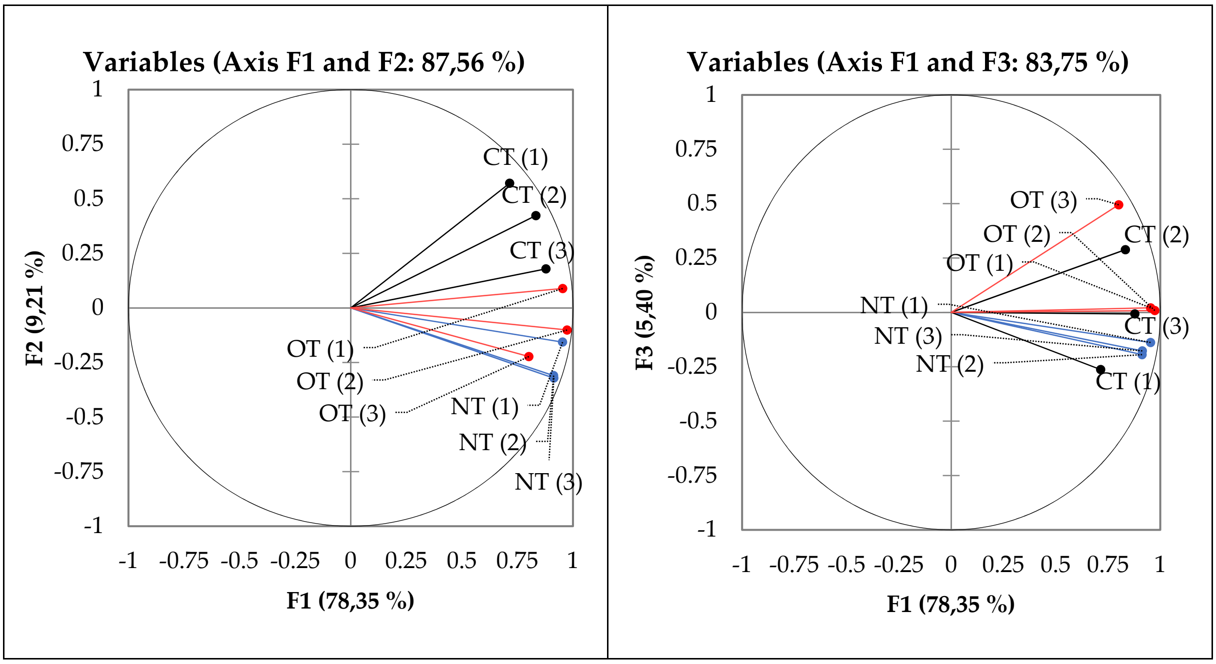


Figure S4: Principal Component Analysis of Portugieser I-ITS2 (top) and Portugieser II-ITS2 (bottom). The correlation circles represent Spearman's Rank correlations ($p < 0,05$) of individual samples. Sample types are color coded: NT=blue, OT=red, CT=black.

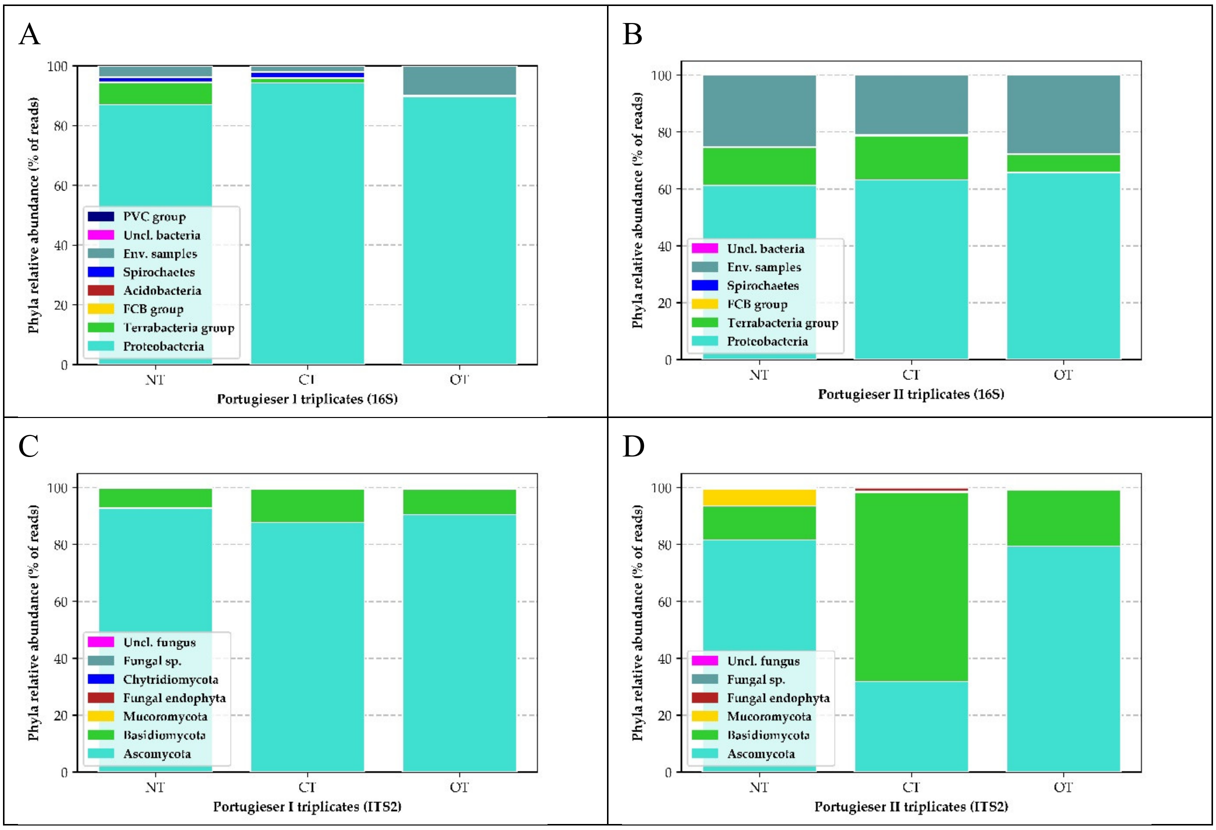


Figure S5: Relative abundance of phyla in Portugieser I and Portugieser II samples expressed in % of the bacterial 16S reads (A, B) and % of the fungal ITS2 reads (C, D). Values derived from sample triplicates after merging and subsampling.

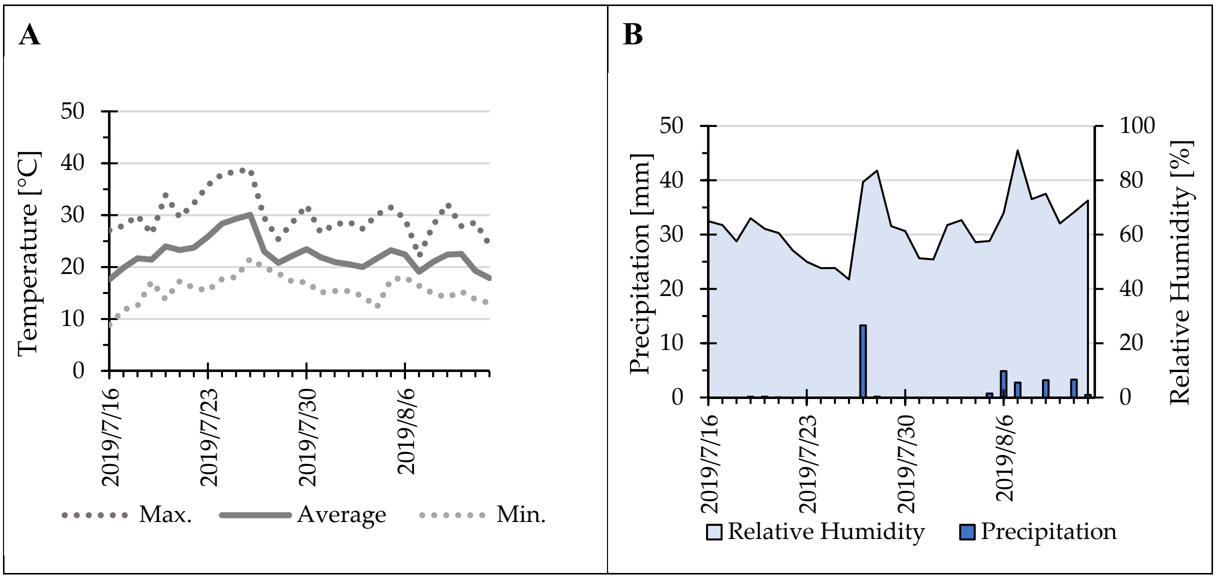


Figure S6: Weather data obtained from the weather station Neustadt (Weinstraße, Rhineland-Palatinate, DE). Source of the daily measures of maximum, average and minimum temperatures (A), precipitation and relative humidity (B) is the government platform Agrarmeteorologie Rheinland-Pfalz (www.Wetter.RLP.de (accessed on 29 March 2022)).