








eDNA-based biomonitoring at an experimental German vineyard to characterize how management regimes shape ecosystem diversity

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Abstract

There is growing interest in the application of sustainable agricultural methods to minimize the environmental impact of farming and thus aiding quantification of the actual benefit that such approaches may confer. We applied DNA metabarcoding with the aim of exploring how the diversity of fungi and arthropods were affected by different agricultural management systems (integrated, organic, biodynamic) at the experimental vineyard of Geisenheim (Rheingau, Germany). Data were generated for the bloom and harvest periods in 2017, using environmental DNA (eDNA) metabarcoding analysis of both soil and vane trap samples. Our data revealed four principal results. (a) Overall richness of vane trap samples was unaffected by the management systems, likely due to the relatively small scale of the plots compared to the ranges of taxa such as the arthropods caught. In contrast, however, the richness of soil-living taxa appeared to be negatively affected by conventional treatments, especially at harvest. (b) Analysis of similarity revealed that the species composition was significantly differentiated by management systems for both fungal and other taxa in both sample types. (c) Taxonomic analysis of fungi revealed that the management system drove differentiation in the abundance patterns for wine-related fungi. Overall, our study reiterates the potential of eDNA techniques as a tool for assessing how biodiversity is affected by different agricultural management regimes, and we hope such approaches will be adopted in future research aimed at guiding vineyard management decisions.

KEYWORDS

arthropods, biomonitoring, eDNA, fungi, high-throughput sequencing, sustainable agriculture, wine production

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1 | INTRODUCTION

Grapevines, and the immediate environment in which they are placed, host a multitude of complex communities, including both fungi and arthropods. The composition of these communities can have a significant effect on the quantity and quality of the wine produced (Gilbert, van der Lelie, & Zarraonaindia, 2014; Hendgen et al., 2018). The composition of these organisms influences the health, yield and vigor of grapevines, but also wine flavor and aroma (and hence are part of terroir). With regard to plant health and yield, the micro- and macrofauna living in vineyards directly affect soil fertility through both decomposition and soil structuring (Bardgett & van der Putten, 2014). Fungi in the soil can also directly affect plant health and growth (Jackson, Bowles, Hodson, & Lazcano, 2012), for example, in grape plants, fungal root symbionts influence nutrient acquisition, and may increase resistance against, or susceptibility to microbial pathogens (Tonelli, Furlan, Taurian, Castro, & Fabra, 2011), which ultimately affects the final grape yields. Above ground, visitation by arthropods can influence the probability of the development of sour rot and other diseases (Madden et al., 2017). Fungal and bacterial composition of grapes are also important for the downstream application of farmed grapes for wine production. Producers are interested in, for example, wild yeasts for uninoculated production, where the fungal composition on the phyllosphere of the grape will affect the final product (Barata, Malfeito-Ferreira, & Loureiro, 2012; Gilbert et al., 2014). Ultimately all these effects, whether below or above ground, and on the grape itself, have the potential to influence the aroma and other characteristics of the resulting wines. Therefore, improving our understanding of how both beneficial and spoiling fungi are vectored around vineyards is crucial for improving wine quality (Barata, González, Malfeito-Ferreira, Querol, & Loureiro, 2008), as is deciphering how different management systems shape these communities. Given that the microbial community composition on grapevines is itself dependent upon their transfer to and from plants by arthropods (Stefanini et al., 2016), we also need to understand how arthropod communities are shaped by different agricultural management systems.

Modern winemakers are increasingly interested in replacing conventional production with organic, and even biodynamic strategies, both of which may influence vineyard-associated fungal and arthropod communities. Gaining an understanding of what the effects are,

however, is by no means simple. One challenge is that there is no simple dichotomy between conventional and organic vineyards, in practice, one encounters a range of growing practices. Secondly, traditional biodiversity assessment methods are very labor intensive, particularly when attempting to generate replicable datasets on poorly studied species (which might, although poorly studied, have large effects). Fortunately, this latter challenge can be resolved through the application of molecular tools, in particular metabarcoding, using relatively generic taxonomic assays to simultaneously profile the composition of whole communities (Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012).

In this study, we aimed to apply metabarcoding to monitor the biodiversity of arthropods and fungi, using vane trap and soil eDNA samples taken at an experimental vineyard where several different management systems are applied. Specifically, we sampled at the trial vineyard in Geisenheim, Germany, that adopts integrated, organic and biodynamic systems, without high spatial variation.

2 | METHODS

2.1 | Experimental site

The experiments were performed at Hochschule Geisenheim University, Geisenheim (49°59'; 7°56'), Rheingau, Germany during two different periods in 2017: before full bloom (referred as "bloom") and immediately prior to harvest (referred as "harvest"). Sampling details are presented in Appendix S1. The experimental vineyard is 0.8 ha in size and was planted in 1991 (cv. 'Riesling', clone Gm 198-30, grafted on *Vitis berlandieri* x *Vitis riparia* cv. 'SO4' and *Vitis riparia* x *Vitis cinerea* cv. 'Börner', respectively). The vines are spaced 1.2 m within rows, and 2 m between rows, using a Vertical Shoot Positioned (VSP) system, and rows are oriented North to South. Until the end of 2005, the vineyard was managed conventionally according to the Code of Good Practice under Regulation (EC) No. 1257/1999. Organic and biodynamic plots were managed according to Regulation (EC) No. 834/2007 and Regulation (EC) No. 889/2008 and according to ECOVIN- and Demeter-Standards, respectively (Table 1). In 2006, management of parts of the plot was converted to organic and biodynamic regimes, and the current experimental site was set up as a complete block design where each management system is replicated

TABLE 1 Distinctions of treatments between management systems (integrated, organic, and biodynamic) modified according to (Döring et al., 2015) and (Hendgen et al., 2018)

Treatments	Integrated management	Organic management	Biodynamic management
Pest and disease management	Use of fungicides, RAK	Use of sulfur, copper, and plant resistant improvers, RAK	
Fertilization	Mineral fertilizers, compost (green waste)	Ploughing cover crop, compost (manure)	Ploughing cover crop, biodynamic compost (manure)
Cover Crop	Grass mixture	Wolff Mixture	
Use of herbicides	Yes	No	
Biodynamic preparation	No		Compost preparations, horn manure, and horn silica

in four blocks. The management systems differ with regard to pest and disease treatments, the usage of herbicides and fertilization, and cover cropping strategy (Table 1). Each replica of one management system consists of four rows, including two buffer-rows and two center-rows, where sampling took place. How growth, yield and grape quality relates to organic and biodynamic management systems has previously been investigated in depth at this site (Döring et al., 2015), and previous soil analysis of this field revealed largely homogenous ground conditions (Hendgen et al., 2018).

2.2 | Sampling

Given the need to be as noninvasive as possible and thus to minimize disruption to the winemakers, we first targeted arthropods using vane traps (SpringStar Inc.) consisting of a 1.9 L plastic container, screw-top funnel and two assemblable plastic vanes, colored either blue or yellow. According to previous studies, these traps have been efficient for collection of pollinators, such as Apidae, Colletidae, Halictidae, and Megachilidae (Hall, 2018). Each trap contained 300 ml of 50% propylene glycol (MP Biomedicals). All traps were geolocated using a GPS-log application (GPS Log) on a cell phone, Appendix S1. Pairs of blue and yellow vane traps were hung on the guiding-wires next to vines at each replicate, at a height of approximately 150 cm from the soil surface. The traps were left for five days before collection. The sampling was undertaken at two time points, the first (bloom) was during early summer (25 May 2017), and the second (harvest) was during early autumn, just prior to harvest (19 September 2017). Arthropods trapped in each pair of different colored vane traps were merged into single replicate samples, then transferred on site, to 50-ml tubes (Sarstedt, Inc.) (hereafter referred to as trap samples) and stored in new 50% propylene glycol at -20°C until processing.

A soil sample was also collected for each pair of vane traps, with an aim of investigating the soil fungal and arthropod communities. Soil samples were collected in 50-ml tubes within the vine stock row, directly under the vine guideline. Collection of the lower layer of topsoil was carried out by removing the first 20 cm top layer of soil before taking the sample to avoid newly rotated soil. Soil samples were immediately stored at -20°C until processing.

2.3 | DNA extraction

All DNA extractions were performed in a dedicated clean laboratory that is isolated from post-PCR laboratories to limit the chance of contamination from previously amplified DNA. DNA was extracted from the trap samples using a method which leaves external arthropod morphology intact (Nielsen, Gilbert, Pape, & Bohmann, 2019). In brief, 15 ml of digestion buffer was added to each trap sample and incubated at 56°C for 12 hr. DNA was then purified from a 200 μl aliquot of the resulting digest, using the standard protocol for PCR purification on a Qiacube Robot (Qiagen).

DNA was extracted from soil samples using the PowerSoil® DNA Isolation Kit (Qiagen) following the manufacturer's protocol. Approximately 0.25 g of soil was used for each extraction. All samples were mixed thoroughly by shaking prior to extraction. Disposable plastic spoons were used to transfer soil into each PowerBead Tube. DNA was eluted in TE buffer and purified using the PowerClean® Pro Clean-Up Kit (Qiagen) following the manufacturer's protocol. All extracts were stored at -20°C until further processing. Extraction blanks were included for both sample types to distinguish laboratory contaminants.

2.4 | Metabarcoding

PCR amplification was carried out using two primer sets, one set aimed at arthropods (Zeale) (Bohmann et al., 2011; Zeale, Butlin, Barker, Lees, & Jones, 2011) and one aimed at fungi (D2) (O'Donnell, 1993; Putignani et al., 2008), as detailed in Appendix S2. All metabarcoding was performed in triplicate using uniquely indexed primers, to enable removal of PCR based errors in the subsequent OTU assignment (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018).

Real-time PCR (qPCR) was performed on all extracts prior to metabarcoding, to optimize the subsequent metabarcoding process. Specifically, all DNA extracts were prescreened using SYBR Green qPCR (Schnell, Bohmann, & Gilbert, 2015) with each primer sets to (a) screen for contamination in extraction negatives, (b) identify the potential presence of PCR inhibitors, and (c) optimize the cycles needed for metabarcoding PCRs. qPCR for trap samples was performed in 25 μl reactions containing 2 μl DNA template, 1 U AmpliTaq Gold Polymerase, 1X PCR Buffer II and 2.5 mM MgCl_2 (all from Applied Biosystems), 0.5 mg/ml bovine serum albumin (Bio Labs), 0.2 mM dNTP Mix (Invitrogen), 0.4 μM each of the 5' nucleotide tagged Zeale forward and reverse primers, and 1 μl of SYBR Green/ROX solution (Invitrogen). qPCR amplifications were performed on an Mx3005 qPCR machine (Agilent Technologies) with the following cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min. For amplification of the fungal D2 regions, DNA was amplified with 2 min at 95°C , followed by 40 cycles of 15 s at 95°C , 15 s at 55°C , and 45 s at 68°C .

Arthropod targeted metabarcoding used Zeale primers that were 5' tagged (Binladen et al., 2007; Bohmann et al., 2011). PCR was performed in 25 μl reactions, consisting of 1 μl of 1:1 DNA, and 1 U AmpliTaq Gold Polymerase (Applied Biosystems) with 1X PCR buffer II (Applied Biosystems), 2.5 mM MgCl_2 (Applied Biosystems), 20 mg/ml Bovine Serum Albumin (BSA) (Bio Labs), 0.25 mM dNTP Mix, 0.1 μM forward, and reverse primers. PCR amplifications were carried out using an Applied Biosystems 2,720 Thermal Cycler with the following conditions: 95°C for 10 min, followed by 36 cycles of 15 s at 95°C , 30 s at 52°C , and 30 s at 72°C , followed by 72°C for 7 min. PCR products were visualized using gel electrophoresis (GE). PCR replicates were pooled into a single pool based on gel intensity, then converted to Illumina sequencing libraries using the TagSteady PCR-free library protocol (Carøe & Bohmann, 2020) that

minimizes pooling bias and tag jumping between amplicons (Schnell et al., 2015). Library quality was assessed using NEBNext Library Quant Kit Quick Protocol (NEB) following the manufacturer's standard protocol (Feld, Nielsen, Hansen, Aamand, & Albers, 2016).

Fungal targeted metabarcoding of both soil and trap DNA extracts was carried out using the Nextera system (Illumina). PCR was carried out in 25 μ l reaction volumes containing 1 U AmpliTaq Gold Polymerase with 1X PCR Buffer II (Applied Biosystems), 2.5 mM MgCl₂ (Applied Biosystems), 20 mg/ml BSA (Bio Labs) and 10 mM dNTPs and 2 μ l combined 0.6 mM D2 forward and reverse primers together with 2 μ l 1:1 DNA template. Amplification was carried out for 2 min at 95°C, followed by 35 cycles of 15 s at 95°C, 15 s at 55°C, 40 s at 68°C, and 4 min of 68°C final extension. PCR products were visualized by GE and pooled together based on gel intensity. Subsequently, all amplicons were uniquely indexed through (Feld et al., 2016), using Accuprime Supermix II (Invitrogen) following the standard protocol with 2 μ l of Nextera Illumina i7 index Primer and 2 μ l Nextera Illumina i5 index Primer for 12 cycles. All indexed libraries were visualized by GE. PCR blanks and library preparation blanks were added for both sample types and primer sets in order to detect laboratory contaminants.

All indexed libraries were pooled based on gel intensity and the pools purified using Qiagen's QIAquick kit. Purified libraries were quantified on an Agilent BioAnalyzer 2,100 to ensure correct concentration and library size. DNA sequencing was carried out at the National High-Throughput DNA Sequencing Centre, University of Copenhagen, using an Illumina MiSeq v2 platform aiming for at least 25,000 reads per replicate with 250bp PE sequencing.

2.5 | Data analysis

Data related to the Zeale marker were quality checked using FastQC/v0.11.5 (Andrews, 2010). All samples were trimmed, and adaptors and low-quality reads were removed using AdapterRemoval/2.1.7 (Lindgreen, 2012). Filtered and trimmed reads were sorted according to primers and tags, using Begum (<https://github.com/shyams/Begum>), an updated version of the toolkit DAME that was originally developed for the preprocessing of PCR replicated metabarcoding sequence datasets (Zepeda-mendoza, Bohmann, Carmona Baez, Thomas, & Gilbert, 2016) and subsequently optimized for eliminating tag jumps and faulty sequences (Yang et al., 2020). Sequences were then filtered so that only sequences present in at least two out of three PCR replicates were kept (Alberdi et al., 2018). Chimeric sequences and sequences identical to those found in blanks were removed. Reads were clustered into OTUs with a 97% clustering threshold using SUMACLUSt (Mercier, Boyer, Bonin, & C. E., 2013), and OTU copy numbers were normalized. Assignment of taxonomy for Zeale primer derived OTUs was carried out using the GBIF server, which queries against a 99% clustered version of the International Barcode of Life project (iBOL) public data (COI-5P sequences) (Ratnasingham & Hebert, 2007). To increase assignment success rate, we then used blast/v2.2.26 to manually assign OTUs that iBOL failed to identify. The lowest common taxonomic level was used when multiple hits of identical similarity were recovered. If

species assignment returned multiple hits for any OTU, a logistic approach was applied that assessed if the species distribution correlated with sample locality, and the lowest common level was used if this was not possible. OTUs with no assigned kingdom were excluded from this study.

Fungal data were quality checked, using FastQC/v0.11.5. Before trimming and adaptor-removal, all read pairs were merged and filtered into maximum 400 bp sequences, using Usearch32/v9.0.2132 (Edgar, 2010). Length distribution was checked before and after merging and filtering. Adaptors were removed, using Usearch32. Dereplication (using full-length matching), and removal of singletons was performed, resulting in 8.56 x 10⁵ unique reads for trap samples and 61.51 x 10⁵ unique reads for soil samples. OTUs were further clustered with 97% similarity, using Vsearch/v2.1.2 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). OTUs were assigned to taxonomy, using blast/v2.2.26 and Qiime/v1.9.1 (Caporaso et al., 2010) with 99.99% similarity against a D2 designed database, generated from NCBI (04/30/2018) to optimize the D2 assignment. Database is available at: <https://sid.erd.dk/public/archives/1e9e4303c5b82b63d505eaa1e585fc5/published-archive.html>. Unassigned OTUs, and OTUs with fewer than 1,000 reads were filtered out. Furthermore, OTU copy numbers were normalized across samples by scaling reads to the sample with the highest number of reads. OTUs with abundances of less than 0.1% of a sample's total reads were discarded. Any fungal sequences identified in extraction or PCR negatives were discarded.

Postclustering curation was applied to all OTUs from each primer sets to minimize false positives using LULU (Frøslev et al., 2017). Sufficient sequencing depth was assessed for all samples with rarefaction, using R-package *vegan* (Dixon, 2003).

2.6 | Statistical analyses

Comparison of richness, extrapolation, and similarity was assessed using unweighted Hill numbers (Hill, 1973), as implemented by the R-package *hilldiv* (Alberdi & Gilbert, 2019). Hill numbers were also used to analyze composition, using $q = 0$ to minimize biased effects of relative abundance (Alberdi & Gilbert, 2019; Jost, 2006). Richness of OTUs was analyzed using ANOVA. Permutational multivariate analyses of variance (PERMANOVA) were conducted, using *vegan*, based on Jaccard distances with 999 permutations. The R-package *metacoder* (Foster, Sharpton, & Grünwald, 2017) was applied to compare differential abundance of OTUs on different taxonomic levels between management systems.

3 | RESULTS

3.1 | Data processing and taxonomic assignment

We characterized the relative taxonomic diversity of trap and soil samples using the Zeale and D2 primers, that are reported to principally target a short region of the arthropod mitochondrial

cytochrome c oxidase I (COI) gene (Zeale primers) and the D2 region of 28S rRNA for fungal species (D2 primers). As with all universal primers, the performance of both sets almost certainly varies between taxa, thus when interpreting the results it is critical to bear in mind that they likely do not provide a full picture of the arthropod and fungal content of the samples, but rather a biased, yet hopefully consistently biased, picture.

In total, 69.6 million reads were obtained from two MiSeq runs, resulting in 19.6 million reads postfiltering and sorting. After data processing, the D2 amplifications contained many distinct OTUs, with 579 fungal OTUs recovered from the trap samples, representing 49 different fungal orders, and 740 fungal OTUs recovered from the soil samples, representing 43 different fungal orders. Only fungal phyla were described because of our mapping against the D2 the specific fungal database.

The Zeale primers yielded fewer OTUs in general, specifically 247 OTUs from trap samples, which represented 12 identifiable arthropod orders, as well as 213 OTUs from the soil samples, which represented 22 phyla, including 45 identifiable orders. Unfortunately, though perhaps unsurprisingly considering our above comments on primer specificity, we found that in addition to yielding the expected arthropod taxa, large numbers of Amoebozoa, Rotifera, and Oomycetes sequences were also amplified from the soil samples using the Zeale primers. Indeed, the relative proportions of these versus the arthropod were sufficiently large, that while our initial aim had been to profile only the arthropod community in the Zeale datasets, we expanded our subsequent analyses to consider the broader community recovered. Henceforth unless specifically stated in the text, subsequent analyses on the Zeale data from soil samples are not solely restricted to arthropod OTUs.

Proportions of successful assignments were calculated at different taxonomic levels. With the Zeale primers, we were able to assign species to 37% of the OTUs in the trap samples and 21% of the OTUs in the soil samples. Fungal OTUs amplified with the D2 primers were assigned to genus level for 85% of the OTUs in the trap samples and 82% of the OTUs in the soil samples. We only considered assignments to genus level for fungi, since this has been shown to increase reliability of identifications in other (ribosomal) markers when reference databases are incomplete (Kocher et al., 2017). Taxonomy assignment efficiency for each primer set and sample type are reported in Appendix S3. The OTU taxonomic assignments are presented as KRONA interactive pie charts (Ondov, Bergman, & Phillippy, 2011) in Appendix S4. OTU assignments for all sample types and primer sets were listed in Appendix S5.

3.2 | Rarefaction curves and extrapolation of cumulative species diversity reveals sampling completeness of traps and soil samples, using Zeale and D2 primers

Rarefaction was used to analyze the data output for both primers and sample types, and the resulting rarefaction curves indicate that

the amount of data appears sufficient to describe the diversity of the samples, see Appendix S6. We also analyzed the observed cumulative species diversity for both sample types and PCR markers, which provides an indication of how efficient the sampling was for the different sample types. We found that the sample completeness for the traps at bloom when metabarcoded with Zeale primers was estimated as 60.8%, 72.3%, and 48.2%, for integrated, organic, and biodynamic management samples, respectively. The sample completeness dropped for these samples at harvest, resulting in 44.7%, 48.58%, and 41.0% in integrated, organic, and biodynamic management, respectively (Figure 1a). A similar analysis of the Zeale amplified soil samples at bloom resulted in an estimated sample completeness of 60.5%, 55.1%, and 70.9% in integrated, organic, and biodynamic managements, respectively. Lastly, the estimated sample completeness for harvest in the soil samples was 59.7%, 75.8%, and 57.9% for integrated, organic, and biodynamic respectively (Figure 1c). Overall, therefore we observed that the sampling completeness was more consistent through time for the soil samples than trap samples.

Analysis of D2 OTUs in trap and soil samples revealed a higher sample completeness, with values > 70% regardless of sampling time and sample type. This indicates that the sample coverage of fungi remains stable with time across both traps (Figure 1b) and soil samples (Figure 1d).

3.3 | The effect of season and management systems on Zeale primer amplified OTU richness

Richness was investigated in traps and soil, using the total identifiable OTUs derived from the Zeale (this section) and D2 (next section) amplicons. For the Zeale primers, the number of OTUs in trap samples ranged from 7 to 64 OTUs, while those in soil samples ranged from 2 to 15. Our ANOVA analysis next revealed that when the sample types are analyzed independently, there is no significant variation in richness by vineyard blocks, indicating there is no spatial effect on richness (Table 2). Interestingly, we did find a noticeable, sample specific, effect of management systems on Zeale OTU richness (Table 2). Overall, we observed higher OTU richness (Figure 1e) in trap samples for integrated (46.5) and organic (46.5), then biodynamic management (34.25) samples, at bloom. The richness of Zeale OTUs in traps dropped dramatically during harvest, resulting in significantly lower richness for all managements. Nevertheless, at this time point, the organically managed samples exhibited a higher mean richness than the integrated and biodynamic management (Table 2 and Figure 1a). In contrast, in soil samples, biodynamic and organic management systems yielded a significantly higher mean richness of Zeale OTUs (9 and 11, respectively), than found under integrated management (7.75).

We next explored how the Zeale OTU richness changed over the season in the different sample types and found that soil richness appears more stable than trap sample richness. Overall, therefore, there is a positive effect of integrated management on Zeale OTU

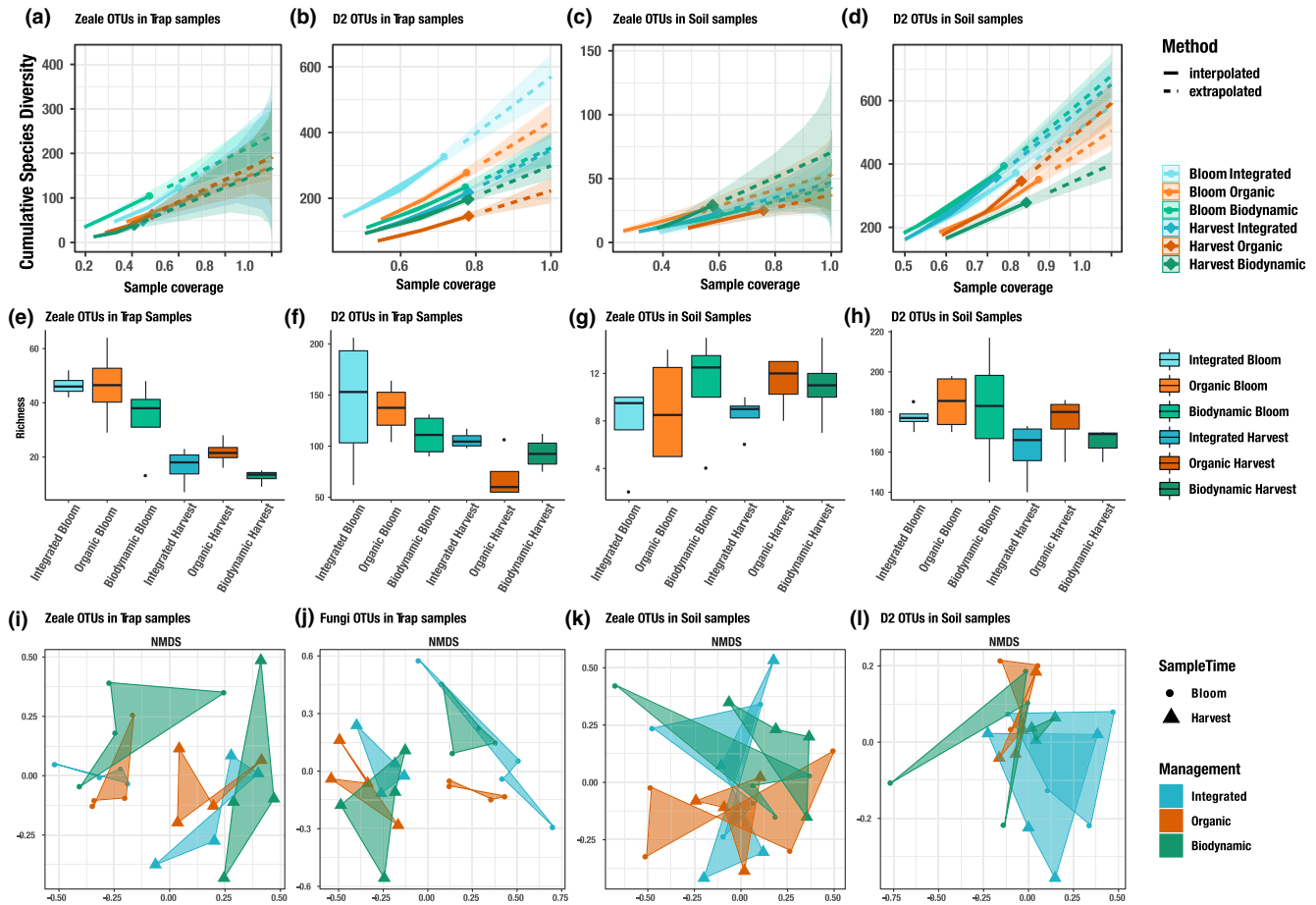


FIGURE 1 Biodiversity measurements of Zeale and D2 OTUs communities in trap and soil samples at bloom and harvest. Completeness-based sampling extrapolated curves based on unweighted Hill numbers for (a) Zeale OTUs in trap samples, (b) D2 OTUs in trap samples, (c) Zeale OTUs in soil samples, and (d) D2 OTUs in soil samples. Boxplots describing the richness of Zeale and D2 OTUs across managements at both bloom and harvest. (e) Zeale OTUs in trap samples, (f) D2 OTUs in trap samples, (g) Zeale OTUs in soil samples, and (h) D2 OTUs in soil samples. For figure (a–h), light and dark green indicate integrated management at bloom and harvest, respectively. Light and dark orange indicate organic management at bloom and harvest, respectively. Light and dark blue indicate biodynamic management at bloom and harvest, respectively. Ordination analysis, using nonmetric multidimensional scaling (NMDS), of communities derived from Zeale and D2 OTUs across management at bloom and harvest. (i) Zeale OTUs in trap samples, (j) D2 OTUs in trap samples, (k) Zeale OTUs in soil samples, and (l) D2 OTUs in soil samples. For NMDS plots, green indicated integrated management, orange indicated organic management, and blue indicated biodynamic management. Shapes indicated sampling time, where circles indicated bloom and triangles indicated harvest [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 ANOVA of OTU richness across integrated, organic, and biodynamic management systems in the vineyard. Richness was tested for Zeale markers and D2 markers in soil and trap samples. Tests were also divided according to sampling period, described as bloom and harvest

PCR Marker	Management	Sampling type	Sampling Period	Vineyard block	Management:Sampling Type
Zeale	ns	0.000 ***	0.000 ***	ns	0.046 *
D2	ns	0.000 ***	0.000 ***	ns	ns

Note: Significance code: [ns]> $\alpha = 0.05$; [•]< $\alpha = 0.1$; [*] < $\alpha = 0.05$; [**] < $\alpha = 0.01$; [***] < $\alpha = 0.001$.

richness in trap samples during bloom (Figure 1a), and a negative effect on soil-associated Zeale OTU (Figure 1c,g), especially during harvest, where richness tended to be higher for organic and biodynamic managements. If extrapolation of cumulative diversity is considered to correct for lower sample coverage, the effect of management seems to increase, with organic and biodynamic managements exhibiting the highest diversity (Figure 1c).

3.4 | The effect of season and management system on fungal OTU richness

The number of D2 fungal OTUs varied from 55 to 206 in trap samples (Figure 1f) and from 140 to 217 in soil samples (Figure 1h). Although both sample type and sampling period strongly affected the overall fungal richness, the effect of spatial variation was insignificant

(Table 2). In general, we observed a significantly higher mean fungal species richness (174.60) in soil, compared to trap samples (109.86). The mean richness of all fungal OTUs at bloom (130) also showed a significantly higher species richness compared to harvest (89.75) (Figure 1f).

We also found that the richness of D2 OTUs was higher in trap samples from integrated management (143.5/106), than both organic (135.75/70.25) and biodynamic management (110.75/93) at both bloom and harvest, respectively (Figure 1f and Table 2). Additionally, we identified an additive effect on impact of management from the extrapolation of cumulative diversity, where samples from integrated management had a higher richness of D2 OTUs (Figure 1b). The richness of D2 OTUs in soil was found to be unaffected by both sampling periods and management regime, indicating there was a high stability of OTU richness in the soil (Figure 1d,h).

3.5 | Zeale and D2 primer profiled OTU compositions are affected both by seasonal changes and management systems

We then compared the similarity based on binary Jaccard distance matrices among management systems for the trap and soil samples, using PERMANOVA (Table 3). Our analysis showed that management systems contributed between 8.0% and 16% to the variance (Table 3) and that sampling periods contributed between 5% and 11% to the variance. A nonmetric multidimensional scaling (NMDS) plot based on Jaccard similarities showed differences among management systems and sampling periods, indicating different species compositions among trial plots (Figure 1i–l). The NMDS further revealed that significant differences exist in the communities recovered from each management system, especially at harvest time (Figure 1i–l). This is also evident from the PERMANOVA analyses, where differences were significant

PERMANOVA of similarities among management systems and sampling periods

Precursors of variance	Sample type	df	Sum of Squares	R ²	F	p-value
Management	Zeale Traps	2	1.0	9%	1.20	0.085 ns
Sampling Time		1	0.9	9%	2.22	0.001 **
Block		3	1.4	13%	1.10	0.204 ns
Management:Sampling Time		2	1.0	10%	1.23	0.069 ns
Residual		15	6.3	59%		
Total		23	10.7	100%		
Management	D2 Traps	2	0.8	8%	1.04	0.368 ns
Sampling Time		1	1.0	11%	2.81	0.001 **
Block		3	1.3	14%	1.19	0.164 ns
Management:Sampling Time		2	0.7	8%	0.99	0.474 ns
Residual		15	5.6	59%		
Total		23	9.4	100%		
Management	Zeale Soil	2	0.6	8%	1.04	0.357 ns
Sampling Time		1	0.7	9%	2.28	0.002 **
Block		3	1.2	17%	1.41	0.031 *
Management:Sampling Time		2	0.5	7%	0.92	0.616 ns
Residual		15	4.3	59%		
Total		23	7.3	100%		
Management	D2 Soil	2	0.6	16%	2.06	0.014 *
Sampling Time		1	0.2	5%	1.34	0.160 ns
Block		3	0.6	17%	1.43	0.107 ns
Management:Sampling Time		2	0.2	6%	0.82	0.634 ns
Residual		14	2.0	55%		
Total		22	3.6	100%		

TABLE 3 PERMANOVA of similarities across integrated, organic, and biodynamic management systems. Similarity was tested for the Zeale and D2 OTUs in soil and trap samples. Tests were applied for seasonal changes, between bloom and harvest, spatial variance (Block), management systems, and seasonal specific variation between managements (Management:Sampling Time)

Note: Significance code: [ns] > $\alpha = 0.05$; [*] < $\alpha = 0.05$; [**] < $\alpha = 0.01$; [***] < $\alpha = 0.001$.

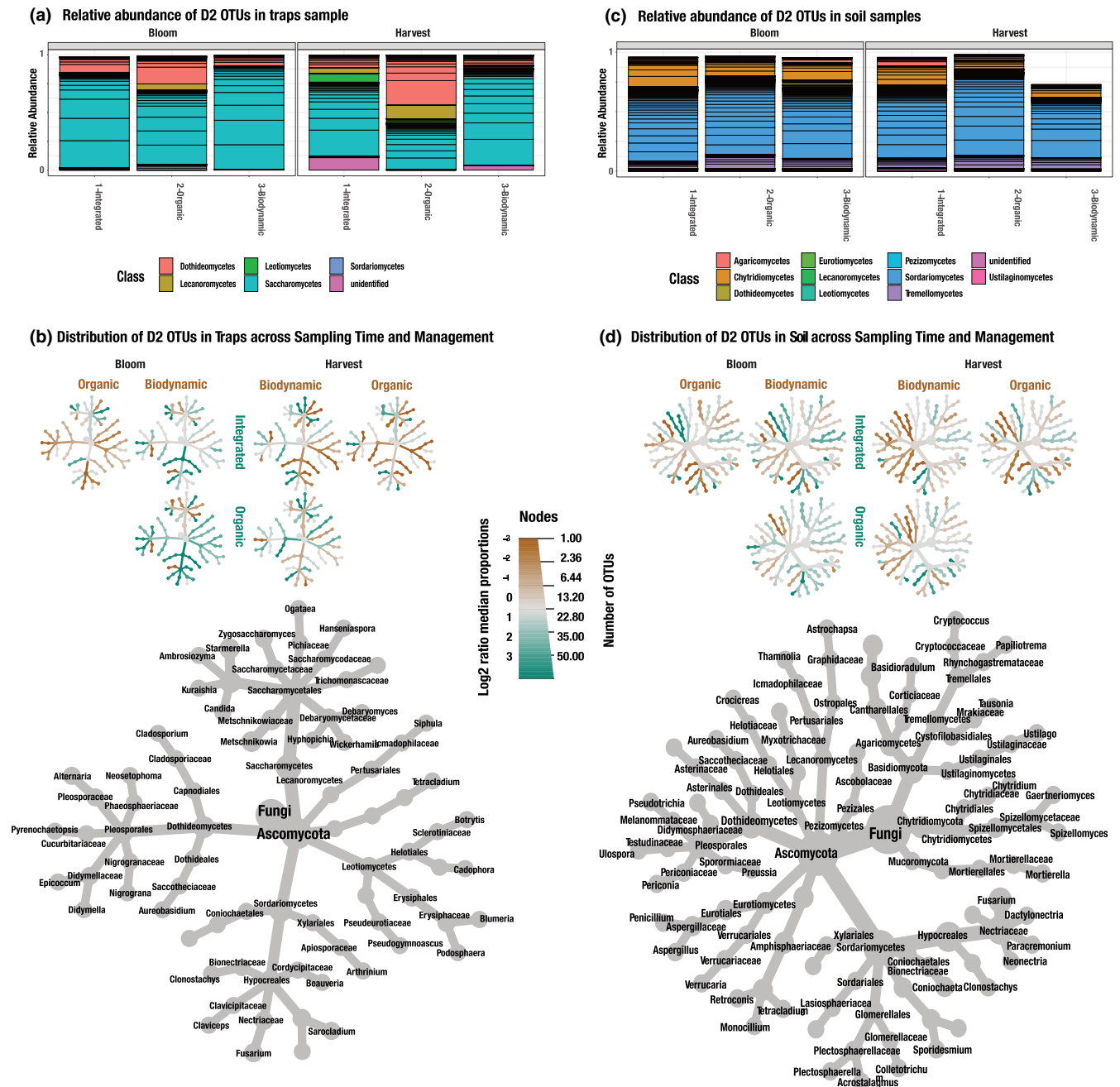


FIGURE 2 (a) Relative abundance of fungal classes, based on the 50 most abundant OTUs in the trap samples. (b) Heat tree of species composition of the 50 most abundant fungi in trap samples combined with pairwise comparison between management systems at bloom and harvest. (c) Relative abundance of fungal classes based on the 50 most abundant OTUs in the soil samples. (d) Heat tree of species composition of the 50 most abundant fungi in soil combined with pairwise comparison between management systems at bloom and harvest. The gray tree on the lower left functions as a taxonomic reference for the smaller unlabeled trees. The color of each taxon represents the log₂ ratio of median proportions of reads observed at each management system. Log₂ changes are colored, determined using a Wilcoxon rank-sum test followed by a Benjamini–Hochberg (FDR) correction for multiple comparisons. Size of nodes relates to the number of OTUs found within the given taxonomy. Nodes were left blank if taxa were unidentified [Colour figure can be viewed at wileyonlinelibrary.com]

between sampling periods for all sample types, except for the soil D2 OTUs (Table 3 and Figure 1i–k). We also found that composition of soil communities, as identified using the Zeale primers, were significantly affected by spatial variation (Table 3).

The composition of the Zeale OTUs in traps tended to significantly differ as an effect of management, especially when sampling

time was considered, which indicates there is some management specific alteration on the Zeale OTU composition (Table 3 and Figure 1i). We also recorded a close clustering of Zeale OTU composition in soil, during both bloom and harvest, underpinning our earlier findings that the composition of Zeale-related OTUs in soil is stable (Figure 1k). Furthermore, we identified a significant effect of

management on the D2 OTU community in soil samples, indicating that the different managements are affecting the composition of D2 primer profiled OTUs in soil (Table 3 and Figure 1l).

3.6 | The dynamics of putatively wine-related fungal OTUs

We then explored the taxonomic composition of the top 50 most abundant OTUs in both trap and soil samples, to look for taxa of potential relevance to wine production. Five relevant fungal classes were common in the trap samples. These were predominantly Saccharomycetes and Dothideomycetes (in both sampling periods), with the other classes including Lecanoromycetes, Leotiomycetes, and Sordariomycetes (Figure 2a). In soil samples, we found ten relevant fungal classes, with the Sordariomycetes dominating the community, followed by Tremellomycetes and Dothideomycetes. The other classes present included Agaricomycetes, Chytridiomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes, Ustilaginomycetes, and Pezizomycetes (Figure 2c). In order to explore the dynamics of these fungal communities, we applied differential abundance tests and analyzed the \log_2 median ratio between genera of these top 50 most abundant fungal OTUs, at both sampling periods. The results revealed clear patterns, both relating to the effect of system and season. Phylogeny heatmaps were used to visualize the dynamics of the fungal community structure in trap and soil samples in relation to management systems, and in particular to explore what happened to taxa relevant for viticulture and wine production. The Wilcoxon rank-sum test was used to assess for significant differences in the \log_2 median values (Figure 2b,d).

The differential abundance analysis revealed an elevated abundance of wine relevant fungal genera in both organic and biodynamic management trap samples, including both beneficial and pathogenic taxa. These differences were particularly apparent at harvest time, when we, for example, observed that the plant pathogenic genera *Podosphaera*, *Epicoccum*, *Didymella*, and *Arthrinium* were more abundant in organic and biodynamic trap samples. Furthermore, putatively beneficial wild yeasts, such as *Botrytis*, *Metschnikowia*, *Hyphopichia*, and *Hanseniaspora* were found to be most abundant in organic and biodynamic. In contrast, we also observed that the wild yeasts, *Zygosaccharomyces* and *Ogataea*, were most abundant in integrated management samples (Figure 2b).

In contrast to the ordination plot of the D2 OTUs, we found fewer significant differences in differential abundance between fungal genus in the soil samples although some possible trends could be observed. Indicating that the variation of the composition, explained in PERMANOVA, are not explained by the top 50 fungal OTUs in soil. One example is how the community differs between sampling periods. Specifically, the difference in abundance was found to be more dissimilar between all management systems at harvest, than at bloom. Secondly, we observed that biodynamic and organic management soil samples shared an increased abundance of several genera, such as *Crocicreas*, *Pseudotrichia*, *Aureobasidium*, and *Monocillium*.

The fungal class Xylariales was also found to be more abundant in biodynamic and organic management (Figure 2d).

4 | DISCUSSION

4.1 | OTU richness in trap and soil samples

In general, our data yield several observations. In the context of trap samples, firstly we overall found the mean Zeale primer amplified arthropod OTU richness was higher at bloom (42.4 ± 12.6) than harvest time (17.0 ± 6.0), independent of the management systems applied. We hypothesize that this might be driven by the fact that the life cycles of many arthropods finish prior to harvest time, thus removing them from the community. Secondly, at bloom the integrated plots exhibited higher diversity as revealed using the Zeale primers, than either organic or biodynamic plots, although interestingly this pattern was not seen in the harvest samples. This observation is at first curious, as in this experimental plot the management systems differ only in the fungicides applied, with no insecticides applied during the growing season at all (Table 1). We hypothesize that the results suggest that the different fungicides applied could still affect the canopy arthropods. For instance, sulfur applied in organic and biodynamic viticulture against powdery mildew (*Erysiphe necator*) is known to also kill beneficial arthropods such as predatory mites (Gadino et al. 2011). In addition, fungicides might indirectly affect some arthropod species via effects on their symbiotic fungi.

Thirdly, we observed a trend (though not with statistical significance) that the fungal richness recovered from trap samples was the highest under an integrated management approach at both bloom and harvest (Figure 1c,k). While this could be a biological signal, we caveat that it could also be a technical artifact derived from the fact that DNA from very common taxa can drown out the signal of that from rarer taxa during the metabarcoding process (Alberdi et al., 2018).

With regard to the soil samples, we firstly found that in contrast to the trap samples, the overall richness of Zeale primer profiled taxa did not differ substantially between bloom and harvest. This indicates that in contrast to the canopy where the traps were placed, the population of soil-associated arthropods, rotifers, and ameba is relatively stable, between seasons. Secondly, in general we found that the richness of soil-associated Zeale OTUs in the organic and biodynamic plots was higher than in integrated plots (Figure 1 cb and gj). We believe this derives from differences in the cover crop mixture grown between the vines on the plots. While integrated treatment uses grass, a more diverse range of plants grow under vines under organic and biodynamic management, which could provide a more diverse range of conditions for their soil-associated arthropods. That a difference in richness is not seen when comparing trap samples under different management systems, probably derives from the much greater capacity of above ground arthropods for dispersal, considering the relatively small geographic scale of the experimental plot. No major differences could be observed in the richness of fungi

in soil samples, either during the season or among management systems, indicating stable communities. This is in accordance with findings of Hendgen et al. (2018), who did not observe differences in species richness of fungi among management systems in the same field trial in August 2015. In contrast, (Di Giacinto et al., 2020) found phospholipid fatty acids (PLFAs) of fungi and enzymatic activity of several enzymes in the soil to be significantly higher in organic and biodynamic plots in the same field trial throughout the growing season 2016.

4.2 | Species composition in trap and soil samples

In the trap samples, we observed major seasonal differences in the community composition of Zeale primer profiled taxa, as well as a strong effect of management on the similarity of both the community types profiled with the Zeale and D2 primers. A similar observation could be seen in the fungi (D2) but not Zeale primer profiled communities in the soil samples (Table 3). That the composition of soil-associated Zeale primer profiled taxa did not seem to be influenced by the different management systems is striking, given that there was a higher species richness for these same samples in the organic and biodynamic plots. We suggest this might imply that the difference in covering crops may not significantly change the kinds of arthropods (in particular) that are able to inhabit the area under the vines, but certainly increases the diversity within these communities.

While there is little variation in this composition between the three management systems during bloom, by harvest they have not only all diverged away from the bloom communities, but also from each other (Figure 1g). While we saw no major differences in the richness of the fungal OTUs obtained from the soil samples, the species compositions varied significantly among management systems, in particular during harvest. This is consistent with both the results of prior monitoring of the soil in the same field trial in 2015 (Hendgen et al., 2018) and the observations from trap samples, where significantly different compositions of the Zeale primer profiled community among management types were observed for both high and low abundant species.

Although the soil fungal community contained a high number of genera, these were mainly dominated by the fungal classes Sordariomycetes, followed by Tremellomycetes, Dothideomycetes, Agaricomycetes, Chytridiomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes, and Pezizomycetes (Figure 2d). All of these classes are commonly found in European agricultural soil (Klaubauf et al., 2010; Kovanci, Kovanci, & Gencer, 2007; Morrison-Whittle, Lee, & Goddard, 2017; Zhou et al., 2017). Our analysis of fungal OTUs revealed a reduction of several genera within Ascomycota, using an integrated management. This could be related to the use of systemic fungicides, which might affect Ascomycota more specifically than the sulfur and copper treatments that are used in organic and biodynamic managements (Döring et al., 2015; Hendgen et al., 2018).

In addition to providing insights at the community level, metabarcoding is a powerful tool for profiling which taxa are present in any sample. For example, the most abundant arthropod families recovered from the Zeale primer profiled community data generated on the trap samples were Staphylinidae, Cecidomyiidae, Sciaridae, Chironomidae, Muscidae, and Aphididae, which all are common families of arthropods in Central Europe. Surprisingly, no OTUs were recovered belonging to the family Apidae, even though we observed arthropods belonging to this family in most of the trap samples. Although this might simply derive from differences in efficiency in the primers binding to different taxa (Brandon-Mong et al 2015; Hamad, Delaporte, Raoult, & Bittar, 2014), we did, however, recover a number of OTUs from the order Hymenoptera. As such, an alternate explanation could be the reference database is incomplete for this taxon (Jusino et al., 2017). Ultimately, future studies based around other primer sets might resolve this. Arthropods in soil were mainly dominated by Eupodidae, Delphacidae, Acrididae, and Arachnida, which were commonly known families in Central Europe.

5 | CAVEATS AND CONCLUSIONS

In this study, we used metabarcoding to analyze 48 samples from three different viticultural management systems, including both soil and trap samples. Although metabarcoding is increasingly being used for biodiversity assessment, it still faces a number of limitations and biases. In light of this, we applied a relatively conservative approach to minimize the introduction of false positives. We included PCR triplicates to increase detection of lowly abundant species and reduce false procedural positives (Alberdi et al., 2018; Ficetola, Taberlet, & Coissac, 2016). We also minimized spurious tag combinations by using a PCR-free library construction method (Schnell et al., 2015) and adopted a strict bioinformatic filtering pipeline to reduce false procedural positives (Alberdi et al., 2018). We acknowledge, however, that our conservative approach could potentially have removed rare species. A second point is that extrapolation analyses at all time points indicated sample completeness did not fully saturate (Figure 1a–d) although given the several clear patterns observed we feel our data provides a reasonable survey. To fully understand the biodiversity in vineyards, more different sample type would ideally have been taken as a reference, such as malaise traps, and several additional primer sets could have been included to improve both taxonomic coverage and species level assignments (Alberdi et al., 2018; Elbrecht, Vamos, Steinke, & Leese, 2018). A third point is that we used the presence/absence data from metabarcoding as quantitative diversity data, which can be biased from the PCR amplifications (Alberdi et al., 2018; Amend, Seifert, & Bruns, 2010). A more reliable, but costly, approach could be to use shotgun metagenomic sequencing to generate abundant data for fully understanding the dynamics of biodiversity within wineries and across vineyards (Faust & Raes, 2012).

Overall sample size is also an obvious limitation to this study, as it ultimately limits the statistical analyses that can be done on our data. With regard to depth, our assessment of the data, using extrapolation, revealed that sample coverage in traps and soil, using Zeale primers was between 41% and 75.8%, which clearly indicates that some improvements are needed for the setup for assessing arthropods. Sample coverage of fungi in traps and soil were in general above 70%, indicating a quite high coverage, but improvements should be implemented. With regard to sample size, had we had access to more samples, several more powerful analyses could have been performed, aimed at investigating the community of both arthropods and fungi more deeply, using more powerful statistical approaches. For example, possible tools that could have been used had we had more data include joint species distribution models to investigate species–species interactions between the fungi and in particular arthropod OTUs recovered (Tikhonov, Abrego, Dunson, & Ovaskainen, 2017). Additionally, more powerful multivariate analysis could be applied, using Bayesian ordination on constrained datasets (Hui, 2016).

Nevertheless, despite these limitations, we believe that the results presented here are valid and provide insights into the fungal, arthropod, and other communities found in vineyards under different managements at this experimental vineyard in Germany, and we hope that our work can provide a framework for future diversity assessments in vineyards to able to make more universal conclusions.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION


JAR, MN, SSTM, and MTPG designed the study. JAR and MN carried out fieldwork, laboratory work and data analysis. JD helped out initial structure and planning of fieldwork and carried out analysis of richness. FK curated the D2 database with input from SG. JAR, MN, SSTM, JD, RRD, and MTPG wrote the first draft and all authors contributed to revisions and accepted the final version.

DATA AVAILABILITY STATEMENT

Sequencing data are available from the Electronic Research Data Archive (ERDA) repository, <https://doi.org/10.17894/ucph.c6b0edea-91ec-4175-9d7a-edaffb66572c>. Tag sequences and

tag combinations in libraries for bioinformatic processing and scripts are available from https://github.com/JacobAgerbo/Rasmussen_et_al_2020a.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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