

# MOLECULAR ECOLOGY RESOURCES

Supplemental Information for:

## Microbial networks inferred from metabarcoding data for biomonitoring ecosystem change: strengths and pitfalls

Didac Barroso-Bergada<sup>1§</sup>, Charlie Pauvert<sup>2§</sup>, Jessica Vallance<sup>3,4</sup>, Laurent Delière<sup>3,5</sup>, David A. Bohan<sup>2</sup>,  
Marc Buée<sup>6</sup>, Corinne Vacher<sup>1\*</sup>

**To cite this article:** Barroso-Bergada D., Pauvert C., Vallance J., Delière L., Bohan D.A., Buée M., Vacher C. 2020. Microbial networks inferred from environmental DNA data for biomonitoring ecosystem change: strengths and pitfalls. *Molecular Ecology Resources*, DOI: [10.1111/1755-0998.13302](https://doi.org/10.1111/1755-0998.13302)

<sup>1</sup>BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France

<sup>2</sup>Agroécologie, AgroSup Dijon, INRA, Université Bourgogne, Université Bourgogne Franche-Comté, Dijon, France

<sup>3</sup>INRA, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, F-33882 Villenave d'Ornon, France

<sup>4</sup>Université de Bordeaux, Bordeaux Sciences Agro, UMR 1065 SAVE, F-33175 Gradignan, France

<sup>5</sup>INRA, UE 1442 Vigne Bordeaux, F-33883 Villenave d'Ornon

<sup>6</sup>INRA, UMR 1136 Interactions Arbres/Micro-Organismes, F-54280, Champenoux, France

§ Co-first authors

\* Corresponding author

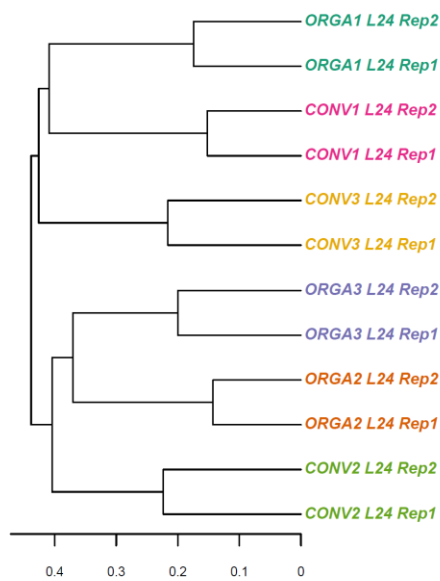
### Table of Contents:

Figure S1	Page 2
Figure S2	Page 3
Figure S3	Page 4
Figure S4	Page 5
Figure S5	Page 6
Table S1	Page 7
Table S2	Page 8
Table S3	Page 8
Table S4	Page 9
Table S5	Page 10
Table S6	Page 11
Table S7	Page 11
Table S8	Page 12
Table S9	Page 12
Table S10	Page 13
Table S11	Page 13

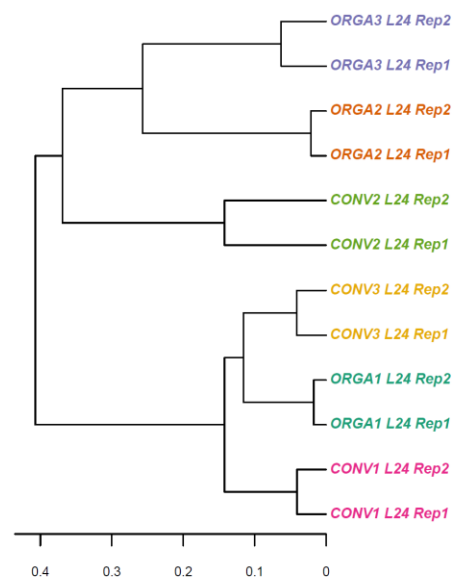
# MOLECULAR ECOLOGY RESOURCES

**Figure S1 - Dendrogram plot of compositional dissimilarities between technical replicates for sequencing.** Technical replicates were created by splitting six lots of PCR products in half and sequencing the two halves independently. The PCR products used were those corresponding to the leaf collected on vine #24 (L24) in each of the six plots studied (ORGA1, ORGA2, ORGA3, CONV1, CONV2, CONV3; see Figure 1). Compositional dissimilarities between samples were computed with (A) the binary Jaccard index and (B) the quantitative Jaccard index. The dendrogram was built using a hierarchical clustering algorithm (complete linkage method). Compositional dissimilarities between the two technical replicates of the same sample were significantly smaller than the dissimilarities among samples (PERMANOVA:  $F = 39.98$ ;  $R^2 = 0.97$ ;  $p = 0.001$ ).

A

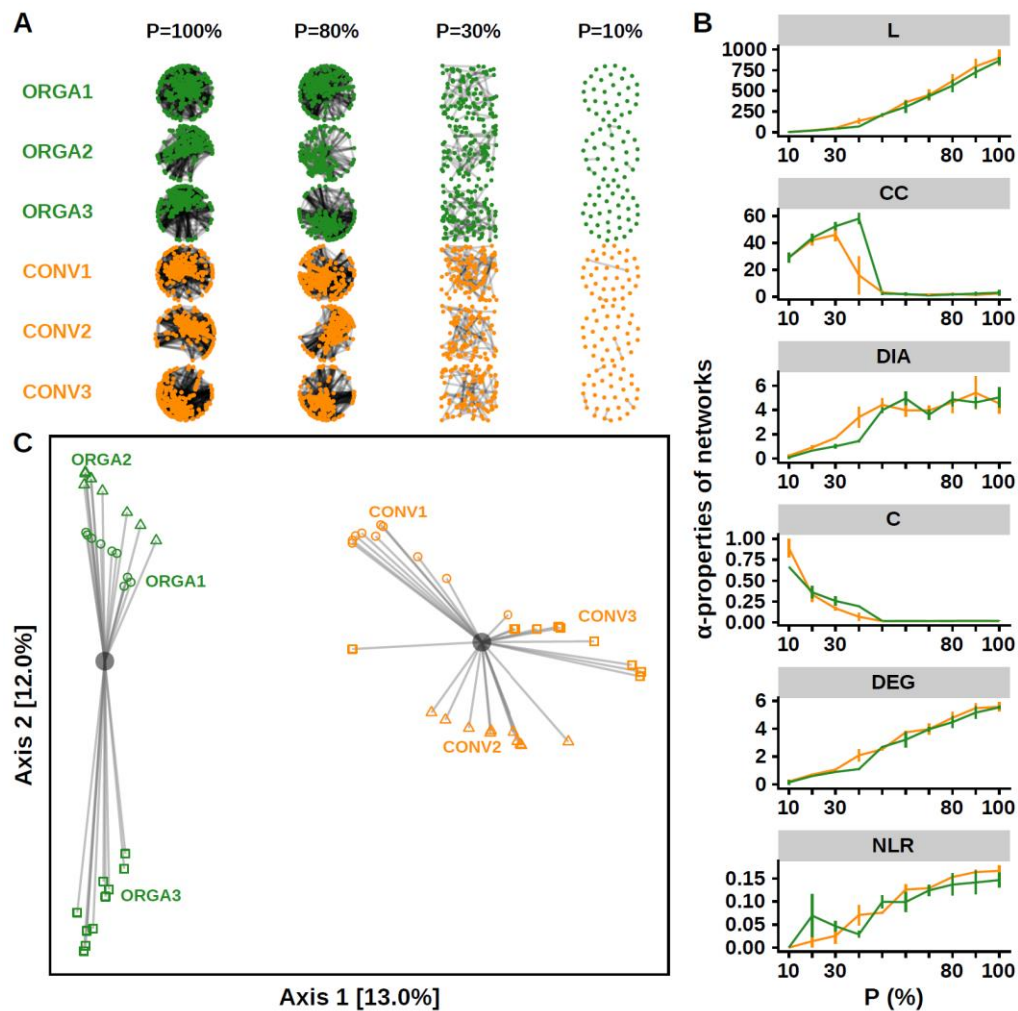


B



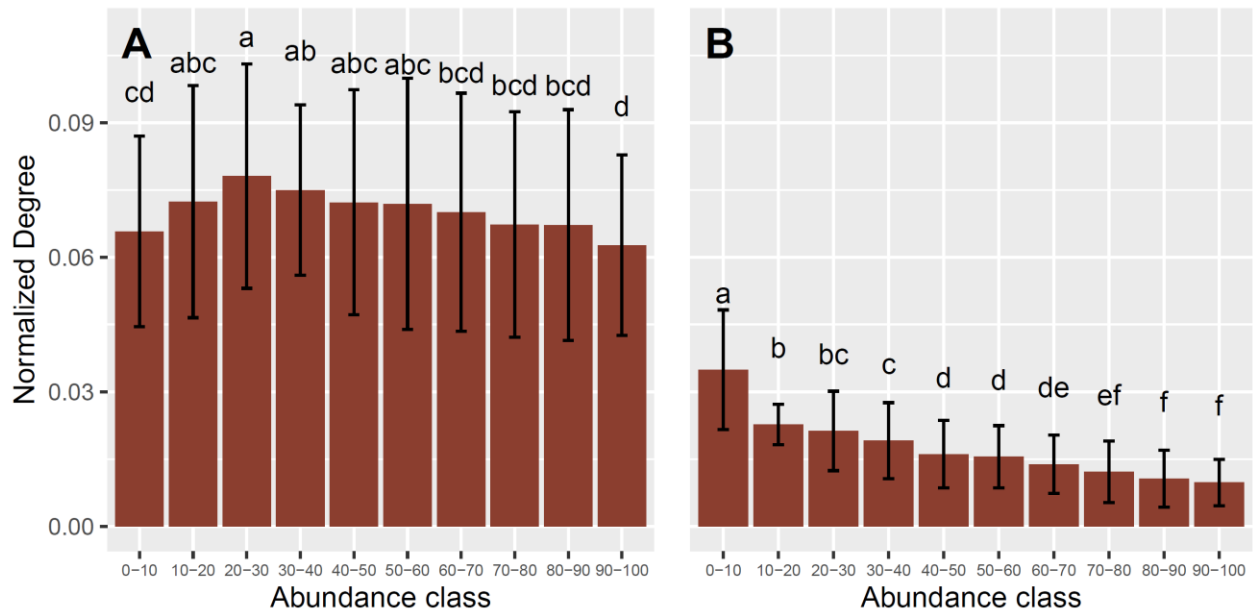
# MOLECULAR ECOLOGY RESOURCES

**Figure S2 - Effect of cropping system — conventional (CONV) versus organic (ORGA) — on the  $\alpha$ -properties and  $\beta$ -propertie of grapevine foliar fungal networks.** (A) Association networks inferred from fungal metabarcoding data with SPIEC-EASI (Kurtz et al, 2015). A total of 60 networks were inferred, corresponding to 2 cropping systems  $\times$  3 replicates (blocks)  $\times$  10  $P$  values, with  $P$  the percentage of most abundant ASVs used for network inference. Only four values of  $P$  are shown on the figure. (B) Variations in network  $\alpha$ -properties. The following properties (Table 1) were calculated for each network: the number of links ( $L$ ) and connected components ( $CC$ ), the network diameter ( $DIA$ ) and connectance ( $C$ ) and the mean degree ( $DEG$ ) and negative link ratio ( $NLR$ ). The percentage  $P$  of ASVs used for network reconstruction had a significant influence on all properties (Table S10), whereas the cropping system did not (Table S8). (C) Principal coordinate analysis (PCoA) represents dissimilarities between networks, measured with the  $\beta_{OS}$  index (Poisot et al, 2012) calculated with the binary Jaccard index.  $\beta_{OS}$  measures the dissimilarity between two networks in terms of the presence-absence of associations between shared ASVs. The centroids for each cropping system are represented by gray circles. The effect of the cropping system on  $\beta_{OS}$  was significant, in interaction with the percentage  $P$  of most abundant ASVs used for network inference (Table S11). Networks were inferred with SPIEC-EASI (Kurtz et al, 2015).



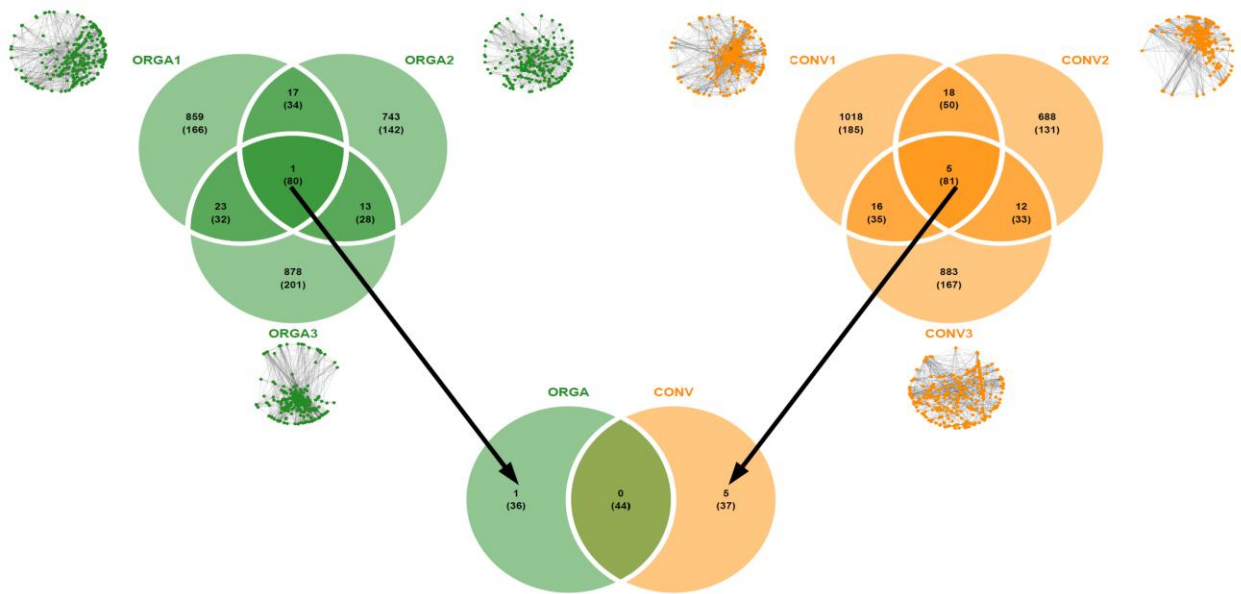
# MOLECULAR ECOLOGY RESOURCES

**Figure S3 - Normalized degree of nodes in networks inferred with (A) SparCC or (B) SPIEC-EASI.** Nodes were classified according to the relative abundance of their corresponding ASVs. Abundance class 0-10 corresponds to the 10% most abundant nodes, while abundance class 90-100 corresponds to the 10% less abundant nodes. Normalized degree was obtained by dividing the node degree by  $n-1$ , where  $n$  is the total number of nodes in the network. The effect of abundance class on the normalized node degree was analyzed with ANOVA followed by post-hoc Tukey's test. Effect of abundance class was significant in both cases (SparCC:  $F = 6.797$ ,  $p < 0.001$ ; SPIEC-EASI:  $F = 173.8$ ,  $p < 0.001$ ).



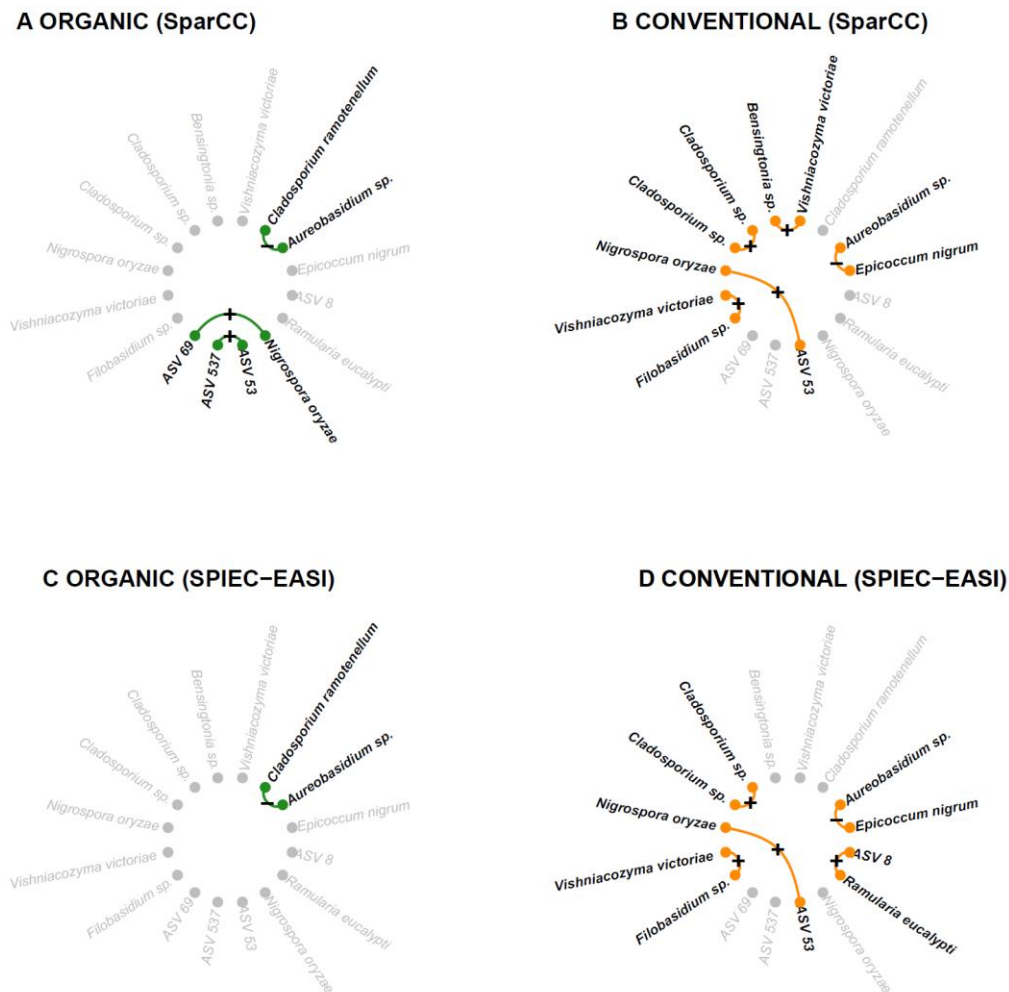
# MOLECULAR ECOLOGY RESOURCES

**Figure S4 - Venn diagrams showing the number of fungal associations common to network replicates.** (A) Associations common to the three network replicates inferred for the organic cropping system (ORGA1, ORGA2, ORGA3) and (B) the three network replicates inferred for the conventional cropping system (CONV1, CONV2, CONV3), regardless of the sign of the association, in the situation in which all ASVs were used for network construction ( $P=100\%$ ). (C) Associations common to the six networks. Networks were inferred with Spiec-Easi (Kurtz et al, 2015). The number of nodes shared by the network replicates is indicated into brackets.



# MOLECULAR ECOLOGY RESOURCES

**Figure S5 - Consensus networks between the three network replicates for the organic (ORGA) and the conventional (CONV) cropping systems depending on the method for network inference.** Network nodes represent fungal ASVs and links represent significant positive (+) or negative (-) associations common to the three network replicates (Fig. 6 and S4). The fungal ASVs absent from a network are indicated in gray. Networks were inferred with SparCC (Friedman & Alm, 2012) or Spiec-Easi (Kurtz et al, 2015).



# MOLECULAR ECOLOGY RESOURCES

**Table S1 - List of phytosanitary products and active ingredients applied in the year of the sampling campaign, together with their normalized dose (also referred to as the treatment frequency index). PM = powdery mildew, caused by the fungal pathogen *Erysiphe necator* and DM = downy mildew, caused by the oomycete pathogen *Plasmopara viticola*. Leaf sampling was performed on September 10, 2015, more than one month after the last phytosanitary treatment and a couple of hours before grape harvest. The treatment frequency index did not differ between cropping systems (ANOVA:  $df = 21$ ;  $F = 0.436$ ;  $p = 0.516$ ).**

Date	Cropping System	Fungicides	Active ingredients	Target disease	
				PM	DM
2015-04-30	ORGA	Heliocuvivre©	Copper		0.145
2015-04-30	ORGA	Citrothiol DG©	Micronized sulfur	0.371	
2015-05-07	CONV	Chaoline©	Fosetyl aluminum + metirame		0.292
2015-05-07	CONV	Dynali©	Cyflufenamid + difenoconazole	0.289	
2015-05-13	ORGA	Heliocuvivre©	Copper		0.167
2015-05-13	ORGA	Citrothiol DG©	Micronized sulfur	0.400	
2015-05-19	CONV	Cabrio Top©	Metirame-zinc + pyraclostrobin	0.500	
2015-05-28	ORGA	Citrothiol DG©	Micronized sulfur	0.800	
2015-05-28	ORGA	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.533
2015-06-04	CONV	Vivando©	Metrafenone	0.833	
2015-06-04	CONV	Chaoline©	Fosetyl aluminum + metirame		0.708
2015-06-09	ORGA	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.533
2015-06-09	ORGA	Citrothiol DG©	Micronized sulfur	0.600	
2015-06-25	ORGA	Citrothiol DG©	Micronized sulfur	0.600	
2015-06-25	CONV	Citrothiol DG©	Micronized sulfur	0.600	
2015-07-01	ORGA	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.533
2015-07-01	CONV	Cabrio Top©	Metirame-zinc + pyraclostrobin	0.750	
2015-07-17	ORGA	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.400
2015-07-17	ORGA	Heliocuvivre©	Copper		0.083
2015-07-17	CONV	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.400
2015-07-17	CONV	Heliocuvivre©	Copper		0.083
2015-08-03	ORGA	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.533
2015-08-03	CONV	Bouillie Bordelaise RSR® Disperss® NC	Copper		0.533

# MOLECULAR ECOLOGY RESOURCES

**Table S2 - Effect of cropping system — conventional (CONV) versus organic (ORGA) — on the incidence and severity of foliar disease symptoms at harvest time (2015-09-07).** Disease incidence is defined as the percentage of leaves displaying symptoms, whereas disease severity is defined as the percentage leaf damage. Symptom incidence and severity were estimated visually on 40 grapevines for each plot (40 × 3 per cropping system). The mean values are reported for each cropping system as a percentage. Wald  $\chi^2$  tests were used for comparisons after linear mixed model analysis with cropping system as a fixed effect and block as a random effect.

Disease		ORGA (%)	CONV (%)	$\chi^2$	p-value
<b>Downy</b>	Incidence	0.749	0.688	0.57	0.450
	Severity	0.037	0.030	1.93	0.164
<b>Powdery</b>	Incidence	0.113	1.346	12.49	<b>&lt;0.001</b>
	Severity	0.003	0.102	7.97	<b>0.005</b>
<b>Black rot</b>	Incidence	0.188	0.354	19.02	<b>&lt;0.001</b>
	Severity	0.007	0.014	5.49	<b>0.019</b>

**Table S3 - Primer pairs used to amplify the fungal ITS1 region**

1 <sup>st</sup> PCR with regular primers (bold)	
Forward	ITS1F: 5'- <b>CTTGGTCATTTAGAGGAAGTAA</b> -3'
Reverse	ITS2: 5'- <b>GCTGCGTTCCTTCATCGATGC</b> -3'
2 <sup>nd</sup> nested PCR with pre-tagged primers (italics)	
Forward	ITS1F-pre-tag: 5'- <i>CTTCCCTACACGACGCTCTCCGATCTCTTGGTCATTTAGAGGAAGTAA</i> -3'
Reverse	ITS2-pre-tag: 5'- <i>GGAGTTCAGACGTGTGCTCTCCGATCTGCTGCGTTCCTTCATCGATGC</i> -3'



# MOLECULAR ECOLOGY RESOURCES

**Table S4 - Effect of cropping system — conventional (CONV) versus organic (ORGA) — on community  $\alpha$ -properties.** Generalized linear mixed models included the cropping system as a fixed treatment effect and the sampling depth as an offset. For every community  $\alpha$ -property (as defined in Table 1), we compared the likelihood of a full model including the block and its interaction with the cropping system as random effects and a simplified model including only the block factor as random effect. Only the results of the best model are shown. The ORGA system was taken as the reference.

Fixed effects	Estimate	SE	z	P(> z )
<b>Richness</b>				
(Intercept)	-6.9569	0.0466	-149.4	<2e-16
Cropping_System (CONV)	-0.1206	0.0554	-2.2	<b>0.029</b>
<b>Diversity</b>				
(Intercept)	-9.5533	0.0655	-145.7	<2e-16
Cropping_System (CONV)	-0.3079	0.1070	-2.9	<b>0.004</b>
<b>Evenness</b>				
(Intercept)	-11.6042	0.0675	-171.9	<2e-16
Cropping_System (CONV)	-0.2810	0.0787	-3.6	<b>&lt;0.001</b>

Random effects	Variance	SD
<b>Richness</b>		
Block	0.0019	0.0433
Residual		
<b>Diversity</b>		
Block	0.000	0.000
Residual	2.126	1.458
<b>Evenness</b>		
Block	0.0025	0.0504
Residual	0.0194	0.1393

# MOLECULAR ECOLOGY RESOURCES

**Table S5 - Effect of cropping system — conventional (CONV) versus organic (ORGA) — on community  $\alpha$ -properties.**

Generalized linear mixed models included the cropping system and the proportion of reads assigned to the *Erysiphe* genus as fixed effects and the sampling depth as an offset. For every community  $\alpha$ -property (as defined in Table 1), we compared the likelihood of a full model including the block and its interaction with the cropping system as random effects and a simplified model including only the block factor as random effect. Only the results of the best model are shown. The ORGA system was taken as the reference.

Fixed effects	Estimate	SE	z	P(> z )
<b>Richness</b>				
(Intercept)	-6.9571	0.0469	-148.4	<2e-16
Cropping_System (CONV)	-0.1255	0.0567	-2.2	<b>0.027</b>
Erysiphe reads %	0.2352	0.6048	0.4	0.697
<b>Diversity</b>				
(Intercept)	-9.5541	0.0652	-146.5	<2e-16
Cropping_System (CONV)	-0.3347	0.1110	-3.0	<b>0.003</b>
Erysiphe reads %	1.3255	1.0417	1.3	0.203
<b>Evenness</b>				
(Intercept)	-11.6060	0.0694	-167.2	<2e-16
Cropping_System (CONV)	-0.3043	0.0802	-3.8	<b>&lt;0.001</b>
Erysiphe reads %	1.2734	0.7344	1.7	0.083

Random effects	Variance	SD
<b>Richness</b>		
Block	0.00197	0.04439
Residual		
<b>Diversity</b>		
Block	0	0
Residual	2.10467	1.4507
<b>Evenness</b>		
Block	0.00281	0.05303
Residual	0.01905	0.13801

# MOLECULAR ECOLOGY RESOURCES

**Table S6 - Effect of cropping system —conventional (CONV) versus organic (ORGA) — on the level of stochasticity in community assembly.** The relative contribution of deterministic and stochastic processes to community assembly was assessed for each cropping system with the Normalized Stochasticity Ratio (NST) defined by Ning et al. (2019), that ranges from 0 to 100, where 0 means a completely deterministic assembly process and 100 a completely stochastic assembly process. NST was calculated using the tNST function with the quantitative and binary Jaccard dissimilarity indices, the FE null model, and other parameters by default values. Differences in NST values between both cropping systems were tested using permutational analysis of variance.

ASV	Dissimilarity index	NST value (%)		F	p-value
		ORGA	CONV		
all	Quantitative Jaccard	29.28	33.62	11.6	0.416
all	Binary Jaccard	78.38	94.80	404.9	<b>0.001</b>

**Table S7 - Effect of cropping system on the  $\alpha$ -properties of fungal association networks inferred with SparCC.** Properties (as defined in Table 1) were compared between cropping systems for every value of the percentage  $P$  of the most abundant ASVs used for network inference. The  $U$  and  $p$ -values of Wilcoxon rank-sum tests are reported. The  $p$ -value is not available (NA) for situations in which property values were equal for all networks. The  $p$ -values after Benjamini-Hochberg adjustment are not reported because all were equal to one.

P (%)	L	CC	DIA	C	DEG	NLR
10	U = 1; p = 0.19	U = 9; p = 0.077	U = 3; p = 0.505	U = 8; p = 0.19	U = 1; p = 0.19	U = 5; p = 1
20	U = 1; p = 0.19	U = 6.5; p = 0.48	U = 7; p = 0.354	U = 2; p = 0.383	U = 2; p = 0.383	U = 9; p = 0.081
30	U = 1; p = 0.19	U = 6; p = 0.505	U = 6.5; p = 0.48	U = 0; p = 0.081	U = 1; p = 0.19	U = 7; p = 0.383
40	U = 1; p = 0.19	U = 6; p = 0.505	U = 5.5; p = 0.814	U = 2; p = 0.383	U = 1; p = 0.19	U = 5; p = 1
50	U = 1; p = 0.19	U = 4.5; p = NA	U = 3; p = 0.505	U = 2; p = 0.383	U = 2; p = 0.383	U = 6; p = 0.663
60	U = 1; p = 0.19	U = 4.5; p = NA	U = 6; p = 0.505	U = 2; p = 0.383	U = 1; p = 0.19	U = 5; p = 1
70	U = 3; p = 0.663	U = 4.5; p = NA	U = 3; p = 0.619	U = 4; p = 1	U = 2; p = 0.383	U = 4; p = 1
80	U = 3; p = 0.663	U = 4.5; p = NA	U = 6; p = 0.505	U = 2; p = 0.383	U = 2; p = 0.383	U = 4; p = 1
90	U = 3; p = 0.663	U = 4.5; p = NA	U = 6; p = 0.505	U = 2; p = 0.383	U = 2; p = 0.383	U = 2; p = 0.383
100	U = 3; p = 0.663	U = 4.5; p = NA	U = 6; p = 0.505	U = 3; p = 0.663	U = 4; p = 1	U = 4; p = 1

# MOLECULAR ECOLOGY RESOURCES

**Table S8 - Effect of cropping system on the  $\alpha$ -properties of fungal association networks inferred with SPIEC-EASI.** Properties (as defined in Table 1) were compared between cropping systems for every value of the percentage  $P$  of the most abundant ASVs used for network inference. The  $U$  and  $p$ -values of Wilcoxon rank-sum tests are reported. The  $p$ -value is not available (NA) for situations in which property values were equal for all networks. The  $p$ -values after Benjamini-Hochberg adjustment are not reported because all were equal to one.

P(%)	L	CC	DIA	C	DEG	NLR
10	U = 2; p = 0.376	U = 5; p = 1	U = 2; p = 0.354	U = 0.5; p = 0.617	U = 2; p = 0.376	U = 1.5; p = NA
20	U = 0; p = 0.081	U = 5; p = 1	U = 3; p = 0.663	U = 5.5; p = 0.814	U = 1; p = 0.19	U = 7; p = 0.354
30	U = 1; p = 0.19	U = 6.5; p = 0.507	U = 0; p = 0.081	U = 7; p = 0.383	U = 0; p = 0.081	U = 7; p = 0.383
40	U = 1; p = 0.19	U = 9; p = 0.081	U = 0; p = 0.081	U = 9; p = 0.077	U = 0; p = 0.081	U = 2; p = 0.383
50	U = 5; p = 1	U = 3; p = 0.653	U = 3; p = 0.663	U = 7; p = 0.383	U = 8; p = 0.19	U = 6.5; p = 0.507
60	U = 3; p = 0.663	U = 5; p = 1	U = 8; p = 0.19	U = 3; p = 0.663	U = 3; p = 0.663	U = 2; p = 0.383
70	U = 4; p = 1	U = 1.5; p = 0.188	U = 2; p = 0.383	U = 5; p = 1	U = 6; p = 0.663	U = 4; p = 1
80	U = 4; p = 1	U = 4; p = 1	U = 4; p = 1	U = 3; p = 0.663	U = 4; p = 1	U = 3; p = 0.663
90	U = 3; p = 0.663	U = 5; p = 1	U = 4; p = 1	U = 4; p = 1	U = 2; p = 0.383	U = 3; p = 0.663
100	U = 3; p = 0.663	U = 4; p = 1	U = 4; p = 1	U = 6; p = 0.663	U = 3; p = 0.663	U = 2; p = 0.383

**Table S9 - Effect of the percentage  $P$  of the most abundant ASVs used for network inference on the  $\alpha$ -properties of fungal association networks inferred with SparCC.** Spearman's correlation coefficient and the results of Spearman's rank correlation tests are reported for each network property (as defined in Table 1). The  $p$ -values are reported after Benjamini-Hochberg adjustment.

Property	Correlation ( $\rho$ )	S	$p$ -value
L	0.98	839	<0.001
CC	-0.63	58685	<0.001
DIA	-0.84	66296	<0.001
C	-0.71	61374	<0.001
DEG	0.95	1647	<0.001
NLR	-0.57	56550	<0.001

# MOLECULAR ECOLOGY RESOURCES

**Table S10 - Effect of the percentage  $P$  of the most abundant ASVs used for network inference on the  $\alpha$ -properties of fungal association networks inferred with SPIEC-EASI.** Spearman's correlation coefficient and the results of Spearman's rank correlation tests are reported for each network property (as defined in Table 1). The  $p$ -values are reported after Benjamini-Hochberg adjustment.

Property	Correlation ( $\rho$ )	S	$p$ -value
L	0.98	621	<0.001
CC	-0.69	60722	<0.001
DIA	0.79	7723	<0.001
C	-0.69	55067	<0.001
DEG	0.97	971	<0.001
NLR	0.84	5158	<0.001

**Table S11– Effect of cropping system — conventional *versus* organic — on the  $\beta$ -properties of grapevine foliar fungal networks inferred with SPIEC-EASI.** The D index quantifies the topological dissimilarity between networks (Schieber et al, 2017) whereas the other three metrics ( $\beta_{WN}$ ,  $\beta_{OS}$  and  $\beta_{ST}$ ), which were calculated with the binary Jaccard index, quantify differences in associations between networks (Poisot et al, 2012). The effect of the percentage  $P$  of the most abundant ASVs used for network inference, and the effect of cropping system on the dissimilarities between networks were evaluated in permutational analysis of variance (PERMANOVA). The number of permutations was set to 999 and permutations were constrained by block.

Dissimilarity index	PERMANOVA				
	Variable	Df	F	R2	Pr(>F)
Topological dissimilarity (Schieber's D)	Percent_ASV (P)	1	100.89	0.65	<0.01
	Cropping_System (CS)	1	0.99	0.01	0.31
	P × CS	1	0.31	0.00	0.68
	Residuals	54		0.35	
	Total	57		1	
Overall dissimilarity of associations ( $\beta_{WN}$ )	Percent_ASV (P)	1	2.689	0.04	<0.01
	Cropping_System (CS)	1	5.060	0.08	<0.01
	P × CS	1	2.547	0.04	<0.01
	Residuals	54		0.84	
	Total	57		1	
	Percent_ASV (P)	1	3.863	0.06	<0.01

# MOLECULAR ECOLOGY RESOURCES

Dissimilarity of associations between shared ASVs ( $\beta_{os}$ )	Cropping_System (CS)	1	8.799	0.13	<0.01
	P × CS	1	3.145	0.05	<0.01
	Residuals	54		0.77	
	Total	57		1	
Dissimilarity of associations due to ASV turnover ( $\beta_{st}$ )	<b>Variable</b>	<b>Df</b>	<b>F</b>	<b>R2</b>	<b>Pr(&gt;F)</b>
	Percent_ASV (P)	1	0.2790	0.01	1.00
	Cropping_System (CS)	1	0.2259	0.01	1.00
	P × CS	1	0.2948	0.01	1.00
	Residuals	54		0.97	
	Total	57		1	