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BRIEF REPORT

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Soil and bark biodiversity forms discrete islands between vineyards that are not affected by distance or management regime

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Abstract

Within geographic regions, the existing data suggest that physical habitat (bark, soil, etc.) is the strongest factor determining agroecosystem microbial community assemblage, followed by geographic location (site), and then management regime (organic, conventional, etc.). The data also suggest community similarities decay with increasing geographic distance. However, integrated hypotheses for these observations have not been developed. We formalized and tested such hypotheses by sequencing 3.8 million bacterial 16S, fungal ITS2 and non-fungal eukaryotic COI barcodes deriving from 108 samples across two habitats (soil and bark) from six vineyards sites under conventional or conservation management. We found both habitat and site significantly affected community assemblage, with habitat the stronger for bacteria only, but there was no effect of management. There was no evidence for community similarity distance–decay within sites within each habitat. While communities significantly differed between vineyard sites, there was no evidence for between site community similarity distance–decay apart from bark bacterial communities, and no correlations with soil and bark pH apart from soil bacterial communities. Thus, within habitats, vineyard sites represent discrete biodiversity islands, and while bacterial, fungal and non-fungal eukaryotic biodiversity mostly differs between sites, the distance by which they are separated does not define how different they are.

INTRODUCTION

Biodiversity underpins ecosystem services and it is therefore important to understand the forces that govern the spatial distribution of species that comprise communities (Tilman et al., 2014). Microbes are a large subset of total biodiversity and are an important part of all global habitats as they play essential roles in ecosystem functions such as nutrient turnover (Graham et al., 2016; Martiny et al., 2015). Understanding the reasons for differences in microbial community assemblages in different places is indispensable for an integrated understanding of ecosystems. Like macroorganisms, microbes are non-uniformly distributed in space; however, microbes do not always appear to follow the same distribution patterns as plants or large animals (Dickey et al., 2021). Several biodiversity patterns have been recognized for microbes (Zhou & Ning, 2017), and these include species–area relationships (Horner-Devine et al., 2004), latitudinal diversity patterns (Andam et al., 2016; Hillebrand, 2004), island biogeography (Li et al., 2020) and community similarity distance–decay (Green et al., 2004; Green & Bohannan, 2006; Morlon et al., 2008).

Two prevailing theories attempt to explain biogeographic patterns generally and these also apply to microbes: the niche (selection) and neutral (stochastic) theories. The niche theory simply suggests that microbial populations are extremely large with no barriers to dispersal and thus communities are predominantly assembled by the deterministic operations of natural selection: this is the classic Baas Becking theory 'Everything is everywhere, and the environment selects' (Baas Becking, 1934; van der Gast, 2015). According to this theory, a niche is a set of abiotic and biotic conditions to which a species is adapted, and that species differ in their respective extents of adaptation to different niches due to evolutionary trade-offs (Adler et al., 2007; Wiens & Donoghue, 2004). In short, those species better adapted to the conditions in a habitat prevail and this defines the types of species in communities in a given habitat. The ecological neutral theory (Hubbell, 1997, 2005) counterbalances the niche theory and explains the observed variance in biodiversity as a function of stochastic birth, death, colonization, immigration, extinction and speciation events (Zhou & Ning, 2017). Here, different taxa are ecologically functionally equivalent, but dispersal limitations (among other things) contribute to the emergence of biogeographic patterns. The neutral theory predicts that similarity distance–decay relationships would emerge without differences in environmental conditions (Bell, 2010; Hubbell, 2005).

There are an increasing number of studies empirically describing biogeographic patterns in bacterial and fungal microbial communities and populations (e.g., Green et al., 2008; Griggs et al., 2021) with evidence for spatial patterns in bacteria (e.g. Zarraonaindia et al., 2015), fungi (e.g., Miura et al., 2017) and other microbial eukaryotes (e.g., Bates et al., 2013) across scales ranging from a few centimetres (e.g., O'Brien et al., 2016) to hundreds of kilometres (e.g., Morrison-Whittle & Goddard, 2015) and from various natural and managed habitats (e.g., Bokulich et al., 2014; Karimi et al., 2018). Non-bacterial and non-fungal microbes (such as mesofauna) also play key roles in ecosystems; however, there is very little data available to understand the spatial patterns of these communities (e.g., Jiang et al., 2015). Considering the importance of all microbes, it is valuable to understand the relative roles of 'selective' versus 'stochastic' forces that govern microbes' spatial distribution, but this is not well characterized and appears to depend on geographic scale, strength of environmental gradients (Hanson et al., 2012) and type of organism (Bahram et al., 2016; Soininen et al., 2007). Further, there is evidence that the drivers of microbial biogeographic patterns differ at different spatial scales (Hanson et al., 2012; Martiny et al., 2011; Meyer et al., 2018; Whitaker et al., 2003), but there are very few studies that test whether community assembly processes vary between taxa at different spatial scales (but see Feng et al., 2019).

Terrestrial agricultural ecosystems provide 97% of the world's food calories and microbes play key roles in providing food as they underpin soil fertility and crop diseases (Kopittke et al., 2019). One agroecosystem that has been utilized to evaluate microbial community patterns is vineyards as these are good model systems to study the effect of distance on microbial communities as vineyards comprise relatively similar environments with similar plants managed in similar ways but are separated by varying distances (Morrison-Whittle & Goddard, 2015). Miura et al. (2017) studied bacteria and fungi on leaves and fruits of Chilean vineyards at regional scales of 35 km and found fungal community composition differed between six vineyards for both leaves and berries and that the dissimilarity of fungal communities increased with distance. The results for bacteria were less straightforward: while bacterial community composition on leaves differed among sites, berry bacterial community composition did not. In addition, leaf bacterial communities were generally not increasingly dissimilar with distance. These results indicate that fungi may be limited in their dispersal at relatively fine scales (in a 0.25 km^2 area), but that bacteria may not be (Belisle et al., 2012; Miura et al., 2017; Peay & Bruns, 2014). Setati et al. (2012) demonstrated that intra-vineyard variation can be greater than inter-vineyard variation for yeast communities on grapes at distances of <10 km. Other work has shown significant biogeographic patterns for fungi and bacteria across New Zealand (NZ) vineyards, both at large (>100 km; Giraldo-Perez et al., 2021; Morrison-Whittle & Goddard, 2015; Taylor et al., 2014) and small (<1 km; Knight et al., 2020) scales, with strong community differences between soil, bark and fruit habitats (Morrison-Whittle et al., 2017; Morrison-Whittle & Goddard, 2015). Morrison-Whittle and Goddard (2015) found fungal community composition differed more greatly by habitat than the distance across 1000 km and inferred these patterns were influenced by both selective (niche) and stochastic (neutral) processes, but that selection was four times stronger than stochastic processes such as dispersal limitation. The use of pesticides sprays, especially fungicides, may affect microbial diversity in agroecosystems; while some work shows this may have a marked effect on biodiversity (Hendgen et al., 2018; Ortiz-Álvarez et al., 2021), other work shows only a weak effect of conservation versus conventional management on vineyard biodiversity (Giraldo-Perez et al., 2021; Morrison-Whittle et al., 2017). However, whether microbial spatial patterns are affected by different management regimes is unknown to our knowledge. In addition, while there is evidence for differences in microbial biodiversity between vineyard soil, root, bark, leaf and fruit habitats (Marasco et al., 2022; Miura et al., 2017; Vitulo

et al., 2019; Zarraonaindia et al., 2015), it is not clear if microbial spatial patterns differ between these habitats. Overall, despite advances in the study of patterns underlying the spatial distribution of microbes, our knowledge of microbial biogeography patterns remains limited, especially for non-bacterial and non-fungal microbes, and especially at fine (<100 m) to medium scales (>100 m and <10 km).

To start to address these gaps in knowledge here we quantify the effect of habitat, location and management regime across <100 m and <10 km spatial scales on broad biodiversity patterns in permanent bark and soil vineyard habitats. Collectively, the existing data suggest there is a hierarchy in the strength of forces that determine microbial community assemblage: ecological habitat (bark, soil, etc.) appears the strongest, followed by geographic location (site), and then management regime (conventional, conservation, etc.). The available data also suggest that community similarities decay with distance within habitats. To assess this, we formulate and then test a 'hierarchical-decay' hypothesis for patterns in microbial community assemblage within regions. This hypothesis has two predictions (P), that within regions (<10 km): (P1) the hierarchy of community assemblage structuring force strength is ordered habitat>site>management regime for communities across all taxa; and (P2) within habitats, there is a constant decay of community similarity with geographic distance, that is, there is no scale at which communities become homogenized. To test this hypothesis, we gathered 3.8 million bacterial 16S, fungal ITS2 and non-fungal eukaryotic COI DNA sequences that were derived from 108 samples across two habitats (soil and bark) from six differentially managed vineyards sites (either conventional or conservation that differ threefold in agrochemical applications; Giraldo-Perez et al., 2021) from the Marlborough region in New Zealand as this is the largest grapevine growing and wine producing region in the country.

EXPERIMENTAL PROCEDURES

Study sites and sampling

One hundred and eight samples of vine bark and soil were collected in April 2019 from six Sauvignon Blanc vineyard sites in the Marlborough region in the South Island of New Zealand, approx. 41°S, 173°E (Figure 1 and Table S1). Three vineyards were under conventional management and were not limited in their use of pesticides, and the other three were under conservation management and did not use any synthetic pesticides and only used phytosanitary products approved under organic agriculture, and Giraldo-Perez et al. (2021) have shown there is approximately a threefold difference in agrochemical inputs between these. All samples were collected into sterilized tubes and taken from a random angle and distance in a 16-m radius from each of nine predefined dispersed sample points within each vineyard following Giraldo-Perez et al. (2021). About 150 g of bulk soil with plant material removed was taken from 20 cm depth soil cores from both under-vine and inter-row at each sample point and then combined to a total of 54 soil samples. Pilot work showed the biodiversity between under-vine and inter-row did not significantly differ (data not shown). Bark was sampled according to Morrison-Whittle et al. (2017): approximately 50 g of bark was peeled from the vine trunk at a height of 30 cm at each sample point to a total of 54 bark samples. All samples were transferred to the University of Auckland on ice and then frozen at −80°C until further processing. Bark and soil pH was measured in the laboratory in water for each sampling point and homogenized soil samples per vineyard site were sent to Hill Laboratories (www.hill-laboratories.com) for analyses of anaerobically mineralizable nitrogen (AnMiN), potentially available nitrogen (PotN), total nitrogen (TotN), total carbon (TotC), Organic Matter (OrgM), Olsen phosphorous (OlsP) and volume weight (VolW, also known as bulk density; Table S13).

DNA extraction

DNA was extracted following Morrison-Whittle and Goddard (2015) for bark samples and Giraldo-Perez et al. (2021) for soil samples. Soil and bark samples were dried at 70°C until their weight stabilized; soil was sieved through a 2-mm mesh and bark was cut with sterilized scissors to roughly 1 mm size particles. DNA was extracted from each sample using a Quick-DNA Faecal/Soil Microbe DNA Miniprep Kit from Zymo Research (D6010), following the manufacturer's protocol with a few minor modifications of the workflow: after the addition of BashingBead™ Buffer samples were incubated at 60°C for 10 min. Qiagen TissueLyzer II (Qiagen) was used for the bead-beating step at 30 Hz frequency for 7 min. Finally, after the addition of DNA Elution Buffer directly to the column matrix, samples were incubated at room temperature for 10 min. DNA was eluted from spin columns with 80 μL of Elution Buffer and stored at −20°C until further analysis.

DNA amplification and sequencing

Amplification and sequencing of 16S, ITS2 and COI barcodes were performed for the analysis of bacterial, fungal, and non-fungal eukaryotes, respectively, following Giraldo-Perez et al. (2021). The V3-V4 domain of the bacterial 16S rRNA gene was amplified using primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-

FIGURE 1 Location of vineyard sites in Marlborough, New Zealand; each vineyard was sampled at nine within vineyard sample points. The distance between the two furthest sample points is 13.2 km, the distance between the two closest is 10 m, the mean distance between points within vineyards is 34 m and the mean distance between vineyards is 8 km. The dark green circle symbols show vineyards with organic certification under a conservation management regime. Map used with a permission from Wine Marlborough.

3′; Herlemann et al., 2011). The Internal Transcribed Spacer 2 (ITS2) region of the fungal 18S rRNA gene was amplified using primers ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′; White et al., 1990). The mitochondrial Cytochrome C Oxidase subunit I gene (COI) of eukaryotes was amplified using mlCOIintF (5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′; Folmer et al., 1994) and jgHCO2198R (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′; Geller et al., 2013) primers. Illumina adapters were added to all primers and each barcode from each sample was amplified in triplicate with KAPA HiFi HotStart ReadyMix DNA polymerase (Roche) and then combined. Negative PCR controls (blanks) included in all PCR batches revealed the polymerase was contaminated with a mixture of bacterial DNA. All sequences that were present in the negative control were subsequently removed from all samples with a post-sequencing bioinformatics removal step using the microDecon R package (McKnight et al., 2019). PCR products were cleaned using AMPure XP (Agencourt) and quality-checked using Qubit® dsDNA HS Assay kit and Agilent 2100 Bioanalyzer High Sensitivity DNA® kits. The University of Auckland Genomics sequencing facility conducted Illumina paired-end library preparation, cluster generation, and 600 cycles of 2 × 300 bp paired-end sequencing run on Illumina MiSeq.

Bioinformatics

Raw Illumina fastq files were delivered demultiplexed by the sequencing centre. Following the DNA-barcode biodiversity standard analysis method (DNA-BSAM) (Fernández-Huarte et al., 2023), sequences were then quality filtered and analysed using QIIME v2.2020.11 (Bolyen et al., 2019). Reads were trimmed from primers and truncated at any site containing more than three consecutive bases with a quality score <30 Phred. The Divisive Amplicon Denoising Algorithm (DADA) 2 (Callahan et al., 2016) was used to remove any low-quality sequences and to filter out chimeric sequences,

and the resulting amplicon sequence variants (ASVs) were clustered to 97% identity, and these merged-ASVs (mASVs) represent taxonomic units that approximate differences between 'species' (Alberdi et al., 2018; Giraldo-Perez et al., 2021) and here we refer to these as 'phylotypes'.

The default *decon* function of the MicroDecon package was used on bacterial, fungal and non-fungal eukaryotic animal datasets separately with sequenced negative PCR controls for bark and soil habitats (McKnight et al., 2019), and this resulted in removing 92 mASVs (16S: 91, ITS2: 1 and COI: 0) and 442,694 sequences (16S: 128806, ITS2: 313888 and COI: 0). Representative 16S and ITS2 mASVs were taxonomically classified in QIIME2 using a scikit-learn naive Bayes machine-learning classifiers generated with QIIME2 and a representative taxonomic database. We used a subset of the SILVA database release 138 (https://www.arb-silva.de/documentation/release-138/; Quast et al., 2013) to classify the 16S mASVs and the UNITE fungal ITS database version 8.2 (https://unite.ut.ee/repository.php; Abarenkov et al., 2020) for ITS2 mASVs classification. The SILVA database was prepared using the RESCRIPt package (Robeson et al., 2021) in QIIME2. Representative COI mASVs were classified by using BLASTn and the MIDORI2 COI database (http://www.reference-midori.info/, GenBank release 252, October 2022; Leray et al., 2022).

Any mASV representing less than 0.001% of the total filtered sequences was removed. 16S and ITS2 data sets were filtered to include only sequences that respectively classified to Bacteria or Fungi at the kingdom level, and all mASVs unidentified at the phylum level were removed. Reads that matched as mitochondria or chloroplasts (6% of total 16S reads) were filtered out with phyloseq (McMurdie & Holmes, 2013). For COI, all mASVs assigned to Fungi, Chordata or unassigned at the phylum level were removed such that the COI data set subsequently comprised only eukaryote non-chordate metazoans and protozoa. The code to perform these operations is supplied in Supplemental File 1.

Statistical analysis

Samples with less than 500 sequences were removed and all data were standardized to median sequencing depth following the recommendation in phyloseq (McMurdie & Holmes, 2013). We follow DNA-BSAM (Fernández-Huarte et al., 2023), developed by Giraldo-Perez et al. (2021), Morrison-Whittle et al. (2017), and Morrison-Whittle and Goddard (2018), which analyses and quantifies the differences in the numbers, types and relative abundances of phylotypes between variables. Here, the discrete variables are habitat, vineyard site and management regime, and 'distance' is a continuous variable.

All data handling and statistical analyses were performed using R Statistical Software v4.0.2 (R Core Team, 2020), *phyloseq* v1.32.0 (McMurdie & Holmes, 2013), *microbiome v1.10.0* (Lahti & Shetty, 2022) and *vegan* v2.5.7 (Oksanen et al., 2022). Figures were generated with *ggplot2 v3.3.5* (Wickham et al., 2022), *venn v1.10* (Dusa, 2021) and *ggpubr 0.4.0* (Kassambara, 2020). Differences in the numbers of phylotypes (Chao1 diversity estimate) were evaluated with Kruskal–Wallis tests and differences in the types and abundances of phylotypes were evaluated with permutational multivariate analysis of variance (PERMANOVA; Anderson, 2008), with 999 permutations, using binary Jaccard distances and the Ružička index for types and abundances respectively. The experimental design of analysing working commercial vineyards means that each site is managed in only one of two ways, and this means that a full factorial analysis which includes analysing for interactions between habitat, site and management factors is not possible. This is because all vineyard sites were managed as a whole and not split between management approaches; that is, all samples from any one site are also managed in the same way. We accounted for this in analyses: when testing habitat or management regime factors, the permutation scheme was modified with the 'strata' option in vegan's 'adonis2' function to accommodate the nested characteristics of this experiment such that the observations from the same vineyard were kept together as the habitat or management label was permuted (Table S3).

η^2 from Kruskal–Wallis tests and R^2 from permANOVA and variation partitioning analysis (VPA; Peres-Neto et al., 2006) quantified effect sizes. For Kruskal–Wallis and permANOVA tests, spatial factors were represented by individual vineyard sites. For VPA, spatial components were either a simple linear spatial gradient or a distance-based Moran Eigenvector Maps (dbMEM) following Borcard et al. (2011) for each scale separately. The dbMEM matrix was created from the geographic coordinates of sites (or individual sample points) and only dbMEM with a significant Moran index was selected for variation partitioning analysis. To calculate the VPA, we used function *varpart* in vegan with adjusted R^2 coefficient. Variance partitions were tested with an ANOVA-like permutation test for Redundancy Analysis on Hellinger transformed community data, geographic cartesian coordinates, habitat and management variables, and if significant, dbMEM was used as well.

The effect of geographic distance was analysed with Mantel tests (Pearson correlation, 700–9999 permutations) and variation partitioning. For Mantel tests, arcsine transformed Jaccard dissimilarities and Ružička indices were correlated with Haversine distances either within-vineyard or between-vineyard scales for separate habitats. For variation partitioning, we used either linear distance or significant dbMEMs

and quantified the comparable effect of habitat at the within-vineyard scale. To test the significance of variation partitioning at a medium scale, we used a modified permutation scheme as in the adonis2 tests. The effect of medium (>100 m and <10 km) scale distances was analysed with a custom function to exclude intra-vineyard dissimilarities from distance matrices and then correlate biodiversity to geographic distance dissimilarity matrices.

Raup–Crick (RC) dissimilarity metrics (Chase et al., 2011), from the *raupcrick* function in the *vegan* package (Oksanen et al., 2022), were used to estimate the proportion of selective and stochastic forces contributing to community assembly within and between habitats. The function uses presence/absence data to calculate the probability of the number of co-occurring species in particular habitats given overall species frequencies, and this indicates whether co-occurring species in a particular habitat are more dissimilar (approaching 1), or more similar (approaching 0) than expected under neutral processes. The result provides a quantitative estimate of the degree to which deterministic processes have created communities deviating from stochastic model expectations (Chase et al., 2011; Vellend et al., 2007). The code to perform these operations is supplied in Supplemental File 1.

RESULTS

After quality filtering, 1,347,321 16S, 1,442,707 ITS2 and 1,115,438 COI sequences were obtained from the vine bark and soil habitat samples from six Sauvignon Blanc vineyard sites in the Marlborough region on the South Island of New Zealand (Figure 1 and Table S1).

Three COI soil samples (from sites 1, 3 and 5) were removed due to low sequence depth, and one random COI soil sample from each of the three remaining vineyard sites (2, 4 and 6) was excluded to regain a balanced design and result in $n = 8$ sample points per vineyard site for the soil COI data, but the 16S and ITS2 samples remained at $n = 9$ (Figure S1). A total of 3941, >97% genetic identity mASVs (these are analogous to OTUs) were revealed in the data, with 1959 16S mASVs assigned to the Bacterial kingdom, 1220 ITS2 mASVs assigned to the Fungal kingdom and 762 COI mASVs assigned to a range of non-fungal non-chordate eukaryotes (COI mASVs assigned to Fungi, Chordata or unassigned at Phylum level were removed). Previous studies comparing the analysis of 16S and ITS2 ASVs and 97% OTU-based community composition revealed that both methods recover similar ecological results (Glassman & Martiny, 2018). From this point on, the manuscript will use >97% mASVs and phylotypes interchangeably. A total of 24, 10 and 30 phyla were identified for 16S, ITS and COI mASVs, respectively; the two most abundant phyla were Proteobacteria and Firmicutes, Ascomycota and Basidiomycota, and Discosea and Arthropoda for 16S, ITS2 and COI mASVs respectively, and the complete mASV tables are in Tables S2A–C. Following Fernández-Huarte et al. (2023), Giraldo-Perez et al. (2021), Morrison-Whittle et al. (2017) and Morrison-Whittle and Goddard (2018), biodiversity will be evaluated using three main metrics and these are differences in the numbers, types, and abundances of phylotypes.

Testing P1: The hierarchy of community assemblage structuring force strength is ordered habitat>site>management regime for communities across all phylotypes

Independent Kruskal–Wallis tests revealed a consistently significant effect of habitat, an inconsistent effect of the site (only Bacteria were affected), and no effect of management on the numbers of phylotypes (Table 1 and Figure 2). As revealed by permANOVA tests, habitat and vineyard site had a consistently significant effect on both the types and relative abundances of all phylotypes, but there was no effect of management on any phylotypes by any metric (Table 1 and Figures 2 and 3).

Since there were significant differences in biodiversity between habitats, we went on to analyse whether communities differed by site and management within each habitat separately. This revealed significant differences between vineyard sites for all measures for bark and soil habitats separately except for the number of soil fungal phylotypes (Table S3). However, there were no significant differences between management regimes within either habitat for any barcode by any measure ($p > 0.1$; Table S3). Soil had 20% more phylotypes than bark overall (2517 and 2019, respectively, Figure 2), and just 13% of phylotypes were present in both bark and soil, but these shared phylotypes represented an average of 66% of total sequences (44%, 84% and 71% for 16S, ITS2 and COI, respectively, Table S4). While habitat-specific non-fungal eukaryote phylotype richness was approximately equal between bark and soil habitats, there were more bacterial than fungal phylotypes in soil, and more fungal than bacterial phylotypes in the bark: bacterial habitat-specific richness was threefold greater in soil, and fungal habitat-specific richness fourfold greater in bark (Figure 2).

Post hoc Dunn's tests revealed that Vineyard 5 had a significantly lower number of phylotypes than all the other vineyards when both habitats were analysed together (Table S5). Post hoc pairwise comparisons for the types and abundances across all phylotypes revealed consistent differences between sites for phylotypes inhabiting bark (70 of all possible 90 bark pairwise distances $p\text{adj} < 0.05$; Table S6), but for soil, there were mostly differences in only bacterial

TABLE 1 Results of tests for differences in the numbers, types and relative abundances of phylotypes between habitats, sites and management regimes for bacteria, fungi and non-fungal eukaryotes.

	Numbers	Types	Abundances
	By Kruskal–Wallis tests	**By PermANOVA on Jaccard distances**	**By PermANOVA on Ružička distances**
Bacteria			
Habitat ($n = 2$)	$\mathbf{p = 0.049; \eta^2 = 0.027}$	$\mathbf{p = 0.001; R^2 = 0.316}$	$\mathbf{p = 0.001; R^2 = 0.301}$
Site ($n = 6$)	$\mathbf{p = 0.001; \eta^2 = 0.157}$	$\mathbf{p = 0.001; R^2 = 0.121}$	$\mathbf{p = 0.004; R^2 = 0.088}$
Management ($n = 2$)	$p = 0.165; \eta^2 = 0.009$	$p = 0.413; R^2 = 0.027$	$p = 0.297; R^2 = 0.021$
Fungi			
Habitat ($n = 2$)	$\mathbf{p \leq 0.001; \eta^2 = 0.275}$	$\mathbf{p = 0.001; R^2 = 0.0805}$	$\mathbf{p = 0.001; R^2 = 0.077}$
Site ($n = 6$)	$p = 0.065; \eta^2 = 0.053$	$\mathbf{p = 0.001; R^2 = 0.107}$	$\mathbf{p = 0.001; R^2 = 0.073}$
Management ($n = 2$)	$p = 0.421; \eta^2 = -0.003$	$p = 1; R^2 = 0.00966$	$p = 0.897; R^2 = 0.012$
Non-fungal eukaryotes			
Habitat ($n = 2$)	$\mathbf{p = 0.005; \eta^2 = 0.069}$	$\mathbf{p = 0.001; R^2 = 0.042}$	$\mathbf{p = 0.001; R^2 = 0.05}$
Site ($n = 6$)	$p = 0.198; \eta^2 = 0.024$	$\mathbf{p = 0.001; R^2 = 0.066}$	$\mathbf{p = 0.001; R^2 = 0.075}$
Management ($n = 2$)	$p = 0.634; \eta^2 = -0.007$	$p = 0.898; R^2 = 0.01$	$p = 0.796; R^2 = 0.012$

Note: Factors were tested separately. Bold font highlights statistically significant results at $p < 0.05$, and η^2 and R^2 effect sizes are shown.

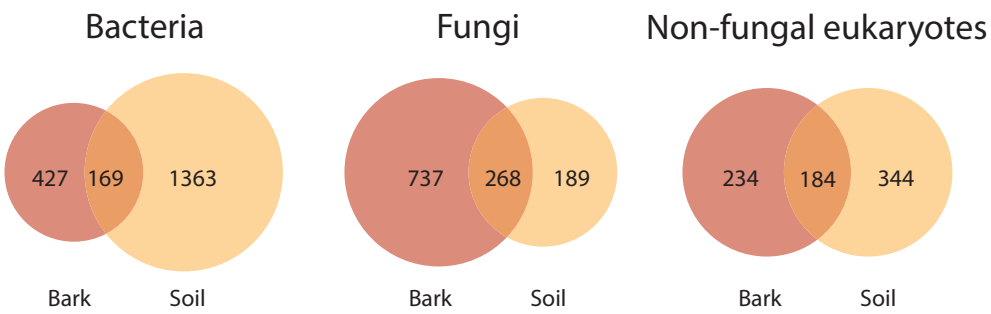

FIGURE 2 Venn diagrams showing the number of unique and shared types of mASVs between bark and soil habitats for phylotypes across all barcodes. Circle sizes are not to scale. mASV, merged amplicon sequence variant.

communities among sites (25 of the 35 significant soil differences were for bacterial communities; Table S6).

Inspection of η^2 and R^2 effect size values (Table 1) shows on average, habitat had the greatest effect size (mean 14%), followed by site with a mean effect size of 10% (Figure S2). However, the relative differences in effect sizes between habitat and site varied between biodiversity metrics and barcodes (Table 1): for the seven cases where there was a significant effect of both habitat and site on a biodiversity metric, only two showed more than a twofold greater effect size for habitat than site (bacterial types and relative abundances) and for the other five instances effect sizes are either comparable or greater for site. Overall, the hypothesis that community assemblage structuring force strength is ordered habitat>site>management is supported. However, the more accurate reformulation of the hypothesis given the data is that all communities consistently differ between soil and bark habitats, but that there are also significant differences between sites, with cases of equal or greater effect size for the site depending on phylotypes and biodiversity metric; management does not affect soil and bark biodiversity.

Testing P2: Within habitats, there is a constant decay of community similarity with geographic distance

We first evaluated if there is a community similarity distance–decay effect at distances below 100 m within vineyard sites. Variation partitioning analyses (VPAs) allow the mean linear distance between within-site points (ranging from 10 to 97 m) to be evaluated as a continuous variable and thus test for evidence of community similarity distance–decay. Within each habitat,

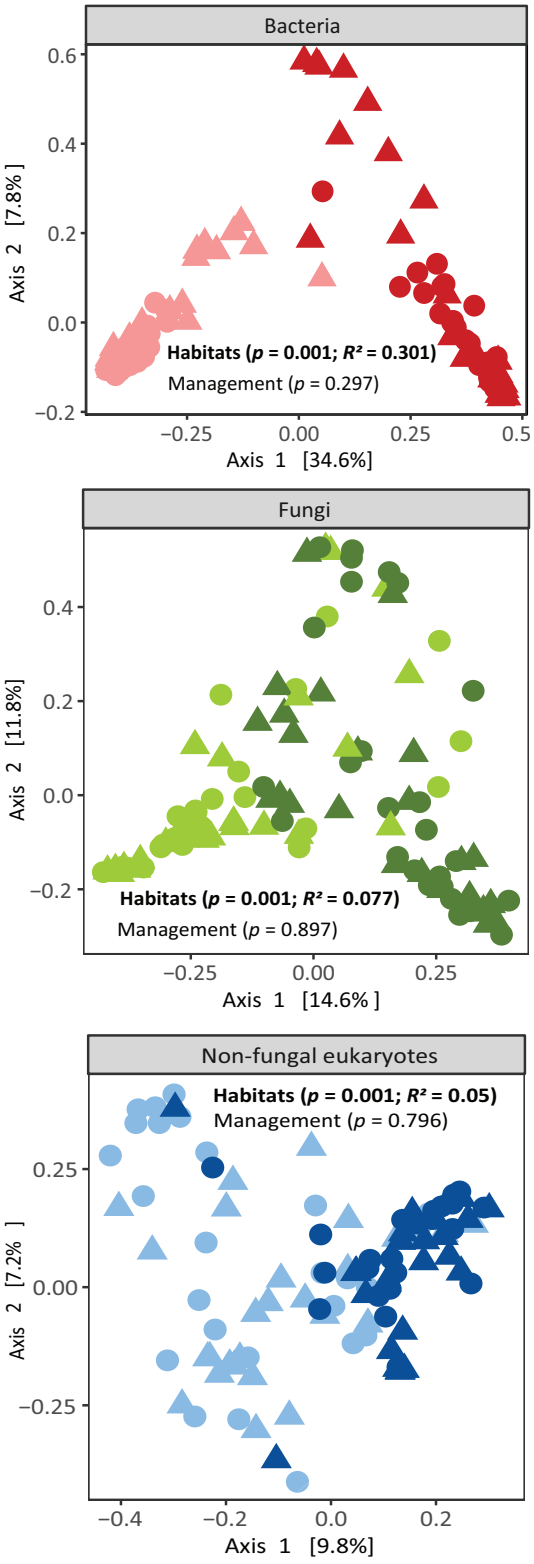

FIGURE 3 Visualization of PCoA ordination of community composition measured by Ružička distance (phylotypes abundances) for each barcode (16S = bacteria; ITS2 = fungi; COI = non-fungal eukaryotes) with percent variation explained by each axis shown. The shade shows habitat (bark = light; soil = dark) and symbols show management regimes (circle = conventional, triangle = conservation).

VPA revealed no effect of linear distance between 10 and 100 m (Table S7), and there were no spatial dbMEM vectors that correlated with community structure (Table S8). Only 7 of the 72 (10%) within vineyard Mantel tests for correlations between linear distance and community similarity for types and abundances across all phylotypes revealed significant positive correlations ($p < 0.05$). However, all seven significant community similarity distance–decay patterns were from conservation-managed vineyards, but with no clustering by phylotypes (Table S9, Figure 4A). The observation of 7 and 0 significant correlations from the 72 overall tests being respectively assigned to the conservation and conventionally managed sites suggest a non-random association between distance–decay and management approach, but the low number of observations hinders robust analysis of this.

Thus overall, for distances below 100 m, there is no clear distance–decay patterns revealed for any phylotypes in either habitat. There is a weak suggestion that vineyards managed under a conservation approach may be more spatially structured for soil and bark biodiversity compared to conventional vineyards, but note the majority of communities in sites managed with a conservation approach showed no distance–decay (29 out of 36 comparisons). Altogether, it appears that within vineyards, most soil and bark communities do not show distance–decay and are effectively not spatially structured: this is not in line with P2.

We next analysed for community similarity distance–decay patterns between sites at distances greater than 100 m. VPA of phylotypes abundances again revealed the strongest predictor of community differences was habitat, accounting for an average of 21.5% of variation ($p < 0.001$ for all phylotypes, Table S10). When each habitat was analysed separately (Table S11), only two of the six comparisons were significantly affected by absolute distance (bark bacterial and non-fungal eukaryotic communities, $p = 0.009$ and 0.008, respectively; Table S11). Mantel tests revealed only 3 of the 12 community types or abundances similarity and geographic distance correlations to be marginally significant, but all three were for bark communities (types of bark fungal phylotypes, $p = 0.045$, and bark non-fungal eukaryotes, $p = 0.026$, and abundance of bark bacterial phylotypes, $p = 0.048$; Figure 4B and Table S12). Therefore, while there are significant differences in biodiversity between sites in each habitat, there is no strong evidence for community similarity distance–decay between sites at distances between 100 m and 10 km. However, there is a suggestion that some bark communities may be weakly structured by community similarity distance–decay whereas soil communities are not. Taken together, these analyses reject P2 and instead show that within habitats, there is not a constant decay of

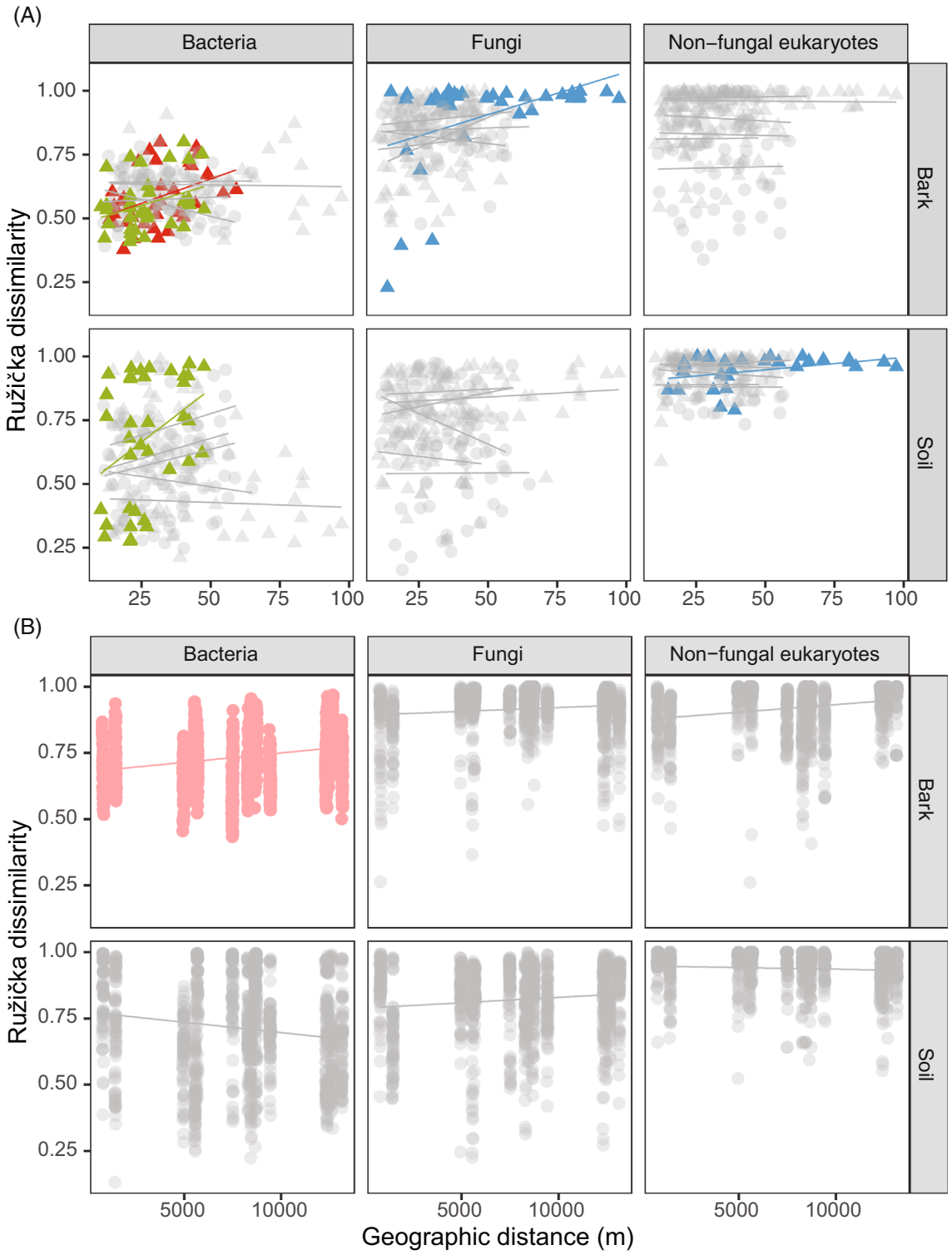

FIGURE 4 Distance–decay plots between community dissimilarity (Ružička index) and geographic distance for bark and soil communities for each phylotype (16S = bacteria; ITS2 = fungi; COI = non-fungal eukaryotes) and for (A) within and (B) between vineyard sites. The significance of distance–decay relationships was tested with Mantel tests and only significant Pearson correlations are represented in colour, others are in grey. For (A), symbols show management regimes (circle = conventional, triangle = conservation) and colours represent vineyard sites with significant distance–decay relationship (red = vineyard 4, blue = vineyard 5 and green = vineyard 6).

community similarity with geographic distance: within vineyards at fine scales <100 m, biodiversity within bark and soil habitats show no overall distance–decay and while biodiversity significantly differs between vineyard sites, there is no strong correlation between community similarity and absolute distance

between vineyards (with the possible exception of bacterial bark communities). It appears that within each habitat and vineyard site, there are no barriers to dispersal (Figure S3), and while vineyard sites are differentiated from one another in their bark and soil biodiversity, the distance they are separated by does not strongly define how different they are. This suggests that factors other than dispersal limitation play a significant role in driving these variations.

THE DRIVERS OF COMMUNITY DISSIMILARITY

The data and analyses show that communities vary more greatly by habitat than distance for all phylotypes and from a comparison of effect sizes in Table 1 this suggests that selection is one and a half times as strong as stochastic processes in assembling these bark and soil communities (mean fold-difference in effect sizes of habitat and site across numbers, types and abundances for 16S, ITS2 and COI are 1.7, 1.6 and 0.8, respectively). The modified Raup–Crick dissimilarity metric (βRC) is another way to evaluate the likely mechanisms of community assemblage as this calculates the probability of species co-occurrence. The neutral community assemblage process predicts species will co-occur in habitats with a probability defined by their overall cross-habitat underlying frequencies, but the deterministic actions of selection predict species adapted to specific habitats will more likely co-occur together in these habitats (Raup & Crick, 1979). In line with the above inferences, the clearest signals were for the operations of selection between habitats and stochastic processes within habitats at both fine and medium scales (Figure 5). However, while bacterial community assemblage between habitats appears to be driven predominantly by selection, there is a suggestion for an equal contribution of both selective and stochastic forces defining the assemblage of fungal and non-fungal eukaryote communities between habitats (Figure 5). The picture within habitats is a little more complex: community composition within both habitats for most phylotypes was mostly driven by stochastic forces at both scales (βRC is towards 0) (Figure 5).

DISCUSSION

Understanding the patterns and mechanisms of community assembly is an important topic in microbial ecology (Antwis et al., 2017). The focus of this study was to understand and evaluate the effects of habitat, location and management regime on biodiversity in bark and soil habitats in vineyard ecosystems at regional spatial scales. In addition, studies that have analysed taxonomically broader aspects of microbial biodiversity are rare and here we evaluate bacterial, fungal, and eukaryote non-chordate metazoans and protozoa.

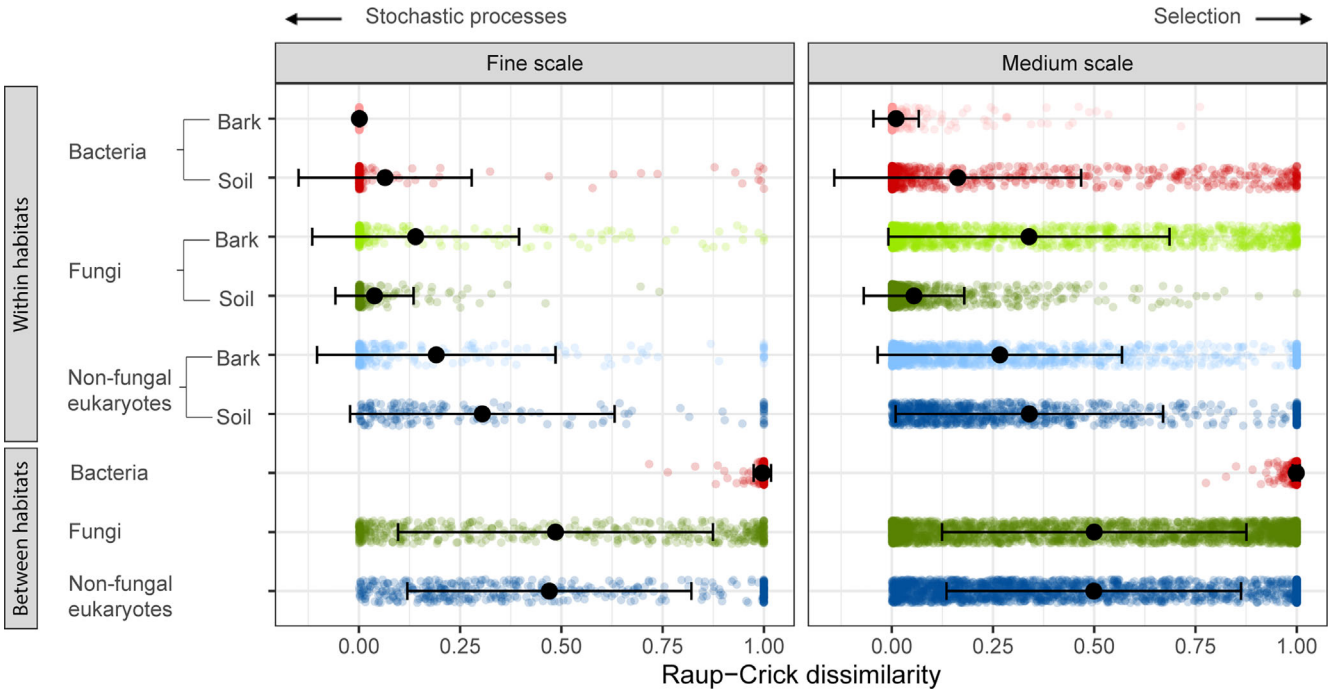

FIGURE 5 Raup–Crick dissimilarity measuring the deviation of communities from a null model of community composition. Values near 0 indicate stochastic processes and values near 1 indicate composition is driven more by selection. The plots show differences at fine (within vineyards) and medium scale (between vineyards), within and between habitats, for different phylotypes. Mean and SD are shown for each.

Overall, the data and analyses support P1: within regions, the hierarchy of community assemblage structuring force is ordered habitat>site>management (Table 1 and Figures 2 and 3), and the effects of habitat were on average 40% greater than site. However, management had little to no detectable effect on any phylotypes in either habitat (only bacterial abundances on bark were revealed as being affected by management with VPA). Analyses also revealed that different phylotypes displayed different hierarchies of community assemblage structuring force strength, with bacteria following habitat>site>management, but for fungi and non-fungal eukaryote phylotypes, the effect of habitat and site were more equivalent. The data rejected the second prediction from the hypothesis (P2) and instead showed that at scales <100 m communities of all phylotypes show no distance–decay patterns, but there was a weak indication that conservation-managed sites might show more overall heterogeneity at this scale (Figure 4A). While biodiversity significantly differs between vineyard sites (Figures S2 and S3), there is no strong correlation between biodiversity dissimilarity and absolute distance between vineyards except for a weak signal for bacterial bark communities (Figure 4B). Overall, this supports the conclusion that there are microbiome differences between habitats and that for each habitat, vineyard sites represent separate homogenized microbiome islands that are differentiated from one another in their biodiversity, but the distances they are separated by do not strongly define how different they are.

Even though the strength of environmental filtering on community assembly is relatively well studied, it has not previously been put in the context of multiple spatial scales and tested using a range of phylotypes. We attempted to understand which ecological forces might drive these differences in community assemblage, and the greater dissimilarity of communities overall by habitat than by distance revealed here suggests that selection is one and a half times as strong as stochastic processes in assembling communities. This is in line with fungal data from NZ vineyards reported by Morrison-Whittle and Goddard (2015), which found that habitat explained four times more variance in fungal community composition than geographic separation. The VPAs conducted here showed a similar trend, at least for bacterial communities, as habitat explained an average of 10 times more variance in community composition than geographic distance (Table S10). Within habitats, the inference from βRC analyses is that assemblage is mostly driven by stochastic forces. In addition, the PCoA ordination plots (Figure 3) showed a notable horseshoe effect which can indicate the presence of a strong environmental gradient linked with habitat differentiation (Bay et al., 2020; Morton et al., 2017) and the degree to which the horseshoe effect was manifested in plots correlated with the strength of ordering by habitat between phylotypes groups (Bacteria>Fungi>Non-fungal eukaryotes). Bark and soil habitats impose vastly different selective conditions for organisms (Griggs et al., 2021). In line with the data presented here, different habitats, albeit close in space, often exhibit different environmental filtering and have the potential to create strong selective pressure on residing communities (Griggs et al., 2021; Zarraonaindia et al., 2015). This inference is also in line with previous data for other vineyard habitats such as flowers, fruits, leaves, roots, bark and soil (Marasco et al., 2022; Martins et al., 2013; Morrison-Whittle et al., 2017; Morrison-Whittle & Goddard, 2015; Vitulo et al., 2019; Zarraonaindia et al., 2015). Also, in line with the findings here, Vitulo et al. (2019) found habitat—grape and bark—to be a major determinant of the bacterial community structure, explaining 44% of the variance. Similarly, Zarraonaindia et al. (2015) also found that the habitat type (bulk soil, root zone soil, root, flower, leaf or grape) explained 45% of the variability in bacterial community composition. Here, we explored community spatial patterns in permanent habitats, and it would be interesting to determine if similar patterns are apparent in ephemeral niches, like leaves and fruit, where the community assembles every growing season completely anew.

While selection is inferred to play a main role in biodiversity differences between habitats, the forces that drive the significant differentiation of microbiomes between sites in the same habitats appear to be a balance of both stochastic and selective forces. Distance did not define the extent of differences in microbiomes between sites, that is, there was no pattern of distance–decay, except for bacterial communities on bark. This is in line with data from Knight et al. (2020) who analysed soil fungal microbiomes from vineyards only a few 100 m apart, and the variance in phylotypes types and relative abundances between sites was relatively higher (∼25%) than was found here. Similar results were reported for soil bacterial communities in Australian vineyards that were an average of ∼12 km apart (Zhou et al., 2021), where bacterial communities were not structured by geographic distance, and the study concluded this was due to environmental heterogeneity between sites. The data here are also in line with the findings for rhizosphere bacterial and fungal communities between two Argentinian vineyards, separated by 6 km (Aguilar et al., 2020). In contrast to these results, Miura et al. (2017) reported significant leaf and berry fungal community similarity distance–decay, but bacterial communities did not show this and so are in line with the data here. We are aware of no vineyard bark spatial studies for comparisons, but the data reported here are in line with the classic study by Bell (2010) which found little evidence of bacterial community distance–decay relationship in bark-associated tree hole ecosystems at fine scales (<600 m), and this is

similar to findings from other microbial biogeographic surveys that report weak or non-existent distance–decay relationship over meters to hundreds of meters (e.g. Horner-Devine et al., 2004).

The lack of dispersal limitation between sites implies the operations of selection are driving the biodiversity differences between sites, and this may be due to numerous factors (e.g., soil types, rootstocks, microclimates, the precise nature of management, the surrounding ecosystems, etc.). For soils, there is an understanding of some parameters that influence microbial communities (Bahram et al., 2016) and pH is well documented to affect bacteria and fungi (Fernández-Huarte et al., 2023; Fierer & Jackson, 2006; Rousk et al., 2009). Fungi also may be influenced by the C/N ratio (Di Lonardo et al., 2020), and COI communities potentially by biotic interactions (Oliverio et al., 2020; Seppey et al., 2017). Marasco et al. (2022) reported that soil type was the main determinant of grapevine root microbial community diversity. Soil chemistry measurements (pH, anaerobically mineralizable nitrogen, potentially available nitrogen, total nitrogen, total carbon, organic matter, Olsen phosphorous, and volume weight, also known as bulk density; Table S13) were determined for the sites and Mantle tests revealed there is no correlation between similarities in soil chemistry profiles and soil biological communities, except for the abundances of soil fungi (Table S14). pH has been documented to significantly affect soil bacterial, and to a lesser extent fungal communities (Fernández-Huarte et al., 2023; Fierer & Jackson, 2006; Rousk et al., 2009), and correlations between soil and bark pH and their respective community compositions revealed that only similarities in soil bacterial communities were correlated with similarities in soil pH (Table S15). This suggests that other than pH for soil bacteria and soil chemistry profiles for fungal abundances, selection due to other environmental parameters may be driving community differentiation between these sites.

It is worth noting the sites studied here were not connected: the space between vineyards may represent a barrier to dispersal, and the fragmentation of environments can contribute to dispersal decay (Peay et al., 2007). In this sense, individual vineyards may be considered as islands, and insufficient dispersal between islands would create heterogeneity between communities in each vineyard, as observed here. The effect of primary colonizers and the history of each site, or each site's historical contingency, is also recognized as an important factor that can define community composition (Fukami et al., 2010) and is possibly responsible for heterogeneity between vineyard units. Priority effects in combination with dispersal limitation, where first colonizers exclude later arriving species, can persist after the establishment of an initial community (Bell, 2010; Vellend, 2010). As Vellend et al. (2007) suggest, the human land-use spatial pattern of biodiversity can remain both within and across sites for decades or centuries. The vineyards evaluated here were planted between 16 and 24 years ago, and differences in historical contingency might have influenced the current biodiversity composition in each. Since the relatively high community dissimilarity between vineyards was not strongly explained by distance or soil chemistry, we tested if the age of vineyards correlated with community differences. Mantel tests indicated a significant relationship between only bark bacterial community dissimilarity and vineyard age ($p < 0.022$, Table S16). However, it is also conceivable that each vineyard developed in relative isolation with the current microbiome being shaped by the starting community. This may be especially true for the communities in soil that differed by neither absolute distance nor age of the vineyards, where the priority effect of previous communities has a long history. For bark communities, however, the starting community developed only recently with the plantation of vines, and differences in bark physiochemical priorities (including morphology, physiology, exudates, etc.) other than the simple pH measured here may have played a role in modulating the recruitment and selection of members of the microbial community.

Finally, agricultural management regimes have the potential to manipulate community composition, but work in NZ, including in the same sites as analysed here, shows the overall strength of the effect on biodiversity is relatively small for soil microbiomes at least (Giraldo-Perez et al., 2021; Morrison-Whittle et al., 2017). This is in contrast to recently published literature from other countries, including studies from Spain, United States and Slovenia (Burns et al., 2016; Likar et al., 2017; Ortiz-Álvarez et al., 2021). In our analyses, we found a small significant effect of management only for bark bacterial phylotypes and only with variation partitioning analysis. While we did not observe a strong management effect, it is conceivable the low replication of each management type in this study ($n = 3$) does not allow this to be evaluated with a high degree of certainty. Additionally, as previously discussed by Giraldo-Perez et al. (2021), vineyards under conventional management in New Zealand rely on both synthetic and non-synthetic chemistry, overlapping with conservation management practices, and generally limit the use of synthetic chemistry on their sites. These aspects of NZ conventional management could contribute to the smaller difference between conventional and conservation management regimes compared to other countries.

This study contributes to our understanding of the patterns of biodiversity of major microbial taxonomic groups in bark and soil habitats at different spatial scales in agricultural systems and helps to uncover the underlying processes governing species distributions at

these scales. Overall, these data show that differences in agricultural microbiomes are most strongly defined by habitat but within habitat, communities form more discrete microbiome islands between sites within regions. While vineyard sites are differentiated from one another in their microbial biodiversity, the distance they are separated by does not strongly define how different they are. If present at all, any effect of agricultural management on total soil and bark microbiomes is very weak and subsidiary to the effects of habitat and site. These patterns and hierarchy of effects hold across bacterial, fungal, and eukaryote non-chordate metazoans and protozoa phylotypes, but the absolute size of effects of habitat and site differs between these major taxonomic groups.

AUTHOR CONTRIBUTIONS

Lucie Jiráska: Conceptualization (equal); data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); project administration (lead); software (lead); visualization (lead); writing – original draft (equal); writing – review and editing (equal). **Beatrix Jones:** Formal analysis (supporting); methodology (supporting); software (supporting); supervision (supporting); writing – review and editing (equal). **Sarah Knight:** Conceptualization (equal); formal analysis (equal); methodology (supporting); project administration (supporting); supervision (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Jed Lennox:** Conceptualization (equal); investigation (supporting); writing – review and editing (equal). **Matthew Goddard:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); methodology (equal); project administration (supporting); supervision (lead); visualization (supporting); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequence data are available at the NCBI database: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA906189 and the published article contains tables derived from the raw sequence data.

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SUPPORTING INFORMATION

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