# Supporting information

# **Appendix 1** Soil sampling, DNA extraction, sequencing, bioinformatics and sequence preprocessing

5 In each country, soil samples were taken at least one season after experimental plot preparation (2016 6 and/or 2017) and at minimum 6 weeks after tillage/herbicide application around the time of grapevine 7 flowering. Eight subsamples were taken in the two central inter-rows of each plot to a depth of 10 cm 8 approximately 30 – 50 cm apart from each other and were pooled afterwards into one mixed sample, 9 summing up to a total of 405 soil samples. The number comprises n= 54 samples for Austria 2016, France 2017, Romania 2017, Germany 2016 and Germany 2017, but n = 77 for Switzerland 2016 and n = 58 for Switzerland 2017. Thus, we included samples for bacteria and fungi for each country for at least one season (2016 for Austria, 2017 for France and Romania) or two seasons for Switzerland and Germany. DNA extraction from each soil sample was either performed directly after soil sampling or samples were stored frozen at -20° C until extraction. DNA was extracted from 0.25 g of mixed soil sample per inter-row by using the DNeasyPowerSoil Kit® following the manufacturer's protocol (QIAGEN N.V., Venlo, Netherlands).

 DNA sequencing was conducted for the bacterial V4 region of the 16S rDNA using the primers 515f: 5'- GTGYCAGCMGCCGCGGTAA-3' and 806rB: 5'-GGACTACNVGGGTWTCTAAT-3' (1), and for the fungal Internal transcribed spacer ITS2 using the primer pair ITS4: 5'-TCCTCCGCTTATTGATATGC-3' and fITS7: 5'-GTGARTCATCGAATCTTTG-3' (2, 3). Sequencing of 250bp paired-end amplicons was conducted on a MiSeq Illumina machine at Génome Quebec Innovation Centre (Montreal, Canada).

 Raw Illumina fastq reads were quality controlled with FastQC (4), generally showing good quality. The reads were cleaned and filtered using sickle (5).

 For Bacteria, the software package Mothur (version 1.39.5) was used for sequence analysis (6) while following the Standard Operating Procedure outlined on http://www.mothur.org/wiki/MiSeq\_SOP.

 Briefly, the two overlapping paired-end reads were combined using make.contig. Then, each unique sequence was aligned with align.seqs to the SILVA reference alignment release 132 (7). A distance matrix was calculated allowing for four mismatches. Chimeric sequences were identified using chimera.uchime and removed. Sequences matching "Chloroplast-Mitochondria-unknown-Archaea- Eukaryota" were also removed. Next, sequences were clustered using the opticlust clustering algorithm (8) to build operational taxonomic units (OTUs). The resulting file was parsed to separate the data for each sample. OTUs were assigned a taxonomic group with classify.seqs using the RDP reference file release 11 (9) and a cut-off of 80% of the bootstrap value. For the description of the community, the sequences are split at the order level and OTUs with the same taxonomy were clustered together at cut-off level 0.03. Mothur was used to convert data into biom format files.

 Sequence analysis for fungi was conducted using the software package PIPITS (version 1.3.x, (10)). This was necessary because Mothur relies on alignment of all sequences which is not possible for fungi. PIPITS generates a biom file for OTU with the UNITE fungal ITS reference set based on the RDP classifier (11).

 Downstream sequence preprocessing was done in R version 3.4.2 (12) with the package phyloseq (13). Unassignable sequences not belonging to the kingdom of bacteria or fungi were removed. OTU tables were rarefied (set.seed(631)) and low abundances (< 0.1%) removed. DNA samples with number of sequences lower than 50 % of the mean number of sequences of all samples per country were removed 44 from the analysis (bacteria:  $n = 6$ ; fungi:  $n = 4$ ).

#### Appendix 2: Microbial taxa responding to different disturbance levels

#### Methods

 To evaluate which microbial taxa were associated with different disturbance levels, we used the Indicator Value approach on the bacterial and fungal abundance data sets at the European level and for each country (14). The approach allows to identify taxa with a preference or sensitivity to a certain disturbance level. The Indicator Value shows associations of taxa with samples belonging to certain  target groups (in our case different disturbance levels) by calculation of specificity, which signifies the probability of the site belonging to the target group, given that the taxon has been at that site, and sensitivity, which signifies the probability of a taxon occurring at a site of the target group. We conduct the Indicator Value analysis using the multipatt function of the indicspecies package (14). We tested all soil disturbance levels as target groups including their combinations and applied permutation tests (n = 10000) using 'vineyard' as block variable to test for significance of associations between taxa and groups. We only reported taxa with Indicator Values > 0.6 and p < 0.05.

#### Results

 Using the Indicator Value approach, we identified two bacterial genera but no fungi that were associated with soil disturbance at the European level (Table S9). The bacterial genera *Hymenobacter* and *Pontibacter* were identified as indicator taxa for the combined disturbance levels 'intermediate + high', suggesting that these genera were less abundant in soils of low disturbance.

 At the level of countries, we found various taxa associated with different disturbance levels (Table S9). Here, we only report taxa that show a consistent pattern in more than one country . Four bacterial taxa and three fungal taxa were assigned as indicators for disturbance levels in more than one country, however, no taxon was found that was assigned as indicator in all countries. The bacterial genus *Dyadobacter* was associated with the combined disturbance level 'low + intermediate' in Austria and Switzerland, indicating that *Dyadobacter* were sensitive too high disturbance. The bacterial genera *Hymenobacter*, *Pontibacter* (in accordance with the European level) and the assemblage of unclassified members of the phylum *Armatimonadetes* were identified as indicators for the combined disturbance level 'intermediate + high' in Austria, Germany, and France.

 The fungal family *Glomeraceae* was identified as indicator taxon for the combined disturbance level 'low + intermediate' in Austria and Germany suggestion susceptibility of this family to high disturbance.

 The species *Sarocladium strictum* was associated with disturbance level 'low + intermediate' in Germany and France. The species *Spizellomyces dolichospermus* was an indicator for the combined soil disturbance 'intermediate + high' for Germany and France.

#### Discussion

 The Indicator Value approach evaluates the predictive power of taxa for different habitats (in our case soil disturbance levels). The approach is suitable to find taxa that show contrasting patterns between different habitats rather than identifying small changes of taxa abundances (14). At the level of individual countries, we identified several taxa with a prediction power for different disturbance levels. This shows that the changes in community composition indicated by PERMANOVA was accompanied by strong changes of abundances of individual taxa.

 However, we only found 4 bacterial and 3 fungal taxa that were identified as indicators for different disturbance levels in more than one country. This is a low number, given that we found a total number of 504 bacterial and 916 fungal taxa across all countries. This corroborates our conclusion that responses of microbial communities in one region cannot be simply extrapolated to other regions because of different local community composition and environmental conditions.

 The bacterial genus *Dyadobacter* was identified as indicator for low and intermediate disturbance, i.e., it is rare in highly disturbed soils. The genera *Pontibacter* and *Hymenobacter* as well as the group of unclassified *Armatimonadetes* were associated with samples under intermediate to high soil disturbance. The functions of these bacteria, commonly found in soil, are largely unknown. However, the phylum *Armatimonadetes* has previously been found to be positively correlated with tilled soils and negatively with cover crops in Californian vineyards (15).

 The fungal family *Glomeraceae*, whose members are known to interact with plant roots forming arbuscular mycorrhiza, was associated with low to intermediate disturbed soils. It is known that arbuscular mycorrhizal fungi are susceptible to tillage because of disruption of mycelial networks (16).

 Similarly, the application of Glyphosate caused a decline of arbuscular mycorrhizal fungi in green house experiments (17). Grapevines also benefit from mycorrhizal symbiosis (16), thus, supporting Glomeromycotan diversity by reducing soil disturbance practices might be beneficial for grapevine growth.

 The fungal species *Sarocladium strictum* and *Spizellomyces dolichospermus* were also identified as indicator species. While *S. dolichospermus* indicated intermediate to highly disturbed soils, *S. strictum*, which is known as a plant pathogen in many crops (18), was associated with low to intermediate disturbed soils.

#### Appendix 3 Relative abundances of taxa

 The most abundant bacterial phyla (> 1% total relative abundance) were *Acidobacteria* (~30%), *Proteobacteria* (~21%), *Actinobacteria* (~14%), *Planctomycetes* (~5%), *Bacteroidetes* (~4%), *Verrucomicrobia* (~3.5%), *Chloroflexi* (~1.5%), *Gemmatimonadetes* (~1.3%) and unclassified *bacteria*  (~17%).

 The most abundant fungal orders (> 1% total relative abundance) were *Hypocreales* (~18%)*, Pleosporales* (~11%), *Sordariomycetes* (~9%), *Helotiales*(~5.1%), *Dothideomycetes* (~4%), *Xylariales*  (~3.3%), *Mortierellales* (~4.4%), *Tremellales* (~3.2%), *Sordariales* (~3.2%), *Agaricales* (~2%), *Capnodiales* (~1.8), *Chaetothyriales* (~1.5%), *Pezizales* (~1.3%). Sequences not assignable to order level, like *Ascomycota* (~11%) and 'unclassified fungi' (~13%) did also contribute to a relevant extent to fungal community composition.

# **Appendix 4** Supplementary figure and tables





 Fig. S1 Map showing localities of vineyards in vine-growing regions of five different countries (red circle: AT, Austria (Kamptal, Kremstal, Leithaberg); purple inverse triangle: CH, Switzerland (Valais); green diamond: DE, Germany (Rheinhessen); ocher square: FR, France Bordeaux, Libournais; blue triangle: RO, Romania (Dobrogea))

#### Supplementary tables

- Table S1 Details of vineyards used for the study.
- Table S2 Implementation of vineyard soil disturbance levels "low", "intermediate", "high" in the
- countries involved in the study.
- Table S3 Ranges, mean values and standard deviations of soil covariates.
- Table S4 Summary of general linear mixed model fit by maximum likelihood for each soil covariate.
- Table S5 Summary of coefficients from linear mixed effect model fitted by maximum likelihood for
- bacterial OTU richness.
- Table S6 Summary of coefficients from linear mixed effect model fitted by maximum likelihood for bacterial OTU Shannon Index.
- Table S7 Summary of coefficients from linear mixed effect model fitted by maximum likelihood for
- fungal richness fungal OTU richness.
- Table S8 Summary of coefficients from linear mixed effect model fitted by maximum likelihood for
- 146 fungal fungal OTU Shannon Index.
- Table S9 Results of Indicator species analysis of bacterial and fungal communities at European and country level.
- Table S10 Coefficients from linear mixed effect model fitted by maximum likelihood for microbial respiration.
- Table S11 Summary coefficients from linear mixed effect model fitted by maximum likelihood for
- decomposition of labile substrate.
- Table S12 Summary coefficients from linear mixed effect model fitted by maximum likelihood for
- decomposition of recalcitrant substrate.
- Table S13 ANOVA table of db-RDA results (a) and variance inflation factors (VIF, b) at the European and
- country scale. Table S14: Variance partitioning for soil bacterial and fungal communities based on db-
- RDA.
- 

Details of vineyards used for the study. Countries: AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania; initial state of inter-row treatment before installation of experimental plots: ah, alternating herbicide, at, alternating tillage; cc, complete cover; ti, complete tillage; ch, complete herbicide. Details regarding inter-row treatments are given in Table S2.





 $1$  Vineyards were organically managed

Implementation of vineyard soil disturbance levels "low", "intermediate", "high" in the countries involved in the study (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania). Denomination, in italics, and description of soil treatments are given.

![](_page_9_Picture_249.jpeg)

Range, mean values and standard deviation for the included soil parameter covariates (OC [%] = organic carbon; C/N ratio = carbon/nitrogen ratio; Cu [mg/kg] = bioavailable soil copper content; pH = soil pH; clay = clay content in %) at the different disturbance levels (1 = low; 2 = intermediate, 3 = high) in each country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania).

![](_page_10_Picture_227.jpeg)

Summary of general linear mixed model fit by maximum likelihood for the response of each soil covariate (OC [%] = organic carbon; C/N ratio = carbon/nitrogen ratio; Cu [mg/kg] = bioavailable soil copper content;  $pH = soil pH$ ; clay = clay content in %) to the disturbance gradient (low = 1; intermediate = 2; high = 3). t-tests used Satterthwaite's method ['lmerModLmerTest'].

![](_page_11_Picture_53.jpeg)

Summary of coefficients from linear mixed effect model fitted by maximum likelihood for bacterial OTU richness. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at p < 0.001; '\*\*' significant at  $p < 0.01$ ; '\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_12_Picture_218.jpeg)

Summary coefficients from linear mixed effect model fitted by maximum likelihood for bacterial OTU shannon index. Plot within vineyard was included in the models as nested random factor (1|vineyard/plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at p < 0.001; '\*\*' significant at  $p < 0.01$ ; "\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_13_Picture_212.jpeg)

Summary coefficients from linear mixed effect model fitted by maximum likelihood for fungal OTU richness. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at p < 0.001; '\*\*' significant at  $p < 0.01$ ; "\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_14_Picture_222.jpeg)

Summary coefficients from linear mixed effect model fitted by maximum likelihood for fungal OTU shannon index. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at  $p < 0.001$ ; '\*\*' significant at  $p < 0.01$ ; '\*' significant at  $p < 0.05$ ; '.' significant at p < 0.1) L.

![](_page_15_Picture_297.jpeg)

Results of Indicator species analysis of bacterial and fungal communities at European and country level (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania). Values for specitivity (Value A) and sensitivity (value B) and indicator values (Ind. Val. )follow De Cáceres, Legendre, 2009. P-values based on permutation tests ('\*\*\*' significant at p < 0.001; '\*\*' p < 0.01; '\*' p < 0.05; '.' p < 0.1). Relative abundances correspond to total sequence numbers in the given data set. Indicator taxa for individual or combined levels of soil disturbance are shown, if they met the pre-defined criteria (Ind. Value > 0.6, p < 0.05). Indicator taxa for the European level or for at least two countries are given in bold.

![](_page_16_Picture_212.jpeg)

![](_page_17_Picture_322.jpeg)

![](_page_18_Picture_292.jpeg)

![](_page_19_Picture_264.jpeg)

## **soil disturbance 'low + intermediate'**

![](_page_19_Picture_265.jpeg)

#### **soil disturbance 'intermediate**

![](_page_20_Picture_193.jpeg)

Coefficients from linear mixed effect model fitted by maximum likelihood for microbial respiration. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at p < 0.001; '\*\*' significant at  $p < 0.01$ ; '\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_21_Picture_219.jpeg)

Summary coefficients from linear mixed effect model fitted by maximum likelihood for decomposition of labile substrate. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at  $p < 0.001$ ; '\*\*' significant at  $p < 0.01$ ; '\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_22_Picture_218.jpeg)

Summary coefficients from linear mixed effect model fitted by maximum likelihood for decomposition of recalcitrant substrate. Plot within vineyard was included in the models as nested random factor (1|vineyard/ plot/year) and country (AT, Austria; CH, Switzerland; DE, Germany; FR, France; RO, Romania) was included as interaction term for all analyses. The symbol ":" in the fixed factor collumn, indicates the interaction of the fixed factor with the country. (Significances were obtained by t-tests using Satterthwaite's method; '\*\*\*' significant at  $p < 0.001$ ; '\*\*' significant at  $p < 0.01$ ; '\*' significant at  $p < 0.05$ ; '.' significant at  $p < 0.1$ )

![](_page_23_Picture_345.jpeg)

ANOVA table of db-RDA results (a) and variance inflation factors (VIF, b) at the European and country scale. db-RDA results were obtained by stepwise selection of variables using the AIC criterion. Only retained vairables shown here. OC: organic carbon; C/N: carbon/nitrogen ratio; Cu: bioavailable soil copper content; pH; clay = clay content, dist = soil disturbance. Df: degrees of freedom; SumOfSqs: Sum Squares; F: pseude-F value based on 9999 permutations.

![](_page_24_Picture_148.jpeg)

#### **Appendix 5** Detailed acknowledgements

 The German team would like to thank Olivia Herczynski for soil DNA extraction and is grateful for the student assistants who supported us during field work and in the lab. The contract of Brice Giffard (France) was financed in 2017 via a grant from the PromESSinG project. BG thanks Benjamin Joubard for soil sampling and DNA extraction, and Pauline Tolle (Bordeaux Sciences Agro) for setting up the management types in vineyards and coordination of the French vineyard network. The Austrian team wants to thank Lisa Cibej, Rudi Rizzoli, Sarhan Khalil and Bettina Schlossnikel for their help in data acquisition during different periods of the project. The Romanian team is grateful to Oana Paula Popa for soil DNA extraction, Sergiu Ene, Sabina Vlad, Razvan Zaharia for their help with data acquisition, and to Oana Belu, Aurora Ranca and Ion Vladoi for their collaboration and interest in the project and for granting us the access to their fields and for their collaboration in the project. The Swiss team is grateful to Andy Brown, Anne-Laure Fragnière, Franziska Keller and Mervi Laitinnen, Lara Volery and Divija Jatavallabhula as well as Nina Häner and Alix Badel for their dedication to the project and their help in data acquisition.

## Supplementary references

- 176 1. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M et al. Evaluation of general 16S
- ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity
- studies. Nucleic Acids Res 2013; 41(1):e1.
- 2. Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J et al. New primers to
- amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities.
- FEMS Microbiol Ecol 2012; 82(3):666–77.
- 3. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA et al. Nuclear ribosomal
- internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A 2012; 109(16):6241–6.
- 4. FastQC: A quality control tool for high throughput sequence data.; 2010. Available from: URL: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
- 5. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files. Version 1.33; 2011.
- Available from: URL: https://github.com/najoshi/sickle.
- 6. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al. Introducing mothur:

open-source, platform-independent, community-supported software for describing and comparing

- microbial communities. Appl Environ Microbiol 2009; 75(23):7537–41.
- 7. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P et al. The SILVA ribosomal RNA gene
- database project: improved data processing and web-based tools. Nucleic Acids Res 2013;
- 41(Database issue):D590-6.
- 8. Westcott SL, Schloss PD. OptiClust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units. mSphere 2017; 2(2).
- 9. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 2014; 42(Database issue):D633-42.
- 10. Gweon HS, Oliver A, Taylor J, Booth T, Gibbs M, Read DS et al. PIPITS: an automated pipeline for
- analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods in Ecology and Evolution 2015; 6(8):973–80.
- 11. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M et al. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 2013; 22(21):5271–7.
- 12. R Core Team. R: A language and envrionment for Statistical Computing; 2019. Available from: URL: https://www.R-project.org/.
- 13. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 2013; 8(4):e61217.
- 14. Cáceres M de, Legendre P. Associations between species and groups of sites: indices and statistical inference. Ecology 2009; 90(12):3566–74.
- 15. Burns KN, Bokulich NA, Cantu D, Greenhut RF, Kluepfel DA, O'Geen AT et al. Vineyard soil
- bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard
- management. Soil Biology and Biochemistry 2016; 103:337–48.
- 16. Trouvelot S, Bonneau L, Redecker D, van Tuinen D, Adrian M, Wipf D. Arbuscular mycorrhiza
- symbiosis in viticulture: a review. Agron. Sustain. Dev. 2015; 35(4):1449–67.
- 215 17. Zaller JG, Heigl F, Ruess L, Grabmaier A. Glyphosate herbicide affects belowground interactions
- between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Sci Rep 2014; 4:5634.
- 18. The University of Adelaide School of Biological Sciences. Sarocladium | Mycology Online; 2021
- 218 [cited 2021 Jan 29]. Available from: URL:
- https://mycology.adelaide.edu.au/descriptions/hyphomycetes/sarocladium/.
-