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Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems

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ABSTRACT

There is increased need to identify sustainable agricultural methods which avoid environmental degradation. Previous studies have focused on the effect of specific agricultural interventions on large organisms, but we have fewer data evaluating how microbes, which are key components of ecosystems, might be affected. Additionally, previous studies have been constrained as they only examined one habitat in an ecosystem and have not gone on to evaluate the effect of agricultural approach on harvested crops. Here we take an ecosystems approach and evaluate the net effect of conventional versus biodynamic management on agricultural ecosystems by quantifying fungal communities in multiple habitats using metagenomics. We go on to measure biodiversity in the crop and key chemical quality parameters in the product consumed by humans. We find that the method of management significantly affects communities in soil, on plant structures, and on the developing crop in subtle but importantly different ways in terms of number, type, and abundance of species. However, management approach has no effect on communities in the final harvested juice, nor on product traits aligned with quality. This shows that while management approach impacts different habitats in the environment in different ways, this does not automatically flow onto the harvested crop.

1. Introduction

How biodiversity, and the ecosystem services it provides, responds to the way we manage natural and agricultural ecosystems is a key area of modern ecology; it impacts both conservation efforts and the cultivation of crop species which provide essential food resources (Tanentzap et al., 2015). It is commonly asserted that agriculture conflicts with natural environments, and sustainable approaches to agriculture are now receiving greater attention (Edwards et al., 2015). While we may more readily perceive how various human-mediated ecosystem interventions impact larger plants and animals, we have a poor idea about how microbial communities respond, if they respond at all, to various management approaches. Microbial communities perform essential functions in all ecosystems and play a role in directly modulating plant health, productivity, and development (Lau and Lennon, 2012; Panke-Buisse et al., 2015; Sugiyama et al., 2013). Studies to date have reported that the structure and composition of microbial communities often vary considerably over different spatial and ecological gradients (Hanson et al., 2012; Martiny et al., 2006; Nemergut et al., 2013). While the main drivers of microbial diversity may differ between ecosystems, it is generally held that terrestrial microbial communities are mostly driven by natural selection to specific habitats present in any particular environment (which would include selection pressures imposed by agrochemicals), though the significance of stochastic (neutral) effects in defining microbial community composition should not be ignored (Morrison-Whittle and Goddard, 2015; Stegen et al., 2012, 2013).

Modern agricultural management practices do not involve just one treatment but instead comprise a range of different biological, physical, and chemical treatments applied to cultivated land to maximise the health, resilience, and productivity of crop species. There is significant and seemingly growing public concern surrounding the use of agrochemical interventions, though the science evaluating their effects at the ecosystems level is sparse (Edwards et al., 2015; Tanentzap et al., 2015). Due to concerns about environmental impacts of agrochemicals, alternative philosophies to agricultural management have emerged. These alternative approaches include “organic” and “biodynamic” styles of management that, while very similar to “conventional” practices, often differ in a few notable ways. At their core, organic and biodynamic practices are primarily shaped by their philosophical opposition to the use of agrichemical pesticides and herbicides, both of which are routinely used in conventional management (Tilman et al.,

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2002). In practice, this can often manifest as differential constraints on what specific treatment decisions can be made for any one site. Organic and biodynamic practices are constrained in what they may use by local commercial-certification bodies such as BioGro^{NZ} or Demeter^{Int}. As a part of formal certification from these agencies, companies are required to conform to approved codes of practice which either heavily restricts or forbids the use of most pesticide and fertiliser products.

The subject of alternative land management philosophies is a popular albeit controversial one, and has provoked a considerable shift in many industry practices globally. It is imperative that we objectively, quantitatively assess whether different practices differentially affect ecosystems, and the crops and products that derive from them. As huge areas of the planet have been dedicated to cultivating plant species, and realising that these are not completely isolated from surrounding natural ecosystems, any effect of different management approaches to cultivation may have significant implications for the diversity and functioning of ecosystems generally. Fungal communities form a core component of natural and agricultural ecosystems, and this where we focus here as a first step.

We currently have no clear idea whether organic/biodynamic or conventional practices translate to real variation in microbial communities, nor their effect on the products deriving from these systems. Many studies have found that specific agricultural interventions can significantly impact microbial diversity in agricultural ecosystems (Čadež et al., 2010; Gomiero et al., 2011; Hartmann et al., 2015; Martins et al., 2012, 2014; Perazzolli et al., 2014; Saison et al., 2006). However, very few studies have tested whether overall treatments culminate in detectable differences in biodiversity between different agricultural philosophies. One recent long-term study found organic farming increased richness, decreased evenness, and shifted the structure of the soil microbiota compared to conventional approaches (Hartmann et al., 2015). This is an excellent study producing an important result, but only examines soil: one, albeit important, habitat in agricultural systems. To achieve a more holistic picture of the effects of different management approaches on agricultural ecosystems requires examining multiple habitats in these ecosystems. Importantly, for the consumer, the status of the produce that is cropped also needs evaluation.

Here we take an approach that samples multiple habitats in vineyard ecosystems, including the harvested juice and wine, and use DNA sequencing to enumerate the fungal communities in multiple habitats from six conventional as well as six “biodynamically” managed vineyards. We test the null hypothesis that there is no difference in the effect of management approach on microbial biodiversity across this agricultural ecosystem. We do this by breaking down and analysing different components of microbial diversity in different habitats. Since fungi are the key component that drives the fermentation of juice to wine, and produce many key quality flavour and aroma compounds as they do so, we also go onto analyse fungal diversity in juice and key fungal-derived quality flavour compounds in the wines: varietal thiols (Anfang et al., 2009; Harsch and Gardner, 2013; Masneuf-Pomarède et al., 2006; Santiago and Gardner, 2015). By quantifying community structure across multiple vineyard habitats, and key microbe-derived compounds in wine, we can more powerfully assess the ecosystem level effects of management approach that would not be possible by characterising one habitat or aspect of the ecosystem in isolation.

2. Methods

2.1. Sampling viticulture ecosystems

Soil, bark, and ripe fruit habitats were sampled from 12 commercial sauvignon blanc vineyards managed by nine different companies across the Wairau valley in the Marlborough region on the South Island of New Zealand, approx. 41°S, 173°E. The experimental design was such that $n = 6$ for each habitat for each management type; thus, 36 samples were collected from vineyards comprising six biodynamic and six conventionally managed vineyards for a fully-balanced design. All biodynamically managed vineyards had achieved BioGro™ organic certification. Approximately two weeks before harvest, around 30 g of each habitat was aseptically collected. Each sample comprised three pooled sub-samples taken across each vineyard. All samples were taken at least 5 m into the vineyard to avoid edge effects. Soil samples were taken 50 cm away from a grapevine trunk at a depth of ~10 cm. Bark samples were taken from at least 30 cm above the soil, and whole bunches of fruit were cut into sterile bags. All samples were taken with sterile tools and placed into sterile containers, and transported on ice to the laboratory for processing. Microbes were washed off fruit samples by immersion in sterile water with rocking for 30 min. The resulting solution was then centrifuged at 3000 rpm, and the resulting pellet re-suspended in 500 µl of sterile water. Soil and bark samples were homogenised mechanically using aseptic technique to increase surface area for DNA extraction.

We also collected commercially harvested juice from these same vineyards. Approximately 10 L of juice was transferred into sterile jerry cans at each winery and transported to the laboratory on ice. 50 ml of homogenised juice was centrifuged at 3000 rpm and the resulting pellets suspended in 500 µl of sterile water. Twelve juice samples directly deriving from the six biodynamic vineyards and six conventionally managed vineyards were collected. Thus, in total 48 samples were collected from the twelve vineyards and the juice derived from them.

2.2. Extraction and sequencing

All samples were frozen at −20 °C prior to processing. DNA was extracted using the Zymo Research Soil Microbe DNA MiniPrep™ kits. We empirically determined this kit was sufficient to extract DNA from all substrates. Fungal communities were characterised and enumerated by 454-sequencing of the D1/D2 region of 26S ribosomal RNA, and amplified using NL1 and NL4 primers described in Kurtzman and Robnett (2003) with unique multiplex identifiers added as appropriate. Sequencing this locus provides an effective method for taxonomic identification down to at least genus level as well as the quantification of the relative richness and abundances of fungal communities (Morrison-Whittle and Goddard, 2015; Taylor et al., 2014). All PCR products were cleaned using AmpureXP beads and their quality checked by Agilent DNA1000 chips. Juice samples were uni-directionally sequenced on a 454-junior instrument by New Zealand Genomics Limited. Vineyard communities were sequenced on a full plate of a 454 Life Sciences GS FLX instrument by Macrogen (Korea).

2.3. Sequencing pipeline

Sequence processing was carried out using Mothur v.1.30 (Schloss et al., 2009). Primers and sequences < 200 bp were removed. Low quality reads were removed using the pyronoise algorithm. Chimeric sequences produced during PCR were identified and removed using the uchime algorithm. Once the remaining high-quality sequences were bioinformatically assigned labels based on their multiplex identifier sequence, they were merged and analysed together. Unique sequences were compared to a reference database of fungal sequences. Sequences that were not identified as fungal were removed (11,105, 7.26% of all reads). The remaining 141, 940 fungal sequences were then aligned using a fungal reference database and clustered at > 98% identity.

The 98% identity threshold was used to approximate clusters of fungal species (Kurtzman and Robnett, 2003; Romanelli et al., 2010) and was the lowest level of molecular operational taxonomic unit (MOTU) in this study. Any MOTU that was represented by a single read (a singleton) was conservatively removed from the sequence pool. To effect equal sampling effort for these DNA sequences, reads were sub-sampled (rarefied) to the sample with the lowest read count, resulting in 509 reads per sample. Representative sequences of each MOTU were

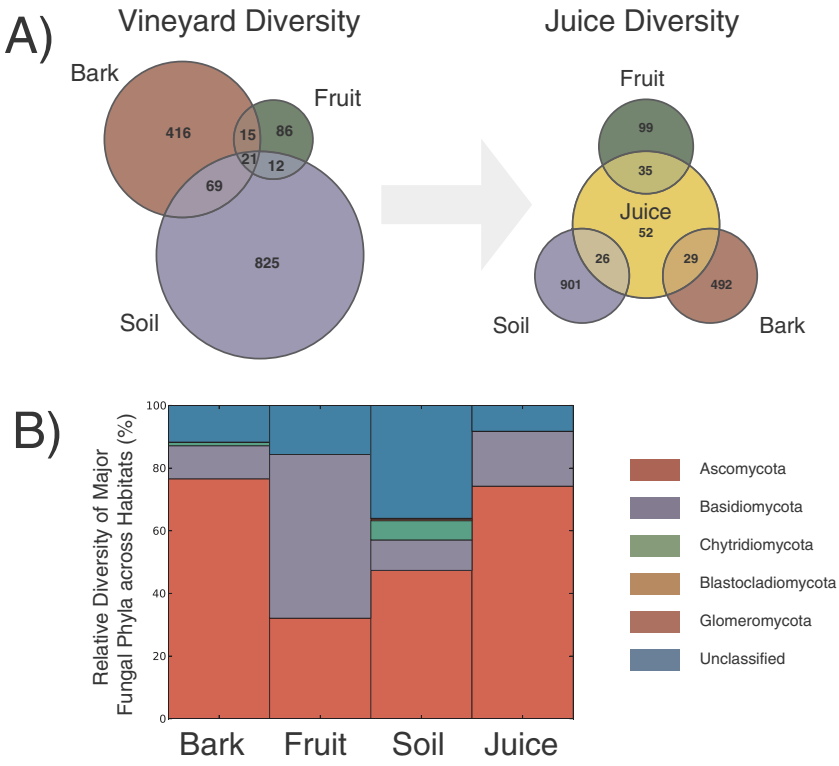

Fig. 1. (A) Overlap of community diversity across vineyard habitats (bark, fruit, soil) and the overlap of separate vineyard communities to those found in juice. (B) The relative diversity of each of the five detected fungal phyla across all four habitats in 12 vineyards (six biodynamic and six conventionally managed vineyards).

then classified against a fungal taxonomic database using a Bayesian approach. Each MOTU was classified to the genus level and above using the 'classify.seqs' command in Mothur. Sequences were listed as unclassified at any one taxonomic level if their sequence match fell below 70%.

2.4. Winemaking and thiol analyses

No additions were made to juices once they arrived in the laboratory, where they were fitted with an air-lock and allowed to spontaneously ferment at 15 °C, the standard temperature for sauvignon blanc ferments in Marlborough. Ferment progression was monitored by weight loss, and once complete the varietal thiols 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were quantified to ng L^{-1} by the standard commercial GC–MS service available at Hill Laboratories (http://www.hill-laboratories.com).

2.5. Statistical analysis

The effect of vineyard management on total species richness was tested using a two-way ANOVA with management and habitat as fixed effects followed by Tukey's Honestly Significant Differences (Tukey HSD) adjustment of pairwise comparisons. Differences in relative species abundance and community structure was evaluated with two-way full factorial permutational multivariate ANOVA (permanova) of Jaccard dissimilarities (Anderson, 2001) with management and habitat as fixed effects. Variation in community structure of bark, fruit, soil, and juice habitats across conventional and biodynamic vineyards was visualised using classic multidimensional scaling of Jaccard community dissimilarities. All statistical testing was conducted using R (R Core Team, 2016), including tests available in the 'vegan' package (Dixon, 2003). The relative abundance of fungal taxa in biodynamic and conventional soil and fruit was visualised using CYTOSCAPE 3.4 (Shannon et al., 2003). To explore the importance of different fungal taxa in influencing significant patterns of diversity, we examined the effect of removing each taxa from the dataset. Wherever significant differences in fungal diversity were found, we repeated the analysis while removing one fungal taxonomic level from the dataset at a time for each phylum, class, order, family, and genus levels. Whenever removing a fungal taxonomic level resulted in a test going from significant to non-significant, we considered that as contributing to the original difference.

3. Results

Characterising fungal diversity using amplicon sequencing is not quantitative in terms of evaluating the total numbers of organisms from any given sample. However, by ensuring equal biological and analytical sampling effort of communities across habitats and vineyard management systems, we can quantify relative differences in fungal diversity. We point out that, as with most ecological studies, we do not sample all organisms in any given habitat, but we randomly sub-sample the same number of individuals (DNA sequences) and use these to make comparative inferences. For example, while one habitat may contain ten times more individuals than another, one randomly sub-samples the same number of individuals from both, and uses these for analyses.

3.1. Overview of fungal diversity

The analyses of DNA from the 48 samples spanning four habitats (soil, bark, fruit, and juice) revealed the presence of 1496 fungal MOTUs – hereafter referred to as species. Raw sequences for each sample are available in GenBank (accession number: SRP106145).

Overall, we recovered five phyla, 25 classes, 66 orders, 143 families, and 268 genera of fungi. The most diverse and abundant phylum overall was Ascomycota, which comprised 55.5% of species and 70.6% of reads, followed by Basidiomycota, Chytridiomycota, Glomeromycota, and Blastocladiomycota which comprised 13.1%, 4.0%, 0.2%, and 0.3% of species respectively (19.3%, 1.6%, 0.0%, and 0.1% of reads respectively); see Fig. 1. Ascomycota were the most diverse and abundant phylum in all habitats except fruit, where Basidiomycota were the most diverse (52.2% of all fruit species) and abundant (56.7% of all fruit reads). Diversity was greatest in soil which contained 927 species, followed by bark with 521 species, then fruit

with 134 species, and least diverse in juice with 97 species. Analyses of variance revealed that soil communities had significantly greater numbers of species than any other habitat ($F_{3,44} = 176.8$, $P < 0.001$, Tukey HSD: $P < 0.001$). The bark habitat harboured significantly greater numbers of species than both fruit and juice (Tukey HSD: $P < 0.001$), and the fruit and juice habitats contained the lowest numbers of species, which did not numerically significantly differ from one other (Tukey HSD: $P = 0.63$). In total only 16 species (1.1%) were found across all four habitats with 117 species (7.8%) found across at least two habitats. Conversely, 1363 species (91.1% of all species) were exclusively found in one habitat only. Of the four habitats sampled, soil had the highest proportion of habitat-specific species (88.8% of all soil species) followed by bark, fruit, and juice (79.1%, 56.7%, and 53.6% respectively).

3.2. Evaluating the effect of management on communities

Given the same random sampling effort from all habitats, we define three possible types of differences among communities: 1 – absolute species richness: the difference in number of species; 2 – relative species richness: the difference in types of species present in the sub-sample; and 3 – community composition: the differential abundances of species in the sub-sample. The difference between these is important and using these we may test whether management approach changes the numbers of species present, the types of species that are present, the relative abundances of species, or all of these (Fig. 2).

We first simultaneously analysed the effect of habitat and management approach on absolute numbers of fungal species using a two-way full-factorial ANOVA. This revealed habitat significantly affects species numbers (see Table 1A), but the effect of management approach on the number of species was much weaker. We found no significant interaction between these two main factors, meaning management does not dramatically differentially affect species richness in the different habitats. We then analysed how habitat and management approach affects the types of fungal species present using a 2-way PERMANOVA on a Jaccard community similarity matrix, and this revealed a similar pattern: that overall, habitat significantly influenced the types of species present ($R^2 = 0.287$, $P < 0.001$) but management approach had no effect ($R^2 = 0.021$, $P < 0.087$), again with no significant interaction between habitat and management. Lastly, we analysed how habitat and management approach affects fungal community composition, and this again revealed a similar pattern: habitat significantly influenced the relative abundances of species in fungal communities ($R^2 = 0.346$, $P < 0.001$) but management approach did not ($R^2 = 0.017$, $P < 0.263$), with no significant interaction between habitat and management approach. The relationships between communities deriving from different habitats and management approaches are shown in Fig. 3.

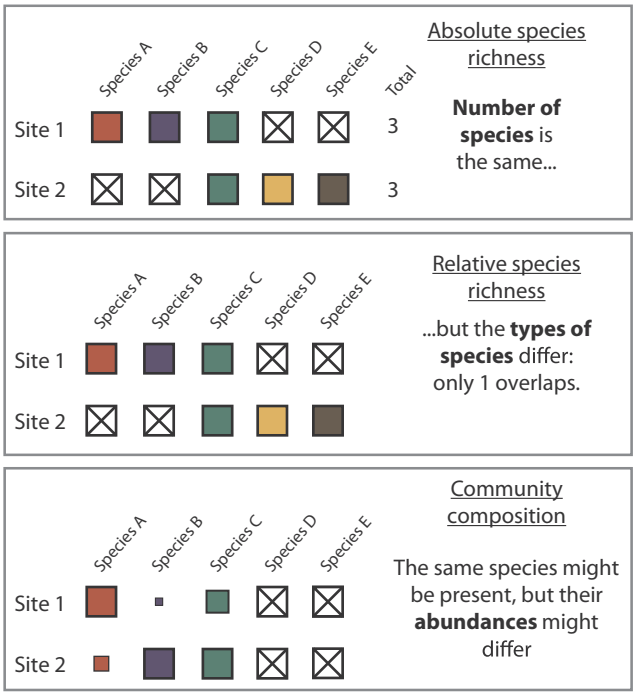

Fig. 2. The three measures of biological diversity examined across four habitats (bark, fruit, soil, and juice) and across two vineyard management practices (six biodynamic and six conventionally managed vineyards). Note these differences are relative measures and based on identical sampling effort in each habitat for each management system.

Table 1
(A) Results of two-way full-factorial ANOVA of absolute species richness for habitat and vineyard management effects. Results of two-way full factorial PERMANOVA on (B) relative richness – binary Jaccard community dissimilarities (9999 permutations) (C) Community composition – non-binary Jaccard community dissimilarities (9999 permutations).

	Effect	DF	SS	MS	*F*	*P*
(A) Absolute richness	Habitat	3	122,420	40,806	197.13	< 0.001
	Management	1	768	768	3.71	0.061
	Interaction	3	1110	370	1.79	0.165
	Residuals	40	8280	207		

	Effect	Df	SS	MS	Pseudo-*F*	*P*	R^2
(B) Relative richness	Management	1	0.4	0.4	1.33	0.087	0.02
	Habitat	3	5.6	1.9	6.02	< 0.001	0.29
	Interaction	3	1.1	0.4	1.19	0.095	0.06
	Residuals	40	12.4	0.3	0.64		
	Total	47	19.5	1.0			
(C) Community composition	Management	1	0.3	0.3	1.14	0.263	0.02
	Habitat	3	6.9	2.3	7.87	< 0.001	0.35
	Interaction	3	1.0	0.3	1.13	0.217	0.05
	Residuals	40	11.7	0.3	0.59		
	Total	47	20.0	1.0			

The effect of habitat eclipses the effect of management practice in terms of fungal biodiversity, and this result aligns with our previous findings in terms of the strong structuring effect of habitat (Morrison-Whittle and Goddard, 2015). Taking the R^2 values, which indicate the proportion of variance explained by a variable, we estimate that habitat is approximately 17 times stronger than management practice in determining the number, type, and abundance of fungal species across this agricultural ecosystem. However, this does not necessarily mean management approach has no effect on these fungal communities.

3.3. Effect of management on absolute number of species among habitats

We went onto examine each habitat independently as the previous analyses show these differ significantly in terms of biodiversity, and management practices are not discrete effects and may thus differentially affect habitats (e.g. fungicides are sprayed on the crop but not usually on soil). First, we evaluated the effect of agricultural management on the absolute number of species present. One-way ANOVAs revealed that management approach only affected the number of species present in two of the four habitats we analysed. Significantly more fungal species were found on the bark and fruit of biodynamic than conventionally managed vineyards (Bark: $F_{1,10} = 7.524$, $P = 0.020$; Fruit: $F_{1,10} = 11.56$, $P = 0.007$). However, management approach did not significantly affect the number of species in either the soil or juice (Soil: $F_{1,10} = 0.12$, $P = 0.733$; Juice: $F_{1,10} = 0.418$, $P = 0.533$). Overall bark communities in biodynamically managed vineyards had 102 or 35.8% more species than those in conventionally managed ones,

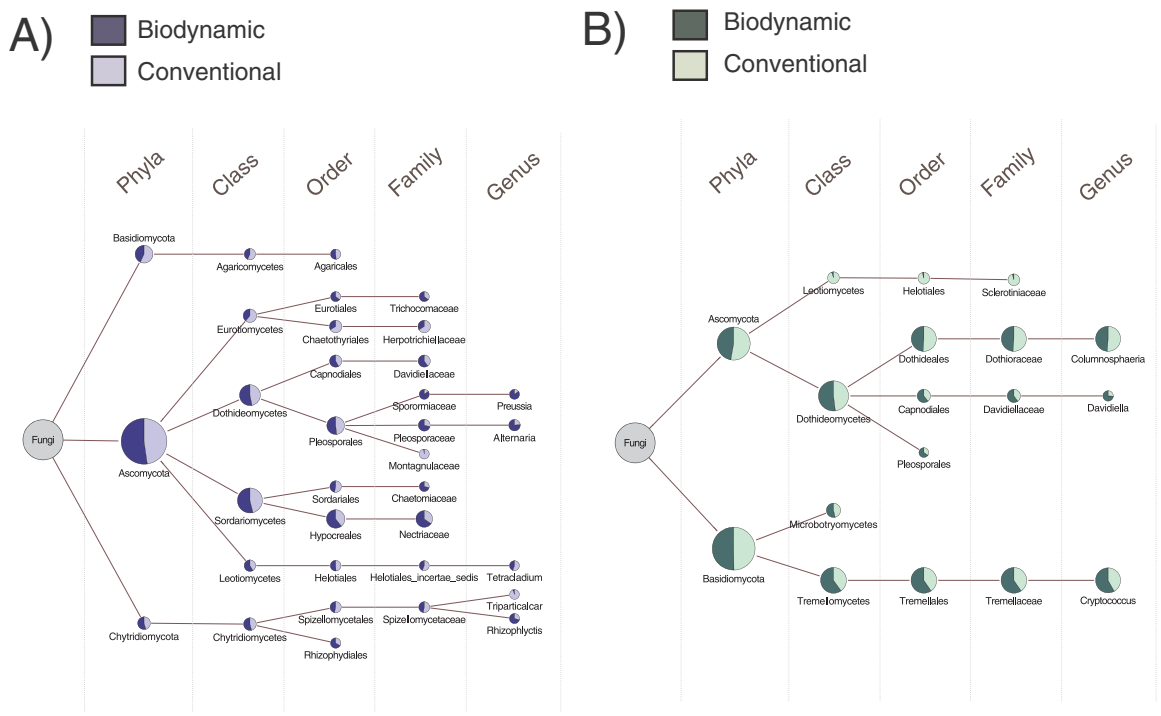

Fig. 3. Relative abundances of identified taxa in biodynamic and conventional samples that compose > 1% of all reads in: (A) soil (B) fruit habitats using CYTOSCAPE. The size of taxa nodes is proportional to their relative abundance in their respective habitat.

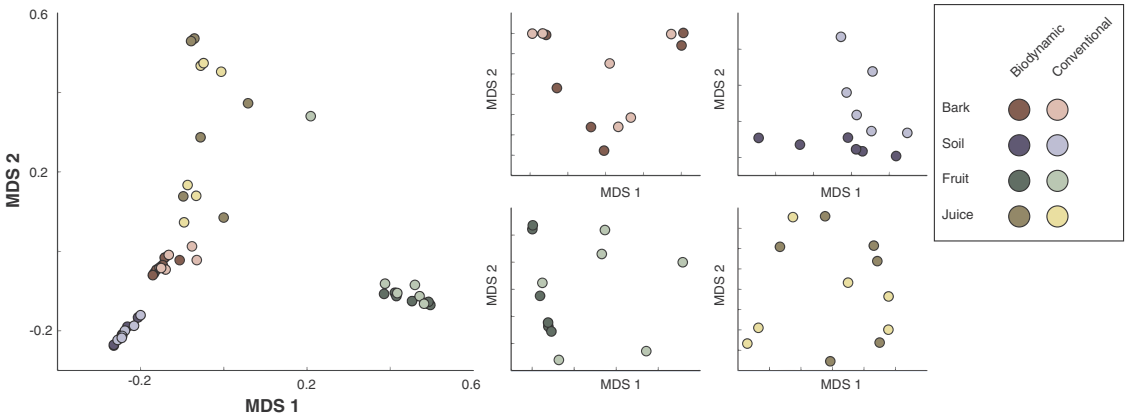

Fig. 4. Classic multidimensional scaling of community composition measures (non-binary Jaccard dissimilarity) across: (A) all samples: bark (red), soil (blue), fruit (green), and juice (yellow) communities; biodynamic communities are represented by darker shades and conventional by lighter shades; and (B) by each habitat.

and fruit communities 41 or 63.1% more species. These represent a reasonable fraction of 19.6% of the 521, and 30.6% of the 134 total species in bark and fruit respectively.

We evaluated the potential contributions of different taxonomic groups underpinning the significant differences we observed, and this revealed the significant differences between biodynamic and conventional bark communities collapsed only when the class Dothideomycetes (largest and most diverse class of ascomycete fungi) and the order Pleosporales were excluded from analysis.

3.4. Effect of management on the types of species among habitats

To examine whether management approach differentially affects the types of species in different habitats, we carried out one-way PERMANOVA on binary (presence/absence) Jaccard dissimilarities for each habitat independently. Management approach had a significant effect on the types of fungal species in soil and fruit (soil: $R^2 = 0.107$, $P = 0.003$; fruit: $R^2 = 0.152$, $P = 0.005$; Table 1), but not in bark or juice (bark: $R^2 = 0.095$, $P = 0.260$; Juice: $R^2 = 0.092$, $P = 0.420$). We evaluated whether there were any consistent patterns in the types of species that differentiate soil and fruit fungal communities of biodynamic and conventional vineyards. We found the significant effect of management on communities in both fruit and soil habitats was not driven by the differential presence of any one taxonomic group as significant differences remained even when every individual class, order, family, and genus was systematically excluded from the dataset.

3.5. Effect of management on community composition among habitats

Lastly, we evaluated the effect of management approaches on the abundance of species in habitats. One-way PERMANOVA on abundance-based Jaccard dissimilarities revealed that the structure and composition of soil and fruit communities significantly differed according to the management approach (soil: $R^2 = 0.113$, $P = 0.013$; fruit: $R^2 = 0.156$, $P = 0.046$). Again, the bark and juice communities showed no significant differences between the two management

approaches (bark: $R^2 = 0.080$, $P = 0.566$; juice: $R^2 = 0.082$, $P = 0.552$). Variation in the structure and composition of fungal communities is represented by classic multidimensional scaling of non-binary Jaccard measures of community dissimilarity in Fig. 4. The significant effect of management on communities in fruit and soil appeared to be differentially underpinned by various taxonomic groups. In soil, only the removal of Sordariomycetes (class) disrupted the significant difference detected. However, differences between biodynamic and conventional fruit communities appeared to be affected by the differential abundance of five separate genera: *Columnosphaeria*, *Davidiella*, *Hanseniaspora*, *Chalara*, and *Trichothecium*.

3.6. Effects of management on fungal-derived quality indicators in wine

Finally, we evaluated the concentrations of volatile thiols in spontaneously fermented wines deriving from these vineyards. Two volatile thiols are important in sauvignon blanc aroma and quality, and these are metabolically liberated by yeasts from aroma-less precursors in juice during fermentation (Anfang et al., 2009; Harsch and Gardner, 2013; Masneuf-Pomarède et al., 2006; Santiago and Gardner, 2015). A simple *t*-test reveals there was no difference in the concentrations of 3MH and 3MHA in wines deriving from vineyards with different management approaches: $P = 0.053$ (*t*-ratio 2.193, 10 d.f.) and $P = 0.706$ (*t*-ratio 0.388, 10 d.f.); see Supplementary Table S1.

In summary, habitat is approximately 17 times more important than management practice in determining the number, type, and abundance of fungal species across this agricultural ecosystem. However, it appears that management approaches also subtly effect fungal communities, and the striking observation is that these effects differ according to habitat in the ecosystem. Communities in all vineyard habitats are affected by management approach in some way, while communities in juice are not affected nor are some important quality parameters in the final wine, and these differences are summarised in Table 2.

4. Discussion

We have shown that conventional and biodynamic agricultural practices significantly differentially influence patterns of fungal diversity in vineyards. Whilst this is not striking, the fact that biodiversity was affected differentially between habitats is of significance. Perhaps most importantly, these data show no difference in biodiversity associated with the harvested products from alternate management systems, and this translated to no effect of management approach for one key fungal-derived quality component in wine. Exploring the impacts of commercial management on microbial diversity is particularly relevant to the practice of commercial winemaking, as the process itself hinges on the activity of naturally occurring fungal species that convert sugars to ethanol and other flavour compounds from harvested grapes (Barata et al., 2012; Swiegers et al., 2005; Zott et al., 2010, 2011).

This study represents a significant step forward as it both quantifies how biodiversity is affected by different agri-management approaches, and evaluates the flow-through effect on the harvested crop and its microbially-derived products. As far as we are aware, this is the first study to test these questions. In characterising diversity across multiple habitats, we show not only that different management practices for the cultivating plant species can significantly impact resident microbial communities, but also that these effects are complex and that communities are differentially affected contingent upon habitat. Additionally, our data show that while we can observe differences in diversity between different management practices, these differences are far less pronounced than differences imposed by selection in different habitats. Understanding the relationship between human interventions and the ecology of microbial ecosystems represents an exciting frontier of research. It is especially relevant for the wine industry as it demonstrates that management decisions in the vineyard can directly affect microbe communities that surround commercially valuable grape vines. Here we show that such impacts on vineyard diversity may not necessarily affect the harvest crop or the products the crop might be transformed into by microbes. Moving forward, these impacts are likely to become the subject of commercial and scientific interest as we understand more about how terrestrial microbial communities can affect the health, development, and resilience of plant species and the crops and products derived from them (Lau and Lennon, 2011, 2012; Panke-Buisse et al., 2015; Sugiyama et al., 2013).

Table 2
Summary of fungal community differences across habitats by agricultural management.

Habitat	Number of species	Types of species	Abundance of species
Soil	No difference	Different	Different
Bark	Biodynamic > conventional	Not different	Not different
Fruit	Biodynamic > conventional	Different	Different
Harvested juice	No difference	Not different	Not different

Another key challenge to understanding the impacts of human intervention on complex microbial ecosystems is the requirement to measure and quantify the impacts on communities in different habitats within larger ecosystems. To date, the vast majority of studies only measure microbial diversity of one habitat at a time – principally soil communities. While the soil microbiome represents a crucial component of terrestrial ecosystems, these data suggest we may not necessarily use it to directly assess other microbial communities in the ecosystem.

Our study reports differential patterns of fungal diversity between various habitats in biodynamic and conventionally managed vineyards, and there are a number of factors that could plausibly be driving these differences. Other studies have reported that a number of specific human interventions can affect microbial diversity in specific habitats of commercially managed ecosystems (Čadež et al., 2010; Gomiero et al., 2011; Hartmann et al., 2015; Martins et al., 2012, 2014; Perazzolli et al., 2014). One central component of biodynamic viticulture is the regular and systematic use of different organic composting techniques which are used extensively over vineyard blocks. Composting techniques are used by conventionally managed vineyards to some degree, but generally are implemented less intensively and less frequently than biodynamic vineyards. Studies have thus reported corresponding significant effects of composting techniques and soil management on soil microbial diversity in agricultural environments (Bossio et al., 1998; Girvan et al., 2003; Gomiero et al., 2011; Hartmann et al., 2014; Hartmann and Widmer, 2006; Saison et al., 2006; Vega-Avila et al., 2015). Another key feature of biodynamic viticulture (and organic viticulture generally) is the heavily reduced use of pesticide sprays. Pesticide use is rare/non-existent in biodynamic viticulture contrasting conventional vineyards who routinely use them to control the spread and development of various fungal diseases. In cases when biodynamic vineyards are permitted to apply pesticides, the number of approved fungicides is considerably fewer than those available to conventionally managed vineyards. The impacts of fungicide sprays on fungal diversity have been documented in terrestrial ecosystems that are commercially managed (reviewed in Bünemann et al., 2006; Barata et al., 2012). In vineyard studies these effects have been reported but have almost exclusively come from examinations of specific fungicides on fruit-associated fungi (Barata et al., 2012; Čadež et al., 2010; Comitini and Ciani, 2008; Martins et al., 2014, 2012; Perazzolli et al., 2014; Schmid et al., 2011).

Broadly, the fungal diversity we observed in fruit and soil habitats appear consistent with previous research examining soil or fruit independently from separate conventional or biodynamic or other organic vineyards (Hartmann et al., 2015; Martins et al., 2014; Saison et al., 2006). Other than our previous report of bark associated fungi at

the landscape scale (Morrison-Whittle and Goddard, 2015), we cannot compare and contrast our findings of fungal communities associated with vineyard bark as such data are lacking.

Not all the patterns commonly associated with organically managed agri-systems were supported by our results. Many studies have reported the tendency of increased levels of biodiversity in organically managed ecosystems (reviewed in Hole et al., 2005; Setati et al., 2012; Martins et al., 2014; Bossio et al., 1998 (biomass not diversity); Gomiero et al., 2011; Hartmann et al., 2015; Saison et al., 2006). While we did see significantly higher species richness in biodynamic fruit and bark communities, this was not detectable in soil communities where this trend is most often reported. Overall species richness did not significantly differ between management approaches in this study.

As we grow more aware of the role of microbial assemblages in the health, development, and productivity of plant species, it will become more imperative that we characterise and manage the way in which we influence plant-associated microbial diversity – intentionally or not. Our approach provides insight into the complex microbial ecosystem surrounding and potentially affecting a commercially valuable plant species and represents a significant step forward in our attempts to understand the impacts of human activities on microbial ecosystems. While it is unsurprising to discover that different management approaches mainly based around the use of anti-fungal sprays and microbially-based fertilisers affect fungal biodiversity, our data reveal that: (1) the way biodiversity is affected by management approach differs between habitats; and (2) that management approach does not necessarily translate to biodiversity differences associated with the harvested product or quality signature which are microbially-derived from them.

By understanding the impacts of specific ecosystem interventions and practices on microbial communities, we glean valuable insight into the ecology of these ecosystems. This provides a baseline by which to objectively develop approaches that safeguard and strategically manage biodiversity and the environment. It may also pave the way for deliberate and targeted manipulation of microbial communities and ecosystems, and to minimise harmful impacts on the environment while maintaining the value of products derived from it.

Conflict of interest

We are not aware of any conflict of interest in carrying out this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2017.05.022.

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