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Authors

Burns, Kayla N

Kluepfel, Daniel A

Strauss, Sarah L

et al.

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Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by geographic features

Kayla N. Burns [a], Daniel A. Kluepfel [b], Sarah L. Strauss [b], Nicholas A. Bokulich [a, c, 1], Dario Cantu [a], Kerri L. Steenwerth [b, *]

[a] Department of Viticulture and Enology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
[b] USDA-ARS Crops Pathology and Genetics Research Unit, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
[c] Foods and Health Institute and Department of Food Science and Technology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

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ABSTRACT

Here, we examine soil-borne microbial biogeography as a function of the features that define an American Viticultural Area (AVA), a geographically delimited American wine grape-growing region, defined for its distinguishing features of climate, geology, soils, physical features (topography and water), and elevation. In doing so, we lay a foundation upon which to link the *terroir* of wine back to the soil-borne microbial communities. The objective of this study is to elucidate the hierarchy of drivers of soil bacterial community structure in wine grape vineyards in Napa Valley, California. We measured differences in the soil bacterial and archaeal community composition and diversity by sequencing the fourth variable region of the small subunit ribosomal RNA gene (16S V4 rDNA). Soil bacterial communities were structured with respect to soil properties and AVA, demonstrating the complexity of soil microbial biogeography at the landscape scale and within the single land-use type. Location and edaphic variables that distinguish AVAs were the strongest explanatory factors for soil microbial community structure. Notably, the relationship with TC and TN of the <53 μm and 53–250 μm soil fractions offers support for the role of bacterial community structure rather than individual taxa on fine soil organic matter content. We reason that AVA, climate, and topography each affect soil microbial communities through their suite of impacts on soil properties. The identification of distinctive soil microbial communities associated with a given AVA lends support to the idea that soil microbial communities form a key in linking wine terroir back to the biotic components of the soil environment, suggesting that the relationship between soil microbial communities and wine *terroir* should be examined further.

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1. Introduction

Wine *terroir*, the set of perceived qualities imparted to a wine by its land of origin (e.g. van Leeuwen et al., 2004), by definition, is related to geographical patterns. This has economic importance, and, accordingly, wine grape-growing regions are legally delimited and regulated. An American Viticultural Area (AVA) is a geographically delimited American wine grape-growing region, defined for its distinguishing features of climate, geology, soils, physical features (topography and water), and elevation. Recent detection of microbial biogeographical patterns in wine grape musts has provided a potential link between microbial biogeography and wine *terroir* by AVA (Bokulich et al., 2014). However, the present study is the first to examine soil microbial biogeography as a function of the features that define an AVA. Here, we lay a foundation upon which to link the *terroir* of wine back to the soil-borne microbial communities.

Wine grape production, a multi-billion dollar global industry, can support a multi-functional landscape that provides many ecosystem services (Viers et al., 2013). Soil microorganisms are an active part of these ecosystem services, for example, through pathogen suppression (Garbeva et al., 2004), nitrogen cycling (Madsen, 2005), and mineralization, contribution, and preservation of soil organic matter (Kögel-Knabner, 2002; Kuzyakov et al.,

* Corresponding author.
E-mail addresses: knburns@ucdavis.edu (K.N. Burns), daniel.kluepfel@ars.usda.gov (D.A. Kluepfel), sarah.strauss@ars.usda.gov (S.L. Strauss), nbokulich@ucdavis.edu (N.A. Bokulich), dacantu@ucdavis.edu (D. Cantu), kerri.steenwerth@ars.usda.gov (K.L. Steenwerth).
[1] Present address: Department of Medicine, New York University Langone Medical Center, New York, NY 10016, USA.

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2009; Grandy and Neff, 2008; Plaza et al., 2013). Indirect effects of soil microorganisms on plant growth, health, and fruit development are particularly pertinent to the role of soil microorganisms in wine *terroir* (Garbeva et al., 2004; Compant et al., 2010). Conceptually, vineyard microorganisms that participate in unique, beneficial interactions could be exploited to improve or modify grapevine performance and enhance wine properties in specific viticultural zones. Likewise, soil microorganisms that negatively impact wine grape production, such as stunting fruit development or promoting excess vigor, could be controlled by both biotic factors and viticultural management practices. to enhance wine grape production.

Understanding the form and function of soil microbial community in the grape perennial cropping system will facilitate our ability to enhance wine grape production. Improvements in next-generation sequencing technology have made high-throughput community analyses affordable, allowing widespread, comprehensive biogeographical surveys of microbial communities. Biogeographical patterns of microbial communities across all systems, including soil systems, are thought to be driven by two general factors: dispersal limitation and contemporary environmental heterogeneity (Fierer, 2008). A driver is "any natural or human-induced factor that directly or indirectly causes a change in an ecosystem" (Nelson et al., 2005). However, because of the paradox of scale, different drivers may dominate at different scales. The hypothesis "everything is everywhere, but the environment selects" (Baas Becking, 1934; De Wit and Bouvier, 2006) suggests environmental heterogeneity alone drives microbial biogeographical patterns. More recent studies also support the importance of dispersal limitation, by showing the power of geographic distance in predicting soil microbial community structure (Martiny et al., 2006). Thus, the drivers of soil microbial community structure include (*a*) environmental heterogeneity itself, (*b*) factors that influence that environmental heterogeneity, and (*c*) geographic distances.

In soils, examples of these drivers include climate, soil properties, land use or management, and topography, including slope and elevation. Soil properties that are potential drivers of soil microbial community biogeographical patterns, include soil texture, pH, water content, carbon (C) and nitrogen (N) content, and C:N ratio, and the ability of soil microorganisms to influence their own environment (Drenovsky et al., 2004; Cookson et al., 2006; Fierer and Jackson, 2006; Hogberg et al., 2007; Lauber et al., 2009; Fierer et al., 2012). Specifically, microbial communities are likely responsible for both contributing and stabilizing fine soil organic matter (SOM) associated with silt and clay particles (<53 μm) (Kögel-Knabner, 2002; Grandy and Neff, 2008; Plaza et al., 2013), thus providing a key mechanism for agricultural producers to participate in mitigation of greenhouse gas emissions. Even though the SOM in fine soil fraction (<53 μm) is often assumed to be mineral associated, Plaza et al. (2013) suggested this may not always be the case. Therefore, we refer to this fraction as 'fine SOM' instead of 'organomineral complexes.' Typically, fine SOM is thought to reflect residues that have been highly decomposed by soil microorganisms. Therefore, it is likely that fine SOM concentrations and measures of soil microbial activity, like respiration and potentially mineralizable nitrogen (PMN), reflect the microbial community structure (Soon et al., 2007; Riches et al., 2013). Although PMN has been found to correlate with bacterial community structure (Cookson et al., 2006), the link between SOM concentration gradients, either in the silt- and clay-sized fraction or in coarser fractions, with soil microbial community structure is less clear. Furthermore, mechanisms behind these myriad drivers of soil microbial community structure named here are not entirely understood, and these factors alone often do not fully explain the spatial patterns observed in soil microbial community structure (Drenovsky et al., 2010).

The distinct features of the Napa Valley AVA of California, its subdivision into 16 smaller AVAs (i.e. sub-appellations) and the well-documented knowledge of the environment, soils, and management provided the opportunity to examine the effects of each of these factors on soil microbial community structure in order to identify key drivers of soil microbial biogeographical patterns. To exploit this opportunity, we measured differences in the soil bacterial and archaeal community structure as a function of soil properties and environmental gradients in Napa Valley. Microbial community structure was examined by sequencing the fourth variable region of the small subunit ribosomal RNA gene (16S V4 rDNA) amplified from total genomic DNA extracted directly from the soil. We acknowledge that while there are limitations to using 16S rRNA sequences in comparison to metagenomics, which can allow for characterization of functional traits, this approach is still an effective and common technique for characterizing the soil bacterial community structure (Lombard et al., 2011). Based on current understanding of soil-microorganism interactions and microbial biogeography, we hypothesized that: (*1*) PMN of whole soil and C and N content of fine SOM are correlated with soil bacterial community structure and (*2*) variations in soil bacterial communities, at the landscape scale, result from gradients in environmental and edaphic properties, which may be represented by AVAs.

2. Materials and methods

2.1. Study system

Soil samples were collected from 57 sites in 19 wine grape vineyards, with three sites per vineyard, throughout the Napa Valley AVA (Fig. 1). Napa County and its AVAs offer a diversity of mesoclimates, soils, and topography, consisting of steep mountains, rolling hills, terraces, and several dividing valleys of various sizes (Lambert and Kashiwagi, 1978). Partitioning around medoids (Kaufman and Rousseeuw, 1990; Hollander, 2012) considering elevation, solar irradiation, soil great group, soil drainage class rank, soil texture by percent sand, silt, and clay, and percent organic matter from the Soil Survey Geographic Database (SSURGO, 2010) was used to determine potential soil sampling sites across eight cluster types in order to capture the diversity of environmental conditions throughout the Napa Valley AVA as part of a larger study. The present study uses a subset of those samples from six of the eight cluster types based on sample fidelity for microbial work. Thus, this study is structured as a completely randomized design (Drenovsky et al., 2010) due to unequal sampling across clusters.

Each site's GPS coordinates were utilized to extract select soil (SSURGO, 2013), climate (PRISM Climate Group, 2012), and topographical data (Towill, 2003) from a geographic information system. The soil type, landscape features, and management practices of the 19 selected vineyards are outlined in Table 1. The 19 vineyards represent 8 of the 16 sub-appellations of the Napa Valley AVA (Fig. 1) and 4 soil great groups (Haploxeralfs, Haploxerolls, Haploxerults, Xerofluvents) of various textures (Table 1).

2.2. Soil sampling and characterization

Soil samples were collected March–June, 2011, at a depth of 0–5 cm, from the centers of the vineyard alleyways. Plant residues and shoots, if present, were removed prior to soil collection. GPS coordinates were recorded for each site. Pairwise geographic distances between sites within each vineyard ranged from 19 m to 270 m, and pairwise geographic distances between sites from vineyard to vineyard ranged from 112 m to 52.97 km (Fig. 1). At each

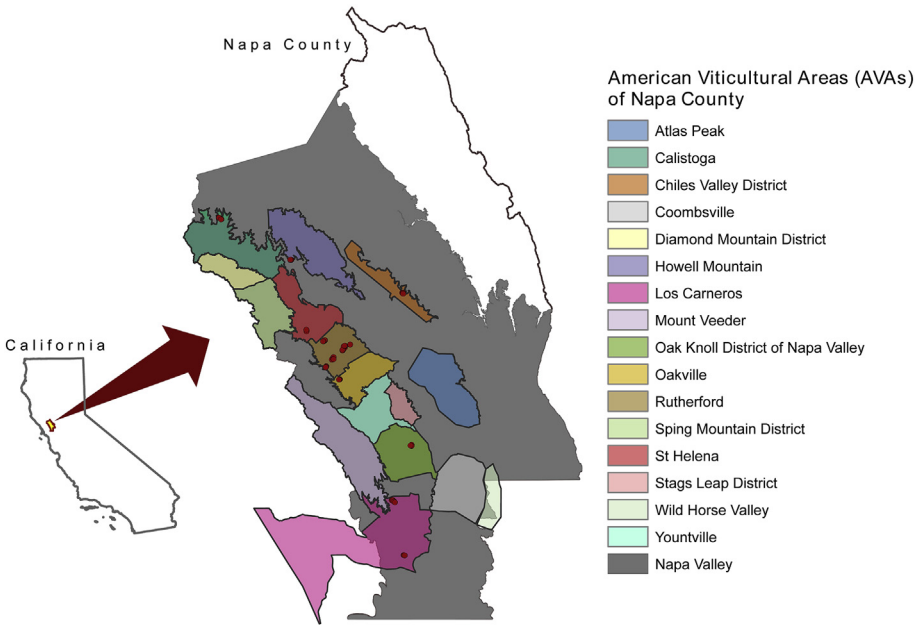

Fig. 1. Map of soil sampling sites throughout Napa Valley. Color-coding is by American Viticultural Area (AVA).

site, three soil samples, approximately 2 m between each, were collected and mixed into a composite sample. Samples were kept on ice (ca. 2–6 h) until representative subsamples were divided for laboratory analyses. For microbial community assessment, 50 g of soil from each composite sample was stored in sealed plastic bags at −80 °C.

2.2.1. Whole-soil physicochemical property determination

Bulk density, soil water content, pH, inorganic nitrogen (N) pools, dissolved organic carbon (DOC), and potentially mineralizable nitrogen (PMN) were determined for each composite sample. During soil sampling, a brass ring (diameter 5.2 cm and height 6.6 cm) was used to collect soil profile cores for bulk density determination. Gravimetric soil water content was determined by drying soil (50 g–300 g) at 105 °C for 24 h. The pH was determined using a 1:1 water to soil ratio. Inorganic N and DOC were extracted using 0.5 M potassium sulfate (K_2SO_4; 30 mL per 12.5 g soil) (Jones and Willett, 2006; Rousk and Jones, 2010). DOC was determined on a Total Organic Carbon Analyzer (TOC-V CSH, Shimadzu Scientific Instruments, CA, USA) (Pella, 1990a,b). PMN was measured by

Table 1
Region, soil type, and select management, landscape, and climate attributes for the vineyard locations (three sites per each) sampled in this study.

Vineyard	AVA[a]	Soil subgroup	Texture[b]	Cover crop[c]	Tillage[d]	Compost[e]	Method[f]	Elev[g] (m)	Slope (%)	Aspect	Precip[h] (mm)
V01	Rutherford	Mollic Haploxeralfs	SL	L + C + M	T	Y	O	50	1.5	S	870
V02	Rutherford	Cumulic Ultic Haploxerolls	L	L + C	NT	N	O	55	1.0	S	870
V03	Oakville	Cumulic Ultic Haploxerolls	L	N	NT	Y	C	65	4.0	SE	882
V04	Rutherford	Mollic Haploxeralfs	SL	L + C + M	T	Y	O	56	2.0	S	882
V05	Rutherford	Mollic Haploxeralfs	SL	N	T	Y	O	57	2.5	SE	895
V06	Rutherford	Cumulic Ultic Haploxerolls	L	L + C	NT	Y	O	58	2.0	S	872
V07	Rutherford	Cumulic Ultic Haploxerolls	L	L + C	NT	N	O	82	11.5	NE	902
V08	Rutherford	Mollic Xerofluvents	SiL	L + C	T	Y	C	48	1.5	NW	868
V09	Rutherford	Mollic Xerofluvents	SiL	M	NT	Y	C	47	1.0	SE	868
V10	Rutherford	Mollic Xerofluvents	SiL	G	NT	Y	C	49	1.0	SW	868
V11	Los Carneros	Typic Haploxeralfs	SCL	L + C	NT	Y	B	94	25.0	SE	754
V12	Los Carneros	Typic Haploxeralfs	L	L + C	T	Y	B	68	12.0	E	731
V13	Los Carneros	Typic Haploxerults	L	L + C	T	Y	B	10	4.0	SW	567
V14	St Helena	Typic Xerofluvents	L	M	T	N	C	78	2.0	E	947
V15	Oak Knoll	Typic Haploxerults	L	G	NT	N	C	22	1.0	S	676
V16	Howell Mtn	Lithic Haploxerolls	L	G	NT	N	C	492	14.5	SE	1033
V17	Chiles Valley	Typic Haploxeralfs	SiL	L	T	N	O	275	6.0	W	954
V18	Calistoga	Mollic Haploxeralfs	L	G	NT	N	C	184	21.0	E	1059
V19	Calistoga	Lithic Haploxerolls	L	G	NT	Y	C	208	27.0	SW	1059

[a] AVA: American Viticultural Area.
[b] Soil texture of the fine-earth fraction. SL: Sandy Loam; L: Loam; SiL: Silty Loam; SCL: Sandy Clay Loam.
[c] L: legumes (unspecified peas, beans, clovers, or vetch); C: cereals; M: mustards; G: grasses (non-cereal); N: resident vegetation or no cover crop.
[d] T: tilled; NT: no-till.
[e] Y: Compost was broadcast in the alley; N: No compost (or compost was banded under vine, and since soil samples were taken in the alley, they did not receive the compost).
[f] General agricultural method as self-defined by vineyard managers. O: organic; C: conventional; B: biodynamic.
[g] Elevation.
[h] Average annual precipitation (1981–2010).

anaerobic incubation (15 mL DDI water per 7 g soil) at 40 °C for 7 days and extracted with 2 M potassium chloride (KCl; 30 mL per 7 g soil, from the addition of 15 mL of 4 M KCl per incubated sample) (Waring and Bremner, 1964; Soon et al., 2007). Nitrate and ammonium concentrations from soil and PMN were determined by colorimetric analysis (Kempers and Kok, 1989; Miranda et al., 2001).

2.2.2. Soil fractionation and chemical property determination

Soil was fractionated into size classes for characterization of organic matter pools (Lee et al., 2009). Air-dried soil was sieved to <2 mm, shaken with 0.5% sodium hexametaphosphate ($Na_6O_{18}P_6$; 100 mL per 30 g soil) for 18 h, wet-sieved into fractions (2000–1000 μm, 1000–250 μm, 250–53 μm, and <53 μm), oven-dried at 65 °C for 3 days, and mechanically ground for 4 h. Total C and total N of each fraction and of the whole soil (<2 mm fraction) were determined by combustion using an Elemental Combustion System (Costech Analytical Technologies, Inc., CA, USA) (Pella, 1990a,b).

2.3. Soil DNA extraction

From each frozen (−80 °C) soil sample stored at −80 °C, a representative subsample was homogenized using a sterile mortar and pestle. DNA from four subsamples (0.25 g field-moist soil each) was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, CA, USA). The manufacturer's protocol was modified slightly by: (i) increasing the vortex time of the PowerBead Tubes to 15 min on a vortex equipped with a 24-place vortex adapter, (ii) extending centrifugation of the PowerBead Tubes to 60 s to for soils with higher clay contents, and (iii) extending the drying time after use of Solution C5 to 2 min. All DNA extracted from soil was checked for quality using gel electrophoresis and a NanoDrop spectrophotometer (Thermo Fisher Scientific, DE, USA).

2.4. DNA library preparation and sequencing

The V4 region of the 16S rRNA gene (Liu et al., 2007) was amplified using the universal primer pair 515F (5′-NNNNNNNNGTGTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Bates et al., 2011; Caporaso et al., 2011), following a procedure similar to that of Bokulich et al. (2012). Specifically, a unique 8-digit barcode sequence on the 5′ end of the forward primer was applied to each sample to allow multiplexed sequencing downstream. Each polymerase chain reaction (PCR) contained 5–25 ng DNA template, 1× Colorless GoTaq Flexi Buffer (Promega Corporation, WI, USA), 0.625 u GoTaq DNA Polymerase (Promega Corporation, WI, USA), 1.25 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.2 μM of forward and reverse primer. Each PCR run included a negative control. Thermal cycler conditions consisted of initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min.

Following PCR amplification, products were checked using gel electrophoresis. Samples exhibiting weak bands were reamplified. PCR products were combined into a single pooled sample on an equimolar basis based on concentrations determined using a Qubit fluorometer (Invitrogen, Life Technologies, CA, USA). The pooled sample was passed over illustra MicroSpin S-300 HR Columns (GE Healthcare Life Sciences, NJ, USA) for PCR purification and submitted to the University of California–Davis Genome Center DNA Technologies Core Facility (Davis, CA, USA) for sequencing using the MiSeq system (Illumina, Inc., CA, USA).

2.5. DNA sequence processing and analysis

Raw Illumina fastq files were demultiplexed and quality filtered using QIIME v1.6.0 and analyzed using QIIME v1.7.0 (Caporaso et al., 2010b) (Table S1). Reads with a Phred quality of <20 were discarded. Operational taxonomic units (OTUs) were assigned using QIIME's UCLUST-based (Edgar, 2010) open-reference OTU-picking workflow, with a threshold of 97% pairwise identity. Sequence prefiltering (discarding sequences with <60% pairwise identity to any reference sequence) and reference-based OTU picking were performed using the Greengenes 16S rRNA gene database (13_5 release) (DeSantis et al., 2006). OTUs were classified taxonomically using a QIIME-based wrapper of the Ribosomal Database Project (RDP) classifier (Wang et al., 2007) and the Greengenes 16S rRNA gene reference database (13_5 release) (McDonald et al., 2012; Werner et al., 2012), using a 0.80 confidence threshold for taxonomic assignment. 16S rRNA gene sequences were aligned using PyNAST (Caporaso et al., 2010a) against a template alignment of the Greengenes core set filtered at 97% similarity, and a phylogenic tree was generated from the filtered alignment using FastTree (Price et al., 2010). Principal coordinates analysis (PCoA, i.e. metric multidimensional scaling), using the weighted and unweighted UniFrac (Lozupone and Knight, 2005) distance matrices of pairwise community dissimilarities (β-diversity) on a randomly selected subset of 6803 sequences per sample (a cutoff based on inspection of the library), showed tight clustering of the replicate samples originating from the same composite soil sample (Fig. S1). For that reason, each replicate was collapsed into its composite sample: OTU tables were split by sample replicate, unique replicate identifiers were removed, and these OTU tables (without replicate identifiers) were merged, forming the OTU table to be used for all further analyses. Any OTU representing less than 0.001% of the total filtered sequences was removed to avoid inclusion of erroneous reads that would otherwise lead to inflated estimates of diversity (Bokulich et al., 2013), as were samples represented by less than 28,008 sequences (a cutoff based on inspection of the library) following all quality-filtering steps.

A randomly selected subset of 28,008 sequences per sample was used for assessments of richness and diversity. The α-diversity (within-sample species richness and evenness) was estimated based on Faith's phylogenetic diversity index (Faith, 1992). Richness was estimated by the number of observed phylotypes (97% similarity OTUs) and by the Chao1 richness estimate, which takes into account the number of singletons present per sample, since they may suggest additional undetected phylotypes (Chao, 1984). Latitude, elevation, slope, average annual precipitation, and soil properties were tested for linear relationships with estimates of α-diversity and richness using Pearson's product moment correlation coefficient (r) with its probability estimate for significance (P, uncorrected).

The β-diversity (between-sample community dissimilarity), using the weighted UniFrac (Lozupone and Knight, 2005) distance between samples, was calculated in QIIME. To enable visualization of sample relationships, the resulting weighted UniFrac distance matrix was used to perform non-metric multidimensional scaling (NMDS) in the R (R Core Team, 2013; RStudio, 2013) vegan package (Oksanen et al., 2013) using four dimensions as determined based on the elbow of the scree (stress *vs.* dimensions) plot in PC-ORD (MjM Software, Gleneden Beach, OR, USA; McCune and Grace, 2006). NMDS is considered the most robust unconstrained ordination method (Minchin, 1987; McCune and Grace, 2006; Oksanen et al., 2013).

To explore relationships between the bacterial communities and each numeric environmental variable, each variable was plotted against each NMDS axis, and Pearson correlation coefficients were

computed and tested for significance. BEST, QIIME's implement of the R vegan BIOENV function, was used to rank the importance of numeric environmental features in influencing β-diversity community comparisons by computing the harmonic (weighted Spearman) rank correlations (Clarke and Ainsworth, 1993). Each environmental feature's significance also was tested using non-parametric permutational multivariate analysis of variance (permutational MANOVA, R vegan ADONIS) (Anderson, 2001) with 999 permutations. For all significant numeric variables based on this analysis, Pearson correlation coefficients and significance were computed in QIIME to determine which relative taxa abundances were correlated.

Soil types and AVAs were tested for differences in soil properties, in α-diversity and richness, and in β-diversity, by differences in spread along NMDS axes, all using the Kruskal–Wallis rank sum test (non-parametric, one-way ANOVA). Like for the numeric environmental features, ADONIS (Anderson, 2001) with 999 permutations was employed to test significant differences between sample groups of AVAs or soil types, based on weighted UniFrac (Lozupone and Knight, 2005) distance matrices of β-diversity.

To determine which relative taxa abundances differed between AVAs or soil types at various levels of taxonomy, one-way analysis of variance (ANOVA) was performed in QIIME. We focused our attention first on our most abundant taxonomic groups and second on taxonomic groups also shown by others to be associated with *Vitis vinifera* rhizosphere, roots, leafs and leaf surfaces, flowers and seeds, berries and berry surfaces, grape musts, or botrytized wine fermentations (Compant et al., 2011; Leveau and Tech, 2011; Barata et al., 2012; Bokulich et al., 2012; Martins et al., 2012; Bokulich et al., 2014) (Table S2). Canonical discriminant analysis (CDA) was performed using the candisc and heplots R packages to graphically reveal differences between sample groups of AVAs or soil types and to identify high-level taxa associated with each AVA and soil type (Friendly, 2007; Gittins, 2011; Fox et al., 2013; Friendly and Fox, 2013).

2.6. Accession numbers

The sequences from this study have been deposited in the QIITA data bank, accession numbers 10082.

3. Results

3.1. Soil physicochemical properties

The soil physicochemical characteristics are presented in Table 2 and Tables S3–S4 (see also Dataset S1). Soil TC and TN varied from 10.61 to 60.64 g kg^{-1} soil and from 0.91 to 4.73 g kg^{-1} soil, respectively. Soil C:N ratios ranged from 9.44 to 16.52. Soil resource pools, i.e. pools of C and N including whole soil TC and TN, DOC, PMN, and TC and TN of the 53–250 μm and <53 μm soil fractions, were positively correlated, along with several other environmental variables (Pearson $r >$ abs(±0.26), $P < 0.05$, Table S5). Notably, elevation was positively correlated with latitude, slope, average annual precipitation, soil TC and TN, DOC, PMN, TC and TN in the 53–250 μm soil fraction, TC (but not TN) in the <53 μm soil fraction, and the C:N ratio of the 53–250 μm soil fraction. Latitude, slope, and average annual precipitation were also positively correlated with soil resource pools, particularly, whole soil TC and TN, DOC, PMN, and TC and TN in the <53 μm soil fraction. In general, spatial and topographic variables in Napa Valley reflect edaphic factors.

Soil properties, especially those that reflect resource availability to soil microorganisms, differed across AVAs and soil types (uncorrected Kruskal–Wallis rank sum tests, Table 2 and Tables S3,S4). The TC, TN, and C:N of the whole soil and each soil fraction, DOC, PMN, pH, and soil water content were each statistically different across AVAs ($P < 0.05$, Table 2). In general, soils from the AVAs Howell Mountain, Oak Knoll, and Calistoga were loams (Table 1) and tended to have the highest C, N, C:N, pH, and soil water content compared to soils from other AVAs. Most notably, soils from Howell Mountain, Oak Knoll, and Calistoga had on average 5.8-fold greater TC in the 53–250 μm soil fraction, 5.0-fold greater TN in the 53–250 μm soil fraction, and 4.8-fold greater PMN than soils from the other AVAs ($P < 0.001$). Xerofluvents, which were predominantly silty loams, had on average 2.9-fold lower TC in the 250–1000 μm soil fraction, 2.7-fold lower TN in the 250–1000 μm soil fraction, and 7.4-fold lower PMN than the other soils ($P < 0.002$). Meanwhile, the Haploxerults, which were all loams, had the highest soil water content, with a mean 1.7-fold greater than the other soils ($P < 0.001$).

Within soil textural classes, on average, loams had 3.1-fold higher TC in the 53–250 μm soil fraction and 2.7 fold higher TN in the 53–250 μm soil fraction when compared to the other soils ($P \leq 0.01$). Loams had on average 1.4-fold higher PMN than the mean PMN of all soils, while silt loams had 4.7-fold higher PMN than the mean PMN of all soils ($P < 0.001$). The loams included soils from all four soil great groups across many AVAs, while the silt loams were predominantly Xerofluvents in the Rutherford AVA (Table 1). Sandy loams, which were all Haploxeralfs in Rutherford, had the lowest DOC with a mean 2.5-fold lower compared to the other soils ($P = 0.005$). Soil water content was 1.8-fold higher on average in loams and sandy loams than in silt loams and the sandy clay loam ($P = 0.002$). In general, soil properties indicative of the soil physical environment and resource availability to soil microorganisms differed with respect to AVA, soil great group, and soil textural class.

3.2. Composition of bacterial communities

In total, we obtained 17,151,254 DNA sequences with a median read length of 242 bp. After quality filtering, 5,743,693 sequences remained, with 28,008 to 208,727 sequences obtained per sample (mean = 100,767 sequences). Of these filtered reads, 99.996% were classifiable to the phylum level, and 43.054% were classifiable to the genus level (Table S6). When grouped at the 97% similarity level, there were 7312 different phylotypes in all of the soils, with an average of 4761 phylotypes per sample. Before final filtering, which removed any OTU representing less than 0.001% of the total filtered sequences, there were 48,227 different phylotypes in all of the soils with an average of 8246 phylotypes per sample (Table S6).

The dominant bacterial groups (relative abundance > 1%) across all soil samples were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, *Verrucomicrobia*, *Gemmatimonadetes*, and *Firmicutes* (Fig. 2 and Table S7). Combined, these nine groups accounted for 96.4% of the sequences. Additional dominant bacterial phyla (relative abundance ≥ 0.05%) were *Cyanobacteria*, *Armatimonadetes*, *WS3*, *Nitrospirae*, *BRC1*, *Chlorobi*, *Elusimicrobia*, *Fibrobacteres*, and *Tenericutes* (Table S7).

3.3. Diversity and richness of bacterial communities

To compare the soil microbial diversity among all samples, the same survey effort level of 28,008 randomly selected sequences was applied to each sample in the sequence library. The Faith's phylogenetic α-diversity ranged from 177 to 258 branches per sample, and phylotype richness ranged from 3184 to 5465, based on the number of observed phylotypes at 97% similarity (Dataset S1). Chao1 richness estimates ranged from 4243 to 6172 (Dataset S1). The diversity between samples (β-diversity) is presented in Dataset S2 as a matrix of pairwise weighted UniFrac distances, or the phylogenetic dissimilarities between samples, weighted based on

Table 2
Means, standard deviations, and Kruskal–Wallis rank sum test of soil properties by American Viticultural Area.

Variable	Calistoga		Chiles Valley		Howell Mtn		Los Carneros		Oak Knoll		Oakville		Rutherford		St. Helena		χ2	P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
GWC	0.12	0.03	0.05	0.02	0.26	0.05	0.11	0.06	0.30	0.06	0.15	0.03	0.17	0.07	0.10	0.01	25.85	<0.001***
pH	6.44	0.31	6.55	0.20	7.07	0.41	6.41	0.29	7.02	0.19	6.16	0.21	6.59	0.45	5.92	0.19	19.05	0.008*
TC	41.74	4.71	14.23	0.99	52.01	13.33	18.22	3.52	46.37	1.62	16.00	3.58	19.92	4.20	16.13	0.50	34.59	<0.001***
DOC	159.68	55.36	45.30	4.25	140.04	58.24	55.65	20.00	107.11	23.67	25.06	3.13	55.98	45.33	38.72	3.92	26.89	<0.001***
TC 250–1000 μm	46.93	7.60	18.16	3.04	55.53	24.52	58.37	58.87	70.94	20.65	11.71	3.32	25.73	29.57	7.69	0.50	25.16	<0.001***
TC 53–250 μm	50.04	12.07	8.91	2.46	58.09	37.42	10.28	6.67	78.56	17.17	9.27	3.52	10.36	2.93	10.76	1.79	29.24	<0.001***
TC < 53 μm	35.72	2.71	13.56	0.55	36.00	15.70	19.53	4.55	34.73	2.03	18.47	2.91	24.01	6.71	26.50	3.38	31.76	<0.001***
TN	3.30	0.54	1.34	0.12	3.96	1.18	1.54	0.29	3.47	0.04	1.45	0.23	1.56	0.30	1.35	0.09	30.34	<0.001***
PMN	203.67	71.26	13.56	6.30	161.80	100.56	64.94	22.47	152.15	34.09	41.11	8.56	29.82	33.92	44.66	5.24	32.89	<0.001***
TN 250–1000 μm	2.48	0.61	1.04	0.16	3.10	1.60	3.13	2.93	3.59	0.94	0.99	0.20	1.44	1.64	0.54	0.01	22.21	0.002**
TN 53–250 μm	2.98	0.78	0.65	0.16	3.70	2.81	0.72	0.38	4.90	0.94	0.80	0.26	0.72	0.22	0.81	0.20	29.10	<0.001***
TN < 53 μm	3.22	0.30	1.33	0.07	2.81	1.44	1.85	0.47	2.82	0.24	1.72	0.27	2.12	0.49	2.34	0.35	28.56	<0.001***
C:N	12.75	1.04	10.67	0.54	13.29	0.71	11.85	0.48	13.35	0.56	10.99	0.90	12.76	1.24	11.97	0.57	24.77	<0.001***
C:N 250–1000 μm	19.37	2.89	17.38	0.67	18.45	1.65	17.47	2.18	19.71	1.23	11.68	1.72	17.82	1.56	14.24	1.05	19.89	0.006*
C:N 53–250 μm	17.02	1.85	13.71	0.80	16.58	1.78	13.85	1.31	15.99	0.57	11.43	0.89	14.47	1.75	13.42	1.17	22.86	0.002**
C:N < 53 μm	11.14	0.57	10.17	0.17	13.12	0.93	10.58	0.46	12.34	0.57	10.76	0.18	11.20	0.98	11.36	0.29	23.08	0.002**

***p < 0.001, **p < 0.005, *p < 0.01, . p < 0.05, ns p ≥ 0.05.
GWC: gravimetric water content (g water g^{-1} dry soil); pH: pH value in water (one-to-one); TC: total carbon (g kg^{-1} dry soil or g kg^{-1} dry fraction); DOC: dissolved organic carbon (mg kg^{-1} dry soil); TN: total nitrogen (g kg^{-1} soil or g kg^{-1} dry fraction); PMN: estimated potentially mineralizable nitrogen (mg kg^{-1} dry soil); C:N: carbon to nitrogen ratio. Note: df is seven for all tests because there are eight AVAs: Calistoga, Chiles Valley, Howell Mountain, Los Carneros, Oak Knoll, Oakville, Rutherford, and St. Helena.

relative phylotype abundances. The UniFrac distances range from 0.0611 (most similar) to 0.3418 (most dissimilar), with an average distance of 0.1717 (±0.0519) between sites within the same vineyard, and an average distance of 0.2032 (±0.0474) between sites in different vineyards. There was a significant increase in phylogenetic distance, or pairwise dissimilarity in the weighted UniFrac distance matrix, with increasing geographic distance (Pearson $r = 0.296$, $P < 0.001$) (Fig. 3), despite the small geographic scale of the present study (<53 km).

3.3.1. Region, environmental attributes, and bacterial alpha-diversity patterns

To address the hypothesis that species richness and biodiversity vary with AVA and environmental attributes, these attributes were tested for relationships with Chao1 richness and Faith's phylogenetic α-diversity (PD). Neither richness nor PD varied with AVA (Kruskal–Wallis rank sum test, $P > 0.05$). Among spatial coordinates, topographical measurements, average annual precipitation, and soil properties, only soil properties showed consistent significant correlations with PD and richness (Table S8). Finer textures, drier soils, and higher TC and TN in coarse fractions (250–1000 μm) were related to higher α-diversity or richness, while pH and other pools of C and N did not show relationships.

3.3.2. Region, environmental attributes, and phylogenetic bacterial beta-diversity patterns

Phylogenetic β-diversity segregates by AVA, based on unconstrained NMDS ordination of the weighted UniFrac distances

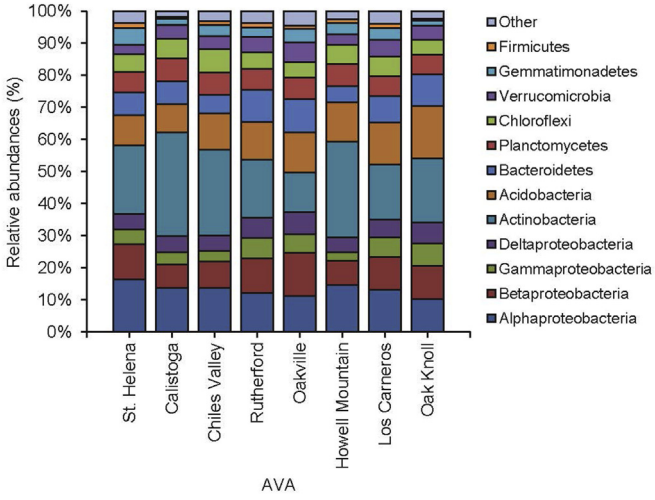

Fig. 2. Relative abundances of the dominant bacterial taxonomic groups separated according to American Viticultural Area (AVA). Bacterial phyla and classes of proteobacteria were assessed for dominance based on their relative abundances across all samples, and dominant groups were chosen for each having greater than 1% relative abundance. Relative abundances were estimated based on frequency of occurrence of sequences classified to each taxonomic group. The "Other" group encapsulates bacteria and archaea belonging to all other phyla (each <1%).

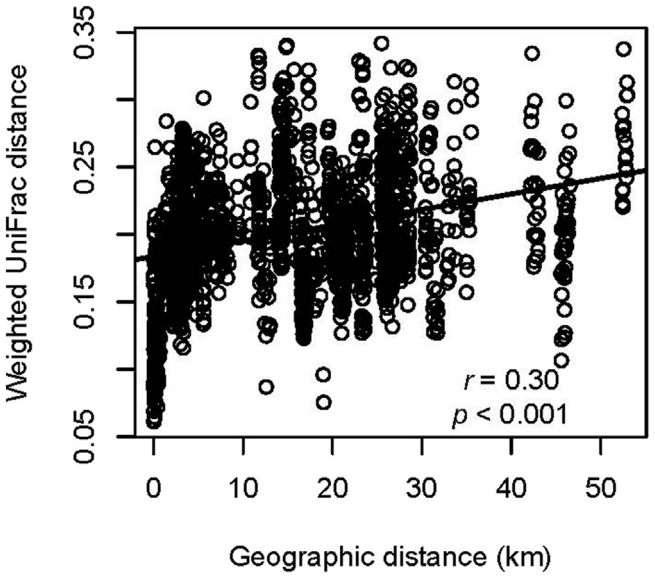

Fig. 3. Relationship between weighted UniFrac distance and geographic distance. Weighted Unifrac distance indicates phylogenetic β-diversity, and geographic distances are Euclidean distances in kilometers, considering dissimilarity in latitude, longitude, and elevation. The relationship was tested using Pearson's product moment correlation coefficient (r) with its probability estimate for significance (p). The line of best fit is from linear least-squares regression.

(Fig. 4). The AVAs each cluster separately and distinctively in NMDS space, with the exception of overlap with the heavily sampled Rutherford AVA and overlap between Howell Mountain and Calistoga AVAs in the space of the first and second axes. The Kruskal–Wallis rank sum tests for sample scores along each axis confirms that sample scores along the first ($P < 0.001$), second ($P = 0.005$), and fourth ($P = 0.022$) NMDS axes show separation with AVA (Fig. 4 and Table S9). Soil type, whether assessed by soil great group or soil textural class, showed statistically significant separation only on the second NMDS axis ($P = 0.023$ and 0.007, respectively, Table S9).

β-diversity patterns with spatial coordinates, climate, topography, and soil properties were also discernible in NMDS ordinations (not shown). These patterns were confirmed by lines of best fit from linear least-squares regression for each NMDS axis versus each numeric environmental feature (not shown) and Pearson's product moment correlation coefficients with their respective probabilities (Fig. 5 and Table S8). The strongest correlations for each axis are as follows: elevation with NMDS axis 1, pH with NMDS axis 2, and the C:N ratio of the <53 μm soil fraction, with NMDS axes 3 and 4 (Fig. 5). Other variables significantly correlated with NMDS axes included precipitation, silt and clay content, C:N ratios of soil fractions, and TC and TN contents in the 53–250 μm and <53 μm ($P < 0.05$) (Table S8).

The explanatory power of the numerical variables in representing the variation in β-diversity was assessed using BEST rankings of the input variables. When soil, topographic, and other location variables were included in the analysis, elevation (which was strongly correlated with several soil C and N pools, as introduced in Section 3.1) ranked the highest, so that the best combination of variables ($\rho = 0.44$) included elevation, clay content, TC in the <53 μm fraction, pH, the C:N ratios of the 53–250 μm and <53 μm fractions, longitude, and soil water content, in that order (Table S10). When only soil variables were included in the analysis, the resulting best combination of soil variables was strikingly different. Despite inclusion of twenty-two

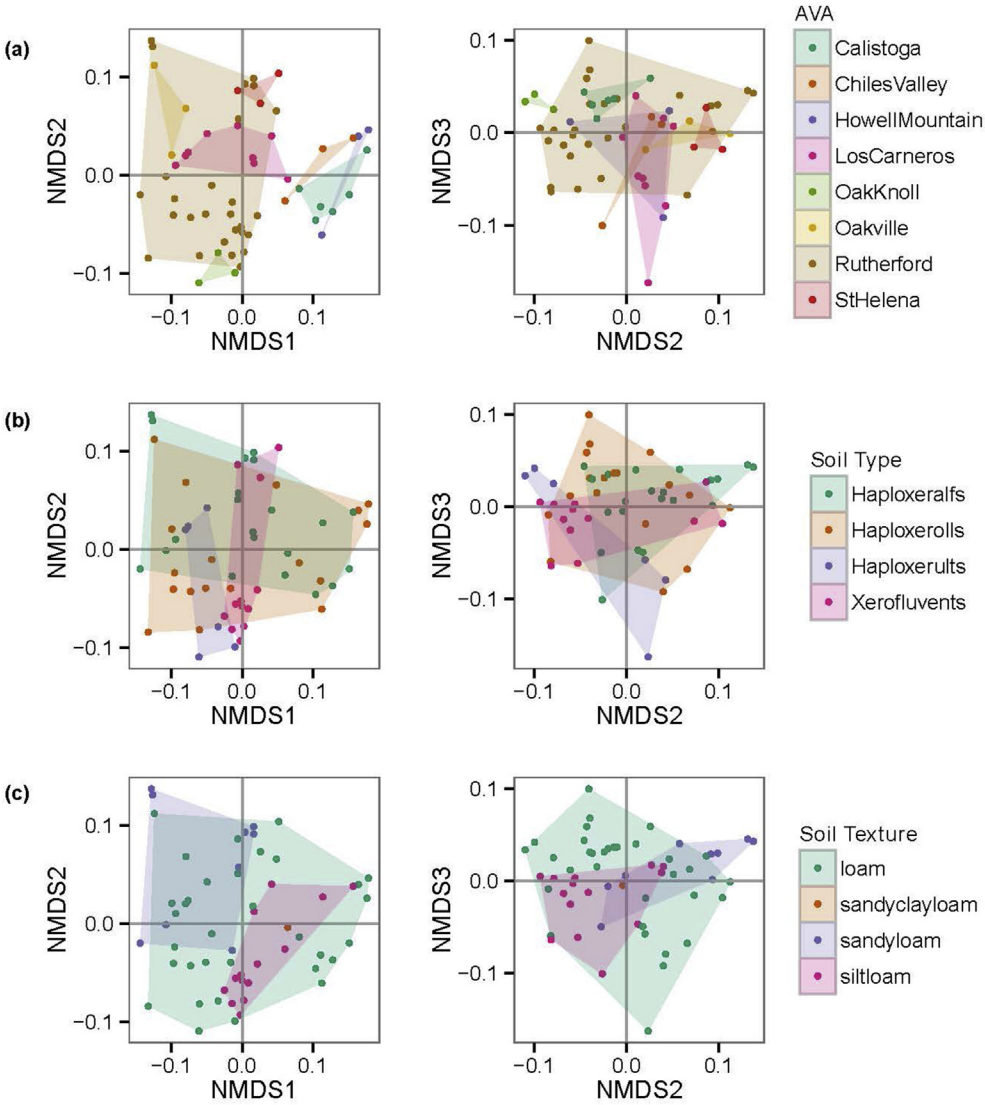

Fig. 4. Non-metric multidimensional scaling (NMDS) unconstrained ordinations of sites based on the weighted UniFrac distance matrix of pairwise phylogenetic dissimilarities between sites (β-diversity). Stress for this four-dimensional solution is 0.06. Lower dimensional solutions are not shown. The left panels show NMDS2 *vs.* NMDS1, the right panels show NMDS3 *vs.* NMDS2, while NMDS3 *vs.* NMDS1 and ordinations using NMDS4 are not shown. Each row is color-coded by a different grouping with convex hulls for the groups: American Viticultural Area (AVA) (*a*); soil great group (*b*); and soil textural class of the fine-earth fraction (*c*). Table S9 shows the mean score of each group along each NMDS axis and shows the Kruskal–Wallis rank sum test for each grouping and axis.

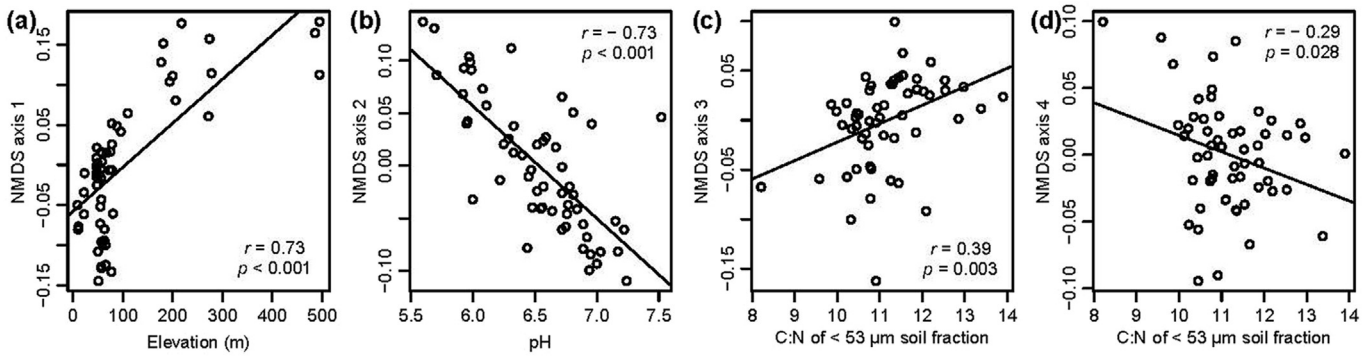

Fig. 5. Select correlations of NMDS scores with numeric variables. Lines of best fit are from linear least-squares regression. Product moment correlation coefficients (r) and their respective probability values (p) are from Pearson correlation. The strongest correlation for each NMDS axis is presented: NMDS1 *vs.* elevation (*a*); NMDS2 *vs.* pH (*b*); NMDS3 *vs.* C:N of <53 μm soil fraction (*c*); and NMDS4 *vs.* C:N of <53 μm soil fraction (*d*). See also Table S8 for additional relationships with each axis.

soil variables in multiple iterations of the analysis, some combination of soil TC and TN, pH, soil water content, TC and TN in the <53 μm fraction, and C:N ratios of the 53–250 μm and <53 μm fractions were consistently ranked the highest (highest ρ's from 0.26 to 0.38, Table S10). That is, as long as some combination of these aforementioned soil resource variables were included, the other soil variables, including clay content, ranked lower in importance in explaining the variation in β-diversity (Table S10). When soil variables were excluded, BEST revealed the following ranking from highest to lowest: elevation, longitude, latitude, precipitation, and slope, where elevation alone has the highest ρ of any of the combinations ($\rho = 0.43$, Table S10).

The ADONIS analysis also confirmed the significance of the relationships of β-diversity patterns with silt and clay, pH, PMN, DOC and TC and TN of soil fractions <250 μm and C:N ratios (Table 3 and Table S11). As ranked by the highest ADONIS R^2-values ($R^2 > 0.1$, $P < 0.002$), the following variables were the most important: vineyard, AVA, followed by elevation, then dissolved organic carbon, slope, soil texture, soil type, precipitation, pH, latitude, clay content, TN, and TC (Table 3 and Table S11).

Table 3
Results from permutational multivariate analysis of variance (ADONIS) for categorical and numeric variables' effects on bacterial β-diversity patterns based on the weighted UniFrac distance matrix.

Variable	df	Sum of Squares	Mean Square	Pseudo-F	R^2	P	
Vineyard	18	0.87	0.05	5.31	0.72	<0.001	***
Region	7	0.47	0.07	4.50	0.39	<0.001	***
Texture	3	0.14	0.05	2.36	0.12	<0.001	***
Soil Type	3	0.15	0.05	2.42	0.12	<0.001	***
GWC	1	0.06	0.06	2.71	0.05	0.021	.
pH	1	0.13	0.13	6.79	0.11	<0.001	***
TC	1	0.12	0.12	6.24	0.10	<0.001	***
DOC	1	0.16	0.16	8.46	0.13	<0.001	***
TC 250–1000 μm	1	0.02	0.02	1.03	0.02	0.369	ns
TC 53–250 μm	1	0.10	0.10	5.01	0.08	<0.001	***
TC < 53 μm	1	0.08	0.08	4.16	0.07	0.001	**
TN	1	0.13	0.13	6.53	0.11	<0.001	***
PMN	1	0.11	0.11	5.49	0.09	<0.001	***
TN 250–1000 μm	1	0.02	0.02	1.05	0.02	0.360	ns
TN 53–250 μm	1	0.09	0.09	4.48	0.08	0.002	**
TN < 53 μm	1	0.09	0.09	4.48	0.08	<0.001	***
C:N ratio	1	0.05	0.05	2.27	0.04	0.030	.
C:N 250–1000 μm	1	0.05	0.05	2.55	0.04	0.010	*
C:N 53–250 μm	1	0.10	0.10	4.83	0.08	<0.001	***
C:N < 53 μm	1	0.04	0.04	1.65	0.03	0.104	ns
Clay	1	0.13	0.13	6.48	0.11	<0.001	***
Silt	1	0.07	0.07	3.59	0.06	0.003	**
Silt + Clay	1	0.10	0.10	4.96	0.08	0.002	**
Latitude	1	0.14	0.14	7.13	0.11	<0.001	***
Longitude	1	0.11	0.11	5.35	0.09	<0.001	***
Elevation	1	0.20	0.20	10.79	0.16	<0.001	***
Slope	1	0.15	0.15	7.85	0.12	<0.001	***
Precipitation	1	0.15	0.15	7.62	0.12	<0.001	***

***p < 0.001, **p < 0.005, *p < 0.01, . p < 0.05, ns p ≥ 0.05.
Region: sub-appellation of Napa Valley American Viticultural Area (AVA); Texture: estimate of soil texture of the fine-earth fraction (National Resource Conservation Service soil survey data); Soil Type: soil suborder by US Soil Taxonomy (National Resource Conservation Service soil survey data); GWC: gravimetric water content (g water g^{-1} dry soil); pH: pH value in water (one-to-one); TC: total carbon (g kg^{-1} dry soil or g kg^{-1} dry fraction); DOC: dissolved organic carbon (mg kg^{-1} dry soil); TN: total nitrogen (g kg^{-1} soil or g kg^{-1} dry fraction); PMN: estimated potentially mineralizable nitrogen (mg kg^{-1} dry soil); C:N: carbon to nitrogen ratio; Clay: estimated percent clay content (National Resource Conservation Service soil survey data); Silt: estimated percent silt content (National Resource Conservation Service soil survey data); Precipitation: average annual precipitation (1981–2010).

Table 4
Location attributes' Pearson's product moment correlation coefficients or one-way ANOVA p-values for taxonomic groups.

Group	Pearson's correlation coefficients (*r*)				ANOVA probabilities (*P*)		
	Lat	Elev	Slope	Precip	AVA	Soil type	Soil texture
Proteobacteria	−0.39**	−0.58***	−0.52***	−0.41**	<0.001***	0.451	0.255
Alphaproteobacteria	0.22	0.34*	0.17	0.24	<0.001***	0.999	0.012*
Rhizobiales	0.57***	0.66***	0.60***	0.62***	<0.001***	0.100	<0.001***
Methylobacteriaceae	0.43***	0.30*	0.57***	0.51***	<0.001***	0.064	<0.001***
Methylobacterium	0.38**	0.14	0.31*	0.42**	0.008**	0.179	0.003**
Rhizobiaceae	0.06	0.07	0.23	0.07	0.001**	0.327	0.781
Agrobacterium	0.03	−0.07	0.12	0.04	0.049*	0.681	0.700
Rhizobium	0.05	0.15	0.27*	0.11	<0.001***	0.442	0.840
Rhodospirillales	0.36**	0.36**	0.25	0.38**	0.007**	0.126	<0.001***
Acetobacteraceae	0.35**	0.18	0.31*	0.44***	<0.001***	0.057	0.029*
Rhodospirillaceae	0.31*	0.32*	0.19	0.30*	0.046*	0.088	<0.001***
Skermanella	0.32*	0.25	0.07	0.27*	0.055	0.108	<0.001***
Sphingomonadales	−0.34**	−0.24	−0.38**	−0.38**	0.008**	0.006**	0.120
Sphingomonadaceae	−0.31*	−0.18	−0.30*	−0.33*	0.009**	0.035*	0.082
Sphingomonas	0.15	0.06	−0.06	0.20	0.016*	0.360	0.081
Betaproteobacteria	−0.38**	−0.49***	−0.46***	−0.37**	<0.001***	0.161	0.064
Burkholderiales	−0.21	−0.38**	−0.35**	−0.21	0.032*	0.262	0.023*
Alcaligenaceae	−0.22	−0.13	−0.06	−0.25	0.919	0.044*	0.813
Achromobacter	−0.34*	−0.15	−0.07	−0.38**	0.449	0.005**	0.943
Burkholderiaceae	0.12	−0.04	0.04	0.18	0.519	0.022*	<0.001***
Burkholderia	0.11	−0.05	0.04	0.17	0.570	0.016*	<0.001***
Comamonadaceae	−0.28*	−0.47***	−0.43***	−0.32*	0.001**	0.035*	0.018*
Variovorax	−0.20	−0.24	−0.22	−0.22	0.102	0.638	0.308
Oxalobacteraceae	−0.02	−0.05	−0.08	0.03	<0.001***	0.733	0.235
Gammaproteobacteria	−0.42**	−0.61***	−0.47***	−0.49***	<0.001***	0.051	0.019*
Alteromonadales	0.07	−0.20	−0.24	0.04	0.344	0.480	0.907
Alteromonadaceae	0.09	−0.18	−0.23	0.06	0.339	0.551	0.807
Cellvibrio	0.09	−0.17	−0.23	0.06	0.348	0.543	0.791
Enterobacteriales	−0.19	−0.17	−0.15	−0.19	0.421	0.053	0.194
Enterobacteriaceae	−0.19	−0.17	−0.15	−0.19	0.421	0.053	0.194
Erwinia	−0.16	−0.12	−0.06	−0.13	0.657	0.227	0.950
Pseudomonadales	−0.17	−0.33*	−0.27*	−0.18	0.048*	0.098	0.021*
Moraxellaceae	−0.01	−0.14	−0.11	−0.01	0.922	0.230	<0.001***
Acinetobacter	0.00	−0.07	−0.05	0.00	0.997	0.628	<0.001***
Pseudomonadaceae	−0.17	−0.33*	−0.27*	−0.18	0.063	0.141	0.025*
Pseudomonas	−0.17	−0.32*	−0.27*	−0.18	0.071	0.124	0.010*
Xanthomonadales	−0.54***	−0.66***	−0.45***	−0.66***	<0.001***	<0.001***	0.073
Xanthomonadaceae	−0.46***	−0.58***	−0.42**	−0.52***	0.001**	0.030*	0.102
Stenotrophomonas	−0.25	−0.20	−0.17	−0.28*	0.871	0.089	0.930
Xanthomonas	−0.18	−0.14	0.02	−0.23	0.589	0.028*	0.756
(Deltaproteobacteria)	−0.18	−0.48***	−0.32*	−0.21	<0.001***	0.422	0.029*
Actinobacteria	0.65***	0.69***	0.56***	0.63***	<0.001***	0.557	0.010*
Actinobacteria	0.64***	0.53***	0.48***	0.59***	<0.001***	0.668	0.002**
Actinomycetales	0.63***	0.53***	0.48***	0.59***	<0.001***	0.670	0.002**
Cellulomonadaceae	0.19	0.09	0.00	0.08	0.002**	0.060	0.058
Cellulomonas	0.23	0.13	0.00	0.13	0.001**	0.038*	0.049*
Kineosporiaceae	0.34**	0.43***	0.71***	0.38**	<0.001***	0.270	0.023*
Microbacteriaceae	0.32*	0.13	0.25	0.23	<0.001***	0.709	0.290
Micrococcaceae	−0.06	−0.10	−0.14	−0.09	0.598	<0.001***	0.270
Arthrobacter	−0.04	−0.06	−0.07	−0.04	0.889	0.154	0.733
Nocardiaceae	0.11	−0.16	−0.10	0.12	0.161	0.007**	0.006**
Rhodococcus	0.00	−0.08	0.07	0.04	0.599	0.175	0.003**
Streptomycetaceae	0.18	0.20	0.22	0.19	0.049*	0.139	0.115
Streptomyces	0.24	0.28*	0.39**	0.26	0.003**	0.681	0.139
Acidobacteria	−0.49***	−0.19	−0.19	−0.55***	<0.001***	0.001**	0.218
Bacteroidetes	−0.23	−0.56***	−0.46***	−0.28*	<0.001***	0.870	0.453
Bacteroidia	−0.35**	−0.11	0.04	−0.31*	0.214	0.472	0.972
Bacteroidales	−0.35**	−0.11	0.04	−0.31*	0.214	0.472	0.972
Flavobacteriia	−0.06	−0.39**	−0.42**	−0.07	<0.001***	0.320	0.093
Flavobacteriales	−0.06	−0.38**	−0.42**	−0.07	<0.001***	0.309	0.078
Flavobacteriaceae	−0.06	−0.38**	−0.41**	−0.06	<0.001***	0.282	0.079
Chryseobacterium	0.00	−0.13	−0.11	−0.03	0.973	0.191	0.105
Flavobacterium	−0.07	−0.39**	−0.43***	−0.06	<0.001***	0.190	0.241
Sphingobacteriia	−0.27*	−0.56***	−0.42**	−0.34**	<0.001***	0.493	0.596
Sphingobacteriales	−0.27*	−0.56***	−0.42**	−0.34**	<0.001***	0.493	0.596
Flexibacteraceae	−0.04	−0.17	−0.28*	−0.15	0.739	0.022*	0.883
Hymenobacter	0.22	0.35**	0.15	0.27*	0.021*	0.648	0.335
Planctomycetes	0.33*	0.22	0.25	0.40**	0.764	0.007**	0.055
Chloroflexi	0.06	0.33*	0.43***	0.17	0.029*	0.231	0.125

Table 4 (*continued*)

Group	Pearson's correlation coefficients (*r*)				ANOVA probabilities (*P*)		
	Lat	Elev	Slope	Precip	AVA	Soil type	Soil texture
Verrucomicrobia	−0.29*	−0.27*	0.11	−0.16	0.001**	0.013*	0.252
Gemmatimonadetes	−0.22	−0.07	−0.17	−0.18	<0.001***	0.512	0.013*
Firmicutes	−0.19	−0.11	−0.30*	−0.14	0.016*	0.007**	0.002**
Bacilli	−0.09	−0.09	−0.35**	−0.08	0.023*	0.005**	0.002**
Bacillales	0.06	−0.10	−0.52***	0.02	0.003**	0.012*	0.006**
Bacillaceae	0.09	−0.16	−0.52***	0.05	<0.001***	<0.001***	0.099
Bacillus	0.07	−0.17	−0.52***	0.03	<0.001***	<0.001***	0.107
Paenibacillaceae	0.07	−0.09	−0.27*	0.07	0.150	0.087	<0.001***
Paenibacillus	0.10	0.01	−0.25	0.09	0.151	0.028*	0.195
Planococcaceae	0.05	−0.02	−0.42**	−0.02	0.320	0.113	0.004**
Sporosarcina	0.04	−0.03	−0.42**	−0.03	0.419	0.105	0.002**
Exiguobacterales	−0.38**	0.01	0.37**	−0.26	<0.001***	0.056	<0.001***
Clostridia	−0.35**	−0.10	0.10	−0.26	0.134	0.227	0.869

***p < 0.001, **p < 0.01, *p < 0.05 (No corrections applied).
Phyla representing > 1% of sequences are listed in order of their relative abundance in our soil samples. Underlined phyla include organisms identified as inhabitants of the surface or interior of *Vitis vinifera* or in botrylized wine fermentations. With the exception of the class Deltaproteobacteria, all of the reported classes, orders, families, and genera include organisms identified as inhabitants of the surface or interior of *Vitis vinifera* or in botrylized wine fermentations. Deltaproteobacteria is reported for its high relative abundance in our soil samples.

3.4. Region, environmental attributes, and bacterial community composition as assessed by taxa abundances

Relative abundances of the dominant, or most highly abundant, phyla showed significant relationships with average annual precipitation, spatial coordinates and topography (elevation, latitude and slope), and soil properties (Pearson correlation, Tables 4 and 5). Soil C and N pools, notably whole soil TC, TN, DOC, and PMN, and TC and TN of the 53–250 μm soil fraction, were negatively correlated with the relative abundances of Proteobacteria (especially Beta- and Gamma-proteobacteria), Bacteroidetes, Gemmatimonadetes, and Firmicutes, while positively correlated with the relative abundances of Actinobacteria. TC and TN of the <53 μm soil fraction were positively correlated with the relative abundances of Actinobacteria and Planctomycetes and negatively correlated with the relative abundances of Proteobacteria (notably Betaproteobacteria) and Gemmatimonadetes. Proteobacteria, Alphaproteobacteria, and Gemmatimonadetes were more abundant in acidic soils, while Acidobacteria were more abundant in neutral soils.

Across the different AVAs of Napa Valley, eight of the nine dominant phyla showed differences in relative abundances: Proteobacteria (as well as the classes Alpha-, Beta-, and Gamma-, and Delta-proteobacteria each individually), Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia, Gemmatimonadetes, and Firmicutes (one-way ANOVA, $P < 0.05$, Table 4; canonical discriminant analysis, Fig. 6). Soil type by soil great group was related to the relative abundances of four of the nine dominant phyla: Acidobacteria, Planctomycetes, Verrucomicrobia, and Firmicutes (ANOVA $P < 0.01$, Table 4; CDA, Fig. 6). Soil texture also was related to the relative abundances of three of the nine dominant phyla: Actinobacteria, Gemmatimonadetes, and Firmicutes, but also Alpha-, Gamma-, and Delta-proteobacteria (ANOVA $P < 0.05$, Table 4; CDA, Fig. S2).

As a reminder, soil taxa were identified for further examination by inspection from other studies surveying taxa in grape musts or botrytized wine. Additional relationships between these soil taxa and soil and environmental variables were found (Tables 4 and 5). Among all four phyla, twenty-five families were identified, and 56%, 36%, and 44% of these families had significant differences among AVAs, soil type by soil great group, and soil texture, respectively. Of the twenty-five families, 48%, 28%, 28% and 36% had significant negative correlations with pH, TC, TN, and slope, respectively, and 20%, 24%, and 20% had significant positive correlations with PMN, C:N of the 53–250 μm soil fraction, and latitude, respectively. Sixty-eight percent of the twenty-five families were significantly correlated with at least one soil C or N pool. Of note, four of the five Alphaproteobacteria families and four of the six Actinobacteria families were associated with higher soil C or N pools, while Sphingomonadaceae (Alphaproteobacteria), Comamonadaceae (Betaproteobacteria), Pseudomonadaceae and Xanthomonadaceae (Gammaproteobacteria), Micrococcaceae and Nocardiaceae (Actinobacteria), Flavobacteriaceae (Bacteriodetes), and Bacillaceae and Paenibacillaceae (Firmicutes) were associated with lower soil C or N pools. This relationship to soil C or N pools also corresponds to relationships with latitude or slope in 70% of cases.

In summary, we found soil bacterial communities were structured with respect to AVA and soil properties (e.g. soil resources), demonstrating the complexity of soil microbial biogeography even at the landscape scale and within a single land-use type.

4. Discussion

4.1. Soil bacterial community composition and diversity

At a sequencing depth of 28,008 sequences per sample, the number of phylotypes, defined at 97% sequence similarity, still increased with increasing sequencing depth, suggesting the bacterial composition of the individual soils was not fully surveyed (Lauber et al., 2009; Liu et al., 2014). Nevertheless, numerous studies have demonstrated that differences among soil samples can be resolved with a sequence effort per sample much lower than we report here (e.g. Lauber et al., 2009; Bates et al., 2011; Liu et al., 2014). Since we were able to resolve differences with our sequencing depth, the sequencing depth of this study was deemed appropriate.

The four most abundant bacterial groups found across all samples were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes*, which correspond to the top four reported by Lauber et al. (2009) in natural ecosystems. The relative abundances of the nine dominant phyla found in our study more nearly correspond to those reported by Liu et al. (2014) for agricultural soils in China, planted to soybean, maize, or wheat. This suggests that the bacterial community compositions, even at a coarse taxonomic level (i.e., phyla), are altered by agriculture (Liu et al., 2014) and that agricultural bacterial communities of perennial and annual systems are more similar to each other than to the bacterial communities found

Table 5
Select soil physicochemical properties' Pearson's product moment correlation coefficients for taxonomic groups.

Group	Whole soil					53–250 μm soil fraction			<53 μm soil fraction	
	pH	TC	TN	DOC	PMN	TC	TN	C:N	TC	TN
Proteobacteria	−0.32*	−0.48***	−0.51***	−0.55***	−0.42**	−0.43***	−0.42**	−0.26	−0.26*	−0.28*
Alphaproteobacteria	−0.41**	0.01	0.01	0.02	0.11	−0.04	−0.06	0.17	0.20	0.18
Rhizobiales	−0.11	0.42**	0.43***	0.56***	0.48***	0.37**	0.35**	0.35**	0.50***	0.53***
Methylobacteriaceae	−0.38**	0.25	0.27*	0.31*	0.44***	0.21	0.19	0.13	0.38**	0.43***
Methylobacterium	−0.53***	0.05	0.04	0.06	0.19	0.05	0.01	0.23	0.22	0.20
Rhizobiaceae	−0.21	−0.01	−0.01	0.24	0.12	−0.06	−0.11	0.32*	0.01	0.03
Agrobacterium	−0.32*	0.03	0.02	0.03	0.17	−0.02	−0.04	0.14	0.10	0.12
Rhizobium	−0.32*	−0.03	0.00	0.13	0.16	−0.08	−0.11	0.14	−0.05	−0.04
Rhodospirillales	0.10	0.18	0.18	0.15	0.11	0.02	−0.02	0.31*	0.16	0.18
Acetobacteraceae	−0.60***	0.07	0.09	0.07	0.29*	0.01	0.01	−0.04	0.20	0.22
Rhodospirillaceae	0.22	0.19	0.18	0.15	0.07	0.02	−0.01	0.32*	0.13	0.15
Skermanella	0.29*	0.22	0.20	0.18	0.08	0.04	0.00	0.34*	0.17	0.19
Sphingomonadales	−0.40**	−0.34**	−0.35**	−0.40**	−0.24	−0.27*	−0.25	−0.23	−0.21	−0.26
Sphingomonadaceae	−0.46***	−0.34**	−0.34**	−0.41**	−0.22	−0.28*	−0.26*	−0.25	−0.21	−0.26
Sphingomonas	−0.40**	−0.16	−0.19	−0.10	−0.07	−0.15	−0.15	−0.05	0.12	0.06
Betaproteobacteria	−0.18	−0.43***	−0.43***	−0.62***	−0.36**	−0.33*	−0.31*	−0.37**	−0.36**	−0.38**
Burkholderiales	−0.36**	−0.36**	−0.37**	−0.56***	−0.25	−0.30*	−0.30*	−0.22	−0.25	−0.28*
Alcaligenaceae	−0.01	−0.06	−0.07	−0.08	−0.06	−0.04	−0.06	0.17	−0.11	−0.09
Achromobacter	−0.19	−0.13	−0.13	−0.10	−0.02	−0.10	−0.10	−0.01	−0.12	−0.11
Burkholderiaceae	−0.69***	−0.12	−0.15	−0.24	−0.02	−0.17	−0.18	0.00	0.10	0.04
Burkholderia	−0.68***	−0.14	−0.16	−0.25	−0.05	−0.18	−0.19	−0.02	0.07	0.01
Comamonadaceae	−0.02	−0.30*	−0.31*	−0.55***	−0.28*	−0.21	−0.20	−0.24	−0.32*	−0.35**
Variovorax	−0.31*	−0.29*	−0.28*	−0.26*	−0.22	−0.29*	−0.28*	−0.26*	−0.22	−0.21
Oxalobacteraceae	−0.49***	−0.23	−0.23	−0.26	−0.08	−0.23	−0.24	−0.10	−0.05	−0.06
Gammaproteobacteria	−0.13	−0.30*	−0.34**	−0.23	−0.34*	−0.30*	−0.30*	−0.14	−0.17	−0.19
Alteromonadales	−0.26	−0.14	−0.16	−0.15	−0.18	−0.17	−0.18	0.01	−0.06	−0.06
Alteromonadaceae	−0.32*	−0.11	−0.13	−0.13	−0.14	−0.15	−0.16	−0.03	−0.03	−0.03
Cellvibrio	−0.33*	−0.12	−0.14	−0.13	−0.15	−0.16	−0.16	−0.04	−0.03	−0.03
Enterobacteriales	−0.23	−0.08	−0.10	−0.14	−0.03	−0.20	−0.20	−0.03	0.00	−0.03
Enterobacteriaceae	−0.23	−0.08	−0.10	−0.14	−0.03	−0.20	−0.20	−0.03	0.00	−0.03
Erwinia	−0.13	−0.05	−0.06	−0.02	−0.02	−0.12	−0.13	0.06	0.03	0.01
Pseudomonadales	−0.28*	−0.34**	−0.36**	−0.20	−0.28*	−0.37**	−0.37**	−0.10	−0.17	−0.18
Moraxellaceae	−0.23	−0.17	−0.18	−0.14	−0.07	−0.17	−0.17	−0.02	−0.09	−0.12
Acinetobacter	−0.20	−0.09	−0.11	−0.03	−0.02	−0.09	−0.10	0.11	−0.03	−0.06
Pseudomonadaceae	−0.26	−0.33*	−0.35**	−0.19	−0.28*	−0.36**	−0.36**	−0.10	−0.16	−0.17
Pseudomonas	−0.30*	−0.32*	−0.34**	−0.22	−0.27*	−0.36**	−0.35**	−0.14	−0.16	−0.17
Xanthomonadales	0.14	−0.16	−0.21	−0.14	−0.29*	−0.08	−0.08	−0.08	−0.10	−0.13
Xanthomonadaceae	−0.36**	−0.27*	−0.30*	−0.36**	−0.28*	−0.24	−0.24	−0.24	−0.17	−0.22
Stenotrophomonas	−0.21	−0.19	−0.21	−0.22	−0.18	−0.17	−0.19	0.03	−0.20	−0.20
Xanthomonas	−0.14	0.02	0.02	−0.10	0.09	0.07	0.06	0.11	0.01	0.02
(Deltaproteobacteria)	0.21	−0.24	−0.26*	−0.17	−0.29*	−0.19	−0.18	−0.13	−0.17	−0.13
Actinobacteria	0.13	0.59***	0.59***	0.62***	0.55***	0.50***	0.46***	0.52***	0.43***	0.43***
Actinobacteria	0.05	0.49***	0.47***	0.53***	0.47***	0.44***	0.38**	0.55***	0.43***	0.43***
Actinomycetales	0.04	0.49***	0.47***	0.53***	0.47***	0.44***	0.38**	0.55***	0.43***	0.43***
Cellulomonadaceae	0.27*	0.39**	0.35**	0.23	0.28*	0.39**	0.35**	0.41**	0.31*	0.28*
Cellulomonas	0.24	0.42**	0.37**	0.22	0.30*	0.40**	0.36**	0.39**	0.34**	0.30*
Kineosporiaceae	−0.16	0.43***	0.43***	0.43***	0.59***	0.54***	0.51***	0.40**	0.35**	0.36**
Microbacteriaceae	0.13	0.41**	0.36**	0.43***	0.31*	0.43***	0.36**	0.61***	0.36**	0.34**
Micrococcaceae	0.22	−0.27*	−0.27*	0.00	−0.30*	−0.22	−0.23	0.09	−0.32*	−0.29*
Arthrobacter	0.22	−0.24	−0.24	−0.05	−0.24	−0.16	−0.17	0.17	−0.32*	−0.31*
Nocardiaceae	−0.37**	−0.20	−0.20	−0.05	−0.10	−0.29*	−0.29*	−0.22	0.00	0.02
Rhodococcus	−0.11	−0.14	−0.14	0.01	−0.07	−0.23	−0.23	−0.21	−0.05	0.00
Streptomycetaceae	−0.28*	0.10	0.09	0.21	0.16	0.02	0.00	0.30*	0.21	0.20
Streptomyces	−0.27*	0.19	0.19	0.29*	0.31*	0.16	0.13	0.37**	0.27*	0.28*
Acidobacteria	0.35**	0.05	0.06	−0.02	−0.03	0.20	0.25	−0.24	0.02	0.01
Bacteroidetes	0.03	−0.25	−0.28*	−0.31*	−0.31*	−0.28*	−0.28*	−0.19	−0.18	−0.18
Bacteroidia	−0.08	−0.11	−0.11	−0.12	−0.02	−0.07	−0.07	−0.03	−0.19	−0.18
Bacteroidales	−0.08	−0.11	−0.11	−0.12	−0.02	−0.07	−0.07	−0.03	−0.19	−0.18
Flavobacteriia	−0.25	−0.29*	−0.30*	−0.36**	−0.28*	−0.37**	−0.36**	−0.34*	−0.17	−0.18
Flavobacteriales	−0.26*	−0.30*	−0.31*	−0.37**	−0.28*	−0.38**	−0.37**	−0.35**	−0.17	−0.18
Flavobacteriaceae	−0.26*	−0.29*	−0.30*	−0.36**	−0.28*	−0.38**	−0.37**	−0.35**	−0.17	−0.18
Chryseobacterium	−0.41**	−0.10	−0.10	−0.09	0.02	−0.14	−0.14	−0.10	−0.02	−0.03
Flavobacterium	−0.19	−0.30*	−0.31*	−0.38**	−0.30*	−0.38**	−0.36**	−0.36**	−0.18	−0.19
Sphingobacteriia	0.16	−0.19	−0.23	−0.25	−0.28*	−0.19	−0.19	−0.10	−0.16	−0.16
Sphingobacteriales	0.16	−0.19	−0.23	−0.25	−0.28*	−0.19	−0.19	−0.10	−0.16	−0.16
Flexibacteraceae	0.30*	0.04	0.00	0.19	−0.09	0.00	−0.01	0.16	0.06	0.05
Hymenobacter	−0.14	0.05	0.01	0.01	0.09	0.05	0.03	0.20	0.09	0.03
Planctomycetes	−0.07	0.20	0.18	0.25	0.10	0.09	0.08	0.20	0.33*	0.32*
Chloroflexi	0.23	−0.02	0.04	0.25	0.02	0.02	0.01	0.12	−0.25	−0.19

Table 5 (*continued*)

Group	Whole soil					53–250 μm soil fraction			<53 μm soil fraction	
	pH	TC	TN	DOC	PMN	TC	TN	C:N	TC	TN
Verrucomicrobia	−0.09	−0.17	−0.14	−0.15	−0.11	−0.22	−0.22	−0.19	−0.19	−0.15
Gemmatimonadetes	−0.37**	−0.40**	−0.36**	−0.36**	−0.22	−0.29*	−0.24	−0.47***	−0.30*	−0.31*
Firmicutes	−0.22	−0.30*	−0.31*	−0.14	−0.28*	−0.41**	−0.39**	−0.22	−0.06	−0.08
Bacilli	−0.21	−0.27*	−0.29*	−0.10	−0.29*	−0.40**	−0.38**	−0.18	0.00	−0.03
Bacillales	−0.24	−0.26*	−0.30*	−0.15	−0.34*	−0.38**	−0.36**	−0.14	0.04	−0.01
Bacillaceae	0.11	−0.27*	−0.31*	−0.11	−0.43***	−0.39**	−0.38**	0.00	−0.02	−0.05
Bacillus	0.11	−0.28*	−0.32*	−0.11	−0.44***	−0.39**	−0.39**	0.00	−0.04	−0.06
Paenibacillaceae	−0.48***	−0.24	−0.26	−0.16	−0.19	−0.28*	−0.27*	−0.13	0.01	−0.04
Paenibacillus	−0.10	−0.17	−0.17	0.01	−0.20	−0.23	−0.21	−0.11	0.01	−0.02
Planococcaceae	−0.30*	−0.15	−0.17	−0.12	−0.20	−0.26	−0.24	−0.12	0.06	0.00
Sporosarcina	−0.30*	−0.14	−0.17	−0.12	−0.19	−0.26	−0.24	−0.11	0.06	0.00
Exiguobacterales	0.08	−0.03	0.00	0.12	0.11	−0.03	−0.03	−0.10	−0.07	−0.02
Clostridia	−0.11	−0.16	−0.14	−0.16	−0.05	−0.15	−0.13	−0.20	−0.21	−0.19

***p < 0.001, **p < 0.01, *p < 0.05 (No corrections applied).
The selected soil physicochemical properties shown were chosen based on their Pearson's correlations ($r \geq 0.35$ and $p < 0.05$) with the first or second NMDS axes (Table S8). Phyla representing >1% of sequences are listed in order of their relative abundance in our soil samples. Underlined phyla include organisms identified as inhabitants of the surface or interior of *Vitis vinifera* or in botrylized wine fermentations. With the exception of the class Deltaproteobacteria, all of the reported classes, orders, families, and genera include organisms identified as inhabitants of the surface or interior of *Vitis vinifera* or in botrylized wine fermentations. Deltaproteobacteria is reported for its high relative abundance in our soil samples.

in natural ecosystems. However, based on phospholipid fatty acid (PLFA) markers, Drenovsky et al. (2010) reported that microbial communities in perennial agricultural soils are more similar to those of natural ecosystems than annual agricultural soils. PLFA accounts for the composition of only the living microbial community, and includes markers for both fungi and bacteria, which may help explain these different outcomes. Together, these findings identify a gap in knowledge, suggesting that soil microbial communities of various agricultural management schemes should be further examined and compared to those found in natural ecosystems.

4.2. Geographic distribution and vineyard influence soil bacterial communities

Even though the present study was at the landscape scale (<53 km), there was still a significant increase in phylogenetic distance with increasing geographic distance, as is found for studies at the continental scale (e.g. Liu et al., 2014). This suggests dispersal limitation is still important, even at this local scale (Green and Bohannan, 2006; Martiny et al., 2006; Fierer, 2008). The principal of dispersal limitation predicts soils more distant should have greater phylogenetic dissimilarities, while soils closer together should have greater phylogenetic similarities (Fierer, 2008). Thus, as expected at this smaller scale, the phylogenetic dissimilarities between sites were smaller than those observed at the cross-country scale by Liu et al. (2014). This could be due to a smaller range in environmental heterogeneity, particularly climatic conditions. However, the overlap in geographic distances (from 24.50 km to 52.97 km) between the two studies, but not in phylogenetic distances, suggests that some other conditions are contributing to the greater similarity between bacterial communities of Napa Valley vineyard soils. Such conditions include the greater sequencing depth in the present study, where more comprehensive surveys yield a higher degree of overlap in phylotypes present at finer levels of taxonomic resolution between pairs of soils (Lauber et al., 2009), leading to smaller phylogenetic distances like those observed here. Another contributing factor could be the long history of perennial agriculture, throughout much of Napa Valley. In some areas, grapevines have been grown for more than 170 years. Cultivation has been shown to have a long-lasting impact on soil microbial community structure (Buckley and Schmidt, 2001), and a long history in one land-use type might assert consistent long-term selective pressures across the landscape.

Within our study, the ranges of phylogenetic distances between sites within the same vineyard (0.07–0.32, median = 0.18) and phylogenetic distances between sites across vineyards (0.06–0.34, median = 0.20) are similar. This suggests intra-vineyard diversity is often just as great as, and in some cases greater than, the inter-vineyard diversity, which could be attributed to variation in geographic distances of within- and between-vineyard samples. That is, the geographic distances between samples within vineyards (19 m–270 m) and between vineyards (112 m–52.97 km) have overlap and inter- and intra-vineyard samples have similar ranges of phylogenetic distances. Rather than precluding the effect of vineyard and vineyard management, the similar intra- and inter-vineyard diversity reflect that adjacent vineyards can receive similar management and sometimes despite their proximity, receive divergent management. For a discussion of vineyard management impacts on patterns of soil microbial community structure, see Burns (2014). Despite our observed similarity in intra- and inter-vineyard diversity, there is still a strong effect (Table 3) of the vineyard on the soil microbial community structure. This may result from a combination of: close geographic distances, similar management practices, and similar climatic, topographic, and edaphic properties within each vineyard.

4.3. Soil microbiota, AVA, and soil properties are intimately linked

Drivers of soil microbial community structure are theorized to be geographic distances, environmental heterogeneity (climate and soil properties), and factors that influence environmental heterogeneity (topography and land use or management) (Baas Becking, 1934; De Wit and Bouvier, 2006; Martiny et al., 2006; Fierer, 2008). Thus, we hypothesized that AVAs, which are geographically delimited and qualitatively associated with certain climatic, topographic, and edaphic features, might be associated with certain soil bacterial community structures. In accord with our hypothesis, AVA was strongly related to the soil microbial communities (Table 3 and Table S11). Soil physicochemical properties varied not only with respect to soil type and texture but also with respect to AVA (Table 2 and Tables S3–S4). Therefore, we suggest that differences in soil properties mediate

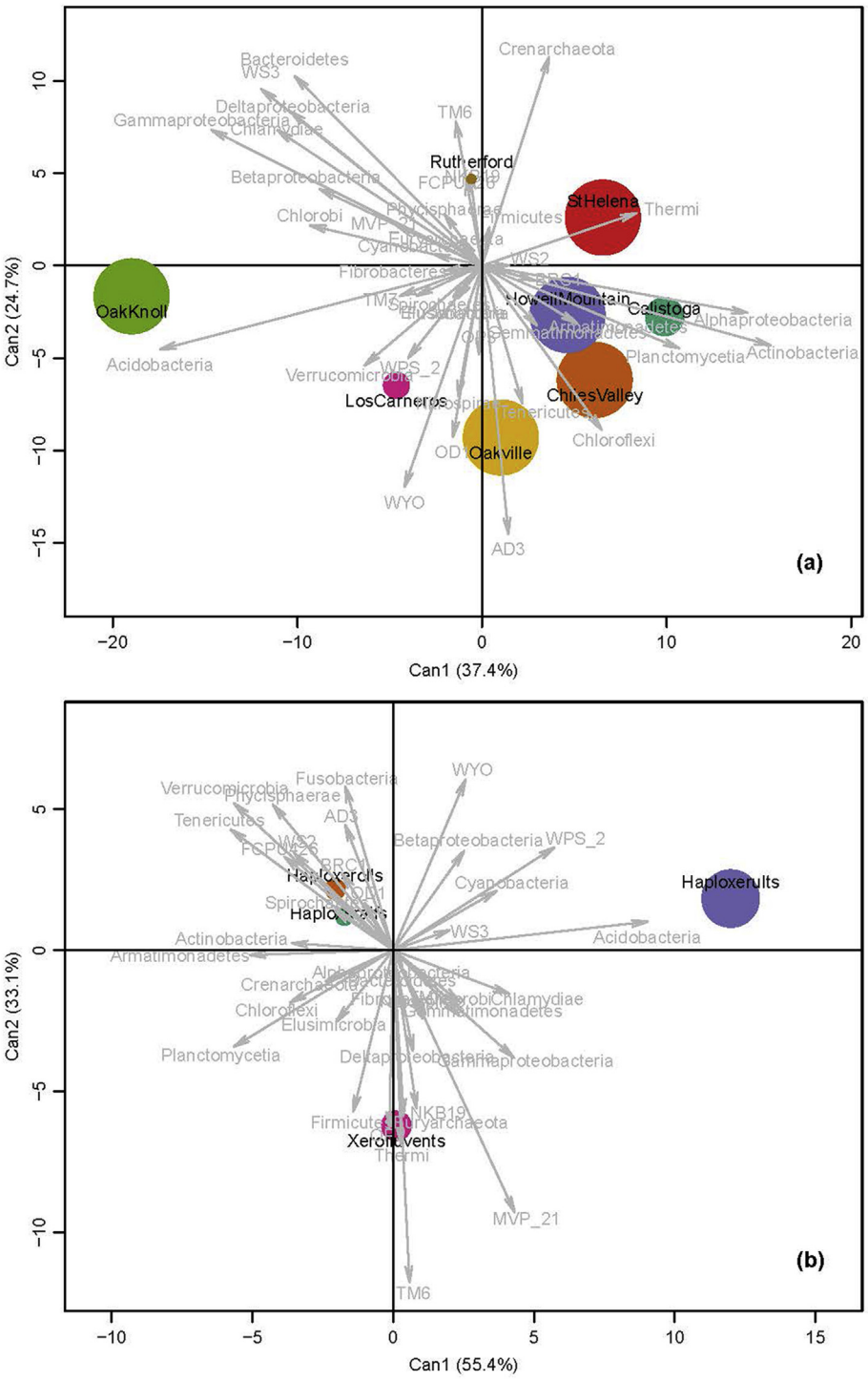

Fig. 6. Canonical discriminant analysis (CDA) of relative taxa abundances by American Viticultural Area (AVA) (*a*) and soil great group (*b*). Circles represent 95% confidence, and no overlap signifies significant differences between groups. Taxonomic groups included in the analysis are phyla or the most highly abundant classes of individual phylum when class-level data was more revealing. Only taxa present across a minimum of 15% of sites were included in the analysis to limit the number of zeros, which would otherwise impair the analysis. See also Tables 4 and 5, Tables S2 and S7, and Fig. S2.

the differences in soil microbial community structure found with these attributes.

We also expect that the relationship of soil microbiota to AVA is related to the differing climate, topography, and soil among the different regions (Tables 1 and 2). Latitude, topography, and average annual precipitation were relatively unimportant in determining the richness and α-diversity of the soil microbial community (Table S8), but they were important in explaining the β-diversity between sites (Table 3). Elevation was positively correlated to latitude, slope, and precipitation, and all of these were positively correlated to soil C and N pools (Table S5). These correlations reflect the topography of Napa Valley, which grades from flat valley floors to rolling hills to steep mountains, and has the highest elevations at higher latitudes. Additionally, the positive relationship between

precipitation, elevation, and SOM across many mountainous regions is commonly known, although the aspect of slope is also important in determining precipitation (Jenny, 1941; Dahlgren et al., 1997). Vineyard management (i.e. tillage), soil rock content, and time since conversion from natural ecosystem to agriculture likely affects the SOM content at higher elevations in Napa Valley, as vineyards at the higher elevations tend to be no-till due to rocky conditions and are usually younger compared to the long-established vineyards of the valley floor, and therefore likely to have differential net C loss (Carlisle et al., 2006). We suggest that elevation was such a strong predictor of soil bacterial community structure because of its integrative connection to soil resources through its positive correlations across so many C and N pools. Thus, we consider the significance of elevation in structuring soil bacterial communities as an indicator of the influence of soil resources, rather than as a direct driver (see Section 3.3.2 and Table S10).

In general, with increases in latitude, elevation, slope, or precipitation, *Actinobacteria*, *Chloroflexi*, and *Planctomycetia* (the dominant class of *Planctomycetes*) relative abundances increased while *Proteobacteria*, *Acidobacteria*, and *Bacteroidetes* relative abundances decreased. Again, we propose that these relationships with specific relative taxa abundances is from the integrative consideration of multiple C and N pools by each latitude, elevation, slope, and precipitation (Table S5), and not a pure effect of any of these geographic variables.

4.4. Soil resources shape soil bacterial communities

Gradients in other indicators of soil chemistry and resources, which are reflected in the correlations between soil properties and geographic variables, underlie soil microbial community structure. Soil pH is consistently associated with distinctions in soil microbial community structure here (Fig. 5, Table 3, and Tables S8, S10, S11) and in other work (Fierer and Jackson, 2006; Lauber et al., 2009). Also consistent with previous work (Drenovsky et al., 2004; Cookson et al., 2006), soil resources that were strongly indicative of soil microbial community structure included PMN, DOC, and TN and TC of whole soil (Table 3 and Table S8). Likewise, TC and TN of the 53–250 μm and <53 μm fractions were correlated with individual taxon abundances and overall soil microbial community structure, although the associations of the fractions were slightly lower than whole soil TC and TN and the fractions were not strongly related to α-diversity or richness (Tables 3–5 and Tables S8 and S10). The relationship with TC and TN of the <53 μm fraction was in accord with our hypothesis that the TC and TN of the <53 μm fraction would be highly related to soil microbial community structure, which we anticipated because of microbial community associations with fine stabilized SOM (<53 μm, clay-or-silt-sized) (Kögel-Knabner, 2002; Grandy and Neff, 2008; Plaza et al., 2013). Distinctions in microbial community structure may occur with respect to soil texture and organic matter because clay and silt content in combination with soil organic matter influence porosity and aggregate formation and stability (Hillel, 1982). Soil porosity, texture and aggregates impact the microclimate experienced by soil microbes, including temperature fluctuations, gas concentrations and exchange, and soil water potential and holding capacity, and can even influence the movement of microorganisms and soil nutrients (Abu-Ashour et al., 1994; Hillel, 1982). In our study, soil texture class, and clay and silt contents were related to soil microbial community structure, as revealed by ADONIS, BEST (clay only), and NMDS (Table 3 and Tables S8 and S10). Clay content, but not silt content, also had positive correlations to α-diversity and richness (Table S8). However, taxa related to clay or silt content were not the same as taxa related to TC or TN of the <53 μm fraction (Tables 4 and 5). Thus, our findings suggest that fine SOM may be derived from many different taxa or consortia of soil microorganisms rather than a few key taxa (Kögel-Knabner, 2002; Grandy and Neff, 2008; Plaza et al., 2013), and that this function is redundant within the microbial community and not dominated by one group over another.

Soil resources also affected the community composition through changes in relative abundances of particular taxa. With increased availability of soil resources, *Proteobacteria* relative abundances decreased while *Actinobacteria* relative abundances increased (Section 3.4). Similarly, most taxa (e.g. *Proteobacteria*) that were related to the C:N ratio of one or more of the soil fractions ($P < 0.05$, Section 3.4) had greater relative abundances with soil fractions of lower C:N ratios and relatively lower C and N pools in whole soil. The one exception was *Actinobacteria*, which had higher relative abundances with higher PMN, C:N in the 53–250 μm soil fraction and other C and N pools (Section 3.4). Together, this suggests that the composition of the *Actinobacteria* population was more copiotrophic, while the composition of the *Proteobacteria* population was more oligotrophic, although both are diverse bacterial groups containing a wide variety of life history strategies (Fierer et al., 2007). Contrastingly, Fierer et al. (2007) found *Betaproteobacteria* to be better classified as copiotrophic, while *Alphaproteobacteria* and *Actinobacteria* could not be assigned to either category. These contrasting outcomes suggest that the life history strategies of organisms in these taxonomic groupings should be studied further.

4.5. Soil-borne microorganisms and wine terroir

Previous studies show that microbial communities from vineyard wine grape musts also differ with AVA, both among appellations of Northern California and among the sub-appellations of the Napa Valley AVA (Bokulich et al., 2014, Unpublished results). Recent work also has shown that endophytic bacteria colonize grapevine berries (Compant et al., 2011; Bokulich et al., 2014) and that most endophytic bacteria are soil-borne (Hardoim et al., 2008; Compant et al., 2010, 2012; Mitter et al., 2013). Zarraonaindia et al. (2015) recently demonstrated that a 'native' microbial fingerprint, relatively constant even with respect to other edaphic factors, exists between the microorganisms of the wine grape must, grapevine vegetative structures and fruit, and the microorganisms of the vine row soil. Such findings show strong support for the soil as a reservoir for endophytes and epiphytes that colonize grapevine structures. Martins et al. (2013) submit that dispersal of vineyard soil microorganisms occurs through rain splash, dust from tillage and air movement, suggesting that the soil microbial communities in the alley in this study can serve as a soil reservoir for grapevine microbial communities. Distinct from these other studies, the current findings demonstrate that AVA, the components that legally define it, and soil properties indicative of soil functions can all structure soil-borne microbial communities. Together, our findings and those from others discussed here highlight the possibility that biogeographical patterns of soil-borne microorganisms could influence wine quality, both directly and indirectly. However, it is currently unclear whether patterns in soil microbial community structure only correlate with edaphic, climatic, and regional factors, which exert an overwhelming effect on regional wine qualities (*terroir*), or if these microbiota substantially alter plant growth, health, and fruit development. Further work is also needed to identify to what extent functional redundancy exists among these regional microbiota, and hence whether the soil microbiota selected in one vineyard or region exert unique effects on plant development and, indirectly, wine properties—or whether

microbiota selected under different conditions fill the same niches for plant–microbe interaction.

4.6. Conclusions

The soil bacterial communities of this study were structured with respect to soil properties (e.g. soil resources) and AVA, demonstrating the complexity of soil microbial biogeography even at the landscape scale and within a single land-use type. As ranked by the highest ADONIS R^2-values ($R^2 > 0.1$, $P < 0.002$), the following variables were the most important: AVA, followed by elevation, then dissolved organic carbon, slope, soil texture, soil type, precipitation, pH, latitude, clay content, TN, and TC. We reason that AVA, climate, and topography each affect soil microbial communities through their suite of impacts on soil properties, but more studies are required to understand the mechanisms behind these drivers. The identification of distinctive microbial communities with AVA lends support to the idea that soil microbial communities of wine grapes form a key in linking wine *terroir* back to the biotic components of the soil environment. By providing a fundamental background on landscape-scale soil microbial community biogeography in vineyards, this work has opened the door for future research on soil-borne microorganism-related wine *terroir*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.09.002.

References

Abu-Ashour, J., Joy, D.M., Lee, H., Whiteley, H.R., Zelin, S., 1994. Transport of microorganisms through soil. Water, Air and Soil Pollution, 75, pp. 141–158.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.
Baas Becking, L.G.M., 1934. Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon, Den Haag, Netherlands, 263 pp.
Barata, A., Malfeito-Ferreira, M., Loureiro, V., 2012. The microbial ecology of wine grape berries. International Journal of Food Microbiology 153, 243–259.
Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. The ISME Journal 5, 908–917.
Bokulich, N.A., Joseph, C.M., Allen, G., Benson, A.K., Mills, D.A., 2012. Next-generation sequencing reveals significant bacterial diversity of botrytized wine. PLOS ONE 7, e36357.
Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nature Methods 10, 57–59.
Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proceedings of the National Academy of Sciences Unites States America 111, E139–E148.
Buckley, D.H., Schmidt, T.M., 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology 42, 11–21.
Burns, K., 2014. Bacterial Communities of Vineyard Soils as Revealed by a 16S rDNA Sequencing Survey (Unpublished Thesis).
Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010b. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences Unites States America 108, 4516–4522.
Carlisle, E.A., Steenwerth, K.L., Smart, D.R., 2006. Effects of land use on soil respiration: conversion of oak woodlands to vineyards. Journal of Environmental Quality 35, 1396–1404.
Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11, 265–270.
Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92, 205–219.
Compant, S., Clement, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biology & Biochemistry 42, 669–678.
Compant, S., Mitter, B., Colli-Mull, J.G., Gangl, H., Sessitsch, A., 2011. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microbial Ecology 62, 188–197.
Compant, S., Sessitsch, A., Mathieu, F., 2012. The 125th anniversary of the first postulation of the soil origin of endophytic bacteria – a tribute to M.L.V. Galippe. Plant and Soil 356, 299–301.
Cookson, W.R., Marschner, P., Clark, I.M., Milton, N., Smirk, M.N., Murphy, D.V., Osman, M., Stockdale, E.A., Hirsch, P.R., 2006. The influence of season, agricultural management, and soil properties on gross nitrogen transformations and bacterial community structure. Australian Journal of Soil Research 44, 453–465.
Dahlgren, R.A., Boettinger, J.L., Huntington, G.L., Amundson, R.G., 1997. Soil development along an elevational transect in the western Sierra Nevada, California. Geoderma 78, 207–236.
De Wit, R., Bouvier, T., 2006. 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Environmental Microbiology 8, 755–758.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72, 5069–5072.
Drenovsky, R.E., Steenwerth, K.L., Jackson, L.E., Scow, K.M., 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecology and Biogeography 19, 27–39.
Drenovsky, R.E., Vo, D., Graham, K.J., Scow, K.M., 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microbial Ecology 48, 424–430.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation 61, 1–10.
Fierer, N., 2008. Microbial biogeography: patterns in microbial diversity across space and time. In: Zengler, K. (Ed.), Accessing Uncultivated Microorganisms: From the Environment to Organisms and Genomes and Back. American Society for Microbiology, Washington, DC, USA, pp. 95–115.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences United States of America 103, 626–631.
Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–1017.
Fox, J., Friendly, M., Monette, G., 2013. heplots: Visualizing Tests in Multivariate Linear Models. R package version 1.0-11. http://CRAN.R-project.org/package=heplots.
Friendly, M., 2007. HE plots for multivariate linear models. Journal of Computational and Graphical Statistics 16, 421–444.
Friendly, M., Fox, J., 2013. candisc: Visualizing Generalized Canonical Discriminant and Canonical Correlation Analysis. R package version 0.6-5. http://CRAN.R-project.org/package=candisc.
Garbeva, P., van Veen, J.A., van Elsas, J.D., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology 42, 243–270.
Gittins, R., 2011. Canonical Analysis: a Review With Applications in Ecology. Springer, Berlin, Germany.

Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of The Total Environment 404, 297–307.
Green, J., Bohannan, B.J.M., 2006. Spatial scaling of microbial biodiversity. TRENDS in Ecology and Evolution 21, 501–507.
Hardoim, P.R., van Overbeek, L.S., van Elsas, J.D., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology 16, 463–471.
Hillel, D., 1982. Introduction to Soil Physics. Academic Press, San Diego, USA, p. 364.
Hogberg, M.N., Hogberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601.
Hollander, A.D., 2012. Using GRASS and R for landscape regionalization through PAM cluster analysis. OSGeo Journal 10, 26–31.
Jenny, H., 1941. Factors of Soil Formation: a System of Quantitative Pedology. McGraw-Hill Book Company, Inc., New York, NY, USA, 281 pp.
Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology & Biochemistry 38, 991–999.
Kaufman, L., Rousseeuw, P.J., 1990. Finding Groups in Data: an Introduction to Cluster Analysis. Wiley, New York, NY, USA, 342 pp.
Kempers, A.J., Kok, C.J., 1989. Re-examination of the determination of ammonium as the indophenol blue complex using salicylate. Analytica Chimica Acta 221, 147–155.
Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology & Biochemistry 34, 139–162.
Kuzyakov, Y., Blagodatskaya, E., Blagodatsky, S., 2009. Comments on the paper by Kemmitt et al. (2008) 'Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective' [Soil Biology & Biochemistry 40, 61–73]: The biology of the Regulatory Gate. Soil Biology & Biochemistry 41, 435–439.
Lambert, G., Kashiwagi, J.H., 1978. Soil Survey of Napa County, California. United States Department of Agriculture, Soil Conservation Service, in cooperation with University of California Agricultural Experiment Station.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111–5120.
Lee, J., Laca, E.A., van Kessel, C., Rolston, D.E., Hopmans, J.W., Six, J., 2009. Tillage effects on spatiotemporal variability of particulate organic matter. Applied and Environmental Soil Science 2009, 1–14.
Leveau, J.H.J., Tech, J.J., 2011. Grapevine microbiomics: bacterial diversity on grape leaves and berries revealed by high-throughput sequence analysis of 16S rRNA amplicons. Acta Horticulturae 905, 31–42.
Liu, J., Sui, Y., Yu, Z., Shi, Y., Chu, H., Jin, J., Liu, X., Wang, G., 2014. High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China. Soil Biology & Biochemistry 70, 113–122.
Liu, Z., Lozupone, C., Hamady, M., Bushman, F.D., Knight, R., 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. Nucleic Acids Research 35, e120.
Lombard, N., Prestat, E., van Elsas, J.D., Simonet, P., 2011. Soil-specific limitations for acccess and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology 78, 31–49.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71, 8228–8235.
Madsen, E.L., 2005. Identifying microorganisms responsible for ecologically significant biogeochemical processes. Nature Reviews Microbiology 3, 439–446.
Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M.-L., Soulas, G., Masneuf-Pomarède, I., 2013. Characterization of epiphytic bacterial communities from grapes, leaves, bark and soil of grapevine plants grown, and their relations. PLOS ONE 8, e73013.
Martins, G., Miot-Sertier, C., Lauga, B., Claisse, O., Lonvaud-Funel, A., Soulas, G., Masneuf-Pomarede, I., 2012. Grape berry bacterial microbiota: impact of the ripening process and the farming system. International Journal of Food Microbiology 158, 93–100.
Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A.L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4, 102–112.
McCune, B., Grace, J.B., 2006. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, OR, USA.
McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6, 610–618.
Minchin, P.R., 1987. An evaluation of the relative robustness of techniques for ecological ordination. Vegetatio 69, 89–107.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62–71.
Mitter, B., Brader, G., Afzal, M., Compant, S., Naveed, M., Trognitz, F., Sessitsch, A., 2013. Advances in elucidating beneficial interactions between plants, soil, and bacteria. In: Sparks, D.L. (Ed.), Advances in Agronomy, vol. 121. Elsevier Academic Press, Inc., San Diego, CA, USA, pp. 381–445.
Nelson, G.C., Bennett, E., Berhe, A.A., Cassman, K.G., DeFries, R., Dietz, T., Dobson, A., Dobermann, A., Janetos, A., Levy, M., Marco, D., Nakicenovic, N., O'Neill, B., Norgaard, R., Petschel-Held, G., Ojima, D., Pingali, P., Watson, R., Zurek, M., 2005. Drivers of change in ecosystem condition and services. In: Carpenter, S.R., Pingali, P.L., Bennett, E.M., Zurek, M.B. (Eds.), Ecosystems and Human Well-Being: Scenarios: Findings of the Scenarios Working Group (Millennium Ecosystem Assessment Series). Island Press, Washington, DC, USA, pp. 173–222.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan.
Pella, E., 1990a. Elemental organic analysis. Part 1. Historical developments. American Laboratory 22 (2), 116–125.
Pella, E., 1990b. Elemental organic analysis. Part 2. State of the art. American Laboratory 22 (12), 28–32.
Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology & Biochemistry 57, 124–134.
Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLOS ONE 5, e9490.
PRISM Climate Group, Oregon State University, 2012. 1981–2010 Annual Average Raster Precip and Temp. U.S. Department of Agriculture, Natural Resources Conservation Service, National Geospatial Management Center, Fort Worth, Texas. http://datagateway.nrcs.usda.gov/.
R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Riches, D., Porter, I.J., Oliver, D.P., Bramley, R.G.V., Rawnsley, B., Edwards, J., White, R.E., 2013. Review: soil biological properties as indicators of soil quality in Australian viticulture. Australian Journal of Grape and Wine Research 19, 311–323.
Rousk, J., Jones, D.L., 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts. Soil Biology & Biochemistry 42, 2331–2335.
RStudio, 2013. RStudio: Integrated Development Environment for R (Version 0.98.501). RStudio, Boston, MA, USA.
Soil Survey Staff, Natural Resources Conservation Service (NRCS), United States Department of Agriculture (USDA), 2010-2013. Soil Survey Geographic Database (SSURGO). USDA NRCS National Geospatial Management Center, Fort Worth, Texas. http://datagateway.nrcs.usda.gov/.
Soon, Y.K., Haq, A., Arshad, M.A., 2007. Sensitivity of nitrogen mineralization indicators to crop and soil management. Communications in Soil Science and Plant Analysis 38, 2029–2043.
Towill, Inc, 2003. Napa County Watershed and Valley 2002 Ortho Data. County of Napa ITS Department, Napa, CA, USA. http://gis.napa.ca.gov/giscatalog/catalog.asp.
van Leeuwen, C., Friant, P., Chone, X., Tregoat, O., Koundouras, S., Dubourdieu, D., 2004. Influence of climate, soil, and cultivar on terroir. American Journal of Enology and Viticulture 55, 207–217.
Viers, J.H., Williams, J.N., Nicholas, K.A., Barbosa, O., Kotzé, I., Spence, L., Webb, L.B., Merenlender, A., Reynolds, M., 2013. Vinecology: pairing wine with nature. Conservation Letters 6, 287–299.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261–5267.
Waring, S.A., Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as index of nitrogen availability. Nature 201, 951–952.
Werner, J.J., Koren, O., Hugenholtz, P., DeSantis, T.Z., Walters, W.A., Caporaso, J.G., Angenent, L.T., Knight, R., Ley, R.E., 2012. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. The ISME Journal 6, 94–103.
Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N.A., Mills, D.A., Martin, G., Taghavi, S., van der Lelie, D., Gilbert, J.A., 2015. The soil microbiome influences grapevine-associated microbiota. mBio 6 e02527–02514.